Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 1 **JAN.–FEB. 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *PF* content to Executive Secretariat
USP–NF, 12601 Twinbrook Pkwy.,
Rockville, MD 20852

execsec@usp.org

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

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

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Fax: (301) 816-8148.

STANDARDS DEVELOPMENT

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum* (*PF*), for the development of official pharmaceutical standards.

USP publishes *Pharmacopeial Forum* (*PF*) bimonthly and provides interested parties an opportunity to review and comment on the new or revised standards of the *United States Pharmacopeia* and the *National Formulary* (*USP–NF*).

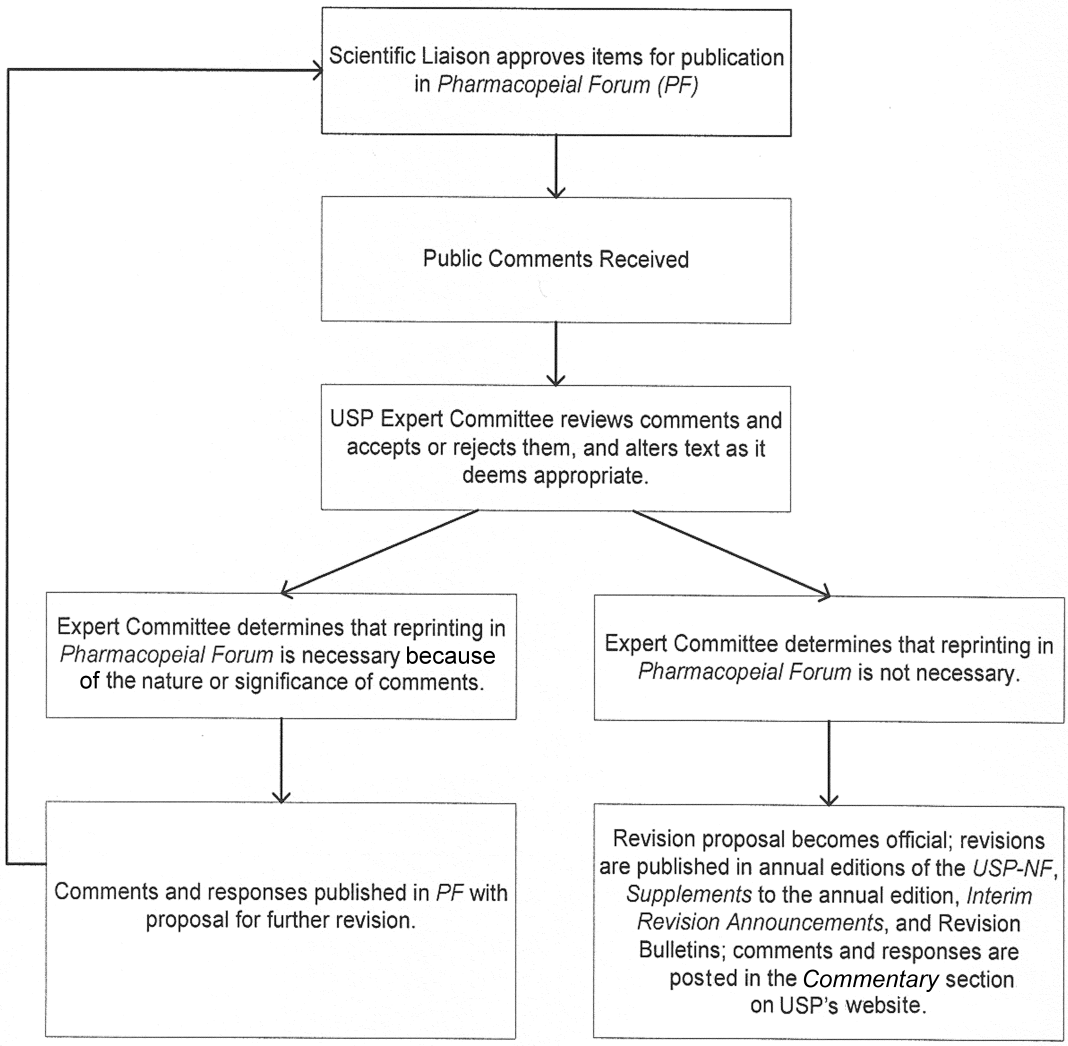
PF includes the following:

1. Potential revisions—entirely new standards, revision ideas, and drafts not yet targeted for official adoption (*Pharmacopeial Previews*)
2. Proposed revisions—new or revised standards targeted for official adoption (*In-Process Revision*)
3. Adopted revisions—new or revised standards that become official and binding before the publication of the next *USP–NF* or *Supplement* (*Interim Revision Announcement*)

USP welcomes comments and data on potential, proposed, or official standards. Comments, along with USP's responses, will be published either in *PF Briefings*, the *Commentary* section of *PF*, the *Commentary* section of *Supplements* to *USP–NF*, or the *Commentary* section of *USP–NF*.

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

This section provides descriptions of the various parts of *PF*. It also includes *Committee Designations* and the *Staff Directory*.

The content of the different sections of *PF* is briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
Pharmacopeial Previews Early ideas for revisions	•BRIEFING: Scientific rationale for potential inclusion or change. May include other information useful to the analyst such as the brand name of the column used in developing the proposed procedure and the USP scientific staff liaison who handled the issue. •Potential revisions not yet targeted for official adoption that require a longer public review and comment process because of issues such as: — the controversial nature of an item; — the application of new technologies that require further study; and — articles produced by multiple sources.	Review drafts and provide comments to the appropriate staff liaison cited in the *Briefing* preceding each *Preview.*
In-Process Revision Revisions targeted for adoption	•BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst such as the brand name of the column used in developing the proposed procedure and the USP scientific staff liaison who handled the issue. •New and revised standards that have been approved for publication by the appropriate USP Committee when it is considering whether to advance standards to official status (see *Standards Development*). New or revised text is marked with symbols (■■ or ●● or ▲▲) to specify the tentative earliest date on which the revision would be officially adopted.	Review material and send comments promptly to USP staff liaison (see the *Staff Directory*) identified at the end of the briefing accompanying each item. For general inquiries or in cases where a particular liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-816-8373. Comment deadlines are found at the end of the *Policies and Announcements* section.
Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to harmonize internationally	•BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. The designated stage of harmonization. The stage determines whether an item appears under *Pharmacopeial Previews* or under *In-Process Revision,* both separate sections of *Harmonization.* •For *In-Process Revision,* new or revised text is marked with symbols (■■) to specify the tentative earliest date on which the revision would be officially adopted.	Review material and provide comments to the appropriate staff liaison cited in the *Briefing* preceding each *Preview* or *In-Process Revision.* Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopeia, with a copy to USP, for a given article. The addresses for the European and Japanese Pharmacopoeias are as follows: EP Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp

Proposed and Adopted Revisions to the *USP–NF* (*Continued*)

Section	Content	How Readers Can Respond
Interim Revision Announcement Adopted standards	Standards that have been adopted and will become officially binding on the specified date. Effective date is specified in the section's introductory page or within parentheses following a particular item. New or revised text is set off by the symbols •.	Review to see if affected by any of the changes. Note effective date when standards become official and ensure compliance.
Pending Proposals	In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *PF* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in either the *USP–NF*, its supplements, or an *IRA*. Those items that have not yet been adopted are still pending.	Review items to track pending proposals.
Canceled Proposals	Canceled proposals are items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption into the *USP–NF*.	Review items to track canceled proposals.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including scientific liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Reagents Used in USP–NF and Pharmacopeial Forum
Update of chromatographic reagents based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* (*Continued*)

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Darrell Abernethy, M.D., Ph.D., Chief Science Officer	dra@usp.org	(301) 816-8184	
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT) B&B Vaccines and Virology (BB-VV)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, Senior Scientific Associate	kxb@usp.org	(301) 816-8462	Food Ingredients (FI)
Colleen Brennan, R.Ph. Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	USP–NF Spanish Edition
Larry N. Callahan, Ph.D., Senior Scientist	lnc@usp.org	(301) 816-8385	B&B Proteins and Polysaccharides (BB PP)
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D.	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards
Anthony DeStefano, Vice President, General Chapters	ajd@usp.org	(301) 998-6303	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	Document Disclosure
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE)
James Griffiths, Ph.D., Vice President, Food and Dietary Supplement Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	USP Spanish Edition; General Chapters (GC), Pharmaceutical Waters
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products— Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexy Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B: Proteins and Polysaccharides (BB PP)
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Vice President, RSO & Applied Compendial Research	psl@usp.org	(301) 816-8337	Reference Standards
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Marcia D. Mayfield, Project Manager, Monograph Procurement	mxm@usp.org	(301) 816-8358	New Monograph Acquisition
Kate Meringolo, Manager, Publications Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Senior Scientific Associate	jm@usp.org	(301)816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Scientist	ktm@usp.org	(301)816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Director, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements Non-Botanicals
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D., Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D., Manager, Pharmacy Compounding EC	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, Director, Excipients and Food Ingredients	cxs@usp.org	(301) 816-8262	Food Ingredients (FI)
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB-VV)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Senior Scientist	dwv@usp.org	(301) 998-6828	Small Molecules Monographs
Mary ''Jeanie'' Waddell	msw@usp.org	(301) 816-8124	Small Molecules Monographs
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	rstech@usp.org	(301) 816-8129	USP Reference Standards
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section includes information about general scientific and policy issues that may have an impact on *USP–NF* standards and processes and announcements about issues being considered by USP. This section also includes publication and comment schedules.

USP IMPLEMENTS GUIDELINE ON THE USE OF ACCELERATED PROCESSES FOR REVISIONS TO THE USP–NF. Sections 9.02 and 9.03 of the Rules and Procedures of the 2005–2010 Council of Experts specify various processes (Accelerated Processes) that can be used to make revisions to the *USP–NF* official more quickly than through USP's standard process (Standard Process). USP, in collaboration with the Compendial Process Improvement Project Team, has posted the Guideline on the Use of Accelerated Processes for Revision to *USP–NF*, with an official date of January 1, 2009. The Guideline is available at www.usp.org.

USP's Standard Process calls for publication of a proposed revision in the *Pharmacopeial Forum (PF)* for a 90-day notice and comment period and, after the revision is approved by the relevant USP Expert Committee, publication in the next *USP–NF* or *Supplement*, as applicable. Accelerated Processes, which include *Errata, Interim Revision Announcements*, and *Revision Bulletins*, do not always require notice and comment, and allow for a revision to become official prior to the next *USP–NF* or *Supplement*.

USP has issued this Guideline to delineate the circumstances under which these Accelerated Processes are utilized. The Guideline includes a Decision Tree that specifies the criteria that are applied by USP in considering whether an Accelerated Process is appropriate rather than USP's Standard Process. This Guideline also addresses the use of delayed official dates for revisions made through the Standard Process, where such revisions have broad industry impact and require additional time to implement.

If you have any questions, contact Susan de Mars, J.D., USP Chief Legal Officer (sdm@usp.org).

USP REDESIGNS *PHARMACOPEIAL FORUM.* USP has begun implementing a redesigned *Pharmacopeial Forum (PF)* as part of an initiative that will result in a completely online, freely available *PF* beginning in January 2011. The redesigned *PF* will no longer be used to publish new official text [*Interim Revision Announcements (IRAs)* and *Errata*], which will instead be posted on USP's website. During 2009, USP will begin posting approved *IRAs, Revision Bulletins*, and *Errata* on www.usp.org in a new ''official text'' section, but also continue to print approved *IRAs* and *Errata* in the *PF*.

USP also will be modifying *PF* beginning with *PF* 35(2) [March–April 2009] to display *In-Process Revision* monographs in the new monograph format that will become effective in *USP 33*.

USP encourages stakeholders to sign up for the Compendial Notices e-mail service in order to receive notices of new postings to the USP website. To sign up for this service, go to http://www.usp.org/support/products/uspNewsletters Request.html?promo=compendial.

If you have any questions about the redesign process, contact Susan de Mars, J.D., USP Chief Legal Officer (sdm@usp.org). If you have any questions about *Pharmacopeial Forum* subscriptions, please contact USP Customer Service [custsvc@usp.org or 301-881-0666, 1-800-227-8772 (U.S. and Canada), or 00-800-4875-5555 (Europe)].

USP ISSUES CALL FOR CANDIDATES FOR 2010–2015 COUNCIL OF EXPERTS, ITS EXPERT COMMITTEES, AND ITS ADVISORY PANELS. In accordance with the Bylaws of the USP Convention, USP is issuing a *Call for Candidates* for the 2010–2015 Council of Experts. The 2010–2015 Council of Experts includes Expert Committees in the areas of Nomenclature, Small Molecules, Biologics and Biotechnology, Excipients, General Chapters, Reference Standards, Compounding, Food Ingredients, and Dietary Supplements. In the 2010–2015 cycle, USP is expanding the number of Advisory Panels that report to Expert Committees. These Expert Committees and Advisory Panels align with the new USP Strategic Plan, which focuses on expanding and enhancing USP's core compendial and standards-setting activities.

The ability to add Advisory Panels according to the needs of USP introduces flexibility and scalability into USP's activities. USP plans to continue to attract global base experts, and therefore encourages any qualified individual to apply. Importantly, this approach also enables USP to closely align USP's documentary and reference standards activities for a more efficient standards-setting process.

Specific Expert Committees and Advisory Panels for which USP is seeking candidates are listed at USP's nominations website (www.usp.org/nominate). The deadline for applications for the Council of Experts (Expert Committee Chair) is **December 31, 2009**. The deadline for applications for Expert Committee members is **May 14, 2010**. Recruitment for Advisory Panels members will be continuous.

For further information, contact Angela G. Long, Vice President, Volunteer and Organizational Affairs (agl@usp.org or nominate@usp.org).

USP REVISES GENERAL NOTICES IN *USP 32–NF 27*. The Council of Experts Executive Committee has approved revisions to the *USP–NF* General Notices, which appear in *USP 32–NF 27*. The first draft of revisions to the *USP–NF* General Notices was posted on USP's website in May of 2007, and made available for public comment until August 31, 2007. Based on comments received on this draft, a second draft was prepared, which then appeared in *Pharmacopeial Forum (PF)* 34(1). This second draft was accompanied by an Explanatory Note, which summarized the comments received and the changes made by USP in response to those comments.

Comments also were received on the second draft, which were carefully considered, and incorporated as deemed appropriate by the Council of Experts Executive Committee into the final

version. Summaries of many of the comments received and USP's responses to the comments, including requests for additional input, are included in a Commentary document.

There also are additional topics for the future that require continuing dialog. These topics are not included in the version effective in *USP 32–NF 27*, but may be considered in the future. These topics include, but are not limited to the following:

- Consider that all compendia published by USP (*USP–NF, Food Chemicals Codex, Pending and Non-US monographs*) be covered by the General Notices because the General Notices include the terms essential for understanding any monograph;
- Consider standardizing language among the compendia to make it easier for users who refer both to *FCC* and *USP–NF*;
- Clarify the distinction between General Chapters intended to be enforceable and those intended to be informational.

The General Notices, Explanatory Note, Commentary, and a Questions and Answers document are posted on USP's website. Please direct questions regarding the General Notices revision to generalnotices@usp.org.

***PHARMACOPEIAL FORUM* PUBLIC REVIEW AND COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 34(6)	February 15, 2009	*USP 33–NF 28*	November 2009	May 1, 2010
PF 35(1)	April 15, 2009			
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section; these revisions are also incorporated in the upcoming *Supplement* or book. The official publication in which an *IRA* is incorporated will depend upon publication deadlines. See table below. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and, therefore, contains all official text up to and including the contents of the latest *Supplement.* The table below outlines the publications and their release and official dates, and the book or supplement which supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
USP 32–NF 27	November 1, 2008	May 1, 2009	*1st Supplement to USP 32–NF 27*
IRA [*PF* 35(1)]	January 1, 2009	February 1, 2009	*2nd Supplement to USP 32–NF 27*
1st Supplement to USP 32–NF 27	February 1, 2009	August 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*

PRIORITY NEW MONOGRAPH ITEMS. In this edition of *PF* the Priority New Monograph Items list has been relocated from the *Pharmacopeial Forum* to http://www.usp.org/USPNF/submitMonograph/newMon.html. It will return to *PF* in 35(2).

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:

- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- New adopted (official) revisions to the *USP–NF* that become effective before the effective date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The effective date for these revisions is stated on the next page.)

Readers should review this section to determine if they are affected by any of the changes.

Symbols—Proposed revisions are shown with language proposed for deletion or replacement crossed off. New text (if any) follows, and is enclosed in symbols and set off from the current official text by a paragraph break and by larger type (print edition only), as shown in the examples below:

— •new text• if slated for an *Interim Revision Announcement;*
— ▲new text▲ if slated for *USP 33–NF 28*; and
— ■new text■ if slated for a *Supplement* to *USP–NF*

The same symbols not set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as •• or ■■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (USP32) indicates that the proposed revision is slated for the *Second Supplement* to *USP 32,* and ▲USP33 and ▲NF28 indicate that the revisions are proposed for *USP 33* and *NF 28*, respectively.

Errata—At the end of the *Interim Revision Announcement* section is a list of errata and corrections to *USP 31–NF 26*. The page number indicates where the item is found in *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement.

INTERIM REVISION ANNOUNCEMENT to *USP 31* and to *NF 26*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

John W. Mauger, *Chair*
USP Board of Trustees

Roger L. Williams, M.D., *Executive Vice President, CEO,* and *Chairman, USP Council of Experts*

Darrell R. Abernethy, M.D., Ph.D., *Chief Science Officer*
William F. Koch, Ph.D., FACB, *Chief Metrology Officer*

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All inquiries and comments regarding *USP 31* text and *NF 26* text should be addressed to the Executive Secretariat, *USP–NF*, 12601 Twinbrook Parkway, Rockville, MD 20852 (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 31* or *NF 26* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard.

USP Flumazenil Related Compound C RS (March 1, 2008)
USP Diluted Isosorbide Mononitrate RS (May 1, 2008)
USP Mibolerone RS (November 1, 2008)
USP Narasin RS (November 1, 2008)
USP Near IR System Suitability RS (May 1, 2008)
USP Powdered St. John's Wort Extract RS (November 1, 2008)
USP Pyrethrum Extract RS (March 1, 2008)
USP Δ^9-Tetrahydrocannabinol RS (May 1, 2008)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 31* or *NF 26* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of October 8, 2008. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at http://www.uspcatalog.com.

USP 23-Epi-26-deoxyactein RS
USP Acarbose RS
USP Acarbose System Suitability Mixture RS
USP Actein RS
USP (*S*)-Adenosyl-L-homocysteine RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Fludeoxyglucose Related Compound B RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Alpha Lipoic Acid RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Propylene Glycol Dilaurate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin RS
USP Valrubicin Related Compound A RS
USP Vasopressin RS

GENERAL CHAPTERS

General Information

⟨1052⟩ BIOTECHNOLOGY-DERIVED ARTICLES—AMINO ACID ANALYSIS

Change to read:

This chapter provides guidance and procedures used for characterization of biotechnology-derived articles by amino acid analysis. This chapter is harmonized with the corresponding chapter in *JP* and *EP*. •Portions of the chapter that are not harmonized with the other two pharmacopeias are marked by the symbol ◆. The footnote below is in the *USP* but is not in the *EP* or *JP*.•| Other characterization tests, also harmonized, are shown in *Biotechnology-Derived Articles—Capillary Electrophoresis* ⟨1053⟩, *Biotechnology-Derived Articles—Isoelectric Focusing* ⟨1054⟩, *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩, *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩, and *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.

Change to read:

REFERENCE STANDARD MATERIAL

Acceptable amino acid standards are commercially available* for amino acid analysis and typically consist of an aqueous mixture of amino acids. When determining amino acid composition, protein or peptide standards are analyzed with the test material as a control to demonstrate the integrity of the entire procedure. Highly purified bovine serum albumin has been used as a protein standard for this purpose.

Change to read:

PROTEIN HYDROLYSIS

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. The glassware used for hydrolysis must be very clean to avoid erroneous results. Glove powders and fingerprints on hydrolysis tubes may cause contamination. To clean glass hydrolysis tubes, boil tubes for 1 hour in 1 N hydrochloric acid or soak tubes in concentrated nitric acid or in a mixture of concentrated hydrochloric acid and concentrated nitric acid (1 : 1). Clean hydrolysis tubes are rinsed with high-purity water followed by a rinse with HPLC grade methanol, dried overnight in an oven, and stored covered until use. Alternatively, pyrolysis of clean glassware at 500° for 4 hours may be used to eliminate contamination from hydrolysis tubes. Adequate disposable laboratory material can also be used.

Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. The acid hydrolysis technique can contribute to the variation of the analysis due to complete or partial destruction of several amino acids. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Application of adequate vacuum (less than 200 µm of mercury or 26.7 Pa) or introduction of an inert gas (argon) in the headspace of the reaction vessel can reduce the level of oxidative destruction. In peptide bonds involving isoleucine and valine, the amido bonds of Ile-Ile, Val-Val, Ile-Val, and Val-Ile are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively. The loss of tryptophan, asparagine, and glutamine during an acid hydrolysis limits quantitation to 17 amino acids. Some of the hydrolysis techniques described are used to address these concerns. Some of the hydrolysis techniques described (i.e., *Methods 4–11*) may cause modifications to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis.

A time-course study (i.e., amino acid analysis at acid hydrolysis times of 24, 48, and 72 hours) is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. By plotting the observed concentration of labile amino acids (i.e., serine and threonine) versus hydrolysis time, the line can be extrapolated to the origin to determine the starting concentration of these amino acids. Time-course hydrolysis studies are also used with amino acids that are slow to cleave (e.g., isoleucine and valine). During the hydrolysis time course, the analyst will observe a plateau in these residues. The level of this plateau is taken as the residue concentration. If the hydrolysis time is too long, the residue concentration of the sample will begin to decrease, indicating destruction by the hydrolysis conditions.

An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. The amino acid in free form may not completely represent the rate of destruction of labile amino acids within a peptide or protein during the hydrolysis. This is especially true for peptide bonds that are slow to cleave (e.g., Ile-Val bonds). However, this technique will allow the analyst to account for some residue destruction. Microwave acid hydrolysis has been used and is rapid but it requires special equipment as well as special precautions. The optimal conditions for microwave hydrolysis must be investigated for each individual protein/peptide sample. The microwave hydrolysis technique typically requires only a few minutes, but even a deviation of 1 minute may give inadequate results (e.g., incomplete hydrolysis or destruction of labile amino acids). Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins. [NOTE—During initial analyses of an unknown protein, experiments with various hydrolysis time and temperature conditions are conducted to determine the optimal conditions.]

Method 1

Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis Solution: 6 N hydrochloric acid containing 0.1% to 1.0% of phenol.

Procedure—

Liquid Phase Hydrolysis—Place the protein or peptide sample in a hydrolysis tube, and dry. [NOTE—The sample is dried so that water in the sample will not dilute the acid used for the hydrolysis.] Add 200 µL of *Hydrolysis Solution* per 500 µg of lyophilized protein. Freeze the sample tube in a dry ice–acetone bath, and flame seal in vacuum. Samples are typically hydrolyzed at 110° for 24 hours in vacuum or inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g., 48 and 72 hours) are investigated if there is a concern that the protein is not completely hydrolyzed.

* •◆•|Suitable standards may be obtained from NIST (Gaithersburg, MD), Beckman Instruments (Fullerton, CA), Sigma Chemical (St. Louis, MO), Pierce (Rockford, IL), or Agilent (Palo Alto, CA).•◆•|

Interim Revision Announcement

Vapor Phase Hydrolysis—This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimized by using vapor phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of *Hydrolysis Solution.* The *Hydrolysis Solution* does not come in contact with the test sample. Apply an inert atmosphere or vacuum (less than 200 µm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110° for a 24-hour hydrolysis time. Acid vapor hydrolyzes the dried sample. Any condensation of the acid in the sample vials is minimized. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

Method 2

Tryptophan oxidation during hydrolysis is decreased by using mercaptoethanesulfonic acid (MESA) as the reducing acid.

Hydrolysis Solution: 2.5 M MESA solution.

Vapor Phase Hydrolysis—About 1 to 100 µg of the protein/peptide under test is dried in a hydrolysis tube. The hydrolysis tube is placed in a larger tube with about 200 µL of the *Hydrolysis Solution.* The larger tube is sealed in vacuum (about 50 µm of mercury or 6.7 Pa) to vaporize the *Hydrolysis Solution*. The hydrolysis tube is heated to between 170° to 185° for about 12.5 minutes. After hydrolysis, the hydrolysis tube is dried in vacuum for 15 minutes to remove the residual acid.

Method 3

Tryptophan oxidation during hydrolysis is prevented by using thioglycolic acid (TGA) as the reducing acid.

Hydrolysis Solution: a solution containing 7 M hydrochloric acid, 10% of trifluoroacetic acid, 20% of thioglycolic acid, and 1% of phenol.

Vapor Phase Hydrolysis—About 10 to 50 µg of the protein/peptide under test is dried in a sample tube. The sample tube is placed in a larger tube with about 200 µL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 µm of mercury or 6.7 Pa) to vaporize the TGA. The sample tube is heated to 166° for about 15 to 30 minutes. After hydrolysis, the sample tube is dried in vacuum for 5 minutes to remove the residual acid. Recovery of tryptophan by this method may be dependent on the amount of sample present.

Method 4

Cysteine-cystine and methionine oxidation is performed with performic acid before the protein hydrolysis.

Oxidation Solution—The performic acid is prepared fresh by mixing formic acid and 30 percent hydrogen peroxide (9 : 1), and incubating at room temperature for 1 hour.

Procedure—The protein/peptide sample is dissolved in 20 µL of formic acid, and heated at 50° for 5 minutes; then 100 µL of the *Oxidation Solution* is added. In this reaction, cysteine is converted to cysteic acid and methionine is converted to methionine sulfone. The oxidation is allowed to proceed for 10 to 30 minutes. The excess reagent is removed from the sample in a vacuum centrifuge. This technique may cause modifications to tyrosine residues in the presence of halides. The oxidized protein can then be acid hydrolyzed using *Method 1* or *Method 2*.

Method 5

Cysteine-cystine oxidation is accomplished during the liquid phase hydrolysis with sodium azide.

Hydrolysis Solution: 6 N hydrochloric acid containing 0.2% of phenol, to which sodium azide is added to obtain a final concentration of 0.2% (w/v). The added phenol prevents halogenation of tyrosine.

Liquid Phase Hydrolysis—The protein/peptide hydrolysis is conducted at about 110° for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the sodium azide present in the *Hydrolysis Solution*. This technique allows better tyrosine recovery than *Method 4,* but it is not quantitative for methionine. Methionine is converted to a mixture of the parent methionine and its two oxidative products, methionine sulfoxide and methionine sulfone.

Method 6

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

Hydrolysis Solution: 6 N hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

Vapor Phase Hydrolysis—The protein/peptide hydrolysis is conducted at about 110° for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the *Hydrolysis Solution*. As an approach to limit variability and to compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine. The response factors from protein/peptide hydrolysates are typically about 30% lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

Method 7

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

Reducing Solution—Transfer 83.3 µL of pyridine, 16.7 µL of 4-vinylpyridine, 16.7 µL of tributylphosphine, and 83.3 µL of water to a suitable container, and mix.

Procedure—Add the protein/peptide (between 1 and 100 µg) to a hydrolysis tube, and place in a larger tube. Transfer the *Reducing Solution* to the large tube, seal in vacuum (about 50 µm of mercury or 6.7 Pa), and incubate at about 100° for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylation reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine to improve accuracy in the pyridylethyl-cysteine recovery. Longer incubation times for the pyridylethylation reaction can cause modifications to the α-amino terminal group and the ϵ-amino group of lysine in the protein.

Method 8

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase pyridylethylation reaction.

Stock Solutions—Prepare and filter three solutions: 1 M Tris hydrochloride (pH 8.5) containing 4 mM edetate disodium (*Stock Solution 1*), 8 M guanidine hydrochloride (*Stock Solution 2*), and 10% of 2-mercaptoethanol in water (*Stock Solution 3*).

Reducing Solution—Prepare a mixture of *Stock Solution 2* and *Stock Solution 1* (3 : 1) to obtain a buffered solution of 6 M guanidine hydrochloride in 0.25 M Tris hydrochloride.

Procedure—Dissolve about 10 µg of the test sample in 50 µL of the *Reducing Solution*, and add about 2.5 µL of *Stock Solution 3*. Store under nitrogen or argon for 2 hours at room temperature in the dark. To achieve the pyridylethylation reaction, add about 2 µL of 4-vinylpyridine to the protein solution, and incubate for an additional 2 hours at room temperature in the dark. The protein/peptide is desalted by collecting the protein/peptide fraction from a reverse-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis.

Method 9

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase carboxymethylation reaction.

Stock Solutions—Prepare as directed for *Method 8*.

Carboxymethylation Solution—Prepare a solution containing 100 mg of iodoacetamide per mL of alcohol.

Buffer Solution—Use the *Reducing Solution,* prepared as directed for *Method 8*.

Procedure—Dissolve the test sample in 50 µL of the *Buffer Solution*, and add about 2.5 µL of *Stock Solution 3*. Store under nitrogen or argon for 2 hours at room temperature in the dark. Add the *Carboxymethylation Solution* in a 1.5 fold ratio per total theoretical content of thiols, and incubate for an additional 30 minutes at room temperature in the dark. [NOTE—If the thiol content of the protein is unknown, then add 5 µL of 100 mM iodoacetamide for every 20 nmol of protein present.] The reaction is stopped by adding excess of 2-mercaptoethanol. The protein/peptide is desalted by collecting the protein/peptide fraction from a reverse-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis. The *S*-carboxyamidomethylcysteine formed will be converted to *S*-carboxymethyl-cysteine during acid hydrolysis.

Method 10

Cysteine-cystine is reacted with dithiodiglycolic acid or dithiodipropionic acid to produce a mixed disulfide. [NOTE—The choice of dithiodiglycolic acid or dithiodipropionic acid depends on the required resolution of the amino acid analysis method.]

Reducing Solution: a solution containing 10 mg of dithiodiglycolic acid (or dithiodipropionic acid) per mL of 0.2 M sodium hydroxide.

Procedure—Transfer about 20 µg of the test sample to a hydrolysis tube, and add 5 µL of the *Reducing Solution*. Add 10 µL of isopropyl alcohol, and then remove all of the sample liquid by vacuum centrifugation. The sample is then hydrolyzed using *Method 1*. This method has the advantage that other amino acid residues are not derivatized by side reactions, and the sample does not need to be desalted prior to hydrolysis.

Method 11

Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during acid hydrolysis. Asparagine and aspartic acid residues are added and represented by *Asx,* while glutamine and glutamic acid residues are added and represented by *Glx.* Proteins/peptides can be reacted with bis(1,1-trifluoroacetoxy)iodobenzene (BTI) to convert the asparagine and glutamine residues to diaminopropionic acid and diaminobutyric acid residues, respectively, upon acid hydrolysis. These conversions allow the analyst to determine the asparagine and glutamine content of a protein/peptide in the presence of aspartic acid and glutamic acid residues.

Reducing Solutions—Prepare and filter three solutions: a solution of 10 mM trifluoroacetic acid (*Solution 1*), a solution of 5 M guanidine hydrochloride and 10 mM trifluoroacetic acid (*Solution 2*), and a freshly prepared solution of dimethylformamide containing 36 mg of BTI per mL (*Solution 3*).

Procedure—In a clean hydrolysis tube, transfer about 200 µg of the test sample, and add 2 mL of *Solution 1* or *Solution 2* and 2 mL of *Solution 3*. Seal the hydrolysis tube in vacuum. Heat the sample at 60° for 4 hours in the dark. The sample is then dialyzed with water to remove the excess reagents. Extract the dialyzed sample three times with equal volumes of *n*-butyl acetate, and then lyophilize. The protein can then be acid hydrolyzed using previously described procedures. The α-, *β*-diaminopropionic and α-, γ-diaminobutyric acid residues do not typically resolve from the lysine residues upon ion-exchange chromatography based on amino acid analysis. Therefore, when using ion-exchange as the mode of amino acid separation, the asparagine and glutamine contents are the quantitative difference in the aspartic acid and glutamic acid assayed contents with underivatized and BTI-derivatized acid hydrolysis. [NOTE—The threonine, methionine, cysteine, tyrosine, and histidine assayed content can be altered by BTI derivatization; a hydrolysis without BTI will have to be performed if the analyst is interested in the •composition•$_{1}$ of these other amino acid residues.]

Change to read:

METHODOLOGIES OF AMINO ACID ANALYSIS GENERAL PRINCIPLES

Many amino acid analysis techniques exist, and the choice of any one technique often depends on the sensitivity required from the assay. In general, about one-half of the amino acid analysis techniques employed rely on the separation of the free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., with ninhydrin or *o*-phthalaldehyde). Postcolumn detection techniques can be used with samples that contain small amounts of buffer components, such as salts and urea, and generally require between 5 and 10 µg of protein sample per analysis. The remaining amino acid techniques typically involve precolumn derivatization of the free amino acids (e.g., phenyl isothiocyanate; 6-aminoquinolyl-*N*-hydroxysuccinimidyl •carbamate•$_{1}$ or *o*-phthalaldehyde; (dimethylamino)azobenzenesulfonyl chloride; 9-fluorenyl-methylchloroformate; and 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole) followed by reverse-phase HPLC. Precolumn derivatization techniques are very sensitive and usually require between 0.5 and 1.0 µg of protein sample per analysis but may be influenced by buffer salts in the samples. Precolumn derivatization techniques may also result in multiple derivatives of a given amino acid, which complicates the result interpretation. Postcolumn derivatization techniques are generally influenced less by performance variation of the assay than precolumn derivatization techniques.

The following *Methods* may be used for quantitative amino acid analysis. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, and chromatographic systems. Specific parameters may vary according to the exact equipment and procedure used. Many laboratories will utilize more than one amino acid analysis technique to exploit the advantages offered by each. In each of these *Methods*, the analog signal is visualized by means of a data acquisition system, and the peak areas are integrated for quantification purposes.

Method 1—Postcolumn Ninhydrin Detection •General Principle•$_{1}$

Ion-exchange chromatography with postcolumn ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic purple or yellow color. Amino acids, except imino acids, give a purple color, and show maximum absorption at 570 nm. The imino acids, such as proline, give a yellow color, and show maximum absorption at 440 nm. The postcolumn reaction between ninhydrin and amino acid eluted from the column is monitored at 440 nm and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition.

Detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for proline. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 1 µg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

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•$_{1}$

Method 2—Postcolumn OPA Fluorometric •Detection General Principle•

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o-Phthalaldehyde• OPA reacts with primary amines in the presence of thiol compound to form highly fluorescent isoindole products. This reaction is utilized for the postcolumn derivatization in analysis of amino acids by ion-exchange chromatography. The rule of the separation is the same as *Method I*. Instruments and reagents for this form of amino acid analysis are available commercially. Many modifications of this method exist.

Although OPA does not react with secondary amines (imino acids, such as proline) to form fluorescent substances, the oxidation with sodium hypochlorite allows secondary amines to react with OPA. The procedure employs a strongly acidic cation-exchange column for separation of free amino acids followed by postcolumn oxidation with sodium hypochlorite and postcolumn derivatization using OPA and thiol compound, such as *N*-acetyl-L-cysteine and 2-mercaptoethanol. The derivatization of primary amino acids are not noticeably affected by the continuous supply of sodium hypochlorite.

Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes of pH and cation strength. After postcolumn derivatization of eluted amino acids with OPA, the reactant passes through the fluorometric detector. Fluorescence intensity of OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limit is considered to be a few tens of pmol level for most of the amino acid derivatives. Response linearity is obtained in the range of a few pmol level to a few tens of nmol level. To obtain good compositional data, a sample greater than 500 ng before hydrolysis is best suited for the amino acid analysis of protein/peptide.

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Method 3—Precolumn •PITC Derivatization General Principle•

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Phenylisothiocyanate• (PITC) reacts with amino acids to form phenylthiocarbamyl (PTC) derivatives, which can be detected with high sensitivity at 254 nm. Therefore, precolumn derivatization of amino acids with PITC followed by a reverse-phase HPLC separation with UV detection is used to analyze the amino acid composition.

After the reagent is removed under vacuum, the derivatized amino acids can be stored dry and frozen for several weeks with no significant degradation. If the solution for injection is kept cold, no noticeable loss in chromatographic response occurs after three days.

Separation of the PTC-amino acids on a reverse-phase HPLC with ODS column is accomplished through a combination of changes in concentrations of acetonitrile and buffer ionic strength. PTC-amino acids eluted from the column are monitored at 254 nm.

Detection limit is considered to be 1 pmol for most of the amino acid derivatives. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, a sample larger than 500 ng of protein/peptide before hydrolysis is best suited for this amino analysis of proteins/peptides.

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Method 4—Precolumn AQC Derivatization •General Principle•

Precolumn derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by reverse-phase HPLC separation with fluorometric detection is used.

AQC reacts with amino acids to form stable, fluorescent unsymmetric urea derivatives (AQC-amino acids) which are readily amenable to analysis by reverse-phase HPLC. Therefore, precolumn derivatization of amino acids with AQC followed by reverse-phase HPLC separation is used to analyze the amino acid composition.

Separation of the AQC-amino acids on an ODS column is accomplished through a combination of changes in the concentrations of acetonitrile and salt. Selective fluorescence detection of the derivatives with an excitation wavelength at 250 nm and an emission wavelength at 395 nm allows for the direct injection of the reaction mixture with no significant interference from the only major fluorescent reagent byproduct, 6-aminoquinoline. Excess reagent is rapidly hydrolyzed ($t_{1/2} < 15$ seconds) to yield 6-aminoquinoline, *N*-hydroxysuccinimide and carbon dioxide, and after 1 minute no further derivatization can take place.

Peak areas for AQC-amino acids are essentially unchanged for at least 1 week at room temperature, and the derivatives have more than sufficient stability to allow for overnight automated chromatographic analysis.

The detection limit is considered to be ranging from about 40 fmol to 320 fmol for each amino acid, except for Cys. The detection limit for Cys is approximately 800 fmol. Response linearity is obtained in the range of 2.5 µM to 200 µM with correlation coefficients exceeding 0.999. Good compositional data can be obtained from the analysis of derivatized protein hydrolysates containing as little as 30 ng of protein/peptide.

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Method 5—Precolumn OPA Derivatization •General Principle•

Precolumn derivatization of amino acids with *o*-phthalaldehyde (OPA) followed by reverse-phase HPLC separation with fluorometric detection is used. This technique does not detect amino acids that exist as secondary amines (e.g., proline).

OPA in conjunction with a thiol reagent reacts with primary amine groups to form highly fluorescent isoindole products. 2-Mercaptoethanol and 3-mercaptopropionic acid can be used as thiol. OPA itself does not fluoresce and consequently produces no interfering peaks. In addition, its solubility and stability in aqueous solution, along with the rapid kinetics for the reactions, make it amenable to automated derivatization and analysis using an autosampler to mix the sample with the reagent. However, lack of reactivity with secondary amino acids has been a predominant drawback. This method does not detect amino acids that exist as secondary amines (e.g., proline). To compensate for this drawback, this technique may be combined with another technique described in *Method 7* or *Method 8*.

Precolumn derivatization of amino acids with OPA is followed by reverse-phase HPLC separation. Because of the instability of the OPA-amino acid derivative, HPLC separation and analysis are performed immediately following derivatization. The liquid chromatograph is equipped with a fluorometric detector for the detection of derivatized amino acids. Fluorescence intensity of the OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

The detection limits as low as 50 fmol via fluorescence have been reported, although the practical limit of analysis remains at 1 pmol.

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Method 6—Postcolumn DABS-Cl Derivatization •General Principle•

Precolumn derivatization of amino acids with (dimethylamino)azobenzenesulfonyl chloride (DABS-Cl) followed by reverse-phase HPLC separation with visible light detection is used.

DABS-Cl is a chromophoric reagent employed for the labeling of amino acids. Amino acids labeled with DABS-Cl (DABS-amino acids) are highly stable and show the maximum absorption at 436 nm.

DABS-amino acids, all 19 naturally occurring amino acid derivatives, can be separated on an ODS column of a reverse-phase HPLC by employing gradient systems consisting of acetonitrile and aqueous buffer mixture. Separated DABS-amino acids eluted from the column are detected at 436 nm in the visible region.

This method can analyze the imino acids, such as proline, together with the amino acids, at the same degree of sensitivity. DABS-Cl derivatization method permits the simultaneous quantification of tryptophan residues by previous hydrolysis of the protein/peptide with sulfonic acids, such as mercaptoethanesulfonic acid, *p*-toluenesulfonic acid, or methanesulfonic acid, described for *Method 2* in *Protein Hydrolysis* •. The other acid-labile residues, asparagine and glutamine, can also be analyzed by previous conversion into diaminopro-

Interim Revision Announcement

pionic acid and diaminobutyric acid, respectively, by treatment of protein/peptide with BTI, described for *Method 11* in *Protein Hydrolysis* •⟨1⟩.

The nonproteinogenic amino acid, norleucine, cannot be used as an internal standard in this method as this compound is eluted in a chromatographic region crowded with peaks of primary amino acids. Nitrotyrosine can be used as an internal standard because it is eluted in a clean region.

The detection limit of DABS-amino acid is about 1 pmol. As little as 2 to 5 pmol of an individual DABS-amino acid can be quantitatively analyzed with reliability, and only 10 ng to 30 ng of the dabsylated protein hydrolysate is required for each analysis.

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Method 7—Precolumn FMOC-Cl Derivatization •General Principle•⟨1⟩

Precolumn derivatization of amino acids with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by reverse-phase HPLC separation with fluorometric detection is used.

FMOC-Cl reacts with both primary and secondary amino acids to form highly fluorescent products. The reaction of FMOC-Cl with amino acid proceeds under mild conditions, in aqueous solution, and is completed in 30 seconds. The derivatives are stable, with only the histidine derivative showing any breakdown. Although FMOC-Cl is fluorescent itself, the reagent excess and fluorescent side-products can be eliminated without loss of FMOC-amino acids.

FMOC-amino acids are separated by reverse-phase HPLC using an ODS column. The separation is carried out by gradient elution varied linearly from a mixture of acetic acid buffer, methanol, and acetonitrile (50 : 40 : 10) to a mixture of acetonitrile and acetic acid buffer (50 : 50), and 20 amino acid derivatives that are separated in 20 minutes. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm.

The detection limit is in the low fmol range. A linearity range of 0.1 µM to 50 µM is obtained for most amino acids.

•⟨1⟩

Method 8—Precolumn NBD-F Derivatization •General Principle•⟨1⟩

Precolumn derivatization of amino acids with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) followed by reverse-phase HPLC separation with fluorometric detection is used.

7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) reacts with both primary and secondary amino acids to form highly fluorescent products. Amino acids are derivatized with NBD-F by heating to 60° for 5 minutes.

NBD-amino acid derivatives are separated on an ODS column of reverse-phase HPLC by employing a gradient elution system consisting of acetonitrile and aqueous buffer mixture, and 17 amino acid derivatives that are separated in 35 minutes. *E*-aminocaproic acid can be used as an internal standard because it is eluted in a clean chromatographic region. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

The sensitivity of this method is almost the same as that for the precolumn OPA derivatization method (*Method 5*), excluding proline to which OPA is not reactive and might be advantageous for NBD-F against OPA.

The detection limit for each amino acid is about 10 fmol. Profile analysis was achieved for about 1.5 mg of protein hydrolysates in the final precolumn labeling reaction mixture for HPLC.

•⟨1⟩

Change to read:

DATA CALCULATION AND ANALYSIS

When determining the amino acid content of a protein/peptide hydrolysate, it should be noted that the acid hydrolysis step destroys tryptophan and cysteine. Serine and threonine are partially destroyed by acid hydrolysis, while isoleucine and valine residues may be only partially cleaved. Methionine can undergo oxidation during acid hydrolysis, and some amino acids (e.g., glycine and serine) are common contaminants. Application of adequate vacuum (less than 200 µm of mercury or 26.7 Pa) or introduction of inert gas (argon) in the headspace of the reaction vessel during vapor phase hydrolysis can reduce the level of oxidative destruction. Therefore, the quantitative results obtained for cysteine, tryptophan, threonine, isoleucine, valine, methionine, glycine, and serine from a protein/peptide hydrolysate may be variable and may warrant further investigation and consideration.

Calculations

Amino Acid Mole Percent—This is the number of specific amino acid residues per 100 residues in a protein. This result may be useful for evaluating amino acid analysis data when the molecular weight of the protein/peptide under investigation is unknown. This information can be used to corroborate the identity of a protein and has other applications. Carefully identify and integrate the peaks obtained as directed for each *Procedure*. Calculate the mole percent for each amino acid present in the test sample by the formula:

$$100r_U / r$$

in which r_U is the peak response, in nmol, of the amino acid under test; and r is the sum of peak responses, in nmol, for all amino acids present in the test sample. Comparison of the mole percent of the amino acids under test to data from known proteins can help establish or corroborate the identity of the sample protein.

Unknown Protein Samples—This data analysis technique can be used to estimate the protein concentration of an unknown protein sample using the amino acid analysis data. Calculate the mass, in µg, of each recovered amino acid by the formula:

$$mM_W/1000$$

in which m is the recovered quantity, in nmol, of the amino acid under test; and M_W is the •⟨1⟩ molecular weight •⟨1⟩, for that amino acid, corrected for the weight of the water molecule that was eliminated during peptide bond formation. The sum of the masses of the recovered amino acids will give an estimate of the total mass of the protein analyzed after appropriate correction for partially and completely destroyed amino acids. If the molecular weight of the unknown protein is available (i.e., by SDS-PAGE analysis or mass spectroscopy), the amino acid composition of the unknown protein can be predicted. Calculate the number of residues of each amino acid by the formula:

$$m/(1000M/M_{WT})$$

in which m is the recovered quantity, in nmol, of the amino acid under test; M is the total mass, in µg, of the protein; and M_{WT} is the molecular weight •⟨1⟩ of the unknown protein.

Known Protein Samples—This data analysis technique can be used to investigate the amino acid composition and protein concentration of a protein sample of known molecular weight and amino acid composition using the amino acid analysis data. When the composition of the protein being analyzed is known, one can exploit the fact that some amino acids are recovered well, while other amino acid recoveries may be compromised because of complete or partial destruction (e.g., tryptophan, cysteine, threonine, serine, methionine), incomplete bond cleavage (i.e., for isoleucine and valine), and free amino acid contamination (i.e., by glycine and serine).

Because those amino acids that are recovered best represent the protein, these amino acids are chosen to quantify the amount of protein. Well-recovered amino acids are, typically, aspartate-asparagine, glutamate-glutamine, alanine, leucine, phenylalanine, lysine, and arginine. This list can be modified based on experience with one's own

analysis system. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. The protein content determined for each of the well-recovered amino acids should be evenly distributed about the mean. Discard protein content values for those amino acids that have an unacceptable deviation from the mean. Typically, a greater than 5% variation from the mean is considered unacceptable •1•. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample. Divide the content of each amino acid by the calculated mean protein content to determine the amino acid composition of the sample by analysis.

Calculate the relative compositional error, in percentage, by the formula:

$$100m / m_S$$

in which m is the experimentally determined quantity, in nmol per amino acid residue, of the amino acid under test; and m_S is the known residue value for that amino acid. The average relative compositional error is the average of the absolute values of the relative compositional errors of the individual amino acids, typically excluding tryptophan and cysteine from this calculation. The average relative compositional error can provide important information on the stability of analysis run over time. The agreement in the amino acid composition between the protein sample and the known composition can be used to corroborate the identity and purity of the protein in the sample.

Add the following:

•⬥APPENDIX

AMINO ACID ANALYSIS PROCEDURES

The examples of the specific procedures for each *Method* described in *Methodologies of Amino Acid Analysis* are shown.

Method 1—Postcolumn Ninhydrin Detection

One method for postcolumn ninhydrin detection is shown below. Many other methods are also available, with instruments and reagents available commercially.

Mobile Phase Preparation—

Solution A—Transfer about 1.7 g of anhydrous sodium citrate and 1.5 mL of hydrochloric acid to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Adjust, if necessary, with hydrochloric acid to a pH of 3.0.

Solution B—Transfer about 1.7 g of anhydrous sodium citrate and 0.7 mL of hydrochloric acid to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Adjust, if necessary, with hydrochloric acid to a pH of 4.3.

Solution C—Prepare a solution containing 5% of sodium chloride, 1.9% of anhydrous sodium citrate, and 0.1% of phenol in water, and adjust to a pH of 6.

Column Regeneration Solution—Prepare a solution containing 0.8% of sodium hydroxide in water, and adjust to a pH of 13.

Mobile Phase—Use variable mixtures of *Solution A, Solution B,* and *Solution C* as directed for *Chromatographic system*.

Postcolumn Reagent—Transfer about 18 g of ninhydrin and 0.7 g of hydrindantin to 900 mL of a solution containing 76.7% of dimethyl sulfoxide, 0.7% of dihydrate lithium acetate, and 0.1% of acetic acid, and mix for at least 3 hours under inert gas, such as nitrogen. [NOTE—This reagent is stable for 30 days if kept between 2° and 8° under inert gas.]

Buffer Solution—Prepare a solution containing 2% of anhydrous sodium citrate, 1% of hydrochloric acid, 0.5% of thiodiglycol, and 0.1% of benzoic acid in water, and adjust to a pH of 2.

Chromatographic System—The liquid chromatograph is equipped with a detector with appropriate interference filters at 440, 570, or 690 nm and a 4.0-mm × 120-mm column that contains 7.5-µm sulfonated styrene-divinylbenzene copolymer packing. The flow rate is about 14 mL per hour. The system is programmed as follows. Initially equilibrate the column with *Solution A;* at 25 minutes, the composition of the *Mobile Phase* is changed to 100% *Solution B;* and at 37 minutes, the composition is changed to 100% *Solution C.* At 75 minutes into the run, the last amino acid has been eluted from the column, and the column is regenerated with *Column Regeneration Solution* for 1 minute. The column is then equilibrated with *Solution A* for 11 minutes before the next injection. The column temperature is programmed as follows. The initial temperature is 48°; after 11.5 minutes, the temperature is increased to 65° at a rate of 3° per minute; at about 35 minutes, the temperature is increased to 77° at a rate of 3° per minute; and finally at about 52 minutes, the temperature is decreased to 48° at a rate of 3° per minute.

Procedure and Postcolumn Reaction—Reconstitute the lyophilized protein/peptide hydrolysate in the *Buffer Solution,* inject an appropriate amount into the chromatograph, and proceed as directed for *Chromatographic System.* As the amino acids are eluted from the column, they are mixed with the *Postcolumn Reagent,* which is delivered at a flow rate of 7 mL per hour, through a tee. After mixing, the column effluent and the *Postcolumn Reagent* pass through a tubular reactor at a temperature of 135°, where a characteristic purple or yellow color is developed. From the reactor, the liquid passes through a colorimeter with a 12-mm flow-through cuvette. The light emerging from the cuvette is split into three beams for analysis by the detector with interference filters at 440, 570, or 690 nm. The 690-nm signal may be electronically subtracted from the other signals for improved signal-to-noise ratios. The 440-nm (imino acids) and the 570-nm (amino acids) signals may be added in order to simplify data handling.

Method 2—Postcolumn OPA Fluorometric Detection

One method of postcolumn OPA fluorometric detection is shown below.

Mobile Phase Preparation—

Solution A—Prepare a solution of sodium hydroxide, citric acid, and alcohol in HPLC grade water having a 0.2 N sodium concentration and containing 7% of alcohol (w/v), adjusted to a pH of 3.2.

Solution B—Prepare a solution of sodium hydroxide and citric acid in HPLC grade water having a 0.6 N sodium concentration, adjusted to a pH of 10.0.

Solution C: 0.2 N sodium hydroxide.

Mobile Phase—Use variable mixtures of *Solution A,* Solution B, and *Solution C* as directed for *Chromatographic System*.

Postcolumn Reagent Preparation—

Alkaline Buffer—Prepare a solution containing 384 mM sodium carbonate, 216 mM boric acid, and 108 mM potassium sulfate, and adjust to a pH of 10.0.

Hypochlorite Reagent—To 1 L of *Alkaline Buffer,* add 0.4 mL of sodium hypochlorite solution (10% chlorine concentration). [NOTE—The hypochlorite solution is stable for 2 weeks.]

OPA Reagent—Transfer 2 g of *N*-acetyl-L-cysteine and 1.6 g of OPA to a 15-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix. Transfer this solution and 4 mL of 10% aqueous polyethylene (23) lauryl ether to a 1-L volumetric flask, dilute with 980 mL of *Alkaline Buffer*, and mix.

Chromatographic System—The liquid chromatograph is equipped with a fluorometric detector set to an excitation wavelength of 348 nm and an emission wavelength of 450 nm and a 4.0-mm × 150-mm column that contains 7.5-µm packing L17. The flow rate is about 0.3 mL per minute, and the column temperature is set at 50°. The system is programmed as follows. The column is equilibrated with *Solution A;* over the next 20 minutes, the composition of the *Mobile Phase* is changed linearly to 85% *Solution A* and 15% *Solution B;* then there is a step change to 40% *Solution A* and 60% *Solution B;* over the next 18 minutes, the composition is changed linearly to 100% *Solution B* and held for 7 minutes; then there is a step change to 100% *Solution C,* and this is held for 6 minutes; then there is a step change to *Solution A,* and this composition is maintained for the next 8 minutes.

Procedure and Postcolumn Reaction—Inject about 1.0 nmol of each amino acid under test into the chromatograph, and proceed as directed for *Chromatographic System*. As the effluent leaves the column, it is mixed with the *Hypochlorite Reagent*. The mixture passes through the first postcolumn reactor which consists of stainless steel 0.5-mm × 2-m tubing. A second postcolumn reactor of similar design is placed immediately downstream from the first postcolumn reactor and is used for the OPA postcolumn reaction. The flow rates for both the *Hypochlorite Reagent* and the *OPA Reagent* are 0.2 mL per minute, resulting in a total flow rate (i.e., *Hypochlorite Reagent, OPA Reagent,* and column effluent) of 0.7 mL per minute exiting from the postcolumn reactors. Postcolumn reactions are conducted at 55°. This results in a residence time of about 33 seconds in the OPA postcolumn reactor. After postcolumn derivatization, the column effluent passes through the fluorometric detector.

Method 3—Precolumn PITC Derivatization

One method of precolumn PITC derivatization is described below.

Mobile Phase Preparation—

Solution A: 0.05 M ammonium acetate, adjusted with phosphoric acid to a pH of 6.8.

Solution B—Prepare 0.1 M ammonium acetate, adjust with phosphoric acid to a pH of 6.8, and then prepare a mixture of this solution and acetonitrile (1 : 1).

Solution C: a mixture of acetonitrile and water (70 : 30).

Mobile Phase—Use variable mixtures of *Solution A, Solution B,* and *Solution C* as directed for *Chromatographic System*.

Derivatization Reagent Preparation—

Coupling Buffer: a mixture of acetonitrile, pyridine, triethylamine, and water (10 : 5 : 2 : 3).

Sample Solvent: a mixture of water and acetonitrile (7 : 2).

Sample Derivatization Procedure—Dissolve the lyophilized test sample in 100 µL of the *Coupling Buffer,* and then dry in a vacuum centrifuge to remove any hydrochloride if a protein hydrolysis step was used. Dissolve the test sample in 100 µL of *Coupling Buffer,* add 5 µL of PITC, and incubate at room temperature for 5 minutes. The test sample is again dried in a vacuum centrifuge, and is dissolved in 250 µL of *Sample Solvent*.

Chromatographic System—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 250-mm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute, and the column temperature is maintained at 52°. The system is programmed as follows. The column is equilibrated with *Solution A;* over the next 15 minutes, the composition of the *Mobile Phase* is changed linearly to 85% *Solution A* and 15% *Solution B;* over the next 15 minutes, the composition is changed linearly to 50% *Solution A* and 50% *Solution B;* then there is a step change to 100% *Solution C,* and this is held for 10 minutes; then there is a step change to 100% *Solution A,* and the column is allowed to equilibrate before the next injection.

Procedure—Inject about 1.0 nmol of each PITC-amino acid under test (10-µL sample in *Sample Solvent*) into the chromatograph, and proceed as directed for *Chromatographic System*.

Method 4—Precolumn AQC Derivatization

One method of precolumn AQC derivatization is shown below.

Mobile Phase Preparation—

Solution A—Prepare a solution having a composition of 140 mM sodium acetate and 17 mM triethylamine, and adjust with phosphoric acid to a pH of 5.02.

Solution B: a mixture of acetonitrile and water (60 : 40).

Mobile Phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic System*.

Sample Derivatization Procedure—Dissolve about 2 µg of the test sample in 20 µL of 15 mM hydrochloric acid, and dilute with 0.2 M borate buffer (pH 8.8) to 80 µL. The derivatization is initiated by the addition of 20 µL of 10 mM AQC in acetonitrile, and allowed to proceed for 10 minutes at room temperature.

Chromatographic System—The liquid chromatograph is equipped with a fluorometric detector set at an excitation wavelength of 250 nm and an emission wavelength of 395 nm and a 3.9-mm × 150-mm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute, and the column temperature is maintained at 37°. The system is programmed as follows. The column is equilibrated with *Solution A;* over the next 0.5 minute, the composition of the *Mobile Phase* is changed linearly to 98% *Solution A* and 2% *Solution B;* then over the next 14.5 minutes to 93% *Solution A* and 7% *Solution B;* then over the next 4 minutes to 87% *Solution A* and 13% *Solution B;* over the next 14 minutes to 68% *Solution A* and 32% *Solution B;* then there is a step change to 100% *Solution B* for a 5-minute wash; over the next 10 minutes, there is a step change to 100% *Solution A;* and the column is allowed to equilibrate before the next injection.

Procedure—Inject about 0.05 nmol of each AQC-amino acid under test into the chromatograph, and proceed as directed for *Chromatographic System.*

Method 5—Precolumn OPA Derivatization

One method of precolumn OPA derivatization is shown below.

Mobile Phase Preparation—

Solution A: a mixture of 100 mM sodium acetate (pH 7.2), methanol, and tetrahydrofuran (900 : 95 : 5).

Solution B: methanol.

Mobile Phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic System*.

Derivatization Reagent—Dissolve 50 mg of OPA in 1.25 mL of methanol (protein sequencing grade). Add 50 µL of 2-mercaptoethanol and 11.2 mL of 0.4 M sodium borate (pH 9.5), and mix. [NOTE—This reagent is stable for 1 week.]

Sample Derivatization Procedure—Transfer about 5 µL of the test sample to an appropriate container, add 5 µL of the *Derivatization Reagent,* and mix. After 1 minute, add not less than 20 µL of 0.1 M sodium acetate (pH 7.0). Use 20 µL of this solution for analysis. [NOTE—Use of an internal standard (e.g., norleucine) is recommended for quantitative analysis because of potential reagent volume variations in the sample derivatization. The sample derivatization is performed in an automated on-line fashion. Because of the instability of the OPA-amino acid derivative, HPLC separation and analysis are performed immediately following derivatization.]

Chromatographic System—The liquid chromatograph is equipped with a fluorometric detector set at an excitation wavelength of 348 nm and an emission wavelength of 450 nm and a 4.6-mm × 75-mm column that contains 3-µm packing L3. The flow rate is about 1.7 mL per minute, and the column temperature is maintained at 37°. The system is programmed as follows. The column is equilibrated with 92% *Solution A* and 8% *Solution B;* over the next 2 minutes, the composition of the *Mobile Phase* is changed to 83% *Solution A* and 17% *Solution B,* and held for an additional 3 minutes; then changed to 54% *Solution A* and 46% *Solution B* over the next 5 minutes, and held for an additional 2 minutes; then changed to 34% *Solution A* and 66% *Solution B* over the next 2 minutes, and held for 1 minute; then over the next 0.3 minute changed to 20% *Solution A* and 80% *Solution B,* and held for an additional 2.6 minutes; and then finally over 0.6 minute changed to 92% *Solution A* and 8% *Solution B,* and held for an additional 0.6 minute.

Procedure—Inject about 0.02 nmol of each OPA-amino acid under test into the chromatograph, and proceed as directed for *Chromatographic System.*

Method 6—Postcolumn DABS-Cl Derivatization

One method for precolumn DABS-Cl derivatization is shown below.

Mobile Phase Preparation—

Solution A: 25 mM sodium acetate (pH 6.5) containing 4% of dimethylformamide.

Solution B: acetonitrile.

Mobile Phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic System*.

Derivatization Reagent Preparation—

Sample Buffer: 50 mM sodium bicarbonate, adjusted to a pH of 8.1.

Derivatization Reagent—Dissolve 1.3 mg of DABS-Cl in 1 mL of acetonitrile. [NOTE—This reagent is prepared fresh shortly before the derivatization step.]

Sample Dilution Buffer—Prepare a mixture of 50 mM sodium phosphate (pH 7.0) and alcohol (1 : 1).

Sample Derivatization Procedure—Dissolve the test sample in 20 µL of *Sample Buffer*, add 40 µL of *Derivatization Reagent*, and mix. The sample container is sealed with a silicon-rubber stopper, and heated to 70° for 10 minutes. During the sample heating, the mixture will become completely soluble. After the derivatization, dilute the test sample with an appropriate quantity of the *Sample Dilution Buffer.*

Chromatographic System—The liquid chromatograph is equipped with a 436-nm detector and a 4.6-mm × 250-mm column that contains packing L1. The flow rate is about 1 mL per minute, and the column temperature is maintained at 40°. The system is programmed as follows. The column is equilibrated with 85% *Solution A* and 15% *Solution B;* over the next 20 minutes, the composition of the *Mobile Phase* is changed to 60% *Solution A* and 40% *Solution B;* over the next 12 minutes, the composition is changed to 30% *Solution A* and 70% *Solution B*, and held for an additional 2 minutes.

Procedure—Inject about 0.05 nmol of the DABS-amino acids into the chromatograph, and proceed as directed for *Chromatographic System.*

Method 7—Precolumn FMOC-Cl Derivatization

One method for precolumn FMOC-Cl derivatization is shown below.

Mobile Phase Preparation—

Acetic Acid Buffer—Transfer 3 mL of glacial acetic acid and 1 mL of triethylamine to a 1-L volumetric flask, and dilute with HPLC grade water to volume. Adjust with sodium hydroxide to a pH of 4.20.

Solution A: a mixture of *Acetic Acid Buffer,* methanol, and acetonitrile (50 : 40 : 10).

Solution B: a mixture of acetonitrile and *Acetic Acid Buffer* (50 : 50).

Mobile Phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic System*.

Derivatization Reagent Preparation—

Borate Buffer—Prepare a 1 M boric acid solution, and adjust with sodium hydroxide to a pH of 6.2.

FMOC-Cl Reagent—Dissolve 155 mg of 9-fluorenylmethyl chloroformate in 40 mL of acetone, and mix.

Sample Derivatization Procedure—To 0.4 mL of the test sample add 0.1 mL of *Borate Buffer* and 0.5 mL of *FMOC-Cl Reagent*. After about 40 seconds, extract the mixture with 2 mL of pentane, and then extract again with fresh pentane. The aqueous solution with amino acid derivatives is then ready for injection.

Chromatographic System—The liquid chromatograph is equipped with a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm and a 4.6-mm × 125-mm column that contains 3-µm packing L1. The flow rate is about 1.3 mL per minute. The system is programmed as follows. The column is equilibrated with *Solution A,* and this composition is maintained for 3 minutes; over the next 9 minutes, it is changed to 100% *Solution B;* then over the next 0.5 minute, the flow rate is increased to 2 mL per minute, and held until the final FMOC-amino acid is eluted from the column. The total run time is about 20 minutes.

Procedure—Inject not less than 0.01 nmol of each FMOC-amino acid under test into the chromatograph, and proceed as directed for *Chromatographic System*. The FMOC-histidine derivative will generally give a lower response than the other derivatives.

Method 8—Precolumn NBD-F Derivatization

One method for precolumn NBD-F derivatization is shown below.

Mobile Phase Preparation—

Solution A: a solution of 10mM sodium citrate containing 75 mM sodium perchlorate, adjusted with hydrochloric acid to a pH of 6.2.

Solution B: a mixture of acetonitrile and water (50 : 50).

Derivatization Reagent Preparation—

Sample Buffer: a 0.1 M boric acid solution, adjusted with sodium hydroxide to a pH of 9.2.

Derivatization Reagent—Dissolve 5 mg of NBD-F in 1.0 mL of alcohol, and mix.

Sample Derivatization Procedure—Dissolve the test sample in 20 µL of *Sample buffer,* add 10 µL of *Derivatization Reagent,* and mix. The sample container is heated at 60° for 5 minutes. After the derivatization, dilute the test sample with 300 µL of *Solution A.*

Chromatographic System—The liquid chromatograph is equipped with a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm and a 4.6-mm × 150-mm column that contains 5-µm particle size ODS silica packing. The flow rate is about 1.0 mL per minute, and the column temperature is maintained at 40°. The system is programmed as follows. The column is equilibrated with 94% *Solution A* and 6% *Solution B;* over the next 16 minutes, the composition is changed linearly to 63% *Solution A* and 37% *Solution B;* over the next 5 minutes, the composition is changed linearly to 62% *Solution A* and 38% *Solution B;* over the next 9 minutes, the composition is changed linearly to 100% *Solution B*, and held for an additional 5 minutes; then finally over 2 minutes, the composition is changed linearly to 94% *Solution A* and 6% *Solution B;* and then the column is allowed to equilibrate before the next injection.

Procedure—Inject about 15 pmol of each NBD-amino acid under test into the chromatograph, and proceed as directed for *Chromatographic System.*◆●1

⟨1053⟩ BIOTECHNOLOGY-DERIVED ARTICLES—CAPILLARY ELECTROPHORESIS

Change to read:

INTRODUCTION

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution under the influence of a direct-current electric field. In this section we are describing four capillary electrophoresis methods: ●*Capillary Zone*●1 *Electrophoresis, Capillary Gel Electrophoresis, Capillary Isoelectric Focusing,* and *Micellar Electrokinetic Chromatography.*

Change to read:

GENERAL PRINCIPLE

The migration velocity of the analyte under an electric field of intensity E is determined by the electrophoretic mobility of the analyte and the electroosmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute (μ_{ep}) depends on the characteristics of the solute (electrical charge, molecular size, and shape) and the characteristics of the buffer in which the migration takes place (type

Interim Revision Announcement

and ionic strength of the electrolyte, pH, viscosity, and additives). The electrophoretic velocity *(v_{ep})* of a solute, assuming a spherical shape, is as follows:

$$v_{ep} = \mu_{ep}E = \left(\frac{q}{6\pi\eta r}\right)\left(\frac{V}{L}\right)$$

in which q is the effective charge of the solute; η is the viscosity of the electrolyte solution; r is the Stoke's radius of the solute; V is the applied voltage; and L is the total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent, called electroosmotic flow, is generated inside the capillary. Its velocity depends on the electroosmotic mobility *(μ_{eo}),* which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electroosmotic velocity *(v_{eo})* is given by the equation:

$$v_{eo} = \mu_{eo}E = \left(\frac{\varepsilon\zeta}{\eta}\right)\left(\frac{V}{L}\right)$$

in which ϵ is the dielectric constant of the buffer; ζ is the zeta potential of the capillary surface; and the other terms are as defined above.

The velocity of the solute (v) is given by the equation:

$$v = v_{ep} + v_{eo}$$

The electrophoretic mobility of the analyte and the electroosmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite direction by the electroosmotic flow and their velocities will be smaller than the electroosmotic velocity. Cations will migrate in the same direction as the electroosmotic flow and their velocities will be greater than the electroosmotic velocity. Under conditions in which there is a fast electroosmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the same run. The time *(t)* taken by the solute to migrate the distance *(l)* from the injection end of the capillary to the detection point (capillary effective length) is as follows:

$$t = \frac{l}{v_{ep} + v_{eo}} = \frac{l(L)}{V\left(\mu_{ep} + \mu_{eo}\right)}$$

in which the other terms are as defined above.

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionized silanol groups in the inner wall. Consequently, the electroosmotic flow is from anode to cathode. The electroosmotic flow has to remain constant from run to run to obtain good reproducibility in the migration velocity of the solutes. For some applications, it might be necessary to reduce or suppress the electroosmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition, and/or the pH of the buffer solution.

After the introduction of the sample into the capillary, each analyte ion of the sample migrates within the background electrolyte as an independent zone according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band results from a different phenomenon. Under ideal conditions, the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case, the efficiency of the zone, expressed as the number of theoretical plates *(N),* is given by:

$$N = \frac{\left(\mu_{ep} + \mu_{eo}\right)(Vl)}{2DL}$$

in which D is the molecular diffusion of the solute in the buffer.

In practice, other phenomena, such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size, and unleveled buffer reservoirs, can also significantly contribute to band dispersion. Separation between two bands (expressed by the resolution, R_S) can be obtained by modification of the electrophoretic mobility of the analytes, by the electroosmotic mobility induced in the capillary, and by increasing the efficiency for the band of each analyte as follows:

$$R_s = \frac{\sqrt{N}\left(\mu_{epb} - \mu_{epa}\right)}{4\left(\bar{\mu}_{ep} + \mu_{eo}\right)}$$

in which μ_{epa} and μ_{epb} are the electrophoretic mobilities of the two analytes to be separated; $\bar{\mu}_{ep}$ is the average electrophoretic mobility of the two analytes calculated as:

$$\bar{\mu}_{ep} = ½\ (\mu_{epb} + \mu_{epa})$$

Change to read:

APPARATUS

An apparatus for capillary electrophoresis is composed of a high voltage controllable direct current power supply; two buffer reservoirs held at the same level and containing specified anodic and cathodic solutions; two electrode assemblies (cathode and anode) immersed in the buffer reservoirs and connected to the power supply; a separation capillary usually made of fused-silica, sometimes with an optical viewing window aligned with the detector, depending on the detector, with the ends of the capillary placed in the buffer reservoirs and the capillary being filled with a solution specified in a given monograph; a suitable injection system; a detector capable of monitoring the amount of substance of interest passing through a segment of the separation capillary at a given time, generally based on absorption spectrophotometry (UV and visible), fluorimetry, conductimetric, amperometric, or mass spectrometric detection, depending on the specific applications, or even indirect detection to detect non-UV-absorbing and nonfluorescent compounds; a thermostatic system capable of maintaining a constant temperature inside the capillary, recommended to obtain good separation reproducibility; a recorder; and a suitable integrator or a computer.

The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum, or electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode.

It is expected that the capillary, the buffer solutions, the preconditioning method, the sample solution, and the migration conditions will be specified in the individual monograph. The electrolytic solution employed may be filtered to remove particles and degassed to avoid bubble formation that could interfere with the detection system or interrupt the electrical contact in the capillary during the separation run. To achieve reproducible migration time of the solutes, it would be necessary to develop, for each analytical method, a rigorous rinsing routine after each injection.

Change to read:

•CAPILLARY ZONE•1 ELECTROPHORESIS

•Principle•1

In •capillary zone•1 electrophoresis, analytes are separated in a capillary containing only buffer without any anticonvective medium. In this technique, separation takes place because the different components of the sample migrate as discrete bands with different velocities. The velocity of each band depends on the electrophoretic mobility of the solute and the electroosmotic flow on the capillary •(see *General Principle*)•1. Coated capillaries ••1 can be used to increase the separation capacity of those substances absorbing on fused-silica surfaces.

This mode of capillary electrophoresis is appropriate for the analysis of small (MW < 2000) and large (2000 < MW < 100,000) molecules. Due to the high efficiency achieved •in capillary zone electrophoresis, separation of•1 molecules having only minute differences in their charge-to-mass ratio can be separated. This •separation mode•1 also allows the separation of chiral compounds by adding chiral selectors to the separation buffer.

•Optimization

Optimization of the separation is a complex process where several separation parameters can play a major role. The main factors to be considered in the development of the separations are instrumental and electrolytic solution parameters.•1

Instrumental Parameters

Voltage—•A Joule heating plot is useful in optimizing the applied voltage and column temperature.•1 The separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, •as a result,•1 viscosity gradients in the buffer inside the capillary, which causes band broadening and decreases resolution.

•**Polarity**—Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electroosmotic flow will move toward the cathode. If the electrode polarity is reversed the electroosmotic flow is away from the outlet and only charged analytes with electro-osmotic mobilities greater than the electroosmotic flow will pass to the outlet.•1

Temperature—The main effect of temperature is observed on buffer viscosity and electrical conductivity, thus affecting migration velocity. In some cases, an increase in capillary temperature can cause a conformational change of some proteins, modifying their migration time and the efficiency of the separation.

Capillary—The length and internal diameter of the capillary affects the analysis time, the efficiency of separations, and the load capacity. Increasing both effective length and total length can decrease the electric fields, at a constant voltage, which will increase migration time. For a given buffer and electric field, heat dissipation (thus, sample band broadening) depends on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected into the capillary and the detection system used.

The adsorption of sample components on the capillary wall limits efficiency; therefore, methods to avoid these interactions should be considered in the development of a separation method. This is critical in samples containing proteins. Strategies have been devised to avoid adsorption of proteins on the capillary wall. •Some of•1 these strategies include both the use of extreme pH and the adsorption of positively charged buffer additives that only require modification of the buffer composition •to prevent protein adsorption•1. Other strategies include the coating of the internal wall of the capillary with a polymer covalently bonded to the silica that prevents interaction between the proteins and the negatively charged silica surface. •For this purpose, ready-to-use•1 capillaries with coatings consisting of neutral-hydrophilic, cationic, and anionic polymers are commercially available.

Electrolytic Solution Parameters

Buffer Type and Concentrations—Suitable buffers for capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimize current generation.

To minimize peak shape distortion, it is important to match buffer–ion mobility to solute mobility whenever possible. The type of sample solvent used is important to achieve on-column sample focusing, which increases separation efficiency and improves detection. Also, an increase in buffer concentration at a given pH will decrease electroosmotic flow and solute velocity.

Buffer pH—The pH of the buffer can affect separation by modifying the charge of the analyte or ••1 additives and by changing the electroosmotic flow. For protein and peptide separation, a change in the pH of the buffer from above the isoelectric point to below the isoelectric point changes the net charge of the solute from negative to positive. An increase in the buffer pH generally increases the electroosmotic flow.

Organic Solvents—Organic modifiers, such as methanol, acetonitrile, and others, •may be•1 added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the ionization degree of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electroosmotic flow.

Additives for Chiral Separations—To separate optical isomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, although in some cases crown ethers, certain polysaccharides, or even proteins can be used. Because chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers, the resolution achieved for the chiral compounds depends largely on the type of chiral selector used. While developing a given separation it may be useful to test cyclodextrins having a different cavity size (α-, β-, or γ-cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxyalkyl, etc.) or ionizable (aminomethyl, carboxymethyl, sulfobutylether, etc.) moities. •When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account because it will influence the selectivity.•1 The resolution of chiral separations is also controlled by the concentration of the chiral selector, the composition and pH of the buffer, and the separation temperature. Organic additives, such as methanol or urea, can also affect the resolution of separation.

Change to read:

CAPILLARY GEL ELECTROPHORESIS

•In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size because smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.•1

Characteristics of ••1 Gels

•Two types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated•1 gels are prepared inside the capillary by reaction of monomers. One example of such a gel is a cross-linked polyacrylamide. This type of gel is •usually•1 bonded to the fused-silica wall and cannot be removed without destroying the capillary. For protein analysis •under reducing conditions•1, the separation buffer usually contains sodium dodecyl sulfate, and the sample is denatured by heating in a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. •When non-reducing conditions are used (for example, analysis of an intact antibody), 2- mercaptoethanol and dithiothreitol are not used.•1 Optimization of separation in a cross-linked gel is ob-

tained by modifying the separation buffer (see •*Capillary Zone Electrophoresis*•) and by controlling the gel porosity during the gel preparation. For a cross-linked polyacrylamide gel, the porosity can be modified by changing the concentration of acrylamide and/or the ratio of the cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease in the mobility of the solutes. Due to the rigidity of this type of gel, only electrokinetic injection can be used.

•Dynamically coated gels• are hydrophilic polymers (i.e., linear polyacrylamide, cellulose derivatives, dextran, etc.) which can be dissolved in aqueous separation buffers, giving rise to a separation medium that also acts as a molecular sieve. These polymeric separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary with no electroosmotic flow. Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the •dynamically coated• gels can be increased by using polymers of higher molecular •mass• (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular •mass•). A decrease in gel porosity leads to a decrease in the mobility of the solute for the same buffer. Both hydrodynamic and electromigration injection techniques can be used because the dissolution of these polymers in the buffer gives low viscosity solutions.

Change to read:

CAPILLARY ISOELECTRIC FOCUSING

•Principle•

•In isoelectric focusing• the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having *pI* values in a wide range (poly-aminocarboxylic acids) dissolved in the separation buffer.

The three basic steps in capillary isoelectric focusing are loading, focusing, and mobilization.

LOADING

•Two methods may be employed.•

Loading in One Step—The sample is mixed with ampholytes and introduced into the capillary by pressure or vacuum.

Sequential Loading—A leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone, and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough so as to not modify the pH gradient.

FOCUSING

When the voltage is applied, ampholytes migrate toward the cathode or the anode according to their net charge, creating the pH gradient from anode (lower pH) to cathode (higher pH). •During this step• the components to be separated migrate until they reach a pH corresponding to their isoelectric point, and the current drops to very low values.

MOBILIZATION

•If mobilization is required for detection, use one of the following methods. Three methods are available.•

Method 1—•Mobilization is accomplished• during *Focusing,* under the influence of the electroosmotic flow when this flow is small enough to allow the focusing of the components.

Method 2—•Mobilization is accomplished• by application of positive pressure after *Focusing.*

Method 3—•Mobilization is achieved• after *Focusing,* by adding salts in the cathode reservoir or the anode reservoir, depending on the direction chosen for mobilization, in order to alter the pH in the capillary when the voltage is applied. As the pH is changed, the proteins and ampholytes are mobilized in the direction of the reservoir, which contains added salts, and pass the detector.

The separation achieved is expressed as Δ*pI* and depends on the pH gradient *(dpH/dx),* the number of ampholytes having different *pI* values, the •molecular• diffusion coefficient *(D),* the intensity of the electric field *(E),* and the variation of the electrophoretic mobility of the analyte with the pH •dμ/dpH):•

$$\Delta pI = 3\sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

••

Optimization ••

The major parameters that need to be considered in the development of separations are •the following:•

Voltage—The use of high fields from 300 V/cm to 1,000 V/cm during *Focusing.*

Capillary—Depending on the *Mobilization* strategy selected (see above), the electroosmotic flow must be reduced or suppressed. Coated capillaries tend to reduce the electroosmotic flow.

Solutions—The anode buffer reservoir is filled with a solution of a lower pH than the *pI* of the most acidic ampholyte, and the cathode reservoir is filled with a solution with a higher pH than the *pI* of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used.

Addition of a polymer, like methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electroosmotic flow by increasing the viscosity. Commercial ampholytes covering many pH ranges are available and may also be mixed to obtain an expanded pH range. Broad pH ranges are used to estimate the isoelectric point, whereas narrower ranges are employed to improve accuracy. Calibration can be made by correlating migration time with the isoelectric point of a series of standard protein markers. During *Focusing,* precipitation of proteins at their isoelectric point can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea, or Zwitterionic buffers. However, depending on the concentration, urea can denature proteins.

Change to read:

MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC)

•Principle•

Separation takes place in an electrolytic solution that contains a surfactant •• at a concentration above the critical micellar concentration •*(cmc).*• The solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed by the micelles according to the solute's partition coefficient. The technique can be considered as a hybrid of electrophoresis and chromatography. It is •a• technique that can be used for the separation of both neutral and charged solutes maintaining the efficiency, speed, and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants •in MEKC is the anionic surfactant,• sodium dodecyl sulfate, although other •• surfactants, such as •cationic surfactant• cetyl trimethyl ammonium salts, have also been used.

•The separation mechanism is as follows.• At neutral and alkaline pH, a strong electroosmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is used as surfactant, the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, because the analyte can partition between the micelle and the aqueous buffer and has no electrophoretic mobility, the analyte migration velocity

will only depend on the partition coefficient between the micelle and the aqueous buffer. In the •electropherogram,•1 the peak corresponding to each uncharged solute is always between that of the electroosmotic flow marker and that of the micelle; and the time elapsed between these two peaks is called the separation window. For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer and on the electrophoretic mobility of the solute in the absence of micelles.

•Because the mechanism in MEKC of neutral and weakly ionized solutes•1 is essentially chromatographic, migration of the solute and resolution can be •rationalized in terms of the retention•1 factor of the solute *(k′),* which is the ratio between the •1 number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, k' is as follows:

$$k' = \frac{t_r - t_0}{t_0\left(1 - t_r/t_m\right)} = K\left(\frac{V_S}{V_M}\right)$$

in which t_r is the migration time of the solute; t_0 is the analysis time of the unretained solute obtained by injecting an electroosmotic flow marker that does not enter the micelle (i.e., methanol); t_m is the micelle migration time measured by injecting a micelle marker, such as Sudan III, which migrates continuously associated in the micelle; K is the partition coefficient of the solute; V_S is the volume of the •micellar•1 phase; and V_M is the volume of the mobile phase.

The resolution between two closely-migrating •solutes•1 *(R_S)* is as follows:

$$R_S = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_b'}{k_b' + 1} \times \frac{1 - \left(\frac{t_0}{t_m}\right)}{1 + k_\alpha' \times \left(\frac{t_0}{t_m}\right)}$$

in which N is the number of theoretical plates for one of the •solutes;•1 α is the selectivity; and k_a' and k_b' are retention factors for both •solutes,•1 respectively *($k_b' > k_a'$).*•1

Similar, but not identical equations give k' and R_S values for electrically charged •solutes.•1

Optimization••1

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

INSTRUMENTAL PARAMETERS

Voltage—Separation time is inversely proportional to applied voltage. An increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross section of the capillary. This effect can be significant with high conductivity buffers, such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.

Temperature—Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelle, the critical micelle concentration, and the viscosity of the buffer. These parameters contribute to the migration time of the solutes. •The use of a good cooling system improves the reproducibility of the migration time for the solutes.•1

Capillary—•As in *Capillary Zone Electrophoresis*•1 length and internal diameter •of the capillary•1 contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electrical fields, working at constant voltage, and will increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation, at a given buffer and electrical field, and •consequently•1 a broadening of the sample band.

ELECTROLYTIC SOLUTION PARAMETERS

Surfactant Type and Concentration—The type of surfactant, as the stationary phase in chromatography, affects the resolution because it modifies separation selectively. The log K' of a neutral compound increases linearly with the concentration of •surfactant•1 in the mobile phase. When K' approaches the value of

$$\sqrt{t_m/t_0}$$

resolution in MEKC reaches a maximum. Modifying the concentration of surfactant in the mobile phase changes the resolution.

Buffer pH—pH does not modify the partition coefficient of non-ionized solutes, but it can modify the electroosmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electroosmotic flow and, therefore, increases the resolution of the neutral solutes •in MEKC, resulting in a•1 longer analysis time.

Organic Solvents—To improve •MEKC•1 separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetonitrile, etc.) can be added to the •1 electrolytic solution. The addition of these modifiers generally decreases migration time and selectivity of the separation. The addition of organic modifiers affects micelle formation, thus, a given surfactant concentration can be used only with a certain percentage of organic modifier before the micellezation •is inhibited•1 or adversely affected, resulting in the absence of micelles and, therefore, the absence of the partition. •The dissociation•1 of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible, because in some cases, the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

Additives for Chiral Separations—•For the separation of enantiomers using MEKC,•1 a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include salts, *N*-dodecanoyl-L-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions that contain micellized achiral surfactants.

Other Additives—Selectivity can be modified by adding chemicals to the buffer. Addition of several types of cyclodextrins to the buffer is also used to reduce the interaction of hydrophobic solutes with the micelle, increasing the selectivity for this type of compound. The addition of substances able to modify solute-micelle interactions by adsorption on the latter has been used to improve the selectivity of the separations in MEKC. These additives may consist of a second surfactant (ionic or non-ionic), which gives rise to mixed micelles or metallic cations that dissolve in the micelle and form co-ordination complexes with the solutes.

•QUANTIFICATION•1

Peak areas must be divided by the corresponding migration time to give the corrected area in order to compensate for the shift in migration time from run to run, thus reducing the variation of the response. Dividing the peak areas by migration time will also compensate for the different responses of sample constituents with different migration times. Where an internal standard is used, check that no peak of the substance to be examined is masked by that of the internal standard.

Calculations—From the values obtained, calculate the content of a component or components being determined. When indicated, the percentage of one (or more) components of the sample to be examined is calculated by determining the •corrected•1 area(s) of the peak(s) as a percentage of the total •of the•1 corrected areas of all the peaks, excluding those due to solvents or any added reagents

Interim Revision Announcement

•(normalization procedure).•1 The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

Change to read:

••1SYSTEM SUITABILITY

•In order to check the behavior of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. They are: retention•1 factor *(K′)* used only for *Micellar Electrokinetic Chromatography,* •apparent•1 number of theoretical plates *(N),* the symmetry factor *(A_S),* and the resolution *(R_S)*. Note that in previous sections, the theoretical expressions for N and R_S have been described, but more practical equations that allow for the determination of these suitability parameters using the •electropherograms•1 are described below.

•Apparent Number of Theoretical Plates—•1The •apparent•1 number of theoretical plates *(N)* may be calculated from the formula:

$$•N = 5.54\ (t_R / w_h)^2•_1$$

in which •t_R•1 is the •migration time or•1 distance ••1 along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak •corresponding to the component•1; and •w_h•1 is the peak width••1 at half-height.

•Resolution—•1The resolution *(R_S)* may be calculated from the formula:

$$•R_S = 1.18\ (t_{R1} - t_{R2}\ ) / (w_{h1} + w_{h2})$$

$$t_{R2} > t_{R1}•_1$$

in which •t_{R1} and t_{R2}•1 are the •migration times or•1 distances along the baseline between the point of injection and the perpendicular dropped from the maxima of two adjacent peaks and •w_{h1} and w_{h2}•1 are the peak widths ••1 at half-height.

•When appropriate,•1 the resolution *(R_S)* may also be calculated by measuring the height of the valley •(H_v)•1 between two partly resolved peaks in a standard preparation, the height of the smaller peak •(H_p)•1 and •calculating the peak-to-valley ratio:

$$p/v = H_p / H_v$$

Symmetry Factor—•1 The symmetry factor of a peak *(A_S)* may be calculated using the formula:

$$•A_s = w_{0.05} / 2d•_1$$

in which •$w_{0.05}$•1 is the width of the peak at one-twentieth of the peak height; and •d•1 is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Other ••1 suitability parameters include tests for area repeatability (••1 standard deviation of areas or of area/migration time) and tests for migration time repeatability (••1standard deviation of migration time).•Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use a migration time relative to an internal standard.

Signal-To-Noise Ratio—•1A test for the verification of the signal-to-noise ratio for a standard preparation or the determination of the limit of •quantification may also be useful for the determination of related substances•1 . The detection limit and •quantification—•1 limit correspond to a signal-to-noise ratio greater than 3 and 10, respectively. The signal-to-noise ratio *(S/N)* is calculated as follows:

$$S/N = 2H/h•_1$$

in which H is the height of the peak corresponding to the component concerned in the •electropherogram•1 obtained with the specified reference solution, •measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height; and h is the range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.•1

⟨1054⟩ BIOTECHNOLOGY-DERIVED ARTICLES—ISOELECTRIC FOCUSING

Change to read:

•GENERAL PRINCIPLE•1

Isoelectric focusing (IEF) is a method of electrophoresis that separates proteins according to their isoelectric points. Separation is carried out in a slab of polyacrylamide or agarose gel that contains a mixture of amphoteric electrolytes (ampholytes). When subjected to an electrical field, the ampholytes migrate in the gel to create a pH gradient. In some cases, gels containing an immobilized pH gradient, prepared by incorporating weak acids and bases to specific regions of the gel network during the preparation of the gel, are used. When the applied proteins reach the gel fraction that has a pH that is the same as their isoelectric point •(pI),•1 their charge is neutralized and migration ceases. Gradients can be made over various ranges of pH, according to the mixture of ampholytes chosen.

Change to read:

•THEORETICAL ASPECTS•1

When a protein is at the position of its isoelectric point, it has no net charge and cannot be moved in a gel matrix by the electric field. It may, however, move from that position by diffusion. The pH gradient forces a protein to remain in its isoelectric point position, thus concentrating it; this concentration effect is called "focusing". Increasing the applied voltage or reducing the sample load results in improved resolution of bands. The applied voltage is limited by the heat generated because the heat must be dissipated. The use of thin gels and an efficient cooling plate controlled by a thermostatic circulator prevents the burning of the gel while allowing sharp focusing. The separation is estimated by determining the minimum pI difference •*(ΔpI),*•1 which is necessary to separate two neighboring bands, as follows:

$$\Delta pI = 3\sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

in which D is the diffusion coefficient of the protein; *dpH/dx* is the pH gradient; E is the intensity of the electric field, in volts per centimeter; and $-d\mu/dpH$ is the variation of the solute mobility with the pH in the region close to the pI. Because D and $-d\mu/dpH$ for a given protein cannot be altered, the separation can be improved by using a narrower pH range and by increasing the intensity of the electric field.

Resolution between protein bands on an IEF gel prepared with carrier ampholytes can be quite good. Better resolution may be achieved by using immobilized pH gradients where the buffering species, which are analogous to carrier ampholytes, are copolymerized within the gel matrix. Proteins exhibiting pI values differing by as little as 0.02 pH units may be resolved using a gel prepared with carrier ampholytes, whereas immobilized pH gradients can resolve protein differing by approximately 0.001 pH units.

Change to read:

•PRACTICAL ASPECTS•

From an operational point, special attention must be paid to sample characteristics and/or preparation. Salt in a sample can be problematic, and it is best to prepare the sample, if possible, in deionized water or 2% ampholytes using dialysis or gel filtration if necessary. •• The time required for completion of focusing in thin-layer polyacrylamide gels is determined by placing a colored protein (e.g., hemoglobin) at different positions on the gel surface and by applying the electric field: the steady state is reached when all applications give an identical band pattern. In some procedures the completion of the focusing is indicated by the time elapsed after the sample application.

The IEF gel can be used as an identity test when migration •pattern• on the gel is compared to a •suitable• standard preparation and IEF calibration proteins; the IEF gel can be used as a limit test when the density of a band on IEF is compared subjectively with the density of bands appearing in a standard preparation, or it can be used as a semiquantitative test when the density is measured using a densitometer or similar instrumentation to determine the relative concentration of protein in the bands •subject to validation.•

Change to read:

APPARATUS

An apparatus for isoelectric focusing consists of a controllable •generator for constant potential, current, and power. Potentials of 2500 V have been used and are considered optimal under a given set of operating conditions. Supply of up to 30 W of constant power is recommended. The apparatus also includes• a rigid plastic isoelectric focusing chamber that contains a cooled plate of suitable material to support the gel; and a plastic cover with platinum electrodes that are connected to the gel by means of paper wicks of suitable width, length, and thickness, impregnated with solutions of anodic and cathodic electrolytes.

Change to read:

•ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GELS: DETAILED PROCEDURE

The following method is a detailed description of an IEF procedure in thick polyacrylamide slab gels, which is used unless otherwise stated in the monograph.•

Preparation of the Gels

•Mold—The mold is• composed of a glass plate (A) on which a polyester film (B) is placed to facilitate handling of the gel, one or more spacers (C), a second glass plate (D), and clamps to hold the structure together (see *Figure 1*).

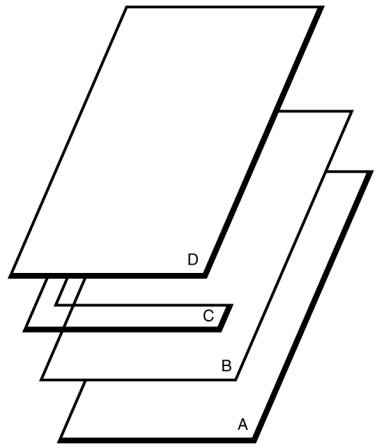

Figure 1. Mold

7.5% Polyacrylamide Gel—Dissolve 29.1 g of acrylamide and 0.9 g of methylenebisacrylamide in 100 mL of water. To 2.5 volumes of this solution, add the mixture of ampholytes specified in the individual monograph, and dilute •• to 10 volumes with water. Mix carefully, and degas the solution.

Preparation of the •Mold—•Place the polyester film on the lower glass plate, apply the spacer, place the second glass plate, and fit the clamps. Before use, place the mixture on a magnetic stirrer, and add 0.25 volumes of a •100 g/L• solution of ammonium persulfate and 0.25 volumes of tetramethylenediamine. Immediately fill the space between the glass plates of the •mold with the solution.•

Fixing Solution for Isoelectric Focusing Polyacrylamide Gel—Mix 35 g of sulfosalicylic acid and 100 g of trichloroacetic acid in 1000 mL of water.

Coomassie Staining Solution and Destaining Solution—Use the same solutions indicated in general information chapter *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.

Procedure

Dismantle the •*Mold*,• and using the polyester film, transfer the gel onto the cooled support wetted with a few mL of a suitable liquid, taking care to avoid forming air bubbles. Prepare the test solutions and reference solutions as specified in the individual monograph. Place strips of paper for sample application, about 10 mm × 5 mm in size, on the gel, and impregnate each with the prescribed amount of the test and reference solutions. •• Also apply the prescribed quantity of a solution of proteins with known isoelectric points as pH markers to calibrate the gel. In some procedures, the gel has precast slots where a solution of the sample is applied instead of using impregnated paper strips. Cut two strips of paper to the length of the gel, and impregnate them with the electrolyte solutions: acid for the anode and alkaline for the cathode. The compositions of the anode and cathode solutions are given in the individual monograph. Apply these paper wicks to each side of the gel several mm from the edge. Fit the cover so that the electrodes are in contact with the wicks (with respect to the anodic and cathodic poles). Proceed with the isoelectric focusing by applying the electrical parameters described in the individual monograph. Switch off the current when the migration of the mixture of standard proteins has stabilized. Using forceps, remove the sample application strips and the two electrode wicks. Immerse the gel in *Fixing Solution for Isoelectric Focusing Polyacrylamide Gel.* Incubate with gentle shaking at room temperature for 30 minutes. Drain off the solution, and add 200 mL of *Destaining Solution.* Incubate with shaking for 1 hour. Drain the gel, and add *Coomassie Staining Solution.* Incubate for 30 minutes. Destain the gel by passive diffusion with *Destaining Solution* until the bands are well visualized against a clear background. Locate the position and intensity of the bands in the electropherogram, as prescribed in the individual monograph.

•Variations to the Detailed Procedure (Subject to Validation)

Where reference to the general method on isoelectric focusing is made, variations in methodology or procedure may be made subject to validation.•1 These variations include the use of commercially available precast gels •and of commercial staining and destaining kits;•1 the use of immobilized pH gradients; the use of rod gels, and the use of cassettes of different dimensions, including ultra-thin (0.2 mm) gels; variations in the sample application procedure, including different sample volumes or the use of sample application masks or wicks other than paper; the use of alternate running conditions, including variations in the electric field depending on gel dimensions and equipment, and the use of fixed migration times rather than subjective interpretation of band stability; the inclusion of a prefocusing step; the use of automated instrumentation; and the use of agarose gels.

Change to read:

Validation of •Isoelectric Focusing•1 Procedures

Where alternative methods to the •detailed procedure•1 are employed, they must be validated. The following criteria may be used to validate the separation: formation of a stable pH gradient of desired characteristics, •assessed for example•1 using colored pH markers of known isoelectric points; comparison with the electropherogram provided with the chemical reference substance for the preparation to be examined; and any other validation criteria as prescribed in the individual monograph.

Change to read:

SPECIFIED VARIATIONS TO THE GENERAL METHOD

Variations to the general method required for the analysis of specific substances may be specified in detail in individual monographs. Variations may include the addition of urea in the running gel (a 3 M concentration is often satisfactory to keep the protein in solution, but up to 8 M can be used). Some proteins precipitate at their isoelectric point. In this case, urea is included in the gel formulation to keep the protein in solution. If urea is used, only fresh solutions should be used to prevent carbamylation of the protein. Other variations include the use of alternative staining methods and the use of gel additives such as nonionic detergents (e.g., octylglucoside) or zwitterionic detergents (e.g., CHAPS or CHAPSO) •and the addition of ampholyte to the sample•1 to prevent proteins from aggregating or precipitating.

Change to read:

•Points To Consider•1

••1

— Samples can be applied to any area on the gel, but ••1 to protect the proteins from extreme pH environments, samples should not be applied close to either electrode. During method development, the analyst can try applying the protein in three positions on the gel (i.e., middle and both ends); the pattern of a protein applied at opposite ends of the gel may not be identical.

— A phenomenon known as cathodic drift, where the pH gradient decays over time, may occur if a gel is focused too long. Although not well understood, electroendoosmosis and absorption of carbon dioxide may be factors that lead to cathodic drift. Cathodic drift is observed as focused protein migrating off the cathode end of the gel. Immobilized pH gradients may be used to address this problem.

— Efficient cooling (approximately 4°) of the bed that the gel lies on during focusing is important. High field strengths used during isoelectric focusing can lead to overheating and affect the quality of the focused gel.

⟨1055⟩ BIOTECHNOLOGY-DERIVED ARTICLES—PEPTIDE MAPPING

Change to read:

This chapter provides guidance and procedures used for characterization of biotechnology-derived articles by peptide mapping. This chapter is harmonized with the corresponding chapter in *JP* and *EP*. •Portions of the chapter that are not harmonized with the other two pharmacopeias are marked by the symbol ◆.•1 Other characterization tests, also harmonized, are shown in *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, *Biotechnology-Derived Articles—Capillary Electrophoresis* ⟨1053⟩, *Biotechnology-Derived Articles—Isoelectric Focusing* ⟨1054⟩, *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩, and *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.

Change to read:

INTRODUCTION

Peptide mapping is an identity test for proteins, especially those obtained by rDNA technology. It involves the chemical or enzymatic treatment of a protein, resulting in the formation of peptide fragments, followed by separation and identification of the resultant fragments in a reproducible manner. It is a powerful test that is capable of identifying single amino acid changes resulting from events such as errors in the reading of complementary DNA (cDNA) sequences or point mutations. Peptide mapping is a comparative procedure because the information obtained, compared to a Reference Standard or Reference Material similarly treated, confirms the primary structure of the protein, is capable of detecting whether alterations in structure have occurred, and demonstrates process consistency and genetic stability. Each protein presents unique characteristics that must be well understood so that the scientific and analytical approaches permit validated development of a peptide map that provides sufficient specificity.

This •chapter•1 provides detailed assistance in the application of peptide mapping and its validation to characterize the desired protein product, to evaluate the stability of the expression construct of cells used for recombinant DNA products, to evaluate the consistency of the overall process, and to assess product stability, as well as to ensure the identity of the protein product or to detect the presence of protein variant. •◆•1 The validation scheme presented differentiates between qualification of the method at an early stage in the regulatory process, the Investigational New Drug (IND) level, and full validation in support of New Drug Application (NDA), Product License Application (PLA), or Marketing Authorization Application (MAA). The validation concepts described are consistent with the general information chapter *Validation of Compendial Procedures* ⟨1225⟩ and with the International Conference on Harmonization (ICH) document on *Analytical Methods Validation*.•◆•1

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THE PEPTIDE MAP

Peptide mapping is not a general method, but involves developing specific maps for each unique protein. Although the technology is evolving rapidly, there are certain methods that are generally accepted. Variations of these methods will be indicated, when appropriate, in specific monographs.

A peptide map may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provide a comprehensive understanding of the protein being analyzed. Four major steps are necessary for the development of the procedure: isolation and purification of the protein, if the protein is part of a formulation; selective cleavage of the peptide bonds; chromatographic separation of the peptides; and analysis and identification of the peptides. A test sample is digested and assayed in parallel with a •Reference Standard or Reference Material.• Complete cleavage is more likely to occur when enzymes such as endoproteases (e.g., trypsin) are used instead of chemical cleavage reagents. A map should contain enough peptides to be meaningful. On the other hand, if there are too many fragments, the map might lose its specificity because many proteins will then have the same profiles.

Isolation and Purification

Isolation and purification are necessary for analysis of bulk drugs or dosage forms containing interfering excipients and carrier proteins and, when required, will be specified in the monograph. Quantitative recovery of protein from the dosage form should be validated.

Selective Cleavage of Peptide Bonds

The selection of the approach used for the cleavage of peptide bonds will depend on the protein under test. This selection process involves determination of the type of cleavage to be employed—enzymatic or chemical—and the type of cleavage agent within the chosen category. Several cleavage agents and their specificity are shown in *Table 1*. This list is not all-inclusive and will be expanded as other cleavage agents are identified.

PRETREATMENT OF SAMPLE

Depending on the size or the configuration of the protein, different approaches in the pretreatment of samples can be used. For monoclonal antibodies, the heavy and light chains will need to be separated before mapping. If trypsin is used as a cleavage agent for proteins with a molecular mass greater than 100,000 Da, lysine residues must be protected by citraconylation or maleylation; otherwise, too many peptides will be generated.

PRETREATMENT OF THE CLEAVAGE AGENT

Pretreatment of cleavage agents, especially enzymatic agents, might be necessary for purification purposes to ensure reproducibility of the map. For example, trypsin used as a cleavage agent will have to be treated with tosyl-L-phenylalanine chloromethyl ketone to inactivate chymotrypsin. Other methods, such as purification of trypsin by HPLC or immobilization of enzyme on a gel support, have been successfully used when only a small amount of protein is available.

PRETREATMENT OF THE PROTEIN

•Under certain conditions, it might be necessary• to concentrate the sample, or to separate the protein from added substances and stabilizers used in the formulation of the product if these interfere with the mapping procedure. Physical procedures used for pretreatment can include ultrafiltration, column chromatography, and lyophilization.

Other pretreatments such as the addition of chaotropic agents (e.g., urea) can be used to unfold the protein prior to mapping. •To• allow the enzyme to have full access to cleavage sites and permit some unfolding of the protein, •it is often necessary to reduce and alkylate the disulfide bonds prior to digestion.•

Digestion with trypsin can introduce ambiguities in the tryptic map as a result of side reactions occurring during the digestion reaction, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, or formation of pyroglutamic groups created from the deamidation of glutamine at the *N*-terminal side of a peptide. Furthermore, peaks may be produced by autohydrolysis of trypsin. Their intensities depend on the ratio of trypsin to protein. To avoid autohydrolysis, solutions of proteases may be prepared at a pH that is not optimal (e.g., at pH 5 for trypsin), which would mean that the enzyme would not become active until diluted with the digest buffer.

ESTABLISHMENT OF OPTIMAL DIGESTION CONDITIONS

Factors that affect the completeness and effectiveness of digestion of proteins are those that could affect any chemical or enzymatic reactions.

pH—The digestion mixture pH is empirically determined to ensure the optimal performance of the given cleavage agent. For example, •when using cyanogen bromide as a cleavage agent,• a highly acidic environment (e.g., pH 2, formic acid) is necessary; however, when using trypsin as a cleavage agent, a slightly alkaline environment (pH 8) is optimal. As a general rule, the pH of the reaction milieu should not alter the chemical integrity of the protein during the digestion and should not change during the course of the fragmentation reaction.

Table 1. Examples of Cleavage Agents

Type	Agent	Specificity
Enzymatic	Trypsin, EC 3.4.21.4	*C*-terminal side of Arg and Lys
	Chymotrypsin, EC 3.4.21.1	*C*-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin A (Pepsin), EC 3.4.23.1	Nonspecific digest
	Lysyl endopeptidase (Lys-C endopeptidase), EC 3.4.21.50	*C*-terminal side of Lys
	Glutamyl endopeptidase (Glu-C endoproteinase; V8 protease); (from *S. aureus* strain V8), EC 3.4.21.19	*C*-terminal side of Glu and Asp
	Peptidyl-Asp metalloendopeptidase (Asp-N endoproteinase), EC 3.4.24.33	*N*-terminal side of Asp
	Clostripain (Arg-C endopeptidase), EC 3.4.22.8	*C*-terminal side of Arg
Chemical	Cyanogen bromide	*C*-terminal side of Met
	2-Nitro-5-thiocyanobenzoic acid	*N*-terminal side of Cys
	O-Iodosobenzoic acid	*C*-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	BNPS-skatole	Trp

Temperature—A temperature between 25° and 37° is adequate for most digestions. The temperature used is intended to minimize chemical side reactions. The type of protein under test will dictate the temperature of the reaction milieu because some proteins are more susceptible to denaturation as the temperature of the reaction increases. For example, digestion of recombinant bovine somatropin is conducted at 4° because at higher temperatures it will precipitate during digestion.

Time—If a sufficient amount of sample is available, a time course study is considered in order to determine the optimum time to obtain a reproducible map and avoid incomplete digestion. Time of digestion varies from 2 to 30 hours. The reaction is stopped by the addition of an acid that does not interfere with the tryptic map, or by freezing.

Amount of Cleavage Agent—Although excessive amounts of cleavage agent are used to accomplish a reasonably rapid digestion time (i.e., 6 to 20 hours), the amount of cleavage agent is minimized to avoid its contribution to the chromatographic map pattern. A protein-to-protease ratio between 20 : 1 and 200 : 1 is generally used. It is recommended that the cleavage agent be added in two or more stages to optimize cleavage. Nonetheless, the final reaction volume remains small enough to facilitate the next step in peptide mapping—the separation step. To sort out digestion artifacts that might be interfering with the subsequent analysis, a blank determination is performed using a digestion control with all the reagents except the test protein.

Chromatographic Separation

Many techniques are used to separate peptides for mapping. The selection of a technique depends on the protein being mapped. Techniques that have been successfully used for the separation of peptides are shown in *Table 2*.

Table 2. Techniques Used for the Separation of Peptides

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)
Ion-Exchange Chromatography (IEC)
Hydrophobic Interaction Chromatography (HIC)
Polyacrylamide Gel Electrophoresis (PAGE), nondenaturating
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
Capillary Electrophoresis (CE)
Paper Chromatography
High-Voltage Paper Electrophoresis (HVPE)

In this section, a most widely used reverse-phase HPLC (RP-HPLC) method is described as one of the procedures of chromatographic separation.

The purity of solvents and mobile phases is a critical factor in HPLC separation. HPLC-grade solvents and water that are commercially available are recommended for RP-HPLC. Dissolved gases present a problem in gradient systems where the solubility of the gas in a solvent may be less in a mixture than in a single solvent. Vacuum degassing and agitation by sonication are often used as useful degassing procedures. The solid particles in the solvents are drawn into the HPLC system; they can damage the sealing of pump valves or clog the top of the chromatographic column. Both pre- and post-pump filtration are also recommended.

Chromatographic Column—The selection of a chromatographic column is empirically determined for each protein. Columns with 100 Å or 300 Å pore size and silica support can give optimal separation. For smaller peptides, •$_{\bullet 1}$ octylsilane chemically bonded to totally porous silica articles 3 to 10 µm in diameter (L7) and of octadecylsilane chemically bonded to porous silica or ceramic microparticles 3 to 10 µm in diameter (L1) •column packings$_{\bullet 1}$ are more efficient than the butyl silane chemically bonded to totally porous silica particles 5 to 10 µm in diameter (L26) •packing.$_{\bullet 1}$

Solvent—The most commonly used solvent is water with acetonitrile as the organic modifier to which less than 0.1% of trifluoroacetic acid is added. If necessary, add isopropyl alcohol or *n*-propyl alcohol to solubilize the digest components, provided that the addition does not unduly increase the viscosity of the components.

Mobile Phase—Buffered mobile phases containing phosphate are used to provide some flexibility in the selection of pH conditions, because shifts of pH in the 3.0 to 5.0 range enhance the separation of peptides containing acidic residues (e.g., glutamic and aspartic acids). Sodium or potassium phosphates, ammonium acetate, and phosphoric acid, with a pH between 2 and 7 (or higher for polymer-based supports), have also been used with acetonitrile gradients. Acetonitrile-containing trifluoroacetic acid is also used quite often.

Gradient Selection—Gradients can be linear, nonlinear, or include step functions. A shallow gradient is recommended in order to separate complex mixtures. Gradients are optimized to provide clear resolution of one or two peaks that will become "marker" peaks for the test.

Isocratic Selection—Isocratic HPLC systems using a single mobile phase are used on the basis of their convenience of use and improved detector responses. Optimal composition of a mobile phase to obtain clear resolution of each peak is sometimes difficult to establish. Mobile phases for which slight changes in component ratios or in pH significantly affect retention times of peaks in peptide maps should not be used in isocratic HPLC systems.

Other Parameters—Temperature control of the column is usually necessary to achieve good reproducibility. The flow rates for the mobile phases range from 0.1 to 2.0 mL per minute, and the detection of peptides is performed with a UV detector at 200 to 230 nm. Other methods of detection have been used (e.g., postcolumn derivatization), but they are not as robust or as versatile as UV detection.

System Suitability—The section *System Suitability* under *Chromatography* ⟨621⟩ provides an experimental means for measuring the overall performance of the test method. The acceptance criteria for system suitability depend on the identification of critical test parameters that affect data interpretation and acceptance. These critical parameters are also criteria that monitor peptide digestion and peptide analysis. An indicator that the desired digestion endpoint was achieved is the comparison with a Reference Standard or Reference Material, which is treated exactly as the article under test. The use of a USP Reference Standard in parallel with the protein under test is critical in the development and establishment of system suitability limits. In addition, a specimen chromatogram should be included with the USP Reference Standard or Reference Material for comparison purposes. Other indicators may include visual inspection of protein or peptide solubility, the absence of intact protein, or measurement of responses of a digestion-dependent peptide. The critical system suitability parameters for peptide analysis will depend on the particular mode of peptide separation and detection, and on the data analysis requirements.

When peptide mapping is used as an identification test, the system suitability requirements for the identified peptides cover selectivity and precision. In this case, as well as when identification of variant proteins is done, the identification of the primary structure of the peptide fragments in the peptide map provides both a verification of the known primary structure and the identification of protein variants by comparison with the peptide map of the USP Reference Standard or Reference Material for the specified protein. The use of a digested USP Reference Standard or Reference Material for a given protein in the determination of peptide resolution is the method of choice. For an analysis of a variant protein, a characterized mixture of a variant and a Reference Standard can be used, especially if the variant peptide is located in a less-resolved region of the map. The index of pattern consistency can be simply the number of major peptides detected. Peptide pattern consistency can be best defined by the resolution of peptide peaks. Chromatographic parameters—such as peak-to-peak resolution, maximum peak width, peak tailing factors, and column efficiency—may be used to define peptide resolution. Depending on the protein under test and the method of separation used, single peptide or multiple peptide resolution requirements may be necessary.

The replicate analysis of the digest of the USP Reference Standard or Reference Material for the protein under test yields measures of precision and quantitative recovery. Recovery of the identified peptides is generally ascertained by the use of internal or external peptide standards. The precision is expressed as the relative standard deviation (RSD). Differences in the recovery and precision of the identified peptides are expected; therefore, the system suitability limits will have to be established for both the recovery and the precision of the identified peptides. These limits are unique for a given protein and will be specified in the individual monograph.

Visual comparison of the relative retention times, the peak responses, the number of peaks, and the overall elution pattern is completed initially. It is then complemented and supported by mathematical analysis of the peak response ratios and by the chromatographic profile of a 1 : 1 (v/v) mixture of sample and USP Reference Standard or Reference Material digest. If all peaks in the sample digest and in the USP Reference Standard or Reference Material digest have the same relative retention times and peak response ratios, then the identity of the sample under test is confirmed.

If peaks that initially eluted with significantly different relative retention times are then observed as single peaks in the 1 : 1 mixture, the initial difference would be an indication of system variability. However, if separate peaks are observed in the 1 : 1 mixture, this would be evidence of the nonequivalence of the peptides in each peak. If a peak in the 1 : 1 mixture is significantly broader than the corresponding peak in the sample and USP Reference Standard or Reference Material digest, it may indicate the presence of different peptides. The use of computer-aided pattern recognition software for the analysis of peptide mapping data has been proposed and applied, but issues related to the validation of the computer software preclude its use in a compendial test in the near future. Other automated approaches have been used that employ mathematical formulas, models, and pattern recognition. Such approaches, for example, the automated identification of compounds by IR spectroscopy and the application of diode-array UV spectral analysis for identification of peptides, have been proposed. These methods have limitations due to inadequate resolutions, co-elution of fragments, or absolute peak response differences between USP Reference Standard or Reference Material and sample fragments.

The numerical comparison of the retention times and peak areas or peak heights can be done for a selected group of relevant peaks that have been correctly identified in the peptide maps. Peak areas can be calculated using one peak showing relatively small variation as an internal reference, keeping in mind that peak area integration is sensitive to baseline variation and is likely to introduce error into the analysis. Alternatively, the percentage of each peptide peak height relative to the sum of all peak heights can be calculated for the sample under test. The percentage is then compared to that of the corresponding peak of the USP Reference Standard or Reference Material. The possibility of autohydrolysis of trypsin is monitored by producing a blank peptide map that is the peptide map obtained when a blank solution is treated with trypsin.

The minimum requirement for the qualification of peptide mapping is an approved test procedure that includes system suitability as a test control. In general, •early in the regulatory process,• qualification of peptide mapping for a protein is sufficient. As the regulatory approval process for the protein progresses, additional qualifications of the test can include a partial validation of the analytical procedure to provide assurance that the method will perform as intended in the development of a peptide map for the specified protein.

Analysis and Identification of Peptides

This section gives guidance on the use of peptide mapping during development in support of regulatory applications.

The use of a peptide map as a qualitative tool does not require the complete characterization of the individual peptide peaks. However, validation of peptide mapping in support of regulatory applications requires rigorous characterization of each of the individual peaks in the peptide map. Methods to characterize peaks range from *N*-terminal sequencing of each peak followed by amino acid analysis to the use of mass spectroscopy (MS).

For characterization purposes, when *N*-terminal sequencing and amino acid analysis are used, the analytical separation is scaled up. Because scale-up might affect the resolution of peptide peaks, it is necessary, using empirical data, to assure that there is no loss of resolution due to scale-up. Eluates corresponding to specific peptide peaks are collected, vacuum-concentrated, and chromatographed again, if necessary. Amino acid analysis of fragments may be limited by the peptide size. If the *N*-terminus is blocked, it may need to be cleared before sequencing. *C*-terminal sequencing of proteins or a combination of carboxypeptidase digestion and matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) MS can also be used for characterization purposes.

The use of MS for characterization of peptide fragments is by direct infusion of isolated peptides or by the use of on-line LC-MS for structure analysis. In general, it includes electrospray and MALDI-TOF analyzers as well as fast atom bombardment (FAB). Tandem MS has also been used to sequence a modified protein and to determine the type of amino acid modification that has occurred. The comparison of mass spectra of the digests before and after reduction provides a method to assign the disulfide bonds to the various sulfhydryl-containing peptides.

If regions of the primary structure are not clearly demonstrated by the peptide map, it might be necessary to develop a secondary peptide map. The goal of a validated method of characterization of a protein through peptide mapping is to reconcile and account for at least 95% of the theoretical composition of the protein structure.

Change to read:

•◆•1 THE USE OF PEPTIDE MAPPING FOR GENETIC STABILITY EVALUATION

A validated peptide map can be used to assess the integrity of the predicted primary sequence of a protein product (i.e., its genetic stability). It can also be used to determine lot-to-lot consistency of the biotechnology-derived product process. Furthermore, the performance of the protein expression of the production system is best assessed by peptide mapping of the expressed protein. Peptide maps of protein produced at various times of the protein expression process, including a point well beyond the normal protein expression time, compared with those of a USP Reference Standard or Reference Material, will evaluate the genetic stability of the expression system as a function of time.

Variant protein sequences can arise from a genetic variation at the DNA level (point mutation) or as an error in the translation process. A validated peptide map is the best approach to the detection of protein variants. However, the limitations of the peptide mapping itself must be taken into consideration. The detection of a structured variant is possible only if the corresponding peptide variant is easily isolated and characterized. To establish genetic stability will require the use of a battery of biochemical methods, provided that the variants have properties different from those of the "normal" protein.•◆•1

Change to read:

•◆•1 VALIDATION

Critical Factors

Validation of peptide mapping requires that a protocol be designed, outlining in detail the experiment to be conducted and the criteria for acceptance of the map. Criteria for acceptance of mapping include detection limit, specificity, linearity, range, accuracy, precision, and reagent stability. Reproducibility of the peptide map is a critical element in the utilization of such a map as an identity test and for confirming genetic stability. Those technical aspects of peptide mapping that influence the reproducibility of the map will be discussed.

The setting of limits, with respect to quantification (peak area or height) and identification (retention times) for the selected group of relevant peaks is based on empirical observations. These limits detect significant differences between the sample and USP Reference Standard or Reference Material within a series of analyses.

Interim Revision Announcement

Another critical issue is the recovery of peptides and its impact on peak area determination and reproducibility and on the establishment of acceptance criteria. The recovery criteria address all aspects of test methodology, from digestion to chromatographic conditions. Determination of peptide recovery includes quantitative amino acid analysis, spike addition, radiolabeling, and UV summation. An overall recovery of about 80% is considered satisfactory. Recovery of individual peptides is more problematic and is handled on a case-by-case basis. The critical factors considered in the validation of a peptide map are as follows.

Written Test Procedures—These procedures include a detailed description of the analytical method in which reagents, equipment, sample preparation, method of analysis, and analysis of the data are defined.

Validation Protocol—A protocol is prepared that contains a procedure for test validation.

Acceptance Criteria—The criteria can be minimal at the early stages, but need to be better defined as validation studies progress.

Reporting of Results—Results from the validation study are documented with respect to the analytical parameters listed in the validation protocol.

Revalidation of the Test Procedure—If the method used requires alteration that could affect the analytical parameter previously assessed in the validation of the procedure, the test procedure must be revalidated. Significant changes in the processing of the article, in laboratories performing the analysis, in formulation of the bulk or the finished products, and in any other significant parameter will require revalidation of the methods.

Requirements

PRECISION

Intratest Precision—This is a measure of the reproducibility of peptide mapping. The two critical steps in peptide mapping are fragmentation (i.e., digestion) and separation of peptides. An acceptable precision occurs where the absolute retention times and the relative peak areas are constant from run to run, and the average variation in retention time is small relative to that of a selected internal reference peak. The reproducibility of the map can be enhanced if a temperature-controlled column oven is used, if an extensive equilibration of the system is performed prior to the start of the test, if a blank (control digest mixture without protein) is run first to minimize ''first run effects,'' and if a USP Reference Standard or a Reference Material digest is interspersed periodically with test samples to evaluate chromatographic drift.

The criteria for validation of the fragmentation step are similar to those described below for separation of peptides, but they are met for consecutive tests of a series of separately prepared digests of the protein under test.

The criteria for validation of the separation of peptides step include the following:

1. The average standard deviation of the absolute retention times of all major peaks for a set of consecutive tests of the same digest does not exceed a specified acceptance criterion.
2. The average standard deviation of absolute peak area for all fully resolved major peaks does not exceed a specified percentage.

Intertest Precision—This is a measure of the reproducibility of the peptide mapping when the test is performed on different days, by different analysts, in different laboratories, with reagents or enzymes from different suppliers or different lots from the same supplier, with different instruments, on columns of different makes or columns of the same make from different lots, and on individual columns of the same make from the same lot. Although it would be desirable, from a scientific perspective, to validate all of these variables in terms of their impacts on precision, a practical approach is to validate the test using those variables most likely to be encountered under operational conditions. Additional variables can be included when needed.

The experimental design allows the analyst to make comparisons using peak retention times and areas that are expressed relative to a highly reproducible internal reference peak within the same chromatogram. The relative peak area is expressed as the ratio of the peak area to that of the internal reference peak. The relative retention time can be expressed as the difference between the absolute retention time and that of the reference peak. The use of relative values eliminates the need to make separate corrections for differences due to injector-to-injector volumes, units of measure for peak areas, column dimensions, and instrument dead volumes. The variability in the retention times and peak areas for the *Intertest Precision* experiments is expected to be slightly higher than the variability observed for *Intratest Precision*.

ROBUSTNESS

Factors such as composition of the *Mobile Phase*, protease quality or chemical reagent purity, column variation and age, and digest stability are likely to affect the overall performance of the test and its reproducibility. Tolerances for each of the key parameters are evaluated and baseline limits established in case the test is used for routine lot release purposes.

Mobile Phase—The composition of the *Mobile Phase* is optimized to obtain the maximum resolution of peptides throughout the elution profile. A balance between optimal resolution and overall reproducibility is desired. A lower pH might improve peak separation but might shorten the life of the column, resulting in lack of reproducibility. Peptide maps at a pH above and below the pH of the procedure are compared to the peptide map obtained at the pH of the procedure and checked for significant differences; they are also reviewed with respect to the acceptance criteria established in the validation protocol.

Protease Quality or Chemical Reagent Purity—A sample of the USP Reference Standard or Reference Material for the protein under test is prepared and digested with different lots of cleavage agent. The chromatograms for each digest are compared in terms of peak areas, shape, and number. The same procedure can be applied to other critical chemicals or pretreatment procedures used during sample preparation, such as reducing and carboxymethylation reagents.

Column Considerations—Column-to-column variability, even within a single lot, can affect the performance of the column in the development of peptide maps. Column size may also lead to significant differences. A USP Reference Standard or Reference Material of the protein under test is digested and the digest is chromatographed on different lots of column from a single manufacturer. The maps are then evaluated in terms of the overall elution profile, retention times, selectivity resolution, and recovery. To evaluate the overall lifetime of the column in terms of robustness, perform a peptide mapping test on different columns and vary significantly the number of injections (e.g., from 10 injections to 250 injections). The resulting maps can then be compared for significant differences in peak broadening, peak area, and overall resolution. As a column ages, an increase in back pressure might be observed that might affect the peptide maps.

A sensible precaution in the use of peptide mapping columns is to select alternative columns in case the original columns become unavailable or are discontinued. Perform a peptide mapping test using equivalent columns from different manufacturers, and examine the maps. Differences in particle shape and size, pore size and volume, carbon load, and end-capping can lead to significant differences in retention times, elution profile selectivity, resolution, and recovery. Slight modifications in the gradient profile may be required to achieve equivalency of mapping when using columns from different manufacturers. [NOTE—The equivalency between instrumentation used for the validation of the test and for routine quality control testing should be considered. It might be preferable to use the same HPLC system for all applications. Otherwise, equivalency of the systems is determined, which may require some changes in the chromatographic test conditions.]

Digest Stability—The length of time a digest can be kept before it is chromatographed, as well as the conditions under which the digest is stored before chromatography, is assessed. Several aliquots from a single digest are stored at different storage conditions and chromatographed. These maps are then evaluated for significant differences.

REPRODUCIBILITY

Determination of various parameters indicated above is repeated using the same USP Reference Standard or Reference Material and test sample in at least two different laboratories by two analysts equipped with similar HPLC systems. The generated peptide maps are evaluated for significant differences. •◆●1

⟨1056⟩ BIOTECHNOLOGY-DERIVED ARTICLES—POLYACRYLAMIDE GEL ELECTROPHORESIS

Change to read:

This chapter provides guidance and procedures used for characterization of biotechnology-derived articles by polyacrylamide gel electrophoresis.• Portions of the chapter that are not harmonized with the other two pharmacopeias are marked by the symbol ◆.●1 This chapter is harmonized with the corresponding chapter in *JP* and *EP*. Other characterization tests, also harmonized, are shown in *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, *Biotechnology-Derived Articles—Capillary Electrophoresis* ⟨1053⟩, *Biotechnology-Derived Articles—Isoelectric Focusing* ⟨1054⟩, *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩, and *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.

Change to read:

INTRODUCTION

•Scope

Polyacrylamide gel electrophoresis (PAGE) is used for the qualitative characterization of proteins in biological preparations, for control of purity and for quantitative determinations. ●1

•Purpose

Analytical gel electrophoresis is an appropriate method with which to identify and to assess the homogeneity of proteins in drug substances. The method is routinely used for the estimation of protein subunit molecular masses and for the determination of the subunit compositions of purified proteins.

Ready-to-use gels and reagents are widely available on the market and can be used instead of those described in this chapter, provided that they give equivalent results and that they meet the validity requirements given below under *Validation of the Test*.●1

Change to read:

•◆●1GENERAL PRINCIPLE OF ELECTROPHORESIS

Under the influence of an electrical field, charged particles migrate in the direction of the electrode bearing the opposite polarity. In gel electrophoresis, the movements of the particles are retarded by interactions with the surrounding gel matrix, which acts as a molecular sieve. The opposing interactions of the electrical force and molecular sieving result in differential migration rates according to the sizes, shapes, and charges of particles. Because of their different physicochemical properties, different macromolecules of a mixture migrate at different speeds during electrophoresis and thus are separated into discrete fractions. Electrophoretic separations can be conducted in systems without support phases (e.g., free solution separation in capillary electrophoresis) and in stabilizing media, such as thin-layer plates, films, or gels.•◆●1

Change to read:

CHARACTERISTICS OF POLYACRYLAMIDE GELS •●1

The sieving properties of polyacrylamide gels are established by the three-dimensional network of fibers and pores that is formed as the bifunctional bisacrylamide cross-links adjacent to polyacrylamide chains. Polymerization is catalyzed by a free-radical-generating system composed of ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine (TEMED).

As the acrylamide concentration of a gel increases, its effective pore size decreases. The effective pore size of a gel is operationally defined by its sieving properties, that is, by the resistance it imparts to the migration of macromolecules. There are limits to the acrylamide concentrations that can be used. At high acrylamide concentrations, gels break much more easily and are difficult to handle. As the pore size of a gel decreases, the migration rate of a protein through the gel decreases. By adjusting the pore size of a gel, through manipulating the acrylamide concentration, the resolution of the method can be optimized for a given protein product. Thus, a given gel is physically characterized by its respective composition of acrylamide and bisacrylamide.

In addition to the composition of the gel, the state of the protein is an important component of electrophoretic mobility. In the case of proteins, electrophoretic mobility is dependent on the pK value of the charged groups and the size of the molecule. It is influenced by the type, the concentration, and the pH of the buffer; by the temperature and the field strength; and by the nature of the support material.

•Denaturing Polyacrylamide Gel Electrophoresis●1

•This method cited is limited to the analysis of monomeric polypeptides with a mass range of 14,000 to 100,000 Da. It is possible to extend the mass range by various techniques (e.g., gradient gels or particular buffer systems), but those techniques are not discussed in this chapter.●1

Denaturing PAGE using sodium dodecyl sulfate (SDS) •(SDS-PAGE)●1 is the most common mode of electrophoresis used in assessing the pharmaceutical quality of protein products •and will be the focus of the example method.●1 Typically, analytical electrophoresis of proteins is carried out •in polyacrylamide gels●1 under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimize aggregation . •Most commonly,●1 the strongly anionic detergent SDS is used in combination with heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind •to●1 SDS, become negatively charged, and exhibit a consistent charge-to-•mass●1 ratio regardless of protein type. Because the amount of SDS bound is almost always proportional to the molecular •mass●1 of the polypeptide and is •●1 independent of its sequence, SDS-polypeptide complexes migrate through polyacrylamide gels •with mobilities dependent on●1 the size of the polypeptide.

The electrophoretic mobilities of the resultant detergent–polypeptide complexes all assume the same functional relationship to •their molecular masses.●1 Migration of SDS •complexes●1 is toward the anode in a predictable manner, with low molecular •mass●1 complexes migrating faster than larger ones. •●1 The molecular •mass●1 of a protein can therefore be estimated from its relative mobility in calibrated SDS-PAGE and •the occurrence of●1 a single band in such a gel is a criterion of purity.

Modifications to the polypeptide backbone, such as *N*- or *O*-linked glycosylation, however, have a significant impact on the apparent molecular •mass of a protein, because●1 SDS does not bind to a carbohydrate moiety in a manner similar to a the polypeptide. Thus, a

consistent charge-to-mass ratio is not maintained. The apparent molecular mass of proteins having undergone post-translational modifications is not a true reflection of the mass of the polypeptide chain.

Reducing Conditions

Polypeptide subunits and three-dimensional structure is often maintained in proteins by the presence of disulfide bonds. A goal of SDS-PAGE analysis under reducing conditions is to disrupt this structure by reducing disulfide bonds. Complete denaturation and dissociation of proteins by treatment with 2-mercaptoethanol or dithiothreitol (DTT) will result in the unfolding of the polypeptide backbone and subsequent complexation with SDS. In these conditions, the molecular mass of the polypeptide subunits can be calculated by linear regression in the presence of suitable molecular mass standards.

Nonreducing Conditions

For some analyses, complete dissociation of protein into subunit peptides is not desirable. In the absence of treatment with reducing agents such as 2-mercaptoethanol or DTT, disulfide covalent bonds remain intact, preserving the oligomeric form of the protein. Oligomeric SDS-protein complexes migrate more slowly than their SDS-polypeptide subunits. In addition, nonreduced proteins may not be completely saturated with SDS and hence may not bind the detergent in a constant mass ratio. This makes molecular mass determinations of these molecules less straightforward than analyses of fully denatured polypeptides, because it is necessary that both standards and unknown proteins be in similar configurations for valid comparisons. However, the staining of a single band in such a gel is a criterion of purity.

Characteristics of a Discontinuous Buffer System Gel Electrophoresis

The most popular electrophoretic method for the characterization of a complex mixture of proteins involves the use of a discontinuous buffer system consisting of two contiguous, but distinct, gels: a resolving or separating (lower) gel and a stacking (upper) gel. The two gels are cast with different porosities, pHs, and ionic strengths. In addition, different mobile ions are used in the gel and electrode buffers. The buffer discontinuity concentrates large volumes of sample in the stacking gel, resulting in improved resolution. When power is applied, a voltage drop develops across the sample solution that drives the proteins into the stacking gel. Glycinate ions from the electrode buffer follow the proteins into the stacking gel. A moving boundary region is rapidly formed with the highly mobile chloride ions in the front and the relatively slow glycinate ions in the rear. A localized high-voltage gradient forms between the leading and trailing ion fronts, causing the SDS-protein complexes to form into a thin zone (stack) and migrate between the chloride and glycinate phases. Within broad limits, regardless of the height of the applied sample, all SDS proteins condense into a very narrow region and enter the resolving gel as a well-defined, thin zone of high protein density. The large-pore stacking gel does not retard the migration of most proteins and serves mainly as an anticonvective medium. At the interface between the stacking and resolving gels, the proteins experience a sharp retardation due to the restrictive pore size of the resolving gel. Once in the resolving gel, proteins continue to be slowed by the sieving of the matrix. The glycinate ions overtake the proteins, which then move in a space of uniform pH formed by tris(hydroxymethyl)aminomethane (Tris) and glycine. Molecular sieving causes the SDS-polypeptide complexes to separate on the basis of their molecular masses.

Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels

GEL STOCK SOLUTIONS

30% Acrylamide–Bisacrylamide Solution—Prepare a solution containing 290 g of acrylamide and 10 g of methylene bisacrylamide per L of warm water, and filter. [NOTE—Acrylamide and methylene bisacrylamide are slowly converted during storage to acrylic acid and bisacrylic acid, respectively. This deamidation reaction is catalyzed by light and alkali. The pH of the solution must be 7.0 or lower. Store the solution in dark bottles at room temperature. Fresh solutions are prepared every month.]

Ammonium Persulfate Solution—Prepare a small quantity of solution having a concentration of 100 g of ammonium persulfate per L, and store at 4°. [NOTE—Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Ammonium persulfate decomposes slowly; therefore, prepare fresh solutions weekly.]

TEMED—Use an electrophoresis-grade reagent. [NOTE—*TEMED* accelerates the polymerization of acrylamide and bisacrylamide by catalyzing the formation of free radicals from ammonium persulfate. Because *TEMED* works only as a free base, polymerization is inhibited at low pH.]

SDS Solution—Use an electrophoresis-grade reagent. Prepare a solution having a concentration of about 100 g of SDS per L, and store at room temperature.

1.5 M Buffer Solution—Transfer about 90.8 g of Tris to a 500-mL flask, dissolve in 400 mL of water, adjust with hydrochloric acid to a pH of 8.8, dilute with water to volume, and mix.

1 M Buffer Solution—Transfer about 60.6 g of Tris to a 500-mL flask, add 400 mL of water, adjust with hydrochloric acid to a pH of 6.8, dilute with water to volume, and mix.

PLATE PREPARATION

Clean two glass plates (size e.g., 10 cm × 8 cm), the polytetrafluroethylene comb, the two spacers, and the silicone rubber tubing (diameter e.g., 0.6 mm × 35 cm) with mild detergent, and rinse thoroughly with water. Dry all items with a paper towel or tissue.

Lubricate the spacers and the tubing with nonsilicone grease. Apply the spacers along each of the two short sides of the glass plate 2 mm away from the edges and 2 mm away from the long side corresponding to the bottom of the gel.

Begin to lay the tubing on the glass plate by using one spacer as a guide. Carefully twist the tubing at the bottom of the spacer, and follow the long side of the glass plate. While holding the tubing with one finger along the long side, twist the tubing again, and lay it on the second short side of the glass plate, using the spacer as a guide.

Place the second glass plate in perfect alignment and hold the gel mold together by hand pressure. Apply two clamps on each of the two short sides of the mold. Carefully apply four clamps on the longer side of the mold, thus forming the bottom of the gel mold. Verify that the tubing is running along the edge of the glass plates and has not been extruded while placing the clamps. The gel mold is now ready for pouring the gel.

PREPARATION OF THE GEL

In a discontinuous buffer SDS-polyacrylamide gel, it is important to pour the resolving gel, let the gel set, and then pour the stacking gel, because the composition of the two gels in the acrylamide–bisacrylamide, the buffer, and the pH are different.

Resolving Gel—In a conical flask, prepare the appropriate volume of solution, containing the desired concentration of acrylamide for the resolving gel using the values given in *Table 1*. Mix the components in the order shown. Where appropriate, before adding the *Ammonium Persulfate Solution* and the *TEMED,* filter the solution if neccessary under vacuum through a cellulose acetate membrane filter

Interim Revision Announcement

(pore diameter 0.45-µm), and keep the solution under vacuum swirling the filtration unit until no more bubbles are formed in the solution. Add appropriate amounts of *Ammonium Persulfate Solution* and *TEMED,* as indicated in *Table 1;* swirl and pour immediately into the gap between the two glass plates of the mold. Leave sufficient space for the stacking gel (the length of the teeth of the comb plus 1 cm). Using a tapered glass pipet, carefully overlay the solution with water-saturated isobutyl alcohol. Leave the gel in a vertical position at room temperature to allow polymerization.

After polymerization is complete (about 30 minutes later), pour off the isobutyl alcohol and wash the top of the gel several times with water to remove the isobutyl alcohol overlay and any unpolymerized acrylamide. Drain as much fluid as possible from the top of the gel, then remove any remaining water with the edge of a paper towel.

Table 1. Preparation of Resolving Gel

	Component Volume (mL) per Gel Mold Volume Below							
Solution Component	5 mL	l0 mL	15 mL	20 mL	25 mL	30 mL	40 mL	50 mL
6% Acrylamide								
Water	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
30% Acrylamide–Bisacrylamide Solution	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 M Buffer Solution	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Acrylamide								
Water	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
30% Acrylamide–Bisacrylamide Solution	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 M Buffer Solution	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Acrylamide								
Water	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
30% Acrylamide–Bisacrylamide Solution	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 M Buffer Solution	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Acrylamide								
Water	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
30% Acrylamide–Bisacrylamide Solution	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 M Buffer Solution	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
14% Acrylamide								
Water	1.4	2.7	3.9	5.3	6.6	8.0	10.6	13.8
30% Acrylamide–Bisacrylamide Solution	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
1.5 M Buffer Solution	1.2	2.5	3.6	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Acrylamide								
Water	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
30% Acrylamide–Bisacrylamide Solution	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 M Buffer Solution	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium Persulfate Solution	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

Stacking Gel—In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide, •using the values given•1 in *Table 2*. Mix the components in the order shown. •Where appropriate,•1 before adding the *Ammonium Persulfate Solution* and the *TEMED,* •filter the solution if neccessary under vacuum through a cellulose acetate membrane filter (pore diameter 0.45-µm), and keep the solution under vacuum swirling the filtration unit until•1 no more bubbles are formed in the solution. Add appropriate amounts of *Ammonium Persulfate Solution* and *TEMED* as •indicated•1 in *Table 2*, swirl, and pour immediately into the gap between the two glass plates of the mold directly onto the surface of the polymerized *Resolving Gel.* Immediately insert a clean •polytetrafluoroethylene•1 comb into the stacking gel solution, being careful to avoid trapping air bubbles. Add more stacking gel solution to fill the spaces of the comb completely. Leave the gel in a vertical position, and allow it to polymerize at room temperature. ••1

Electrophoretic Separation

Sample Buffer 1—Dissolve 1.89 g of Tris, 5.0 g of SDS, 50 mg of bromophenol blue, and 25.0 mL of glycerol in 100 mL of water. Adjust with hydrochloric acid to a pH of 6.8, and dilute with water to 125 mL. Before use, dilute with an equal volume of water or sample, and mix.

Sample Buffer 2 (for reducing conditions)—Prepare as directed in *Sample Buffer 1* except to add 12.5 mL of 2-mercaptoethanol before adjusting the pH. Alternatively, prepare as directed for *Sample Buffer 1* except to start with about 1.93 g of Tris and add a suitable quantity of DTT to obtain a final 100 µM DTT concentration.

Running Buffer—Dissolve 151.4 g of Tris, 721.0 g of aminoacetic acid (glycine), and 50.0 g of SDS in water; dilute with water to 5000 mL; and mix to obtain a stock solution. Immediately before use, dilute this stock solution with water to 10 times its volume, mix, and adjust to a pH between 8.1 and 8.8.

Procedure—•After polymerization is complete (about 30 minutes later), carefully remove the polytetrafluoroethylene comb.•1 Rinse the wells immediately with water or with the *Running Buffer* to remove any unpolymerized acrylamide. If necessary, straighten the teeth of the *Stacking Gel* with a blunt hypodermic needle attached to a syringe. Remove the clamps on one short side, carefully pull out the tubing, and replace the clamps. Proceed similarly on the other short side. Remove the tubing from the bottom part of the gel.

Mount the ••1 gel in the electrophoresis apparatus. Add the electrophoresis buffers to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates. •This•1 is best done with a bent hypodermic needle attached to a syringe. Never prerun the gel before loading the samples, because that will destroy the discontinuity of the buffer systems. Before loading the sample, carefully rinse the slot with *Running Buffer.*

Prepare the test and standard solutions in the recommended *Sample Buffer*, and treat as directed in the individual monograph. Apply the appropriate volume of each solution to the *Stacking Gel* wells.

Start the electrophoresis under the conditions recommended by the manufacturer of the equipment. •Manufacturers of SDS-PAGE equipment may provide gels of different surface area and thickness.•1 Electrophoresis running time and current/voltage may need to •vary as described by the manufacturer of the apparatus•1 in order to achieve optimum separation. Check that the dye front is moving into the *Resolving Gel.* When the dye •is reaching•1 the bottom of the gel, stop the electrophoresis. Remove the gel assembly from the apparatus, and separate the glass plates. Remove the spacers, cut off and discard the *Stacking Gel*, and immediately proceed with staining.

Detection of Proteins in Gels

Coomassie staining is the most common protein staining method, with a detection level on the order of 1 to 10 µg of protein per band. Silver staining is the most sensitive method for staining proteins in gels •and•1 a band containing 10 to 100 ng can be detected. ••1

All of the steps in gel staining are •done•1 at room temperature with gentle •shaking (e.g., on an orbital shaker platform).•1 Gloves must be worn when staining the gels, •because fingertips will stain.•1

REAGENTS

Coomassie Staining Solution—Prepare a solution of Coomassie brilliant blue R-250 having a concentration of 1.25 g per L in a mixture of water, methanol, and glacial acetic acid (5 : 4 : 1). Filter, and store at room temperature.

Destaining Solution—Prepare a mixture of water, methanol, and glacial acetic acid (5 : 4 : 1).

Fixing Solution 1—Prepare a mixture of water, methanol, and trichloroacetic acid (5 : 4 : 1).

Fixing Solution 2—Transfer 250 mL of methanol to a 500-mL volumetric flask, add 0.27 mL of formaldehyde, dilute with water to volume, and mix.

Silver Nitrate Reagent—To a mixture of 40 mL of 1 M sodium hydroxide and 3 mL of ammonium hydroxide, add, dropwise and with stirring, 8 mL of a 200 g per L solution of silver nitrate; dilute with water to 200 mL, and mix.

Developing Solution—Transfer 2.5 mL of a citric acid solution (2 in 100) and 0.27 mL of formaldehyde to a 500.0-mL volumetric flask, dilute with water to volume, and mix.

Stopping Solution—Prepare a 10% (v/v) solution of acetic acid.

Table 2. Preparation of Stacking Gel

Solution Component	Component Volume (mL) per Gel Mold Volume Below							
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	8 mL	10 mL
Water	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
30% Acrylamide–Bisacrylamide Solution	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 M Buffer Solution	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
SDS Solution	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
Ammonium Persulfate Solution	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

COOMASSIE STAINING

Immerse the gel in a •large• excess of *Coomassie Staining Solution,* and •allow to stand• for at least 1 hour. Remove the *Coomassie Staining Solution*. Destain the gel with a •large• excess of *Destaining Solution.* Change the *Destaining Solution* several times, until the stained protein bands are clearly distinguishable on a clear background. The more thoroughly the gel is destained, the smaller the amount of protein that can be detected •by the method.• Destaining can be •speeded up• by including a few •grams• of anion-exchange resin or a small sponge in the *Destaining Solution.* [NOTE—The acid–alcohol solutions used in this procedure do not completely fix proteins in the gel. This can lead to losses of some low molecular •mass• proteins during the staining and destaining of thin gels. Permanent fixation is obtainable by •allowing the gel to stand• in *Fixing Solution 1* for 1 hour before it is immersed in the *Coomassie Staining Solution.*]

SILVER STAINING

Immerse the gel in a •large• excess of *Fixing Solution 2,* and •allow to stand• for 1 hour. Remove *Fixing Solution 2,* add fresh *Fixing Solution 2,* and incubate for at least 1 hour, or overnight if convenient. Discard *Fixing Solution 2,* and wash the gel in a large excess of water for 1 hour. Soak the gel for 15 minutes in a 1% solution of glutaraldehyde (v/v). Wash the gel twice, for 15 minutes each time in a •large• excess of water. Soak the gel in fresh *Silver Nitrate Reagent* for 15 minutes in darkness. Wash the gel three times, for 5 minutes each time, in a •large• excess of water. Immerse the gel for about 1 minute in *Developing Solution* until satisfactory staining has been obtained. Stop the development by incubation in the *Stopping Solution* for 15 minutes. •• Rinse the gel with water. ••

Drying of Gels

•Depending on the method used, the gels are treated in a slightly different way.• For Coomassie staining, after the destaining step, •allow the gel to stand• in a •100 g per L• glycerol solution •• for at least 2 hours. For silver staining, add to the final rinsing step a 5-minute incubation in a •20 g per L• glycerol solution ••.

Immerse two sheets of porous •cellulose film• in water, and incubate for 5 to 10 minutes. Place one of the sheets on a drying frame. Carefully lift the gel, and place it on the •cellulose film.• Remove any trapped air bubbles, and pour a few mL of water around the edges of the gel. Place the second sheet on top, and remove any trapped air bubbles. Complete the assembly of the drying frame. Place in an •• oven, and leave at room temperature until dry. ••

Molecular •Mass• Determination

Molecular •masses• of proteins are determined by comparison of their mobilities with those of several marker proteins of known molecular •mass.• Mixtures of proteins with precisely known molecular •masses• blended for uniform staining are available for calibrating gels. They are available in various molecular •mass• ranges. Concentrated stock solutions of proteins of known molecular •mass• are diluted in an •appropriate• sample buffer and loaded on the same gel as the protein sample to be •studied.•.

Immediately after the gel has been run, the position of the bromophenol blue tracking dye is marked to identify the leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front. After staining, measure the migration distances of each protein band (markers and unknowns) from the top of the *Resolving Gel*. Divide the migration distance of each protein by the distance traveled by the tracking dye. The normalized migration distances so obtained are called the relative mobilities of the proteins (relative to the dye front) and conventionally denoted as R_F. Construct a •• plot of the logarithm of the molecular •masses • (M_R) of the protein standards as functions of the R_F values. •Note that the• graphs are slightly sigmoid. •Unknown molecular masses can be estimated by linear regression analysis of interpolation from the curves of log M_R against R_F as long as the values obtained for the unknown samples are positioned along the linear part of the graph.•

•VALIDATION OF THE TEST

The test is not valid unless the proteins of the molecular mass marker are distributed along 80% of the length of the gel and over the required separation range (e.g., the range covering the product and its dimer or the products and its related impurities); the separation obtained for the relevant protein bands shows a linear relationship between the logarithm of the molecular mass and the R_F. Additional validation requirements with respect to the solution under test may be specified in individual monographs.•

Quantification of Impurities

Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity •should be• prepared by diluting the test solution. For example, where the limit is 5.0%, a reference solution •would be a• 1 : 20 dilution of the test solution. No impurity—any band other than the main band—in the electropherogram obtained from the test solution •may be• more intense than the main band obtained with the reference solution.

Under validated conditions •• impurities may be quantified by normalization to the main band, using an integrating densitometer. In this case, the responses must be validated for linearity.

Interim Revision Announcement

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

USP31–NF26			
Page	**Title**	**Section**	**Description**
48	⟨11⟩ *USP Reference Standards*	*USP Gemfibrozil Related Compound A RS*	Change "[2,2-dimethyl-5-[2,5-dimethyl-4-(propene-1-yl)phenoxy]valeric acid]" to: [(*E,Z*)-2,2-dimethyl-5-[2,5-dimethyl-4-(propene-1-yl)phenoxy]valeric acid]
248	⟨660⟩ *Containers—Glass*	*Introduction*	Second paragraph of introduction: Change "*Containers—Permeation* ⟨671⟩" to: *Containers—Performance Testing* ⟨671⟩
259	⟨681⟩ *Repackaging into Single-Unit Containers and Unit-Dose Containers for Nonsterile Solid and Liquid Dosage Forms*	*Customized Patient Medication Packages*	Line 5 under *Packaging*: Change "*Containers—Permeation* ⟨671⟩" to: *Containers—Performance Testing* ⟨671⟩
1093	*Carbomer 934P*	*Viscosity*	Line 25: Insert footnote 1 following "top of the cylinder to the lower tip of the shaft being 3.02 cm," [1] Available as an RV6 spindle from Brookfield, or the equivalent.
1095	*Carbomer Copolymer*	*Viscosity*	Following *Table 1*: Change "With the spindle rotating at 20 rpm, observe and record the scale reading. Calculate the viscosity, in millipascal seconds, by multiplying the scale reading by the constant for the spindle used at 20 rpm." to: The spindle rotates at 20 rpm. Follow the instrument manufacturer's directions to measure the apparent viscosity.
1329	*Alprazolam Tablets*	*Uniformity of dosage units* ⟨905⟩	Lines 2 and 7 under *Chromatographic system* and *Procedure*: Change "Proceed as directed in the *Assay* under *Alprazolam*. Calculate the quantity, in mg, of $C_{17}H_{13}ClN_4$ in the Tablet by the formula: $CV(R_U/R_S)$ where *V* is the volume, in mL, of *Internal standard solution* in the *Test preparation;* and the other terms are as defined in the *Procedure* for *Alprazolam*." to: Proceed as directed in the *Assay*. Calculate the quantity, in mg, of $C_{17}H_{13}ClN_4$ in the Tablet by the formula: $CV(R_U/R_S)$ where *V* is the volume, in mL, of *Internal standard solution* in the *Test preparation;* and the other terms are as defined in the *Procedure*.
1666	*Cefazolin*	*Assay*	Under *Procedure*: Change the formula "$1000C\ (R_U/R_S)$" to: $500C\ (R_U/R_S)$
USP32–NF27			
145	⟨381⟩ *Elastomeric Closures for Injections*	*Introduction, Testing Procedures*	Second paragraph, and fourth through sixth paragraphs, inclusive: Change the revision official date from "(RB 1-May-2008)" to: (RB 1-May-2009) Three times under *Testing Procedures*, change the revision official date from "(RB 1-May-2008)" to: (RB 1-May-2009)
2874	*Meloxicam Tablets*	*Dissolution*	Line 5 under *Standard solution—For Tablets Labeled to Contain 15 mg*: Change "1000-mL volumetric flask" to: 100-mL volumetric flask

Interim Revision Announcement

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) items that previously appeared under *Pharmacopeial Previews* and are now formally proposed as revisions, (2) proposed revisions placed directly under *In-Process Revision,* or (3) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

> **Name of Item**, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:
>
> (DSN: L. Evans) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. New text (if any) follows, and is enclosed in symbols and set off from the current official text by a paragraph break and by larger type (print edition only), as shown in the examples below:

•new text•

if slated for an *Interim Revision Announcement to USP 30–NF 25* (*IRA*);

▲new text▲*USP31*

if slated for *USP 31–NF 26*; and

■new text■

if slated for a *Supplement to USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as •• or ■■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (*USP 30*) indicates that the proposed revision is slated for the *Second Supplement to USP 30*, and ▲*USP31* and ▲*NF26* indicate that the revisions are proposed for *USP 31* and *NF 26*, respectively.

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

MONOGRAPHS (USP)

BRIEFING

Acetaminophen and Tramadol Hydrochloride Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedures in the tests for *Related compounds* and in the *Assay* are based on analyses performed with the Zorbax Eclipse XBD-Phenyl brand of L11 column. Typical retention times are about 3.5 minutes for acetaminophen and about 9 minutes for tramadol hydrochloride. The chromatographic procedure in the test for *Dissolution* was validated using a Symmetry C8 brand of L7 packing. Using this column, the retention times are about 2.7 minutes for acetaminophen and about 5.3 minutes for tramadol hydrochloride.

(MD-CCA: C. Anthony; BPC: M. Marques) RTS—C58918

Add the following:

▲Acetaminophen and Tramadol Hydrochloride Tablets

» Acetaminophen and Tramadol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ($C_8H_9NO_2$) and tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$).

Packaging and storage—Preserve in tight containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Acetaminophen RS. USP p-Aminophenol RS. USP Tramadol Hydrochloride RS. USP Tramadol Related Compound A RS.*

Identification—The retention times of the peaks for tramadol hydrochloride and acetaminophen in the chromatograms of the *Tramadol assay preparation* and the *Acetaminophen assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate in about 900 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of 2.50, and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (4 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve accurately weighed amounts of USP Tramadol Hydrochloride RS and USP Acetaminophen RS in *Medium* to obtain a final solution having a known concentration of about 0.36 mg per mL of acetaminophen and 0.04 mg per mL of tramadol hydrochloride.

Test solution—Pass a portion of the solution under test through a suitable 0.45-µm filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 15-cm column that contains 5-µm L7 packing. The column temperature is maintained at 25°. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for acetaminophen and 1.0 for tramadol hydrochloride; the resolution, *R,* between acetaminophen and tramadol hydrochloride is not less than 5.0; and the relative standard deviation for replicate injections is not more than 2.0% for both the tramadol hydrochloride and acetaminophen peaks.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for about two times the retention time of tramadol hydrochloride, and measure the responses for the major peaks. Calculate the percentage of acetaminophen and tramadol hydrochloride by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

In-Process Revision

in which r_U and r_S are the peak responses of acetaminophen or tramadol hydrochloride obtained from the *Test solution* and *Standard solution,* respectively; C_S is the concentration, in mg per mL, of acetaminophen or tramadol hydrochloride in the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg, of acetaminophen or tramadol hydrochloride.

Tolerance—Not less than 80% (Q) of the labeled amounts of acetaminophen and tramadol hydrochloride are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Limit of *p*-aminophenol—[NOTE—All Standards, test solutions, and blank solutions have to be mixed with *Basic ferricyanide solution* and analyzed as soon as possible after a 30-minute waiting period.]

Diluent: a mixture of water and methanol (1 : 1).

Basic ferricyanide solution—Dissolve 1 g of sodium ferricyanide and 1 g of anhydrous sodium carbonate in 100 mL of water.

Standard solution—Dissolve an accurately weighed quantity of USP *p*-Aminophenol RS in *Diluent* to obtain a solution having a known concentration of about 0.05 mg per mL. Sonicate if necessary to dissolve. Transfer 5 mL of the resulting solution to a 100-mL volumetric flask, and add about 50 mL of *Diluent* and 5 mL of *Basic ferricyanide solution.* Dilute with *Diluent* to volume, and mix. Let stand for 30 minutes. Pass the solution through a 0.45-µm nylon membrane filter, and use the filtrate.

Test solution—Weigh not fewer than 20 Tablets. Grind the Tablets into a fine powder. Accurately transfer an amount of powder, equivalent to about 5 g of acetaminophen based on the label claim, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate for 15 minutes with intermittent shaking, followed by mechanical shaking for 30 minutes. Add 6 mL of *Basic ferricyanide solution.* Dilute with *Diluent* to volume, mix, and let stand for 30 minutes. Centrifuge a portion of the solution, and filter the clear supernatant through a 0.45-µm nylon membrane filter, and use the filtrate for analysis.

Blank solution—Add about 50 mL of *Diluent* to a 100-mL volumetric flask. Add 5 mL of *Basic ferricyanide solution.* Dilute with *Diluent* to volume, and let stand for 30 minutes. Filter a portion of the solution through a 0.45-µm nylon membrane filter, and use the filtrate for analysis.

Procedure—Concomitantly measure and record the absorbance of the *Standard* and *Test solutions* at about 710 nm in a 1-cm cell against the *Blank solution.* The relative standard deviation of six replicate absorbance readings of the *Standard solution* is not more than 6.0%. The difference between the initial and final absorbance readings of the *Standard solution* differs by not more than 10%. Calculate the percentage of *p*-aminophenol in the portion of Tablets taken by the formula:

$$(C_S / C_U)(r_U / r_S) \times 100$$

in which C_S is the concentration, in mg per mL, of USP *p*-Aminophenol RS in the *Standard solution;* C_U is the concentration, in mg per mL, based on the label claim of acetaminophen in the *Test solution;* and r_U and r_S are the absorbance readings of the *Test solution* and *Standard solution*, respectively: not more than 0.01% of *p*-aminophenol is found.

Related compounds—

Diluent, Mobile phase, Stock assay preparation—Prepare as directed in the *Assay.*

Standard solution—Dissolve accurately weighed quantities of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent* to obtain a solution having known concentrations of about 0.75 µg per mL of tramadol hydrochloride and 0.75 µg per mL each of the related compounds.

Test solution—Pass a suitable volume of *Stock assay preparation* through a 0.45-µm nylon membrane filter. Use the filtrate after discarding the first 4 mL of filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Make sure that the detector monitoring wavelength is set at 216 nm. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure,* identifying the peaks using the relative retention times provided in *Table 1:* the resolution, R, between tramadol related compound A and tramadol hydrochloride is not less than 2.0; the column efficiency is not less than 2000 theoretical plates for tramadol hydrochloride; and the relative standard deviation for six replicate injections is not more than 6.0% for the tramadol hydrochloride peak.

Procedure—Separately inject equal volumes (about 30 µL) of the *Diluent, Standard solution,* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Disregard the peaks due to the *Diluent*. Calculate the percentage of each known and unknown impurity in the portion of Tablets taken by the formula:

$$(C_S / C_U)(r_U / r_S) \times 100$$

in which C_S is the concentration, in µg per mL, of USP Tramadol Hydrochloride RS in the *Standard solution;* C_U is the concentration, in µg per mL, of tramadol hydrochloride in the *Test solution;* r_U is the peak response of each impurity obtained from the *Test solution;* and r_S is the response of the tramadol hydrochloride peak obtained from the *Standard solution.* The impurities are listed in *Table 1*.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, trifluoroacetic acid, and triethylamine (92 : 8 : 0.1 : 0.1). [NOTE—The apparent pH of the final solvent mixture should be between 2.2 and 2.4.] Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent: a mixture of water and methanol (9 : 1).

Standard preparation—Dissolve accurately weighed quantities of USP Acetaminophen RS and USP Tramadol Hydrochloride RS in *Diluent* to obtain a solution having known concentrations of about 0.065 mg per mL and about 0.075 mg per mL for acetaminophen and tramadol hydrochloride, respectively. [NOTE—Sonication may be used to aid dissolution.]

Stock assay preparation—Weigh not fewer than 20 Tablets, and determine the average Tablet weight. Grind the Tablets into a fine powder, and transfer an amount equivalent to one Tablet to a 50-mL volumetric flask. Add about 30 mL of *Diluent* with continuous shaking to disperse the powder. Sonicate for 15 minutes with intermittent shaking, and shake

Table 1

Impurity Name	Relative Retention Time	Limit %
O-Desmethyl-tramadol[1]	0.60	0.2
Tramadol related compound A[2]	0.80	0.2
Tramadol hydrochloride	1.0	n/a
Acetaminophen	0.38	n/a
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.8

[1] 3-{(1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-hydroxycyclohexyl}phenol

[2] (*RS,SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride

the flask on a mechanical shaker for 30 minutes. Dilute with *Diluent* to volume, and mix well. Centrifuge the suspension, and use the supernatant for subsequent dilutions.

Tramadol assay preparation—Quantitatively dilute the centrifuged *Stock assay preparation* with *Diluent* to obtain a final solution having a nominal concentration of about 0.075 mg per mL of tramadol hydrochloride.

Acetaminophen assay preparation—Quantitatively dilute the centrifuged *Stock assay preparation* with *Diluent* to obtain a final solution having a nominal concentration of about 0.065 mg per mL of acetaminophen.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a variable wavelength detector, a 4.6-mm × 15-cm column that contains 5-µm L11 packing. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 50°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure* in the *Assay for tramadol hydrochloride:* the resolution, *R,* between acetaminophen and tramadol hydrochloride is not less than 10.0; the column efficiency is not less than 2000 theoretical plates for each analyte; the tailing factor is not more than 2.0 for each analyte; and the relative standard deviation for five replicate injections is not more than 2.0% for each analyte.

Procedure—

Assay for tramadol hydrochloride—Set the detector wavelength to 216 nm. Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Tramadol assay preparation* into the chromatograph, record the chromatograms for about 4 times the retention time of acetaminophen, and measure the responses for the tramadol peak. Calculate the quantity, in percent label claim, of tramadol hydrochloride, ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$(C_S / C_U)(r_U / r_S) \times 100$$

in which C_S is the concentration, in mg per mL, of USP Tramadol Hydrochloride RS in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, based on the label claim of tramadol hydrochloride in the *Tramadol assay preparation;* and r_U and r_S are the peak responses obtained from the *Tramadol assay preparation* and the *Standard preparation,* respectively.

Assay for acetaminophen—Set the detector wavelength to 249 nm. Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Acetaminophen assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in percent label claim, of acetaminophen ($C_8H_9NO_2$), in the portion of Tablets taken by the formula:

$$(C_S / C_U)(r_U / r_S) \times 100$$

in which C_S is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, based on the label claim of acetaminophen in the *Acetaminophen assay preparation;* and r_U and r_S are the peak responses obtained from the *Acetaminophen assay preparation* and the *Standard preparation,* respectively.▲*USP33*

BRIEFING

Alendronate Sodium Tablets, *USP 31* page 1321, page 3813 of the *Second Supplement*, and the *Interim Revision Announcement* on page 545 of *PF* 34(3) [May–June 2008]. It is proposed to clarify how to run the quantitative step in *Dissolution Test 2*.

(BPC: M. Marques) RTS—C70112

Change to read:

Dissolution ⟨711⟩—

•TEST 1—•3

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Determine the amount of $C_4H_{13}NO_7P_2$ dissolved by employing the following method.

In-Process Revision

Buffer solution and *Mobile phase*—Prepare as directed in the *Assay.*

0.05% 9-Fluorenylmethyl chloroformate solution—Transfer 100 mg of 9-fluorenylmethyl chloroformate to a 200-mL volumetric flask, dilute with acetonitrile to volume, and mix. Prepare this solution fresh.

Borate buffer—Dissolve 6.2 g of boric acid in approximately 950 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.0, and dilute with water to 1 L.

Diluent—Transfer 176.4 g of sodium citrate dihydrate to a 1000-mL volumetric flask, dissolve in and dilute with *Medium* to volume, and mix.

Standard stock solution—Dissolve an accurately weighed quantity of USP Alendronate Sodium RS in *Medium,* and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration corresponding to the concentration that would be obtained by dissolving 1 Tablet in 900 mL of the same *Medium.* Calculate the concentration, *C,* in mg per mL, of anhydrous alendronate sodium in this solution.

Standard solution—Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Borate buffer,* and mix for about 3 minutes. Add 4.0 mL of 0.05% *9-Fluorenylmethyl chloroformate solution,* and agitate for about 30 seconds. Allow the solution to stand at room temperature for 25 minutes. Add 25 mL of methylene chloride, and agitate for about 40 seconds. Centrifuge the mixture for 5 minutes. Use a portion of the clear upper aqueous layer.

Reagent blank—Using 5 mL of water, proceed as directed for *Standard solution,* beginning with "to a 50-mL polypropylene screw-cap centrifuge tube."

Test solution—After 15 minutes, withdraw a portion of the solution under test, and centrifuge immediately. Using 5.0 mL of the clear supernatant, proceed as directed for *Standard solution,* beginning with "to a 50-mL polypropylene screw-cap centrifuge tube."

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the capacity factor, k', is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution,* the *Test solution,* and the *Reagent blank* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of alendronic acid ($C_4H_{13}NO_7P_2$) dissolved by the formula:

$$827.1C(r_U/r_S)$$

in which C is defined under the *Standard stock solution;* and r_U and r_S are the peak areas obtained from the *Test solution* and the *Standard solution,* respectively. [NOTE—827.1 is the molecular weight conversion factor ($C_4H_{13}NO_7P_2/C_4H_{12}NNaO_7P_2$) multiplied by the volume of the *Medium* (900 mL).]

Tolerances—Not less than 80% *(Q)* of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$) is dissolved in 15 minutes. Tablets labeled for weekly dosing: not less than 75% *(Q)* of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$) is dissolved in 15 minutes.

•TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ dissolved ~~as directed in the *Assay*~~

▲employing the following method.

0.1% 9-Fluorenylmethyl chloroformate solution and *Borate solution*—Proceed as directed in the *Assay.*

0.6 M Citrate buffer—Dissolve 176.4 g of sodium citrate dihydrate in 1000 mL of water.

0.05 M Buffer—Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with phosphoric acid to a pH of 8.0, and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of *0.05 M Buffer,* acetonitrile, and methanol (76 : 19 : 5). Make adjustments if necessary (see *System suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Dissolve an accurately weighed quantity of USP Alendronate Sodium RS in *Medium,* and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration corresponding to the concentration that would be obtained by dissolving one Tablet in 900 mL of the same *Medium.* Calculate the concentration, *C,* in mg per mL, of anhydrous alendronate sodium in this solution.

Standard solution—Transfer 5.0 mL of *Standard stock solution* to a 50-mL screw-cap polypropylene centrifuge tube containing 1.0 mL of *0.6 M Citrate buffer* and 5.0 mL of 0.1 M *Borate solution,* and mix for about 3 minutes. Add 4.0 mL of *0.1% 9-Fluorenylmethyl chloroformate solution,* and agitate for about 30 seconds. Allow the solution to stand at room temperature for 30 minutes. Add 25 mL of methylene chloride, and agitate vigorously for about 40 seconds. Centrifuge the mixture for 10 minutes. Use a portion of the clear upper aqueous layer.

Reagent blank—Using 5 mL of water, proceed as directed for the *Standard solution,* beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

Test solution—

For Tablets labeled to contain 5 mg, 10 mg, 35 mg, or 40 mg—After 30 minutes, withdraw 30 mL of the solution under test, and pass through a suitable 0.45-µm filter, discarding the first 10 mL. Using 5.0 mL of the filtrate, proceed as directed for the *Standard solution,* beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

In-Process Revision

For Tablets labeled to contain 70 mg—After 30 minutes, withdraw 30 mL of the solution under test, and pass through a suitable 0.45-µm filter, discarding the first 10 mL. Transfer 6.0 mL of the filtrate to a 10-mL volumetric flask, and dilute with water to volume. Using 5.0 mL of this dilution, proceed as directed for the *Standard solution,* beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

Chromatographic system—Proceed as directed for the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure*: the capacity factor, K', is not less than 2.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Proceed as directed in *Test 1.*▲*USP33*
Tolerances—Not less than 80% *(Q)* of the labeled amount of alendronate sodium ($C_4H_{12}NNaO_7P_2 \cdot 3H_2O$) is dissolved in 30 minutes.•3

BRIEFING

Amantadine Hydrochloride Capsules, *USP 31* page 1374. It is proposed to include a *Dissolution Test 2* for a generic version of this product recently approved by the FDA. The chromatographic procedure in this test was validated using the RTX-1 or DB-1 brand of G1 phase. The retention times for naphthalene and amantadine hydrochloride are approximately 6 and 7 minutes, respectively. In the absence of any adverse comments, it is proposed to implement this revision via the *Interim Revision Announcement* in *PF* 35(3) with an official date of June 1, 2009.

(BPC: M. Marques) RTS—C70389

Add the following:

•**Labeling—**When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.•3

Change to read:

Dissolution ~~*Procedure for a Pooled Sample*~~ ••3 ⟨711⟩—

•TEST 1—*Procedure for a Pooled Sample.*•3
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.

Internal standard solution—Dissolve an accurately weighed quantity of naphthalene in hexane to obtain a solution having a known concentration of about 0.054 mg per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Amantadine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.1 mg per mL. Pipet 15.0 mL of this solution into a 50-mL screw-capped test tube, add 5.0 mL of 5 N sodium hydroxide and 10.0 mL of *Internal standard solution*, and shake for 60 minutes. Collect the hexane layer.
Test solution—Filter 15.0 mL of the solution under test and place into a 50-mL screw-capped test tube. Pipet 5.0 mL of 5 N sodium hydroxide and 10.0 mL of the *Internal standard solution* into the test tube, and shake for 60 minutes. Collect the hexane layer (*Test solution*).
Chromatographic system—Proceed as directed under the *Assay.*
Procedure—Separately inject equal volumes (about 2.5 µL) of the *Standard solution* and the *Test solution*. Record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{10}H_{17}N \cdot HCl$ dissolved.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{17}N \cdot HCl$ is dissolved in 45 minutes.

•TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: water; 900 mL.

Apparatus 2: 75 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.QLA-LLC.com or www.tabletdissolution.com or www.labhut.com.]

Time: 45 minutes.

Standard stock solution—Dissolve an accurately weighed amount of USP Amantadine Hydrochloride RS in *Medium* to obtain a solution with a final concentration of about 0.12 mg per mL.

Internal standard solution—Dissolve an accurately weighed amount of naphthalene in hexanes to obtain a solution with a final concentration of about 0.06 mg per mL.

Working standard solution—Transfer 60.0 mL of the *Standard stock solution* to a 200-mL volumetric flask. Add 20 mL of 5 N sodium hydroxide and 40.0 mL of *Internal standard solution*. Shake the flask for approximately 10 minutes, and allow the layers to separate. Use the top layer for injection. The final concentration is about 0.18 mg per mL.

Test solution—Transfer 3.0 mL of the solution under test to a centrifuge tube. Add 1.0 mL of 5 N sodium hydroxide and 2.0 mL of *Internal standard solution*. Shake the tube for approximately 10 minutes, and allow the layers to separate. Use the top layer for injection.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m column that contains 0.25-µm film of phase G1. The oven temperature is set at 100° for 3 minutes, then to 200° at 10° per minute, and then held at 200° for 2 minutes. The injector is maintained at 250°, and the detector at 300°. The carrier gas is helium at 1.4 mL per minute, and the split flow is 20 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between naphthelene and amantadine hydrochloride is not less than 2; the tailing factor for the amantadine hydrochloride peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for all peaks. Calculate the percentage of amantadine hydrochloride dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times L}$$

in which A_U is the ratio of the peak areas obtained from the *Test solution*; C_S is the concentration, in mg per mL, of amantadine hydrochloride in the *Standard stock solution*; A_S is the average ratio of the peak areas obtained from the *Working standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and *L* is the Capsule label claim in mg.

Tolerances—Not less than 75% (*Q*) of the labeled amount of amantadine hydrochloride is dissolved in 45 minutes.•3

In-Process Revision

BRIEFING

Amlodipine Besylate Tablets, page 1137 of *PF* 34(5) [Sept.–Oct. 2008]. It is proposed to make some corrections to the text of the test for *Dissolution*.

(BPC: M. Marques) RTS—C70406

Add the following:

■Amlodipine Besylate Tablets

» Amlodipine Besylate Tablets contain not less than 90 percent and not more than 110 percent of the labeled amount of amlodipine ($C_{20}H_{25}N_2O_5Cl$).

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Amlodipine Besylate RS. USP Amlodipine Related Compound A RS.*

Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—The spectrum of the *Test solution* corresponds to that of the *Standard solution,* as obtained in the test for *Dissolution*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Change to read:

Dissolution ⟨711⟩**—**

[NOTE—Do not expose any of the solutions to stainless steel because of the degradation of amlodipine.]

Medium: 0.01 M hydrochloric acid; 500 mL.

Apparatus 2: 75 rpm. Use paddles covered with Teflon or made of any inert material except stainless steel.

Time: 30 minutes.

Standard solution A—Transfer about 35.0 mg, accurately weighed, of USP Amlodipine Besylate RS to a 250-mL volumetric flask. Dissolve in 10 mL of methanol, and dilute with *Medium* to volume.

Standard solution B—Prepare as directed for *Standard solution A*.

Working standard solutions A and *B*—

FOR TABLETS LABELED TO CONTAIN 2.5 MG—Transfer 5.0 mL of *Standard solution A* to a 200-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution A*). Transfer 5.0 mL of *Standard solution B* to a 100-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution B*).

FOR TABLETS LABELED TO CONTAIN 5 MG—Transfer 5.0 mL of *Standard solution A* to a 100-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution A*). Transfer 10.0 mL of *Standard solution B* to a 100-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution B*).

FOR TABLETS LABELED TO CONTAIN 10 MG—Transfer 10.0 mL of *Standard solution A* to a 100-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution A*). Transfer 10.0 mL of *Standard solution B* to a 50-mL volumetric flask, and dilute with *Medium* to volume (*Working standard solution B*).

Calculate the absorptivity:

$$A_{1\,cm}^{1\,\%}$$

at 237 nm for each *Working standard solution* using the formula:

$$A_{1cm}^{1\%} = \frac{A_S \times 1000 \times 250 \times DF \times 100}{W_S \times 100 \times 1 \times 1 \times P}$$

in which DF is the dilution factor of the *Working standard solution*; and P is the purity, in percentage, of USP Amlodipine RS.

Test solution—Pass a portion of the solution under test through a suitable 0.45-µm filter.

Procedure—Determine the amount of amlodipine ~~besylate~~▲ ▲*USP33* dissolved by employing UV absorption at the wavelength of maximum absorbance at about 237 nm on portions of the *Test solution* in comparison with the *Standard solution,* using a 1-cm path length cell and *Medium* as the blank. Calculate the percentage of amlodipine dissolved by the formula:

~~$$\frac{A_U \times C_S \times 500 \times 408.9 \times 100}{A_{1cm}^{1\%} \times L \times 567.1}$$~~

▲

$$\frac{A_U \times 1000 \times 500 \times 408.9 \times 100}{\text{mean } A_{1cm}^{1\%} \times 100 \times 1 \times L \times 567.1}$$

▲*USP33*

in which A_U is the absorbance obtained from the *Test solution;* ~~C_S is the concentration, in mg per mL, of the *Standard solution;*~~▲ ▲*USP33* 500 is the volume, in mL, of *Medium*; 408.9 is the molecular weight of amlodipine; 100 is the conversion factor to percentage;

~~$A_{1\,cm}^{1\,\%}$~~

▲

$$\text{mean } A_{1\,cm}^{1\%}$$

▲*USP33*

is the ~~absorptivity previously calculated~~▲average absorptivity of *Working standard solution A* and *B*;▲*USP33* *L* is the Tablet label claim, in mg; and 567.1 is the molecular weight of amlodipine besylate.

Tolerances—Not less than 75% (*Q*) of the labeled amount of amlodipine is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Related compounds—

Buffer solution, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay.*

System suitability solution—Use the *System suitability preparation,* prepared as directed in the *Assay.*

Standard solution—Use the *System suitability preparation,* prepared as directed in the *Assay.*

Test solution—Place a suitable number of Tablets into a 25-mL volumetric flask to obtain a solution having a final nominal concentration of 0.4 mg per mL of amlodipine. Add about 10 mL of *Mobile phase* to the flask. Swirl to disintegrate the Tablet(s) followed by sonication for about 5 minutes for complete dissolution, and then cool the sample to room temperature. Dilute with *Mobile phase* to volume. Stir for an additional 15 minutes using a magnetic stir bar, and pass the sample through a 0.45-µm syringe tip filter, discarding the first 5 mL.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of amlodipine related compound A in the portion of the Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)(\mathrm{MW}_1/\mathrm{MW}_2)$$

in which C_S is the concentration, in mg per mL, of USP Amlodipine Related Compound A RS in the *Standard solution*; C_U is the nominal concentration, in mg per mL, of amlodipine in the *Test solution;* r_U is the peak response for amlodipine related compound A obtained from the *Test solution;* r_S is the peak response of amlodipine related compound A in the *Standard solution;* and MW_1 and MW_2 are the molecular weights of amlodipine related compound A, equal to 406.87, and amlodipine related compound A fumarate, equal to 522.95, respectively. Calculate the percentage of any other individual impurity in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Amlodipine Besylate RS in the *Standard solution*; C_U is the nominal concentration, in mg per mL, of amlodipine in the *Test solution;* r_U is the peak response for each individual impurity obtained from the *Test solution*; and r_S is the peak response of amlodipine obtained from the *Standard solution*. The specified and unspecified impurities meet the limits specified in *Table 1*.

Table 1

Component	Relative Retention Tme	Limit (%)
Amlodipine related compound A*	0.5	1.0
Amlodipine besylate	1	—
Any other individual unspecified degradation product	—	0.10

* 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

Assay—

Buffer solution—Pipet 7.0 mL of triethylamine into a 1000-mL flask containing approximately 900 mL of water. Adjust the solution with phosphoric acid to a pH of 3.0 ± 0.1. Dilute with water to volume, and mix well.

In-Process Revision

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* methanol, and acetonitrile (50 : 35 : 15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability preparation—Transfer accurately weighed known quantities of USP Amlodipine Besylate RS and USP Amlodipine Related Compound A RS into a suitable volumetric flask, and dissolve in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL of amlodipine and 0.002 mg per mL of amlodipine related compound A, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL of amlodipine.

Assay preparation—Place 5 Tablets into a 500-mL volumetric flask. Add 50 mL of *Mobile phase* to the flask, and swirl to disintegrate the Tablets. Add approximately 300 mL of additional *Mobile phase,* insert the stopper into the flask, and shake on a reciprocating shaker for about 30 minutes. Dilute with *Mobile phase* to volume, and mix well. Further dilute quantitatively and stepwise, if necessary, to obtain a concentration of 0.02 mg per mL of amlodipine. Pass the sample through a 0.45-µm syringe tip filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 237-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The run time is about three times the retention of the amlodipine peak. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between amlodipine and amlodipine related compound A is not less than 8.5; the tailing factor is not more than 2.0 for both amlodipine and amlodipine related compound A; and the relative standard deviation for replicate injections is not more than 1.0% for amlodipine and not more than 5.0% for amlodipine related compound A.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for amlodipine. Calculate the percentage of amlodipine free base ($C_{20}H_{25}N_2O_5Cl$), based on the label claim, in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)(1/L)$$

in which C_S is the concentration, in mg per mL, of USP Amlodipine Besylate RS in the *Standard preparation;* C_U is the number of Tablets per mL in the *Assay preparation;* r_U and r_S are the peak responses of amlodipine in the *Assay preparation* and the *Standard preparation,* respectively; and L is the label claim in mg per Tablet.■2S (*USP32*)

BRIEFING

Ampicillin Sodium, *USP 31* page 1420. Elsewhere in this issue of *PF*, in the general chapter *Dimethylaniline* ⟨223⟩, a revision is proposed to replace the packed-column GC method with a capillary GC method to modernize the chapter. On the basis of data obtained during the validation of the new method, it is proposed to revise the test for *Dimethylaniline* in this monograph to delete the specific instructions provided and refer to the solutions in the general chapter.

(MD-ANT: A. Wise) RTS—C68623

Change to read:

Dimethylaniline ⟨223⟩: meets the requirement. ~~the *Internal standard solution*, *Standard preparation*, and *Test preparation* being prepared as follows~~.

~~*Internal standard solution*—Dissolve 75 mg of *N,N* diethylaniline in 25 mL of 1 N hydrochloric acid, and dilute quantitatively and stepwise with water to obtain a solution containing about 30 µg per mL.~~

~~*Standard preparation*— Transfer 50.0 mg of *N,N*-dimethylaniline to a 50-mL volumetric flask, add 25 mL of 1 N hydrochloric acid, swirl to dissolve, dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with water to volume, and mix. To a suitable centrifuge tube add 1.0 mL of this solution, 1.0 mL of 1.25 N sodium hydroxide, 1.0 mL of *Internal standard solution*, and 1.0 mL of cyclohexane, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as the *Standard preparation*.~~

~~*Test preparation*— Transfer 1.0 g of Ampicillin Sodium to a suitable centrifuge tube, add 2 mL of 1.25 N sodium hydroxide, swirl to dissolve the specimen, add 1.0 mL of *Internal standard solution* and 1.0 mL of cyclohexane, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as the *Test preparation*.~~

▲▲*USP33*

BRIEFING

Atenolol Tablets, *USP 31* page 1462. It is proposed to add information on adjusting the pH of the *Medium* in the *Dissolution* test.

(BPC: M. Marques) RTS—C70409

Change to read:

Dissolution ⟨711⟩—

Medium: 0.1 N acetate buffer, pH 4.6, prepared by mixing 44.9 parts (v/v) of 0.1 N sodium acetate with 55.1 parts (v/v) of 0.1 N acetic acid solution,

▲and adjusting with either diluted sodium hydroxide or diluted acetic acid to a pH of 4.6;▲*USP33*

900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{14}H_{22}N_2O_3$ dissolved by employing the following method.

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Atenolol*.

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Atenolol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg per mL.

Test solution—Prepare a filtered portion of the solution under test. Quantitatively dilute an accurately measured volume of the filtrate with *Mobile phase* to obtain a solution estimated to contain about 0.01 mg of atenolol per mL.

Procedure—Proceed as directed in the *Assay*, except to inject the *Test solution* instead of the *Assay preparation.* Calculate the quantity, in mg, of $C_{14}H_{22}N_2O_3$ dissolved by the formula:

$$900CD(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Atenolol RS in the *Standard solution; D* is the dilution factor involved in preparing the *Test solution;* and r_U and r_S are the atenolol peak areas obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{14}H_{22}N_2O_3$ is dissolved in 30 minutes.

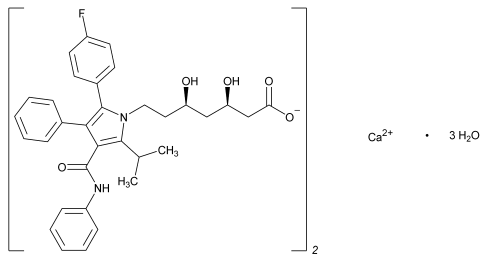
BRIEFING

Atorvastatin Calcium. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedures in the tests for *Related compounds* and the *Assay* are based on analyses performed with the Zorbax Rx-C8 brand of L7 column. The typical retention time for the atorvastatin peak is 26–34 minutes. The liquid chromatographic procedure in the test for *Enantiomeric purity* is based on analyses performed with the Daicel Chiralpak AD brand of L51 column. The typical retention time for the atorvastatin peak is about 44 minutes.

(MD-GRE: E. Gonikberg) RTS—C53875

Add the following:

▲Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$ 1209.42

1*H*-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-, calcium salt (2 : 1), trihydrate [*R*-(*R**,*R**)]-.

Calcium (β*R*,δ*R*)-2-(*p*-fluorophenyl)-β,δ-dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoate (1 : 2), trihydrate [*344423-98-9*].

Anhydrous 1155.34 [*134523-03-8*].

» Atorvastatin Calcium contains not less than 98.0 percent and not more than 102.0 percent of $C_{66}H_{68}CaF_2N_4O_{10}$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

In-Process Revision

USP Reference standards ⟨11⟩—*USP Atorvastatin Calcium RS. USP Atorvastatin Related Compound A RS. USP Atorvastatin Related Compound B RS. USP Atorvastatin Related Compound C RS. USP Atorvastatin Related Compound D RS. USP Atorvastatin Related Compound E RS.*

Identification—*Infrared Absorption* ⟨197K⟩.

Water, *Method Ia* ⟨921⟩**:** not less than 3.5% and not more than 5.5%.

Heavy metals, *Method II* ⟨231⟩**:** not more than 20 ppm.

Related compounds—

Diluent, 0.05 M Ammonium acetate buffer, Solution A, Solution B, Mobile phase, System suitability solution, and *Standard preparation*—Prepare as directed in the *Assay.*

Standard solution—Transfer accurately weighed amounts of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound D RS to a suitable volumetric flask. Dissolve in and dilute with *Diluent* to volume to obtain a solution having known concentrations of about 0.0015 mg per mL of each component.

Test solution—Transfer about 50 mg of Atorvastatin Calcium, accurately weighed, to a 50-mL volumetric flask, dissolve in *Diluent,* using sonication if necessary, and mix well.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* In addition, chromatograph the *Standard solution,* and identify the components based on their relative retention times given in *Table 1*.

Table 1

Name	Relative Retention Time	Limit (%)
Atorvastatin related compound A[1]	0.8	0.3
Atorvastatin related compound B[2]	0.9	0.3
Atorvastatin	1.0	n/a
Atorvastatin related compound C[3]	1.2	0.3
Atorvastatin related compound D[4]	2.1	0.1
Any other individual impurity	—	0.1
Total impurities[5]	—	1.0

[1] Desfluoro impurity, or (3*R,5R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.
[2] 3*S,5R* isomer, or (3*S,5R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.
[3] Difluoro impurity, or (3*R,5R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.
[4] Oxirane impurity, or 3-(4-fluorobenzoyl)-2-isobutyryl-3-phenyloxirane-2-carboxylic acid phenylamide.
[5] Not including atorvastatin related compound E.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each of atorvastatin related compounds A, B, C, and D in the portion of Atorvastatin Calcium taken by the formula:

$$100\ (C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of the relevant atorvastatin related compound in the *Standard solution;* C_U is the concentration, in mg per mL, of Atorvastatin Calcium in the *Test solution;* and r_U and r_S are the peak responses of the relevant atorvastatin related compound obtained from the *Test solution* and the *Standard solution,* respectively. Calculate the percentage of any other individual impurity in the portion of Atorvastatin Calcium taken by the formula:

$$100\ (r_i / r_s)$$

in which r_i is the peak response of any other individual impurity, and r_s is the sum of the responses of all the peaks in the *Test solution.* Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.

Enantiomeric purity—

Mobile phase—Prepare a mixture of hexane, dehydrated alcohol, and trifluoroacetic acid (940 : 60 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution in methanol containing about 5 mg per mL of USP Atorvastatin Calcium RS and 37.5 µg per mL of USP Atorvastatin Related Compound E RS. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume. [NOTE—Atorvastatin related compound E is the 3*S,5S* enantiomer of atorvastatin.]

Test solution—Transfer about 10 mg of Atorvastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 244-nm detector and a 4.6-mm × 25-cm column that contains packing L51. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the elution order is atorvastatin related compound E followed by the atorvastatin peak; and the resolution, *R*, between the peaks is not less than 2.0.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of atorvastatin related compound E in the portion of Atorvastatin Calcium taken by the formula:

$$100\,(r_U / r_s)$$

in which r_U is the peak response for atorvastatin related compound E, and r_s is the sum of the peak responses for atorvastatin related compound E and atorvastatin: not more than 0.3% of atorvastatin related compound E is found.

Assay—

Diluent—Use *N,N*-dimethylformamide.

0.05 M Ammonium acetate buffer—Prepare a solution in water containing 3.9 g of ammonium acetate per L, adjusted with glacial acetic acid to a pH of 5.0 ± 0.1.

Solution A—Prepare a mixture of *0.05 M Ammonium acetate buffer,* acetonitrile, and stabilizer-free tetrahydrofuran (67 : 21 : 12).

Solution B—Prepare a mixture of acetonitrile, *0.05 M Ammonium acetate buffer*, and stabilizer-free tetrahydrofuran (61 : 27 : 12).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in *Diluent* containing about 0.05 mg per mL of USP Atorvastatin Calcium RS and about 0.06 mg per mL of USP Atorvastatin Related Compound B RS.

Standard preparation—Transfer an accurately weighed amount of USP Atorvastatin Calcium RS to a suitable volumetric flask. Dissolve in and dilute with *Diluent* to volume, using sonication if necessary, to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Atorvastatin Calcium, accurately weighed, to a 100-mL volumetric flask. Dissolve in *Diluent,* using sonication if necessary, and mix well.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 244-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing

L7. The flow rate is about 1.5 mL per minute, and the column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–40	100	0	isocratic
40–70	100→20	0→80	linear gradient
70–85	20→0	80→100	linear gradient
85–100	0	100	isocratic
100–105	0→100	100→0	linear gradient
105–115	100	0	re-equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the peaks for atorvastatin related compound B and atorvastatin is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.6, and the relative standard deviation for replicate injections is not more than 0.6%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{66}H_{68}CaF_2N_4O_{10}$ in the portion of Atorvastatin Calcium taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of atorvastatin calcium in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Content of calcium—

Diluent—Prepare a mixture of methanol, water, and hydrochloric acid (75 : 25 : 2).

Calcium standard stock solution—Quantitatively dilute with *Diluent* a suitable amount of a commercially prepared atomic absorption standard solution for calcium to obtain a solution containing 100 µg of calcium per mL.

Standard solutions—Transfer 1.0 and 3.0 mL of *Calcium standard stock solution* to separate 100-mL volumetric flasks, and dilute with *Diluent* to volume. These *Standard solutions* contain 1 and 3 µg of calcium per mL, respectively.

Blank solution—Use *Diluent.*

Test solution—Transfer about 5.0 mg of Atorvastatin Calcium, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.

Procedure—Concomitantly determine the absorbances of the *Standard solution,* the *Blank solution,* and the *Test solution* at the calcium emission line at 422.7 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩), using an air–acetylene flame. Determine the concentration, C_S, in µg per mL, of calcium in the *Test solution*, using the calibration graph. Calculate the percentage of calcium in the portion of Atorvastatin Calcium taken by the formula:

$$100(0.001\, C_S / C_U)$$

where C_S is as defined above; the multiplier of 0.001 is for conversion of µg per mL to mg per mL; and C_U is the concentration, in mg per mL, of atorvastatin calcium in the *Test solution,* corrected for the *Water* content, as determined in the test for *Water.* The calcium content, calculated on the anhydrous basis, is between 3.2% and 3.8%.▲*USP33*

BRIEFING

Benzoin, *USP 31* page 1506. On the basis of comments received, it is proposed to revise the *Botanic characteristics* section for safety consideration. It is also proposed to delete *Identification* test *C* because it requires odor sensing, which is again a safety issue. The General Chapter reference is added to *Identification* test *B* for clarification.

(MD-OOD: F. Mao) RTS—C68574

Change to read:

Botanic characteristics—

Sumatra Benzoin—Blocks or lumps of varying size, made up of tears, compacted together, with a reddish brown, reddish gray, or grayish brown resinous mass. The tears are externally yellowish or rusty brown, milky white on fresh fracture; hard and brittle at ordinary temperatures but softened by heat. ~~and becoming gritty on chewing~~

▲▲*USP33*

Siam Benzoin—Pebble-like tears of variable size and shape, compressed, yellowish brown to rusty brown externally, milky white on fracture, separate or very slightly agglutinated, hard and brittle at ordinary temperatures but softened by heat. ~~and becoming plastic on chewing~~

▲▲*USP33*

Change to read:

Identification—

A: A solution in alcohol becomes milky upon the addition of water, and the mixture is acid to litmus paper.

B:

▲*Identification of articles of botanical origin* ⟨563⟩—▲*USP33*

Heat a few fragments in a test tube: Sumatra Benzoin evolves a sublimate consisting of plates and small, rod-like crystals of cinnamic acid and its esters that strongly polarize light. Siam Benzoin evolves a sublimate directly above the melted mass, consisting of numerous long, rod-shaped crystals of benzoic acid that do not strongly polarize light.

~~**C:** Heat about 500 mg in a test tube with 10 mL of potassium permanganate TS: only the Sumatra variety develops a faint odor of benzaldehyde.~~

▲▲*USP33*

BRIEFING

Bupropion Hydrochloride Extended-Release Tablets, *USP 31* page 1573, page 3623 of the *First Supplement,* page 1123 of the *Interim Revision Announcement* in *PF* 34(5), and page 570 of *PF* 34(3) [May–June 2008]. It is proposed to add a *Dissolution Test 8* for a product recently approved by FDA. In the absence of negative comments, it is proposed to implement this revision through an *Interim Revision Announcement* pertaining to *USP 32–NF 27*, with an official date of June 1, 2009.

(BPC: M. Marques) RTS—C62947

Change to read:

Dissolution ⟨711⟩—

FOR PRODUCTS LABELED FOR DOSING EVERY 12 HOURS—

TEST 1—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Times: 1, 4, and 8 hours.

Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm, using a 1.0-cm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 45%
4	between 60% and 85%
8	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring about 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding about 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5 ± 0.05); 900 mL, deaerated.

Apparatus 1: 50 rpm.

Times: 1, 2, 4, and 6 hours.

Determine the percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing the following method.

Buffer solution—Dissolve 3.45 g of sodium phosphate monobasic monohydrate in 996 mL of water, add 4.0 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 2.80 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (65 : 35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Bupropion Hydrochloride RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration similar to the one expected in the *Test solution.*

Test solution—Use portions of the solution under test, and pass through a 0.45-µm nylon filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 298-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

In-Process Revision

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of bupropion hydrochloride dissolved at each time point.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 50%
2	between 40% and 65%
4	between 65% and 90%
6	not less than 80%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus, and *Procedure*—Proceed as directed for *Test 1,* except using the wavelength of about 250 nm.

Times: 1, 2, 4, and 6 hours.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 30% and 55%
2	between 50% and 75%
4	between 70% and 90%
6	not less than 80%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium and *Procedure*—Proceed as directed for *Test 1*.

Apparatus—Proceed as directed for *Test 1,* except to use a 0.5-cm cell.

Times: 1, 3, and 6 hours.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 35% and 55%
3	between 65% and 85%
6	not less than 80%

•TEST 7—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium, Apparatus 1, and *Times*—Proceed as directed for *Test 2,* including the quantitative chromatographic method, but using as the *Mobile phase* a mixture of *Buffer solution* with methanol (55 : 45).

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 50%
2	between 45% and 70%
4	not less than 70%
6	not less than 80%

•5

FOR PRODUCTS LABELED FOR DOSING EVERY 24 HOURS—

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.

Apparatus 1: 75 rpm.

Time: 2, 4, 8, and 16 hours.

Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 252 nm, using a 1.0-mm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	not more than 20%
4	between 20% and 45%
8	between 65% and 90%
16	not less than 80%

•TEST 6—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium and *Apparatus:* Proceed as directed for *Test 4*.

Times: 1, 2, 4, 8, and 12 hours.

Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm, using a 1.0-cm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 15% and 35%
2	between 25% and 50%
4	between 40% and 65%
8	between 65% and 90%
12	not less than 80%

•6

•TEST 8—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 75 rpm.

Times: 2, 4, 8, and 16 hours.

Standard solution—

FOR TABLETS LABELED TO CONTAIN 150 MG—Prepare a solution containing about 0.1667 mg of USP Buproprion Hydrochloride RS per mL in *Medium*.

FOR TABLETS LABELED TO CONTAIN 300 MG—Prepare a solution containing about 0.3333 mg of USP Bupropion Hydrochloride RS per mL in *Medium*.

Test solution—Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm.

Procedure—Determine the percentage of bupropion hydrochloride dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm on portions of the *Test solution* in comparison with the *Standard solution*, using *Medium* as the blank.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	not more than 10%
4	between 10% and 35%
8	between 45% and 75%
16	not less than 80%

•[3]

Change to read:

Related compounds—

Solution A, Solution B, Mobile phase, System suitability solution 1, System suitability solution 2, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(C/D)F(r_i / r_S)$$

in which C is the concentration, in mg per mL, of bupropion hydrochloride in the *Standard solution; F* is the relative response factor for each impurity (see the accompanying table); D is the concentration, in mg per mL, of bupropion hydrochloride in the *Test solution,* based on the number of Tablets taken, the labeled quantity per Tablet, and the extent of dilution; r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for bupropion hydrochloride obtained from the *Standard solution.* See the accompanying table for limits of individual impurities based upon Tablet strength.

BRIEFING

Capecitabine Tablets, *USP 31* page 1621. It is proposed to change the wavelength used in the quantitative step of the test for *Dissolution* from 325 nm to 304 nm, where the maximum absorbance occurs, for both Tablet strengths.

(BPC: M. Marques) RTS—C60525

Change to read:

Dissolution ⟨711⟩—

Medium: water; 900 mL, degassed.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Standard solution—For Tablets labeled to contain 150 mg: Transfer 17 mg, accurately weighed, of USP Capecitabine RS to a 100-mL volumetric flask, dissolve in and dilute with *Medium* to volume, and mix. For Tablets labeled to contain 500 mg: Transfer 28 mg, accurately weighed, of USP Capecitabine RS to a 50-mL volumetric flask, dissolve in and dilute with *Medium* to volume, and mix.

Test solution—Pass a portion of the solution under test through a 0.45-µm fiberglass filter.

Procedure—Determine the amount of capecitabine ($C_{15}H_{22}FN_3O_6$) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 304 nm ~~(for Tablets labeled to contain 150 mg) and at about 325 nm (for Tablets labeled to contain 500 mg)~~

▲▲*USP33*

on portions of the *Test solution*, suitably diluted with *Medium*, if necessary, in comparison with the appropriate *Standard solution*, using a 1-mm quartz cell. Calculate the amount, in percentage, of $C_{15}H_{22}FN_3O_6$ dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times LC}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of capecitabine ($C_{15}H_{22}FN_3O_6$) in the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{15}H_{22}FN_3O_6$ is dissolved in 30 minutes.

Compound	Relative Retention Time	*F*	Limit (%) 100 mg or less	Limit (%) 150 mg or greater
2-Amino-1-(3-chlorophenyl)-1-propanone	about 0.38	0.80	0.3	0.3
(3*S*,5*S*,6*S*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	about 0.56	0.86	1.0	1.5
(3*S*,5*R*,6*R*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	about 0.78	0.88	0.5	0.4
Bupropion	1.0	—	—	—
Bupropion related compound F	about 1.71	0.55	1.2	2.3
Bupropion related compound C	about 1.75	0.59	0.3	0.3
m-Chlorobenzoic acid	about 1.80	0.24	0.3	0.3
~~Bupropion related compound E~~	about 2.25	1.00	0.4	0.4
■1-(3-Chlorophenyl)-1,2-propanedione■1S (*USP32*)				
Any unspecified impurity	—	1.00	0.2	0.2
Total impurities	—	—	3.2	3.3

In-Process Revision

BRIEFING

Carbidopa, *USP 31* page 1635. It is proposed to specify the type of aluminium chloride to be used in the test for *Specific rotation*.

(HDQ: M. Marques) RTS—C70304

Change to read:

Specific rotation ⟨781S⟩**:** between –21.0° and –23.5°, calculated as the monohydrate.

Test solution: 10 mg per mL, in aluminum chloride solution (2 in 3,

▲prepared using the hexahydrate form of the aluminium salt)▲*USP33*
that has been filtered and then adjusted with 0.25 N sodium hydroxide to a pH of 1.5.

BRIEFING

Clarithromycin Tablets, *USP 31* page 1788. It is proposed to clarify how to prepare the *0.1 M Sodium acetate buffer* used in the *Dissolution* test.

(HDQ: M. Marques) RTS—C70388

Change to read:

Dissolution ⟨711⟩—

0.1 M Sodium acetate buffer— ~~Transfer 13.61 g of sodium acetate trihydrate to a 1-L volumetric flask, add water to dissolve, dilute with water to volume, and mix. Adjust with 0.1 M acetic acid to a pH of 5.0.~~

▲Transfer 13.61 g of sodium acetate trihydrate to a 1-L volumetric flask, and dissolve in and dilute with water to volume. Transfer 5.7 mL of glacial acetic acid to another 1-L volumetric flask, and dilute with water to volume. Combine the two solutions to obtain a pH of 5.0 (the proportion is almost 1 : 1).▲*USP33*

Medium: 0.1 M Sodium acetate buffer; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved in the *Medium,* as directed in the *Assay,* using instead of the *Assay preparation* a filtered portion of the solution under test quantitatively diluted with *Mobile phase* to yield a test solution containing about 125 µg of clarithromycin per mL. Calculate the quantity, in mg, of clarithromycin dissolved by the formula:

$$900(CD)(r_U/r_S)$$

in which D is the appropriate dilution factor used to prepare the test solution, and the other terms are as defined therein.
Tolerances—Not less than 80% *(Q)* of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) is dissolved in 30 minutes.

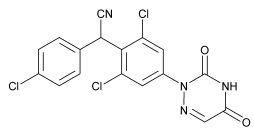
BRIEFING

Diclazuril. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed. The test for *Chromatographic purity* is an HPLC procedure based upon analyses performed with a Hypersil BDS brand of L1 column. The typical retention time is about 14 minutes for diclazuril. Interested parties are invited to submit comments.

(VET: I. DeVeau; T. Sigambris) RTS—C59275

Add the following:

▲Diclazuril



$C_{17}H_9Cl_3N_4O_2$ 407.64

Benzeneacetonitrile, 2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3*H*)-yl)-.

(*p*-Chlorophenyl)[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-*as*-triazin-2(3*H*)-yl)phenyl]acetonitrile [*101831-37-2*].

» Diclazuril contains not less than 97.0 percent and not more than 101.0 percent of $C_{17}H_9Cl_3N_4O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—*USP Diclazuril RS. USP Diclazuril System Suitability Mixture RS.*

Identification—

Infrared Absorption ⟨197K⟩.

In-Process Revision

Loss on drying ⟨731⟩—Dry it at between 100° and 105° under vacuum for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity—

Ammonium formate solution, Solution A, Solution B, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Prepare as directed for the *Standard preparation* in the *Assay.*

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the area percentage of each impurity, relative to diclazuril, in the portion of Diclazuril taken using the relative response factors (*F*) from *Table 1* below, and by the formula:

$$100(1/F)(C_S/C_U)(r_U/r_S)$$

in which F is the relative response factor; C_S is the concentration, in mg per mL, of diclazuril in the *Standard solution*; C_U is the concentration, in mg per mL, of diclazuril in the *Test solution;* r_U is the peak response for each impurity obtained from the *Test solution;* and r_S is the response of the diclazuril peak in the *Standard solution:* in addition to not exceeding the limits in *Table 1,* not more than 1.5% of total impurities is found. Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.

Residual solvents—⟨467⟩**:** not more than 4000 ppm of *N*,*N*-dimethylformamide is found.

Assay—

Ammonium formate solution—Dissolve 6.3 g of ammonium formate in 800 mL of water, adjust with anhydrous formic acid to a pH of 4.0, add 200 mL of water, and mix.

Solution A—Prepare a mixture of water, acetonitrile, and *Ammonium formate solution* (75 : 15 : 10).

Solution B—Prepare a mixture of acetonitrile, *Ammonium formate solution,* and water (85 : 10 : 5).

Table 1

Name	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
6-Carboxylic acid[1]	0.62	0.85	0.50
6-Carboxamide[2]	0.80	0.92	0.50
Diclazuril	1.00	—	—
Ketone[3]	1.03	0.52	0.10
4-Amino derivative[4]	1.09	0.81	0.50
Des-cyano derivative[5]	1.16	1.1	0.50
Trichlorodiphenyl acetonitrile[6]	1.24	0.71	0.50
Any other individual impurity	—	1.0	0.20

[1] (*RS*)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxylic acid.
[2] (*RS*)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamide.
[3] 2-[3,5-Dichloro-4-(4-chlorobenzoyl)phenyl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione.
[4] (*RS*)-2-(4-Amino-2,6-dichlorophenyl)-2-(4-chlorophenyl)acetonitrile.
[5] 2-[3,5-Dichloro-4-(4-chlorobenzyl)phenyl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione.
[6] (*RS*)-2-(4-Chlorophenyl)-2-(2,6-dichlorophenyl)acetonitrile.

In-Process Revision

Mobile phase—Use variable filtered and degassed mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in dimethylformamide containing about 0.5 mg per mL of USP Diclazuril System Suitability Mixture RS.

Standard preparation—Dissolve an accurately weighed quantity of USP Diclazuril RS in dimethylformamide to obtain a solution having a concentration of about 0.5 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Diclazuril in dimethylformamide to obtain a solution having a known concentration of about 0.5 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 10-cm column that contains base-deactivated 3-µm packing L1. It is maintained at a constant temperature of about 35° C. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–20	100→0	0→100	linear gradient
20–25	0	100	isocratic
25–26	0→100	100→0	linear gradient
26–36	100	0	re-equilibrate

Chromatograph the *System suitability solution,* identify the compounds, and record the peak responses as directed for *Procedure*: the resolution, *R,* between diclazuril and diclazuril ketone is not less than 1.9. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the tailing factor for the diclazuril peak is not more than 1.4, and the relative standard deviation for replicate injections for the diclazuril peak is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the percentage of $C_{17}H_9Cl_3N_4O_2$ in the portion of diclazuril taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of diclazuril in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.▲*USP33*

BRIEFING

Megestrol Acetate Oral Suspension, *USP 31* page 2604. It is proposed to include the possibility of diluting both the *Standard solution* and *Test solution* in *Dissolution Test 1*, in order to be within the linearity range of the spectrophotometer.

(BPC: M. Marques) RTS—C70306

Change to read:

Dissolution ⟨711⟩—

TEST 1—
Medium: 0.5% sodium lauryl sulfate in water; 900 mL.
Apparatus 2: 25 rpm.
Time: 30 minutes.
Standard solution—Transfer about 45 mg, accurately weighed, of USP Megestrol Acetate RS to a 250-mL volumetric flask, add about 12 mL of methanol, and place the flask in a warm water bath until the solid is dissolved. Dilute with *Medium* to volume. The final concentration is about 180 µg of megestrol acetate per mL.

▲Dilute with *Medium*, if necessary.▲*USP33*
Procedure—Transfer to the surface of the *Medium* in the dissolution vessel an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 160 mg of megestrol acetate. Determine the amount of $C_{24}H_{32}O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 292 nm on filtered portions of the solution under test,

▲diluted with *Medium*, if necessary,▲*USP33*
in comparison with the *Standard solution.* Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) released by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times V \times LC}$$

in which A_U and A_S are the absorbances obtained from the solution under test and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution; V* is the sample volume, in mL, of Oral Suspension taken; 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the label claim, in mg per mL.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{24}H_{32}O_4$ is dissolved in 30 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.5% sodium lauryl sulfate in water; 900 mL.

Apparatus 2: 25 rpm.

Time: 30 minutes.

Standard solution—Transfer about 45 mg, accurately weighed, of USP Megestrol Acetate RS to a 250-mL volumetric flask. Add about 5 mL of methanol, and mix. Dilute with *Medium* to volume. Transfer 10 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. The final concentration is about 18 µg per mL.

Test solution—[NOTE—Use a separate syringe for each vessel.] Withdraw more than 10 mL of the Oral Suspension, using a 10-mL syringe with a long cannula. Remove air bubbles from the syringe. Adjust the volume to the 10-mL mark on the syringe, and remove the needle. Wipe the tip of the syringe, and accurately weigh (gross weight). Operate the apparatus, and rapidly dispense the Oral Suspension to the side of the vessel at about halfway from the bottom. Similarly dispense the Oral Suspension into other vessels. Accurately weigh each syringe after dispensing the sample (tare weight). Record sample weights. After completion of the dissolution, pass an aliquot through a nylon filter having a 0.45-µm porosity, and dilute 2.0 mL of the filtrate with *Medium* to 50.0 mL to obtain a solution having a theoretical concentration of about 18 µg per mL.

Procedure—Determine the amount of $C_{24}H_{32}O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 292 nm, using 0.5-cm pathlength cuvettes, on the *Test solution* in comparison with the *Standard solution*. Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) released by the formula:

$$\frac{A_U \times C_S \times 900 \times d \times 100}{A_S \times W_U \times LC}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution; d* is the density, in mg per mL, of the Oral Suspension obtained by dividing the weight of Oral Suspension taken by 10 mL; W_U is the weight, in mg, of Oral Suspension taken; 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the label claim, in mg per mL.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{24}H_{32}O_4$ is dissolved in 30 minutes.

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.5% sodium lauryl sulfate in degassed water; 900 mL. Use ultrapure sodium lauryl sulfate with an assay content of not less than 99.0%.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{24}H_{32}O_4$ dissolved by employing the following method.

Mobile phase—Proceed as directed in the *Assay.*

Standard solution—Transfer about 11.5 mg, accurately weighed, of USP Megestrol Acetate RS to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

Test solution—Proceed as directed for *Test 2,* introducing the sample into the vessel over a 10- to 15-second period (about 1 mL per second).

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) released by the formula:

$$\frac{r_U \times C_S \times 900 \times d \times 100}{r_S \times W_U \times LC}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution; d* is the density, in mg per mL, of the Oral Suspension obtained by dividing the weight of Oral Suspension taken by 10 mL; W_U is the weight, in mg, of Oral Suspension taken; 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the label claim, in mg per mL.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{24}H_{32}O_4$ is dissolved in 30 minutes.

BRIEFING

Metformin Hydrochloride Extended-Release Tablets, *USP 31* page 2642, and *Interim Revision Announcement* on page 1403 of *PF* 34(6). It is proposed to include a more generic description of the filters used in *Dissolution Tests 4* and *5*.

(BPC: M. Marques) RTS—C70358

Change to read:

Dissolution ⟨711⟩—

TEST 1—

Medium: pH 6.8 phosphate buffer prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjusting with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1; 1000 mL.

Apparatus 2: 100 rpm, for Tablets labeled to contain 500 mg.

Apparatus 1: 100 rpm, for Tablets labeled to contain 750 mg.

Times: 1, 3, and 10 hours.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a 0.45-µm hydrophilic polyethylene filter, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula:

$$\frac{[C \times (A_U / A_S) \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S)] \times 100}{L}$$

in which C is the concentration, in mg per mL, of the Standard solution; A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_{60} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 1 hour; C_{180} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 3 hours; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

In-Process Revision

Time (hours)	500-mg Tablet, Amount dissolved	750-mg Tablet, Amount dissolved
1	between 20% and 40%	between 22% and 42%
3	between 45% and 65%	between 49% and 69%
10	not less than 85%	not less than 85%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Prepare as directed for *Medium* in *Test 1;* 1000 mL.

Apparatus 2: 100 rpm.

Times: 1, 2, 6, and 10 hours.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a 0.45-µm polyethylene filter, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_t, in mg per mL, in the *Medium* at each time point, *t,* by the formula:

$$\frac{A_U \times C_s \times D_U}{A_s}$$

in which A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; C_S is the concentration of metformin hydrochloride, in mg per mL, in the Standard solution; and D_U is the dilution factor of the solution under test. Calculate the percentage of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point by the following formulas:

Percentage dissolved at the first time point (1 hour):

$$\frac{C_1 \times 1000 \times 100}{L}$$

in which C_1 is the content of metformin hydrochloride, in mg per mL, in the *Medium* at the first time interval; 1000 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Percentage dissolved at the second time point (2 hours):

$$\frac{C_2 \times (1000 - SV_1) + C_1 \times SV_1 \times 100}{L}$$

in which C_2 is the content of metformin hydrochloride, in mg per mL, in the *Medium* at the second time interval; 1000 is the volume, in mL, of *Medium;* SV_1 is the volume, in mL, of the sample withdrawn at 1 hour; C_1 is the content of metformin hydrochloride, in mg per mL, in the *Medium* at 1 hour; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Percentage dissolved at the *n*th time point:

$$\frac{C_n \times [1000 - (n-1)SV] + (C_1 + C_2 + ... + C_{n-1}) \times SV \times 100}{L}$$

in which C_n is the content of metformin hydrochloride, in mg per mL, in the *Medium* at the *n*th time interval; *n* is the time interval of interest; *SV* is the volume, in mL, of sample withdrawn at each time interval; C_1, C_2, C_3, ... C_{n-1} is the content of metformin hydrochloride, in mg per mL, in the *Medium* at each time interval; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 20% and 40%
2	between 35% and 55%
6	between 65% and 85%
10	not less than 85%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus, and *Procedure*—Proceed as directed for *Test 1*.

Times: 1, 2, 5, and 12 hours for Tablets labeled to contain 500 mg; and 1, 3, and 10 hours for Tablets labeled to contain 750 mg.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a 0.45-µm hydrophilic polyethylene filter, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula:

$$\frac{[C \times (A_U / A_S) \times (V - V_S) + (C_{60} \times V_S) + (C_{120} \times V_S) + (C_{300} \times V_S)] \times 100}{L}$$

in which C is the concentration, in mg per mL, of the Standard solution; A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_{60} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 1 hour; C_{120} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 2 hours; C_{300} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 5 hours; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

FOR TABLETS LABELED TO CONTAIN 500 MG:

Time (hours)	Amount dissolved
1	between 20% and 40%
2	between 35% and 55%
5	between 60% and 80%
12	not less than 85%

FOR TABLETS LABELED TO CONTAIN 750 MG:

Time (hours)	Amount dissolved
1	between 22% and 42%
3	between 49% and 69%
10	not less than 85%

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4.*

Medium: Prepare as directed for *Medium* in *Test 1;* 1000 mL.

Apparatus 2: 100 rpm.

Times: 1, 3, 6, and 10 hours.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 250 nm (shoulder) on portions of the solution under test passed through a ~~0.45-µm polyethylene filter,~~

▲suitable 0.45-µm filter,▲*USP33* suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_t, in mg per mL, in the *Medium* at each time point, *t,* by the formulas specified in *Test 2.*

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 20% and 40%
3	between 45% and 65%
6	between 65% and 85%
10	not less than 85%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5.*

Medium: pH 6.8 phosphate buffer prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjusting with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1; 900 mL, deaerated.

Apparatus 1: 100 rpm, with the vertical holder described below.

Times: 2, 8, and 16 hours.

Procedure—Place a vertical sample holder into each basket (see *Figures 1* and *2*). Place one Tablet inside the sample holder, making sure that the Tablets are vertical at the bottom of the baskets. Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 250 nm on portions of the solution under test passed through a ~~0.45-µm polyethylene filter~~

▲suitable 0.45-µm filter,▲*USP33* suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_t, in mg per mL, in the *Medium* at each time point, *t,* by the formulas specified in *Test 2.*

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	500-mg Tablet, Amount dissolved	1000-mg Tablet, Amount dissolved
2	not more than 30%	not more than 30%
8	between 60% and 85%	between 65% and 90%
16	not less than 90%	not less than 90%

TEST 6—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6.*

Medium: pH 6.8 phosphate buffer prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjusting with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.05; 1000 mL, deaerated.

Apparatus 2: 100 rpm, with USP sinker, if necessary.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 233 nm on portions of the solution under test passed through a 0.45-µm hydrophilic polyethylene filter, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula:

$$\frac{[C \times (A_U / A_S) \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S) + (C_{600} \times V_S)] \times 100}{L}$$

in which C is the concentration, in mg per mL, of the Standard solution; A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_{60} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 1 hour; C_{180} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 3 hours; C_{600} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 10 hours; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	500-mg Tablet, Amount dissolved	750-mg Tablet, Amount dissolved
1	between 20% and 40%	between 20% and 40%
3	between 45% and 65%	between 45% and 65%
10	not less than 85%	not less than 85%

TEST 7—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7.*

Medium—Prepare as directed for *Medium* in *Test 1;* 1000 mL.

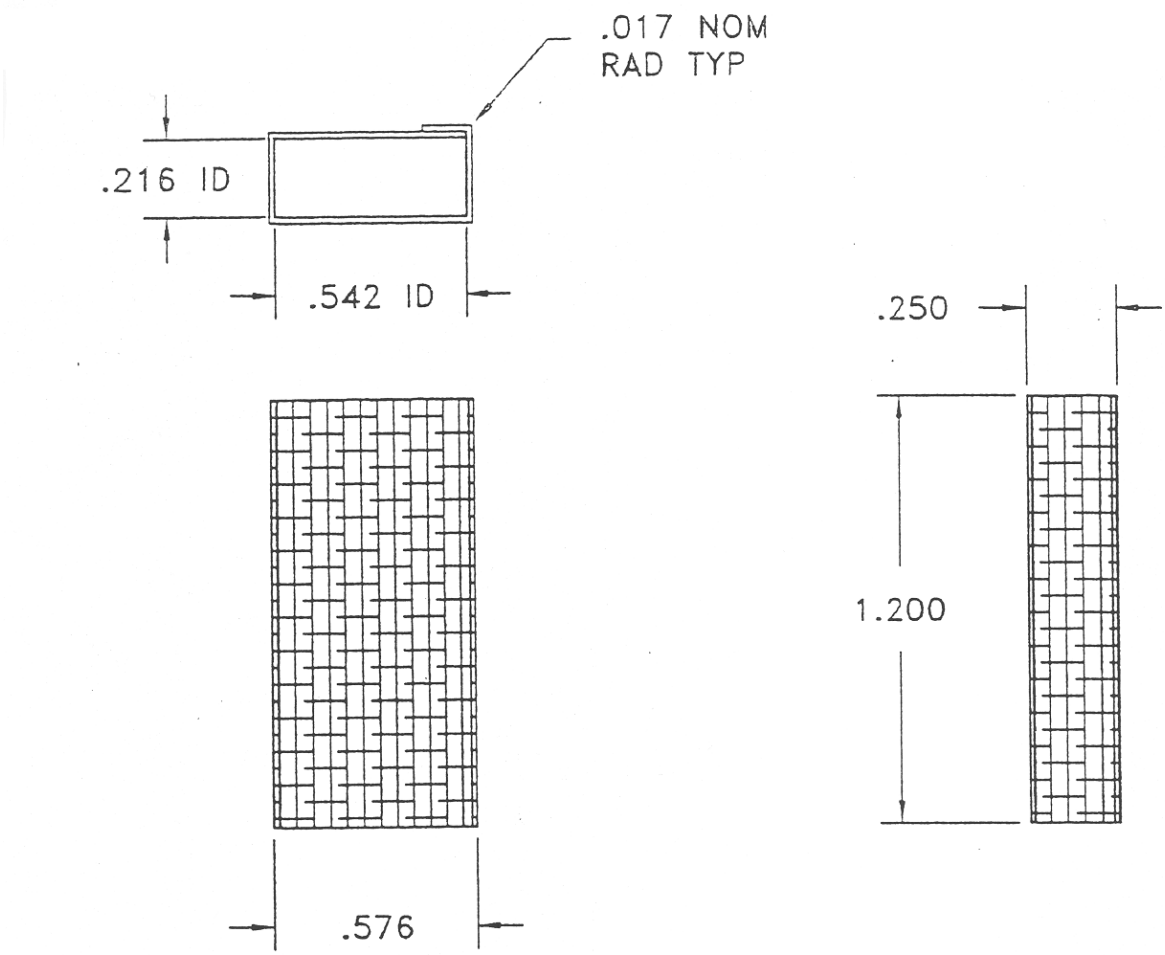
Apparatus 2: 50 rpm, with USP sinker, for Tablets labeled to contain 500 mg.

Apparatus 1: 100 rpm, for Tablets labeled to contain 750 mg.

Times: 1, 3, and 10 hours.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a suitable 0.45-µm filter, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula:

In-Process Revision



NOTES:

1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE +/-.010

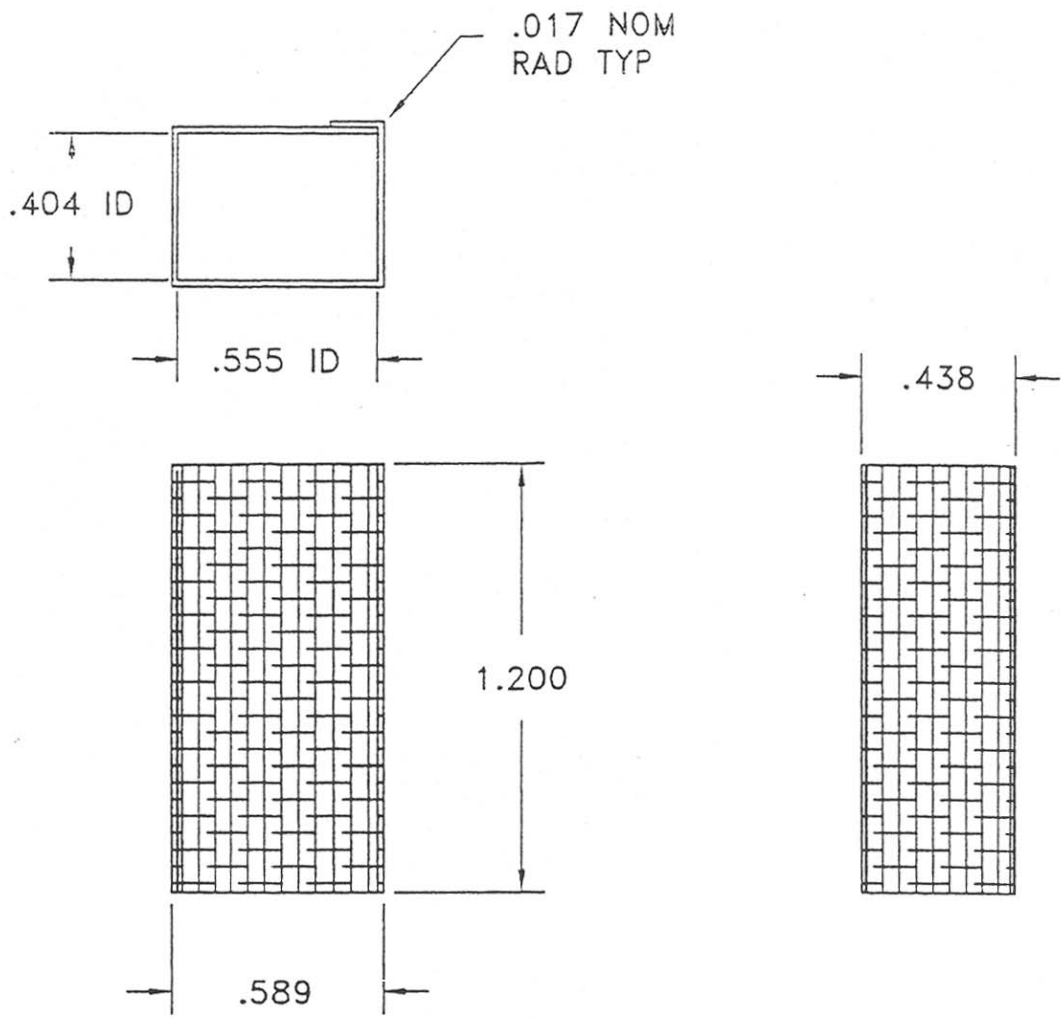
Figure 1

$$\frac{[C\times(A_U/A_S)\times(V-V_S)+(C_{60}\times V_S)+(C_{180}\times V_S)+(C_{600}\times V_S)]\times 100}{L}$$

in which C is the concentration, in mg per mL, of the Standard solution; A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_{60} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 1 hour; C_{180} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 3 hours; C_{600} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 10 hours; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	500-mg Tablet, Amount dissolved	750-mg Tablet, Amount dissolved
1	between 20% and 40%	between 20% and 40%
3	between 45% and 65%	between 40% and 60%
10	not less than 85%	not less than 80%



NOTES:

1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE +/-.010

Figure 2

TEST 8—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8.*

Medium—Prepare as directed for *Medium* in *Test 1;* 1000 mL.

Apparatus 2: 100 rpm, with sinker, for Tablets labeled to contain 500 mg.

Apparatus 1: 100 rpm, for Tablets labeled to contain 750 mg.

Times: 1, 2, 6, and 10 hours

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the solution under test passed through a suitable 0.45-µm filter, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula (1), in which C is the concentration, in mg per mL, of the Standard solution; A_U and A_S are the absorbances of the solution under test and the Standard solution, respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_{60} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 1 hour; C_{120} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 2 hours; C_{360} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 6 hours; C_{600} is the concentration, in mg per mL, of metformin hydrochloride in the *Medium* determined at 10 hours; 100 is the conversion factor to percentage; and L is the tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	500-mg Tablet, Amount dissolved	750-mg Tablet, Amount dissolved
1	between 20% and 40%	between 20% and 40%
2	between 30% and 50%	between 35% and 55%
6	between 65% and 85%	between 75% and 95%
10	not less than 85%	not less than 85%

•TEST 9—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: 0.05 M phosphate buffer, pH 6.8; 1000 mL.

Apparatus 2: 100 rpm.

Times: 1, 5, 12, and 20 hours for Tablets labeled to contain 500 mg; and 1, 4, 10, and 24 hours for Tablets labeled to contain 750 mg.

Standard solution—Transfer about 50 mg, accurately weighed, of USP Metformin Hydrochloride RS to a 100-mL volumetric flask, and dissolve in and dilute with *Medium* to volume.

Test solution—Pass a portion of the solution under test through a filter having a porosity of 0.45 µm.

Procedure—Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by UV absorption at the wavelength of maximum absorbance at about 232 nm on portions of the *Test solution* in comparison with the *Standard solution,* using a 0.01-cm flow cell and *Medium* as the blank. Calculate the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), in percentage, released at each time point by the formula (2), in which C is the concentration, in mg per mL, of the *Standard solution;* A_U and A_S are the absorbances of the *Test solution* and the *Standard solution,* respectively; V is the initial volume, in mL, of *Medium* in the vessel; V_S is the volume, in mL, withdrawn from the vessel for previous samplings; C_1 is the concentration, in mg per mL, of metformin hydrochloride in *Medium* determined at the first timepoint; C_2 is the concentration, in mg per mL, of metformin hydrochloride in *Medium* determined at the second timepoint; C_3 is the concentration, in mg per mL, of metformin hydrochloride in *Medium* determined at the third timepoint; C_4 is the concentration, in mg per mL, of metformin hydrochloride in *Medium* determined at the fourth timepoint; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—The percentages of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

FOR TABLETS LABELED TO CONTAIN 500 MG:

Time (hours)	Amount dissolved
1	between 20% and 40%
5	between 45% and 65%
12	between 70% and 90%
20	not less than 85%

FOR TABLETS LABELED TO CONTAIN 750 MG:

Time (hours)	Amount dissolved
1	between 20% and 45%
4	between 45% and 70%
10	between 70% and 95%
24	not less than 85%

•6

$$\frac{[C \times (A_U / A_S) \times (V - V_S) + (C_{60} \times V_S) + (C_{120} \times V_S) + (C_{360} \times V_S) + (C_{600} \times V_S)] \times 100}{L} \quad (1)$$

$$\frac{\left[C \times (A_U / A_S) \times (V - V_S) + (C_1 \times V_S) + (C_2 \times V_S) + (C_3 \times V_S) + (C_4 \times V_S)\right] \times 100}{L} \quad (2)$$

In-Process Revision

BRIEFING

Mometasone Furoate Cream, *USP 31* page 2727. On the basis of comments received, it is proposed to (1) include the storage conditions in the *Packaging and storage* section, (2) add a test for *Related compounds,* and (3) replace the *Assay* procedure with an improved and validated method that provides better resolution and efficiency. The liquid chromatographic procedures in the test for *Related compounds* and in the *Assay* are based on analyses performed with a Supelcosil-ABZ PLUS brand of L60 column. The typical retention time for the mometasone furoate peak is about 35.2 minutes.

(MD-CCA: C. Anthony) RTS—C41345

Change to read:

Packaging and storage—Preserve in well-closed containers,

▲and store at controlled room temperature.▲*USP33*

Add the following:

▲**Related compounds—**[Note—Protect from light.]

Diluent A, Solution A, Solution B, and *Mobile phase*—Proceed as directed in the *Assay.*

Standard stock solution—Proceed as directed for *Standard stock preparation* in the *Assay.*

Diluent C—Prepare a solution consisting of a mixture of water, acetonitrile, and glacial acetic acid (70 : 30 : 1).

System suitability solution—Quantitatively transfer a quantity of *Standard stock solution* into a suitable container, and dilute quantitatively, and stepwise if necessary, with *Diluent C* to obtain a solution having a known concentration of about 0.1 µg per mL.

Blank solution: a mixture of 5.0 mL of *Diluent A* and 15.0 mL of *Diluent C.*

Test solution—Transfer an accurately weighed portion of Cream, equivalent to about 2.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 minutes. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1 to 2 mL of filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The column temperature is maintained at 25 ± 5°. The chromatograph is programmed as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak area responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Test solution* and the *Blank solution* into the chromatograph, record the chromatogram, and measure the peak area responses. Exclude any peak areas less than those obtained from the chromatogram of the *System suitability solution.* Also exclude any peaks with the same retention time as that observed in the chromatogram of the *Blank solution.* Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph, and therefore are not included in the total specified and unspecified impurities limit. Calculate the percentage of each impurity in the portion of Cream taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak area response for each impurity; and r_s is the sum of the area responses of all the peaks. The impurities meet the requirements specified in the table below.

Compound	Relative Retention Time	Limit (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	NMT 0.1
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	NMT 0.1
21-Chloro-17-hydroxy-16α-methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	NMT 0.1

In-Process Revision

Compound	Relative Retention Time	Limit (%)
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methyl-pregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	NMT 0.6
Mometasone furoate	1.0	—
Unspecified individual impurity		NMT 0.2
Total specified and unspecified impurities		NMT 0.8

▲*USP33*

Change to read:

Assay—

~~*Mobile phase*~~ ~~Proceed as directed under the *Assay* for *Mometasone Furoate*.~~

~~*Internal standard solution*~~ ~~Dissolve a suitable quantity of beclomethasone dipropionate in acetonitrile to obtain a solution containing about 0.53 mg per mL.~~

~~*Standard preparation*~~ ~~Dissolve an accurately weighed quantity of USP Mometasone Furoate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.136 mg per mL. Pipet equal amounts of this solution and the *Internal standard solution*, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having known concentrations of about 0.027 mg of mometasone furoate and 0.106 mg of beclomethasone dipropionate per mL.~~

~~*Assay preparation*~~ ~~Transfer an accurately weighed portion of Cream, equivalent to about 2.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Pipet 15.0 mL of *Internal standard solution* and 15.0 mL of acetonitrile into the tube, and attach the cap. Heat in an 85° water bath until the cream completely melts, and shake by hand for 2 minutes. Repeat the heating and shaking. Place the tube in an ice-methanol bath for 10 minutes. Centrifuge to obtain a clear supernatant, and transfer 10.0 mL of the supernatant layer into a 25-mL volumetric flask. Dilute with acetonitrile to volume, and mix.~~

~~*Chromatographic system* (see *Chromatography* ⟨621⟩)~~ ~~Proceed as directed in the *Assay* under *Mometasone Furoate*.~~

~~*Procedure*~~ ~~Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Cream taken by the formula:~~

$$75C(R_U/R_S)$$

~~in which *C* is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation*, and R_U and R_S are the ratios of the mometasone furoate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.~~

▲[Note—Protect from light.]

Diluent A—Prepare a solution of tetrahydrofuran and glacial acetic acid (100 : 1).

Diluent B—Prepare a solution of water, acetonitrile, and glacial acetic acid (50 : 50 : 1).

Solution A: water.

Solution B: acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of diethyl phthalate in acetonitrile to obtain a solution having a concentration of about 1.4 mg per mL.

Standard stock preparation—Dissolve an accurately weighed quantity of USP Mometasone Furoate RS, and dilute quantitatively, and stepwise if necessary, with *Diluent A* to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Pipet equal quantities of the *Standard stock preparation* and the *Internal standard solution,* and dilute quantitatively, and stepwise if necessary, with *Diluent B* to obtain a solution having a known concentration of about 0.05 mg per mL of mometasone furoate and 0.35 mg per mL of diethyl phthalate.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 1.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B,* mix on a vortex mixer for 1 minute, and centrifuge for 10 minutes. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1–2 mL of filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

In-Process Revision

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	70	30	equilibration
0–2.0	70	30	isocratic
2.0–45.0	70→45	30→55	linear gradient
45.0–46.0	45→70	55→30	linear gradient
46.0–50	70	30	isocratic

Chromatograph the *Standard preparation*, and record the peak area responses as directed for *Procedure:* the relative retention times are about 0.4 for diethyl phthalate and 1.0 for mometasone furoate; the tailing factor for the mometasone furoate peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Cream taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which C_S is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation*; C_U is the concentration, in mg per mL, of mometasone furoate in the *Assay preparation* based on the label claim; and R_U and R_S are the ratios of the mometasone furoate peak response to the diethyl phthalate peak response obtained from the *Assay preparation* and *Standard preparation,* respectively.▲*USP33*

BRIEFING

Mometasone Furoate Ointment, *USP 31* page 2728. On the basis of comments received, it is proposed to (1) include the storage conditions in the *Packaging and storage* section, (2) add a test for *Related compounds,* and (3) replace the *Assay* procedure with an improved and validated method that provides better resolution and efficiency. The liquid chromatographic procedures in the test for *Related compounds* and in the *Assay* are based on analyses performed with a Supelcosil-ABZ PLUS brand of L60 column. The typical retention time for the mometasone furoate peak is about 35.2 minutes.

(MD-CCA: C. Anthony) RTS—C41345

Change to read:

Packaging and storage—Preserve in well-closed containers,

▲and store at controlled room temperature.▲*USP33*

Add the following:

▲**Related compounds—**[Note—Protect from light.]

Diluent A, Solution A, Solution B, and *Mobile phase*—Proceed as directed in the *Assay.*

Standard stock solution—Proceed as directed for *Standard stock preparation* in the *Assay.*

Diluent C—Prepare a solution consisting of a mixture of water, acetonitrile, and glacial acetic acid (70 : 30 : 1).

System suitability solution—Quantitatively transfer an amount of *Standard stock solution* into a suitable container, and dilute quantitatively, and stepwise if necessary, with *Diluent C* to obtain a solution having a known concentration of about 0.1 µg per mL.

Blank solution: a mixture of 5.0 mL of *Diluent A* and 15.0 mL of *Diluent C.*

Test solution—Transfer an accurately weighed portion of Ointment, equivalent to about 2.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A,* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 minutes. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1–2 mL of filtrate.

In-Process Revision

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The column temperature is maintained at 25 ± 5°. The chromatograph is programmed as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak area responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Test solution* and the *Blank solution* into the chromatograph, record the chromatogram, and measure the peak area responses. Exclude any peak area less than that obtained from the chromatogram of the *System suitability solution.* Also, exclude any peaks with the same retention times as those observed in the chromatogram of the *Blank solution.* Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph, and therefore are not included in the total specified and unspecified impurities limit. Calculate the percentage of each impurity in the portion of Ointment taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak area response for each impurity; and r_s is the sum of the area responses of all the peaks. The impurities meet the requirements specified in the table below.

Compound	Relative Retention Time	Limit (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	NMT 0.2
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	NMT 0.2
21-Chloro-17-hydroxy-16α- methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	NMT 0.2
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	NMT 0.3
Mometasone furoate	1.0	—
Unspecified individual impurity		NMT 0.2
Total specified and unspecified impurities		NMT 0.5

▲*USP33*

Change to read:

Assay—

~~*Mobile phase*, *Diluting solution*, *Internal standard solution*, *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Mometasone Furoate*.~~

~~*Assay preparation*—Transfer an accurately weighed portion of Ointment, equivalent to 1.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Pipet 10.0 mL of *Internal standard solution* and 10.0 mL of *Diluting solution* into the tube, and attach the cap. Heat in an 85° water bath until the ointment completely melts, and shake vigorously by hand until the ointment resolidifies. Repeat heating and shaking two more times. Place the tube in an ice-methanol bath for 10 minutes. Centrifuge to obtain a clear supernatant, and transfer 10.0 mL of the supernatant into a 25-mL volumetric flask. Dilute with *Diluting solution* to volume, and mix.~~

~~*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mometasone furoate ($C_{34}H_{44}Cl_2O_8$) in the portion of Ointment taken by the formula:~~

$$\sim\sim 50C(R_u / R_s) \sim\sim$$

~~in which C is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation,* and R_u and R_s are the ratios of the mometasone furoate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.~~

▲[Note—Protect from light.]

Diluent A—Prepare a solution of tetrahydrofuran and glacial acetic acid (100 : 1).

In-Process Revision

Diluent B—Prepare a solution of water, acetonitrile, and glacial acetic acid (50 : 50 : 1).

Solution A: water.

Solution B: acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for the *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of diethyl phthalate in acetonitrile to obtain a solution having a concentration of about 1.4 mg per mL.

Standard stock preparation—Dissolve an accurately weighed quantity of USP Mometasone Furoate RS, and dilute quantitatively, and stepwise if necessary, with *Diluent A* to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Pipet equal amounts of the *Standard stock preparation* and the *Internal standard solution,* and dilute quantitatively, and stepwise if necessary, with *Diluent B* to obtain a solution having a known concentration of about 0.05 mg of mometasone furoate and 0.35 mg of diethyl phthalate per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A,* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B,* mix on a vortex mixer for 1 minute, and centrifuge for 10 minutes. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1–2 mL of filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	70	30	equilibration
0–2.0	70	30	isocratic
2.0–45.0	70→45	30→55	linear gradient
45.0–46.0	45→70	55→30	linear gradient
46.0–50	70	30	isocratic

Chromatograph the *Standard preparation*, and record the peak area responses as directed for *Procedure:* the relative retention times are about 0.4 for diethyl phthalate and 1.0 for mometasone furoate; the tailing factor for the mometasone furoate peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Ointment taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which C_S is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of mometasone furoate in the *Assay preparation* based on the label claim; and R_U and R_S are the ratios of the mometasone furoate peak response to the diethyl phthalate peak response obtained from the *Assay preparation* and *Standard preparation,* respectively.▲*USP33*

In-Process Revision

BRIEFING

Mometasone Furoate Topical Solution, *USP 31* page 2728. On the basis of comments received, it is proposed to make the following changes.
1. Include storage conditions in the *Packaging and storage* section.
2. Add a test for *Related compounds.*
3. Replace the *Assay* procedure with an improved and validated method that provides better resolution and efficiency.

The liquid chromatographic procedures in the test for *Related compounds* and in the *Assay* are based on analyses performed with a Supelcosil-ABZ PLUS brand of L60 column. The typical retention time for the mometasone furoate peak is about 35.2 minutes.

(MD-CCA: C. Anthony) RTS—C41345

Change to read:

Packaging and storage—Preserve in well-closed containers,

▲and store at controlled room temperature.▲*USP33*

Add the following:

▲**Related compounds—**[Note—Protect from light.]

Diluent, Solution A, Solution B, and *Mobile phase*—Proceed as directed in the *Assay.*

Standard solution—Proceed as directed for the *Standard preparation* in the *Assay.*

System suitability solution—Quantitatively transfer an amount of *Standard solution* into a suitable container, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 µg per mL.

Test solution—Proceed as directed for the *Assay preparation* in the *Assay.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The column temperature is maintained at $25 \pm 5^{\circ}$. The chromatograph is programmed as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak area responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Test solution* and *Diluent* into the chromatograph, record the chromatogram, and measure the peak area responses. Exclude any peak areas less than that obtained from the *System suitability solution* chromatogram. Also, exclude any peaks with the same retention times as those observed in the chromatogram of the *Diluent.* Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph, and therefore are not included in the total specified and unspecified impurities limit. Calculate the percentage of each impurity in the portion of Topical Solution taken by the formula:

$$100\,(r_i / r_s)$$

in which r_i is the peak area response for each impurity; and r_s is the sum of the area responses of all peaks. The impurities meet the requirements specified in the table below.

Compound	Relative Retention Time	Limit (%)
9α-Chloro-11β,17,21- trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	NMT 0.3
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	NMT 0.1
21-Chloro-17-hydroxy-16α-methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	NMT 0.1
Mometasone furoate related compound A[1]	0.94	—
Mometasone furoate	1.0	

In-Process Revision

Compound	Relative Retention Time	Limit (%)
Unspecified individual impurity		NMT 0.2
Total specified and unspecified impurities		NMT 1.3

[1] 21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)

▲*USP33*

Change to read:

Assay—

~~*Mobile phase*, *Diluting solution*, *Internal standard solution*, *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Mometasone Furoate*.~~

~~*Assay preparation*—Transfer an accurately weighed portion of Topical Solution, equivalent to 1.0 mg of mometasone furoate, to a 50-mL volumetric flask. Pipet 10.0 mL of *Internal standard solution* into the flask, dilute with *Diluting solution* to volume, and mix.~~

~~*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Topical Solution taken by the formula:~~

~~$$50C(R_U/R_S)$$~~

~~in which *C* is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation,* and R_U and R_S are the ratios of the mometasone furoate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.~~

▲[Note—Protect from light.]

Diluent—Prepare a solution of water, acetonitrile, and glacial acetic acid (50 : 50 : 1).

Solution A: water.

Solution B: acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Mometasone Furoate RS, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately weighed portion of Topical Solution, equivalent to about 2.5 mg of mometasone furoate, to a 25-mL flask. Dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene filter having 0.2-µm porosity, discarding the first 1-2 mL of filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L60. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	70	30	equilibration
0–2.0	70	30	isocratic
2.0–45.0	70→45	30→55	linear gradient
45.0–46.0	45→70	55→30	linear gradient
46.0–50	70	30	isocratic

Chromatograph the *Standard preparation*, and record the peak area responses as directed for *Procedure:* the tailing factor for mometasone furoate is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Topical Solution taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Mometasone Furoate RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of mometasone furoate in the *Assay preparation* based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively.▲*USP33*

In-Process Revision

BRIEFING

Mycophenolate Mofetil, page 3852 of the *Second Supplement*. On the basis of comments received, it is proposed to make the following changes:

1. *Identification* test *B* is revised to replaced the UV method with an HPLC retention time agreement.
2. The test for *Melting range* is deleted, and the melting range information is given in the *Description and Solubility.*
3. In the test for *Related compounds*, the impurity limits are revised, and the disregard limit is added in accordance with the FDA approved specification. The acceptance criteria for the total specified impurities and the total unknown impurities are deleted to be consistent with ICH guidelines.
4. The relative standard deviation requirement for the replicate injections in the *Chromatographic system* under the *Assay* is revised to be consistent with the monograph sponsor's test method.
5. The *Procedure* in the *Assay* is revised to update the calculation formula.

(MD-OOD: F. Mao) RTS—C64392

Change to read:

Identification—

A: *Infrared Absorption* ⟨197K⟩.

~~**B:** *Ultraviolet Absorption* ⟨197U⟩~~

~~*Solution*: 10 µg per mL.~~

~~*Medium*: methanol~~

▲**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the *Standard preparation,* as obtained in the *Assay.*▲*USP33*

Delete the following:

~~▲**Melting range,** *Class Ia* ⟨741⟩**:** between 94° and 98°, but the range between beginning and end of melting does not exceed 2.5°.~~▲*USP33*

Change to read:

Related compounds—

0.3% Triethylamine phosphate buffer and *Mobile phase*—Prepare as directed in the *Assay.*

System suitability solution—Dissolve accurately weighed quantities of USP Mycophenolate Mofetil Related Compound A RS and USP Mycophenolate Mofetil Related Compound B RS in acetonitrile to obtain a solution containing about 10 µg per mL each of USP Mycophenolate Mofetil Related Compound A RS and USP Mycophenolate Mofetil Related Compound B RS, and mix.

Test solution—Use the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 250-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 45°. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B is not less than 1.5.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all peaks.

~~Related Compound~~	~~Relative Retention Time~~	~~Limit (%)~~
~~Mycophenolic acid[1]~~	~~about 0.33~~	~~0.5~~
~~Mycophenolate mofetil related compound A[2]~~	~~about 0.45~~	~~0.1~~
~~Mycophenolate mofetil related compound B[3]~~	~~about 0.49~~	~~0.1~~
~~*N*-Oxide analog[4]~~	~~about 0.60~~	~~0.1~~
~~Impurity A~~	~~about 0.68~~	~~0.1~~
~~1-Morpholinoethoxy analog[5]~~	~~about 0.86~~	~~0.1~~
~~*O*-Methyl analog[6]~~	~~about 1.2~~	~~0.1~~
~~Methyl mycophenolate[7]~~	~~about 1.5~~	~~0.1~~
~~Total specified impurities~~	~~—~~	~~0.5~~
~~Individual unknown impurities~~	~~—~~	~~0.1~~
~~Total unknown impurities~~	~~—~~	~~0.1~~
~~Total impurities~~	~~—~~	~~1.0~~

~~[1] (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.~~

~~[2] 2-morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.~~

~~[3] (*RS*)-7-hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuranyl-1-one.~~

~~[4] 2-Morpholinoethyl-(*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate-*N*-oxide.~~

~~[5] 2-Morpholinoethyl-(*RS*)-(*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-1-(2-morpholinoethoxy)-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.~~

~~[6] 2-Morpholinoethyl-(*E*)-6-(1,3-dihydro-4,6-dimethoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.~~

~~[7] Methyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.~~

▲

Related Compound	Relative Retention Time	Limit (%)
Mycophenolic acid[1]	0.33	0.50
Mycophenolate mofetil related compound A[2]	0.45	0.10
Mycophenolate mofetil related compound B[3]	0.49	0.10
N-Oxide analog[4]	0.60	0.10
1-Morpholinoethoxy analog[5]	0.86	0.10
Mycophenolate mofetil	1.0	—
O-Methyl analog[6]	1.2	0.10
Methyl mycophenolate[7]	1.5	0.10

In-Process Revision

Related Compound	Relative Retention Time	Limit (%)
Any single unspecified impurity	—	0.10
Total impurities	—	0.70

[1] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[2] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[3] (*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuranyl-1-one.
[4] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.
[5] 2-Morpholinoethyl (*RS*)-(*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-1-(2-morpholinoethoxy)-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[6] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dimethoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[7] Methyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

[NOTE—Disregard any unknown peak less than 0.03%.]▲*USP33*

Change to read:

Assay—

0.3% Triethylamine phosphate buffer—Add 4 mL of triethylamine to 1300 mL of water, and adjust with phosphoric acid to a pH of 5.3.

Mobile phase—Prepare a suitable mixture of *0.3% Triethylamine phosphate buffer* and acetonitrile (65 : 35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Mycophenolate Mofetil RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer about 50 mg of mycophenolate mofetil, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 250-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 45°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 8000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than ~~1.0%~~

▲2.0%.▲*USP33*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. ~~Calculate the quantity, in mg, of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil taken by the formula:~~

~~$$50C(r_U/r_S)$$~~

~~in which C is the concentration, in mg per mL, of USP Mycophenolate Mofetil RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.~~

▲Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil taken by the formula:

$$100(C_S / C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of Mycophenolate Mofetil in the *Standard preparation;* C_U is the concentration of mycophenolate mofetil in the *Assay preparation; and* r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.▲*USP33*

BRIEFING

Naratriptan Hydrochloride Oral Suspension. Because there is no existing *USP* monograph for this dosage form, the following new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with a 4.6-mm × 25-cm analytical column that contains 5-µm packing L11. USP has received data indicating that a Spheraclone Phenyl from Phenomenex is suitable. The typical retention time for naratriptan hydrochloride is about 9.7 minutes.

(CRX: R. Schnatz) RTS—C60109

Add the following:

▲Naratriptan Hydrochloride Oral Suspension

» Naratriptan Hydrochloride Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled content of naratriptan hydrochloride. Prepare Naratriptan Hydrochloride Oral Suspension 0.5 mg per mL as fol-

In-Process Revision

lows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Naratriptan Hydrochloride	50 mg
Vehicle: a mixture of Vehicle for Oral Solution, *NF,* (regular or sugar free), and Vehicle for Oral Suspension, *NF,* (1 : 1), a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using Tablets, place the required number of Tablets in a suitable mortar, and comminute the Tablets to a fine powder or add Naratriptan Hydrochloride powder. Add the Vehicle in small portions and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make a naratriptan suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the Vehicle to bring to final volume, and mix well.

Packaging and storage—Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.

Labeling—Label it to state that it is to be well-shaken before use and to state the beyond-use date.

USP Reference standards ⟨11⟩—*USP Naratriptan Hydrochloride RS.*

Beyond-use date: not later than 90 days after the date on which it was compounded, when stored at controlled cold temperature, and not later than 7 days after the date on which it was compounded, when stored at controlled room temperature.

pH ⟨791⟩**:** between 4.0 and 4.5.

Assay—

Mobile phase—Prepare a solution consisting of 12 mM triethylamine phosphate buffer and 2-propanol (90 : 9). Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve about 25 mg of USP Naratriptan Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask. Dilute with *Mobile phase* to obtain a concentration of 0.5 mg per mL.

Standard preparation—Pipet 1 mL of *Standard stock solution* to a 25-mL volumetric flask and dilute with *Mobile phase* to volume to obtain a concentration of 20 µg per mL, and pass through a 0.22-µm filter.

Assay preparation—Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 0.4 mL to a 10-mL volumetric flask. Add 1 mL of 0.1 N sodium hydroxide solution by pipet, and sonicate for five minutes. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 20 µg naratriptan hydrochloride per mL. Centrifuge, and pass the naratriptan hydrochloride solution through a 0.22-µm filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm analytical column that contains 5-µm packing L11. The flow rate is about 1.4 mL per minute. Chromatograph the 20 µg per mL *Standard preparation,* and record the peak responses as directed for *Procedure:* the retention time is about 9.7 minutes, and the relative standard deviation for replicate injections is not more than 4.9%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of naratriptan hydrochloride ($C_{17}H_{25}N_3O_2S \cdot HCl$) in the volume of Oral Suspension taken by the formula:

$$(r_U / r_S)(C_S / C_U) \times 100$$

in which r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively; C_S is the concentration of USP Naratriptan Hydrochloride RS, µg per mL, in the *Sample preparation* on the anhydrous basis; and C_U is the nominal concentration of naratriptan hydrochloride, in µg per mL, in the *Assay preparation*.▲*USP33*

BRIEFING

Nitrofurantoin, *USP 31* page 2811; **Nitrofurantoin Capsules,** *USP 31* page 2812 and page 3655 of the *First Supplement;* **Nitrofurantoin Oral Suspension,** *USP 31* page 2813; and **Nitrofurantoin Tablets,** *USP 31* page 2814. It is proposed to revise the *Packaging and storage* requirement to be consistent with the FDA-approved package inserts.

(MD-AA: H. Ramanathan; B. Davani) RTS—C69658

Change to read:

Packaging and storage—Preserve in tight ~~light resistant~~

▲▲*USP33*
containers,

▲and store at controlled room temperature.▲*USP33*

BRIEFING

Nitrofurantoin Capsules, *USP 31* page 2812 and page 3655 of the *First Supplement*—See briefing under *Nitrofurantoin*.

(MD-AA: H. Ramanathan; B. Davani) RTS—C67481

Change to read:

Packaging and storage—Preserve in tight ~~light resistant~~

▲▲*USP33*
containers,

▲and store at controlled room temperature.▲*USP33*

BRIEFING

Nitrofurantoin Oral Suspension, *USP 31* page 2813—See briefing under *Nitrofurantoin.*

(MD-AA: H. Ramanathan; B. Davani) RTS—C69660

Change to read:

Packaging and storage—Preserve in tight ~~light resistant~~

▲▲*USP33*
containers,

▲and store at controlled room temperature.▲*USP33*

BRIEFING

Nitrofurantoin Tablets, *USP 31* page 2814—See briefing under *Nitrofurantoin*.

(MD-AA: H. Ramanathan; B. Davani) RTS—C69661

Change to read:

Packaging and storage—Preserve in tight ~~light resistant~~

▲▲*USP33*
containers,

▲and store at controlled room temperature.▲*USP33*

BRIEFING

Norethynodrel, *USP 31* page 2830. It is proposed to omit this monograph from USP for the following reasons.

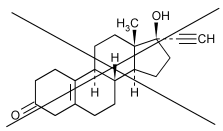
1. All drug products containing norethynodrel have been discontinued in the United States.
2. The drug is currently not used in veterinary medicine in the United States.
3. A source for the bulk drug substance for the development of the USP Reference Standard to support the monograph has not been found.

(MD-PS: D. Bempong) RTS—C68500

In-Process Revision

Delete the following:

▲~~Norethynodrel~~



~~$C_{20}H_{26}O_2$ 298.42~~
~~19-Norpregn-5(10)-en-20-yn-3-one, 17-hydroxy-, (17α)-.~~
~~17-Hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one [*68-23-5*].~~

~~» Norethynodrel contains not less than 97.0 percent and not more than 101.0 percent of $C_{20}H_{26}O_2$.~~

~~**Packaging and storage**— Preserve in well-closed containers.~~
~~**USP Reference standards** ⟨11⟩— *USP Norethindrone RS*. *USP Norethynodrel RS*.~~
~~**Identification,** *Infrared Absorption* ⟨197S⟩—~~
~~*Solution:* 1 in 20.~~
~~*Medium:* chloroform.~~
~~**Specific rotation** ⟨781S⟩: between +119° and +125°.~~
~~*Test solution:* 10 mg per mL, in dioxane.~~
~~**Limit of ethynyl group**— Dissolve 200 mg in about 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10), and titrate with 0.1 N sodium hydroxide VS, using either a glass calomel or a silver-silver chloride electrode system with potassium nitrate filling solution. Each mL of 0.1 N sodium hydroxide is equivalent to 2.503 mg of ethynyl group (−C≡CH). Not less than 8.18% and not more than 8.43% of ethynyl group is found.~~
~~**Limit of norethindrone**—~~
~~*Test preparation*— Prepare a solution of Norethynodrel in chloroform containing 10 mg per mL.~~
~~*Standard solution*— Prepare a solution of USP Norethindrone RS in chloroform to contain 1 mg per mL. Dilute 2 mL of the solution with chloroform to 10 mL.~~
~~*Procedure*— Apply 10-µL volumes of the *Test preparation* and the *Standard solution* (see *Chromatography* ⟨621⟩) to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow not more than 5 minutes between spotting the plate and starting development of the chromatogram. Place the plate in a suitable chromatographic chamber previously equilibrated with a mixture of cyclohexane, ethyl acetate, and methanol (60 : 40 : 2), and allow the solvent front to move 15 cm. Spray the plate with dilute sulfuric acid (1 in 2), heat the plate at 105° for 5 minutes, and view under long-wavelength UV light. Locate any norethindrone impurity in the *Test preparation* by comparison with the R_F value from the *Standard solution*. If present, the norethindrone spot from the *Test preparation* is not larger or more intense than the spot from the *Standard solution* (2.0%).~~
~~**Ordinary impurities** ⟨466⟩—~~
~~*Test solution:* chloroform.~~
~~*Standard solution:* chloroform.~~
~~*Eluant:* ether.~~
~~*Visualization:* 5, followed by viewing under long-wavelength UV light.~~
~~**Organic volatile impurities,** *Method V* ⟨467⟩: meets the requirements.~~
~~*Solvent*— Use dimethyl sulfoxide.~~
~~(Official until July 1, 2008)~~
~~**Assay**—~~
~~*Standard preparation*— Dissolve a suitable quantity of USP Norethynodrel RS, accurately weighed, in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 1 mg per mL.~~
~~*Assay preparation*— Dissolve about 100 mg of Norethynodrel, accurately weighed, in methanol to make 100.0 mL, and mix.~~
~~*Procedure*— Transfer 10.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 100-mL volumetric flasks. To each flask add 40 mL of methanol, then add 5 mL of a mixture of 3 volumes of hydrochloric acid and 2 volumes of water, mix quickly, and allow to stand at a temperature of about 25° for 1 hour, accurately timed. Prior to the end of the 1-hour period, prepare blanks as follows. Add 1.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 100-mL volumetric flasks, each containing a mixture of 50 mL of methanol and 2 mL of water, dilute each with methanol to volume, and mix. At the end of the 1-hour reaction period, dilute each of the acid-containing solutions with methanol to volume, and mix. Transfer 10.0 mL of each into separate 100-mL volumetric flasks, add 2 mL of water to each, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, relative to the corresponding blanks. Calculate the quantity, in mg, of $C_{20}H_{26}O_2$ in the portion of Norethynodrel taken by the formula:~~

~~$$100C(A_u/A_s)$$~~

~~in which C is the concentration, in mg per mL, of USP Norethynodrel RS in the *Standard preparation*, and A_u and A_s are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.~~ ▲*USP33*

BRIEFING

Oxybutynin Chloride Tablets, *USP 31* page 2876. It is proposed to correct the injection volume in the *Dissolution* test *Procedure*.

(BPC05: M. Marques) RTS—C70434

Change to read:

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{22}H_{31}NO_3 \cdot HCl$ dissolved using the method set forth in the *Assay*, making any necessary modifications to the concentration of the *Standard preparation* to correspond to that of the solution under test,

▲and injecting 100 µL of both solutions.▲*USP33*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$ is dissolved in 30 minutes.

BRIEFING

Liquefied Phenol, *USP 31* page 2976. On the basis of comments received, it is proposed to move the *Identification* tests from the *Other requirements* section to the *Identification* section for clarification.

(MD-OOD: F. Mao) RTS—C68576

In-Process Revision

Add the following:

▲**Identification—**

A: To a solution, add bromine TS: a white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added.

B: To 10 mL of a solution (1 in 100), add 1 drop of ferric chloride TS: a violet color is produced.▲*USP33*

Change to read:

Other requirements—It ~~responds to the *Identification* tests, and~~ ▲▲*USP33*
meets the requirements of the tests for *Clarity of solution and reaction* and *Nonvolatile residue,* under *Phenol.*

BRIEFING

Propafenone Hydrochloride, *USP 31* page 3098. On the basis of comments received, it is proposed to revise the test for *Chromatographic purity* to make the following recommended changes.
1. Change the title from *Chromatographic purity* to *Related compounds,* which will be in line with current USP convention.
2. Revise the test procedure so that it is not only specific to the identified impurities, but will also conform with current harmonization efforts.

The proposed liquid chromatographic procedure is validated using a YMC-Pack Pro brand of column containing packing L7. The typical retention time for propafenone is about 6 minutes.

(MD-CV: S. Ramakrishna) RTS—C42543

Change to read:

USP Reference standards ⟨11⟩—*USP Propafenone Hydrochloride RS.*

▲*USP Propafenone Related Compound B RS.*▲*USP33*

Delete the following:

▲~~**Chromatographic purity—**~~

~~*Mobile phase*—Prepare a filtered and degassed mixture of 2.5 mM tetrabutylammonium hydrogen sulfate and acetonitrile (16 : 9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).~~

~~*Diluent*—Prepare a mixture of acetonitrile and water (9 : 1).~~

~~*Standard solution*—Dissolve an accurately weighed quantity of USP Propafenone RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 5 µg per mL.~~

~~*Test solution*—Transfer about 50 mg of Propafenone Hydrochloride, accurately weighed, to a 50 mL volumetric flask, dissolve in 5 mL of *Diluent,* dilute with *Mobile phase* to volume, and mix.~~

~~*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 222 nm detector and a 3.9 mm × 15 cm column that contains packing L10. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections is not more than 15%.~~

~~*Procedure*—Separately inject a volume (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than eight times the retention time of propafenone. Record the chromatogram, and measure the peak responses for all the peaks: the sum of the peak responses, other than that of propafenone, in the chromatogram of the *Test solution* is not more than two times the propafenone response obtained from the *Standard solution* (1.0%); and no other peak response, other than that of propafenone, in the chromatogram of the *Test solution* is greater than the propafenone response obtained from the *Standard solution* (0.5%).~~▲*USP33*

Add the following:

▲**Related compounds—**

Solvent A—Dissolve approximately 3.42 g of monobasic potassium phosphate trihydrate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.5.

Solvent B: acetonitrile.

Mobile phase—Use variable mixtures of *Solvent A* and *Solvent B.*

Diluent—Prepare a mixture of *Solvent A* and *Solvent B* (65 : 35).

Resolution solution—Dissolve a known quantity of each of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS in *Diluent,* and dilute with *Diluent* to prepare a solution having a known concentration of 1 mg per mL each.

Standard solution—Dissolve an accurately weighed known amount of USP Propafenone Hydrochloride RS taken in a suitable volumetric flask in *Diluent*. Dilute quantitatively, and stepwise if necessary, to prepare a solution having a known concentration of about 0.001 mg per mL.

Test solution—Dissolve and dilute a known amount of Propafenone Hydrochloride with *Diluent* to prepare a solution of about 1 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column containing 5-µm packing L7.

In-Process Revision

The flow rate is about 1.0 mL per minute. The column is maintained at a temperature of about 30°. The chromatograph is programmed as follows.

Time (minutes)	*Mobile phase* (A) (%)	*Mobile phase* (B) (%)
0–8	65	35
8–20	65→30	35→70
20–30	30	70
30–31	30→65	70→35
31–36	65	35

Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R*, between propafenone and propafenone related compound B is not less than 3.0; and the relative standard deviation for replicate injections, as determined by the propafenone peak, is not less than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than eight times the retention time of propafenone. Record the chromatogram, and measure the peak responses for all the peaks. Calculate the percentage of each impurity in the portion of Propafenone Hydrochloride taken by the formula:

$$100\ (r_i/r_S)$$

in which r_i is the peak response for each impurity from the *Test solution;* and r_S is the peak response of propafenone as obtained from the *Standard solution:* the specified and unspecified impurities meet the limits described in *Table 1*.

Table 1

Related Compound	Relative Retention Time (minutes)	Limit NMT (%)
Propafenone related compound B[1]	0.8	0.1
Impurity D[2]	2.3	0.1
Impurity G[3]	3.6	0.1
Impurity C[4]	4.1	0.1
Impurity F[5]	5.3	0.1
Individual unspecified impurities	—	0.1
Total impurities	—	0.3

[1] (2*E*)-1-[2-[(2*RS*)-2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one.
[2] 1-[2-[(2*RS*)-2,3-Dihydroxypropoxy]phenyl]-3-phenylpropan-1-one.
[3] 1,1′[Propyliminobis(2-hydroxypropane-3,1-diyl)oxy-2,1-phenylene]bis(3-phenylpropan-1-one.
[4] 1-[2-[[(2*RS*)-Oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one.
[5] 1,1′-[2-Hydroxypropane-1,3-diylbis(oxy-2,1-phenylene)]bis(3-phenylpropan-1-one).

Disregard the peaks below 0.03%.▲*USP33*

BRIEFING

Ritonavir, *USP 31* page 3200 and page 3866 of the *Second Supplement*. It is proposed to eliminate the use of chloroform under the IR *Identification* test due to safety concerns. Additionally, the proposed revision is consistent with that in the *European Pharmacopoeia,* 6th edition.

(MDAA: H. Ramanathan; B. Davani) RTS—C69084

Change to read:

Identification—

A: *Infrared Absorption* ⟨~~197~~⟩
▲⟨197K⟩.▲*USP33*

~~*Test specimen*~~ ~~Dissolve 50 mg of Ritonavir in 1.0 mL of chloroform. Add 1 drop of this solution to the surface of a potassium bromide or a sodium chloride disk, and evaporate to dryness.~~

▲▲*USP33*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* is within 2% of the retention time of the major peak in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

In-Process Revision

BRIEFING

Terbinafine Oral Suspension. Because there is no existing *USP* monograph for this dosage form, the following new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with a 4.6-mm × 25-cm analytical column that contains 5-µm packing L1. USP has received data indicating that a Zorbax RX-C18 from Phenomenex is suitable. The typical retention time for terbinafine is about 5.1 minutes.

(CRX: R. Schnatz) RTS—C61617

Add the following:

▲Terbinafine Oral Suspension

» Terbinafine Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled content of terbinafine hydrochloride. Prepare Terbinafine Oral Suspension 25 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Terbinafine Hydrochloride	2500 mg
Vehicle: a mixture of Vehicle for Oral Solution, *NF,* and Vehicle for Oral Suspension, *NF* (1 : 1), a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder, or add terbinafine hydrochloride powder. Add the Vehicle in small portions, and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make a terbinafine suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the liquid Vehicle to bring to final volume, and mix well.

Packaging and storage—Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.

Labeling—Label it to state that it is to be well-shaken before use, and to state the beyond-use date.

USP Reference standards ⟨11⟩—*USP Terbinafine Hydrochloride RS.*

Beyond-use date: not later than 30 days after the date on which it was compounded, when stored at controlled cold temperature or controlled room temperature.

pH ⟨791⟩**:** between 5.3 and 5.7.

Assay—

Mobile phase—The *Mobile phase* consists of acetonitrile and water (40 : 60), with 0.15% triethylamine and 0.15% phosphoric acid. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve about 25 mg of USP Terbinafine Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask. Dilute with methanol to obtain a concentration of 1.0 mg per mL.

Standard preparation—Pipet 0.5 mL of the *Standard stock preparation* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a concentration of 5 µg per mL, and pass through a 0.22-µm filter.

Assay preparation—Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 25-mL volumetric flask. Dilute with methanol to volume to obtain a nominal concentration of 1 mg of terbinafine hydrochloride per mL. Mix, and centrifuge the solution. Accurately pipet 0.5 mL of the supernatant to a 10-mL volumetric flask, and dilute with methanol to volume to obtain a nominal concentration of 50 µg of terbinafine hydrochloride per mL. Mix the sample

In-Process Revision

again. Accurately pipet 1 mL of the diluted terbinafine hydrochloride solution to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 5 µg of terbinafine hydrochloride per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 224-nm detector and a 4.6-mm × 15-cm analytical column that contains 3.5-µm packing L1. The flow rate is about 0.4 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the retention time is about 5.1 minutes, and the relative standard deviation for replicate injections is not more than 5.8%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of terbinafine hydrochloride ($C_{21}H_{25}N \cdot HCl$) in the volume of Oral Suspension taken by the formula:

$$(r_U / r_S)(C_S / C_U) \times 100$$

in which r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively; C_S is the concentration of USP Terbinafine Hydrochloride RS, in µg per mL, in the *Sample preparation* on the anhydrous basis; and C_U is nominal concentration of terbinafine hydrochloride, in µg per mL, in the *Assay preparation*.▲*USP33*

BRIEFING

Terbutaline Oral Suspension. Because there is no existing *USP* monograph for this dosage form, the following new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with a 3.9-mm × 30-cm analytical column that contains 10-µm microphenyl packing L11. USP has received data indicating that a Microphenyl from Waters Associates, Inc., is suitable. The typical retention time for terbutaline is about 5.0 minutes.

(CRX: R. Schnatz) RTS—C60263

Add the following:

▲Terbutaline Oral Suspension

» Terbutaline Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled content of terbutaline sulfate. Prepare Terbutaline Oral Suspension 1 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Terbutaline Sulfate	100 mg
Syrup, *NF,** a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using Tablets, place the required number of Tablets in a suitable mortar, and comminute to a fine powder, or add terbutaline sulfate powder. Add the Syrup, *NF,* in small portions and triturate to make a smooth paste. Add increasing volumes of the Syrup, *NF,* to make a terbutaline suspension that is pourable. Transfer the contents of the mortar, stepwise and

* Syrup, *NF,* containing 0.2% sodium benzoate.

quantitatively, to a calibrated bottle. Add enough of the Syrup, *NF,* to bring to final volume, and mix well.

Packaging and storage—Package in tight, light-resistant containers. Store at controlled cold temperature.

Labeling—Label it to state that it is to be well-shaken before use and to state the beyond-use date.

USP Reference standards ⟨11⟩—*USP Terbutaline Sulfate RS.*

Beyond-use date: not later than 30 days after the date on which it was compounded, when stored at controlled cold temperature.

Assay—

Mobile phase—Prepare a solution of methanol and 20 mM monobasic potassium phosphate (8 : 92) adjusted with phosphoric acid to a pH of 3.6. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Terbutaline Sulfate RS in water to obtain a concentration of 5 mg per mL.

Standard preparation—Pipet 0.2 mL of the *Standard stock preparation* to a 100-mL volumetric flask, dilute with water to volume to obtain a concentration of 10 µg per mL, and pass through a 0.22-µm filter.

Assay preparation—Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 100 µg terbutaline sulfate per mL. Extract terbutaline sulfate from the suspension with methanol. Accurately pipet 1 mL of Oral Suspension and 3 mL of *Mobile phase* in the barrel of a 5-mL plastic syringe. Shake, and pass through a 0.22-µm filter into a 10-mL volumetric flask. Repeat the process with an additional 2 mL of methanol. Bring to final 10 mL volume with *Mobile phase* to obtain a nominal concentration of 10 µg terbutaline sulfate per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm analytical column that contains 10-µm microphenyl packing L11. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the retention time is about 5.0 minutes, and the relative standard deviation for replicate injections is not more than 2.2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of terbutaline sulfate $((C_{12}H_{19}NO_3)_2 \cdot H_2SO_4)$ in the volume of Oral Suspension taken by the formula:

$$(r_U / r_S)(C_S / C_U) \times 100$$

in which r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively; C_S is the concentration of USP Terbutaline Sulfate RS, µg per mL, in the *Sample preparation* on the anhydrous basis; and C_U is nominal concentration of terbutaline sulfate, in µg per mL, in the *Assay preparation.*▲*USP33*

BRIEFING

Tiagabine Hydrochloride Oral Suspension. Because there is no existing *USP* monograph for this dosage form, the following new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with a 3.0-mm × 15-cm analytical column that contains 5-µm packing L10. USP has received data indicating that a Zorbax CN from MAC-MOD Analytical, Inc., is suitable. The typical retention time for tiagabine is about 3.2 minutes.

(CRX: R. Schnatz) RTS—C60334

In-Process Revision

Add the following:

▲Tiagabine Hydrochloride Oral Suspension

»Tiagabine Hydrochloride Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled content of tiagabine hydrochloride. Prepare Tiagabine Hydrochloride Oral Suspension 1 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Tiagabine Hydrochloride	100 mg
Vehicle: a mixture of Vehicle for Oral Solution, *NF,* and Vehicle for Oral Suspension, *NF* (1 : 1), a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using Tablets, place the required number of Tablets in a suitable mortar, and comminute the Tablets to a fine powder, or add Tiagabine Hydrochloride. Add the Vehicle in small portions and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make a tiagabine suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the Vehicle to bring to final volume, and mix well.

Packaging and storage—Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.

Labeling—Label it to state that it is to be well-shaken before use and to state the beyond-use date.

USP Reference standards ⟨11⟩—*USP Tiagabine Hydrochloride RS.*

Beyond-use date: not later than 90 days after the date on which it was compounded, when stored at controlled cold temperature, and not later than 60 days after the date on which it was compounded, when stored at controlled room temperature.

pH ⟨791⟩**:** between 4.0 and 4.5.

Assay—

Mobile phase—Prepare a solution of 5 mM octanesulfonic acid and acetonitrile (50 : 50). Pass through a 0.45-µm filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve about 25 mg of USP Tiagabine Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask. Dilute with methanol to obtain a concentration of 1.0 mg per mL.

Standard preparation—Pipet 0.2 mL of the *Standard stock preparation* to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume to obtain a concentration of 20 µg per mL. Centrifuge, and pass through a 0.22-µm filter.

Assay preparation—Shake thoroughly by hand each bottle of Oral Suspension. Centrifuge, and pass through a 0.22-µm filter. Accurately pipet 0.2 mL of the Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 20 µg tiagabine hydrochloride per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 3.0-mm × 15-cm analytical column that contains 5-µm packing L10. The flow rate is about 0.4 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the retention time is about 3.2 minutes, and the relative standard deviation for replicate injections is not more than 1.7%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure

the responses for the major peaks. Calculate the percent of tiagabine hydrochloride ($C_{20}H_{25}NO_2S_2 \cdot HCl$) in the volume of Oral Suspension taken by the formula:

$$(r_U / r_S)(C_S / C_U) \times 100$$

in which r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively; C_S is the concentration of USP Tiagabine Hydrochloride RS, µg per mL, in the *Sample preparation* on the anhydrous basis; and C_U is the nominal concentration of tiagabine hydrochloride, in µg per mL, in the *Assay preparation*.▲*USP33*

BRIEFING

Trenbolone Acetate, *USP 31* page 3438. On the basis of comments received, several changes to the *Trenbolone Acetate* monograph are being proposed. The upper *Assay* limit is increased from 101.0% to 103.0% to reflect approved products in the U.S. market. The UV *Identification* test is proposed to be deleted. The TLC-based *Chromatographic purity* and HPLC-based *Limit of trenbolone acetate 17α-isomer* tests are proposed to be deleted and replaced by a single test for *Related compounds*. In addition, the *Assay* has been changed to one that has shorter analytical run times. The new *Related compounds* and *Assay* are HPLC methods, utilizing a 4.6-mm × 10-cm column that contains 3-µm packing L1. USP has received information indicating that a Thermo Scientific Hypersil ODS C18 brand of column is suitable. The typical retention time of trenbolone acetate is about 5 minutes. Interested parties are invited to submit comments.

(VET: I. DeVeau) RTS—C63129

Change to read:

» Trenbolone Acetate contains not less than 97.0 percent and not more than ~~101.0~~

▲103.0▲*USP33*
percent of $C_{20}H_{24}O_3$,

▲calculated on the dried basis.▲*USP33*

Change to read:

USP Reference standards ⟨11⟩— ~~*USP Trenbolone RS.*~~

▲
▲*USP33*
USP Trenbolone Acetate RS.

▲*USP Trenbolone Acetate System Suitability Mixture RS.*▲*USP33*

Change to read:

Identification—

A: *Infrared Absorption* ⟨197K⟩.
B: ~~*Ultraviolet Absorption* ⟨197U⟩—~~
~~*Solution:* 16 µg per mL.~~
~~*Medium:* Alcohol.~~
~~Absorption maxima at about 237 nm and 340 nm. Absorptivity at 340 nm is between 92.0 and 97.6.~~

▲The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*▲*USP33*

~~**C:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a peak for trenbolone acetate, the retention time of which corresponds to that exhibited by the *Standard preparation*.~~

▲
▲*USP33*

Delete the following:

▲~~**Chromatographic purity—**~~
~~*Diluent*— Prepare a mixture of chloroform and methanol (9:1).~~
~~*Standard solutions*— Prepare four solutions in *Diluent* containing USP Trenbolone RS and USP Trenbolone Acetate RS containing in each mL 0.1 mg of each, 0.05 mg of each, 0.02 mg of each, and 0.01 mg of each, corresponding to 1.0%, 0.5%, 0.2%, and 0.1% of impurities, respectively.~~
~~*Test solution*— Prepare a solution of Trenbolone Acetate in *Diluent* containing 10 mg per mL.~~
~~*Procedure*— Separately apply 10 µL of each of the *Standard solutions* and the *Test solution* to a thin layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25 mm layer of chromatographic silica gel mixture as follows. Develop the chromatograms in a solvent system consisting of a mixture of chloroform and acetone (98:2) in an unsaturated chromatographic chamber protected from light until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, dry it for about 15 seconds in a stream of dry nitrogen, and immediately develop the chromatograms a second time until the solvent front has moved about three-fourths of the length of the plate. Examine the plate under short-wavelength UV light. Spray the plate with phosphomolybdic acid TS, and heat the plate at 100° for about 10 minutes. Examine the plate under visible light, and compare the intensities of any secondary spots in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*. No trenbolone spot from the chromatogram of the *Test solution* is larger or more intense than the trenbolone spots from the *Standard solution* containing 0.1 mg of USP Trenbolone RS per mL (1%). Estimate the percentage of each other impurity observed in the chromatogram of the *Test solution* by comparison with the trenbolone acetate spots in the chromatograms of the *Standard solutions*: No other impurity spot is greater than 0.5%, and the total of all other impurities, including that of the 17α-isomer obtained in the test for *Limit of trenbolone acetate 17α-isomer*, is not more than 1%.~~▲*USP33*

Delete the following:

▲~~**Limit of trenbolone acetate 17α isomer—**~~
~~*Mobile phase*, *Resolution solution*, and *Chromatographic system*— Proceed as directed in the *Assay*.~~
~~*Standard solution*— Prepare a solution of USP Trenbolone Acetate RS in *Mobile phase* having a known concentration of 4 µg per mL.~~
~~*Test solution*— Transfer about 20 mg of Trenbolone Acetate, accurately weighed, to a 20-mL volumetric flask, add about 10 mL of *Mobile phase*, swirl to dissolve, dilute with *Mobile phase* to volume, and mix.~~

In-Process Revision

~~*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Trenbolone acetate 17α isomer, if present, has a retention time of about 0.8 relative to that of the trenbolone acetate peak. Calculate the percentage of 17α isomer found in the Trenbolone Acetate taken by the formula:~~

~~$2(C/W)(r_u/r_s)$~~

~~in which *C* is the concentration, in µg per mL, of USP Trenbolone Acetate RS in the *Standard solution*, *W* is the weight, in mg, of Trenbolone Acetate taken to prepare the *Test solution*, r_u is the response of any peak at a retention time of about 0.8 in relation to that of the main trenbolone acetate peak in the chromatogram obtained from the *Test solution*, and r_s is the peak area response of the trenbolone acetate peak in the chromatogram obtained from the *Standard solution*. Not more than 0.5% of the 17α isomer is found.~~ ▲*USP33*

Add the following:

▲Related compounds—

Diluent, Solution A, Solution B, and *Mobile phase*—Proceed as directed in the *Assay.*

System suitability solution—Dissolve an accurately weighed quantity of USP Trenbolone Acetate System Suitability Mixture RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1 mg of the mixture per mL. Sonicate, if necessary, to dissolve.

Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*

Test solution—Prepare as directed for *Assay preparation* in the *Assay.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.2 for trenbolone acetate related compound A and 1.0 for trenbolone acetate; the resolution, *R,* between trenbolone acetate and trenbolone acetate related compound A is not less than 3.0; and the column efficiency, *N,* is not less than 8,000 theoretical plates for the trenbolone acetate peak. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for the trenbolone acetate peak.

Procedure—Inject a volume (about 5 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity peak by the formula:

$$(100 \times r_i)/[(r_c + S) \times F]$$

in which r_i is the peak area for each impurity peak in the *Test solution;* r_c is the peak area of trenbolone acetate in the *Test solution;* *S* is the sum of areas of all impurity peaks, each divided by their respective response factor (*F*) listed in the table below; and *F* is the respective response factor of the individual impurities relative to the trenbolone acetate peak. The reporting level for impurities is 0.05%.

Compound	Approximate Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Related compound A[1]	1.2	0.56	NMT 0.5
Related compound B[2]	0.4	1.04	NMT 1
Related compound C[3]	0.8	1.10	NMT 0.5
Any unspecified impurity	—	1.00*	NMT 0.5
Trenbolone acetate	1.0	—	—
Total specified and unspecified	—	—	NMT 2.0

[1] Conjugated dihydrotrenbolone acetate.
[2] Trenbolone.
[3] Trenbolone acetate 17α-isomer.
* Unless determined otherwise.

▲*USP33*

Change to read:

Assay—

~~*Mobile phase*—Prepare a mixture of acetonitrile and 1% ammonium acetate solution (55 : 45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).~~

~~*Resolution solution*—Prepare a solution in *Mobile phase* containing about 0.2 mg each of USP Trenbolone RS and USP Trenbolone Acetate RS per mL.~~

~~*Standard preparation*—Prepare a solution of USP Trenbolone Acetate RS in *Mobile phase* having a known concentration of about 0.2 mg per mL.~~

In-Process Revision

~~*Assay preparation*—Transfer about 20 mg of Trenbolone Acetate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.~~

~~*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 344-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for trenbolone and 1.0 for trenbolone acetate, and the resolution, *R*, between the trenbolone peak and the trenbolone acetate peak is not less than 25. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 14,000 theoretical plates when calculated by the formula:~~

~~$$5.545(t_R / W_{h/2})^2$$~~

~~the tailing factor is not more than 1.2 when calculated by the formula:~~

~~$$W_{0.1}/2f$$~~

~~in which $W_{0.1}$ is the width of the peak at 10% height, and the relative standard deviation for replicate injections is not more than 2%.~~

~~*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trenbolone acetate ($C_{20}H_{24}O_3$) in the portion of Trenbolone Acetate taken by the formula:~~

~~$$100C(r_U / r_S)$$~~

~~in which *C* is the concentration, in mg per mL, of USP Trenbolone Acetate RS in the *Standard preparation*, and r_U and r_S are the trenbolone acetate peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.~~

▲*Diluent*—Prepare a mixture of acetonitrile, methanol, water, and acetic acid (36.5 : 30 : 33.5 : 0.1), and mix well.

Solution A—Prepare a mixture of acetonitrile, methanol, and water (36.5 : 30 : 33.5). Mix, and degas.

Solution B—Prepare a mixture of acetonitrile and methanol (9 : 1). Mix, and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Trenbolone Acetate RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Sonicate, if necessary, to dissolve.

Assay preparation—Dissolve an accurately weighed quantity of Trenbolone Acetate in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Sonicate, if necessary, to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 10-cm column that contains 3-µm L1 packing. The flow rate is about 1.0 mL per minute. The gradient profile is as follows:

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–6	100	0	isocratic
6–16	100→0	0→100	linear gradient
16–26	0	100	isocratic
26–26.1	0→100	100→0	linear gradient
26.1–30	100	0	re-equilibration

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for the trenbolone acetate peak.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in percent, of $C_{20}H_{24}O_3$ in the portion of trenbolone acetate taken by the formula:

$$(r_U / r_S)(C_S / C_U) \times 100$$

in which r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively; and C_S and C_U are the concentrations, in mg per mL, of USP Trenbolone Acetate RS and trenbolone acetate taken, respectively.▲*USP33*

In-Process Revision

BRIEFING

Valrubicin, *USP 31*, page 3494. On the basis of comments received, it is proposed to revise the monograph as follows:

1. The *Assay* specification was revised to 98.0%–102.0% which is representative of currently marketed products. The *Definition* section was revised to indicate that the *Assay* is calculated on the anhydrous basis to reflect the change in the *Loss on Drying* test (see below).
2. The USP Reference Standards section was updated to replace *USP Valrubicin Related Compound A RS,* which is unstable, with *USP Valrubicin Resolution Mixture RS*.
3. The *Ultraviolet Absorption* test was deleted from the *Identification* section since Infrared spectroscopy and chromatographic retention time comparison are adequate for identification purposes.
4. The *Loss on Drying* test, which provides information about the content of water and volatile solvents, was replaced with a test for *Water*.
5. The *Limit of residual solvents* test was deleted to be consistent with general chapter *Residual Solvents* ⟨467⟩.
6. The *Assay* and the test for *Related compounds* have been revised based on validated methods. The methods are based on analyses performed using a Zorbax SB C18 brand of L1 column. The typical retention time of valrubicin in the *Assay* is about 2.5 minutes. The typical retention time of valrubicin in the test for *Related compounds* is about 34 minutes.

(MD-ANT: A. Wise) RTS—C65734

Change to read:

» Valrubicin contains ~~not less than 95.0 percent and not more than 103.0 percent~~

▲not less than 98.0 percent and not more than 102.0 percent▲*USP33*
of $C_{34}H_{36}F_3NO_{13}$, calculated on the ~~dried basis~~

▲anhydrous and solvent-free basis.▲*USP33*

Caution—Great care should be taken to prevent inhaling particles of Valrubicin and exposing the skin to it.

Change to read:

USP Reference standards ⟨11⟩—*USP Valrubicin RS.* ~~*USP Valrubicin Related Compound A RS.*~~
▲USP Valrubicin Resolution Mixture RS.▲*USP33*

Change to read:

Identification—

A: *Infrared Absorption* ⟨197M⟩.

~~**B:** *Ultraviolet Absorption* ⟨197U⟩—~~
~~*Solution:* 10 mg per mL.~~
~~*Medium:* methanol.~~
~~Absorptivities, calculated on the dried basis, are 555 ± 20 at 233 nm and 382.5 ± 17.5 at 252 nm.~~

▲
▲*USP33*
~~**C:**~~
▲**B:**▲*USP33*

The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Delete the following:

▲~~**Loss on drying** ⟨731⟩ Dry it in vacuum over phosphorus pentoxide at 80° for 4 hours: it loses not more than 3.0% of its weight.~~▲*USP33*

Add the following:

▲**Water,** *Method I* ⟨921⟩—not more than 0.5%.▲*USP33*

Delete the following:

▲~~**Limit of residual solvents**~~

~~*Internal standard solution* Prepare a solution of *n*-propyl alcohol in dimethyl sulfoxide having a concentration of about 0.05 µL per mL.~~

~~*Standard solution* Prepare a solution in *Internal standard solution* having a concentration of 2.5 µg of chloroform, 5.0 µg of dehydrated alcohol, 5.0 µg of acetone, 5.0 µg of butyl alcohol, 5.0 µg of dioxane, 10.0 µg of methylene chloride, 15.0 µg of diisopropyl ether, 20.5 µg of acetonitrile, 50 µg of pentane, and 100 µg of methanol in each mL, and sonicate.~~

~~*Test solution* Dissolve about 200 mg of Valrubicin, accurately weighed, in 4.0 mL of *Internal standard solution,* and sonicate.~~

~~*Chromatographic system* (see *Chromatography* ⟨621⟩) The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m fused silica capillary column coated with a 5-µm film of G2 stationary phase. The carrier gas is helium, flowing at a rate of 30 mL per minute. The column temperature is maintained at 220°. The injection port temperature and the detector block temperature are maintained at 250°. Chromatograph the *Standard solution,* and record the responses as directed for *Procedure:* the relative retention times are about 0.48 for methanol, 0.66 for dehydrated alcohol, 0.71 for acetonitrile, 0.76 for acetone, 0.86 for pentane, 0.92 for methylene chloride, 1.0 for *n*-propyl alcohol, 1.19 for diisopropyl ether, 1.22 for chloroform, 1.35 for butyl alcohol, and 1.52 for dioxane; the component solvent peaks are resolved; and the relative standard deviation of the ratios of the peak area of each solvent to the peak area of *n*-propyl alcohol is not more than 10%.~~

~~*Procedure* Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the concentration, in µg per g, of each residual solvent in the portion of Valrubicin taken by the formula:~~

~~$$4000(C/W)(R_i/R_s)$$~~

~~in which C is the concentration, in µg per mL, of the respective individual solvent in the *Standard solution; W* is the quantity, in mg, of Valrubicin taken to prepare the *Test solution;* and R_i and R_s are the peak area ratios of the respective individual solvent to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution,* respectively: not more than 50 µg per g of chloroform, 100 µg per g of dehydrated alcohol, 100 µg per g of acetone, 100 µg per g of butyl alcohol, 100 µg per g of dioxane, 300 µg per g of methylene chloride, 410 µg per g of acetonitrile, 500 µg per g of diisopropyl ether, 1000 µg per g of pentane, and 2000 µg per g of methanol are found.~~▲*USP33*

Change to read:

Related compounds—

~~*Mobile phase* Prepare as directed in the *Assay*.~~

~~*Resolution solution* Prepare a solution of USP Valrubicin Related Compound A RS and USP Valrubicin RS in acetonitrile having known concentrations of about 0.25 mg per mL and 1 mg per mL, respectively.~~

~~*Test solution* Use the *Assay preparation*.~~

In-Process Revision

~~*Chromatographic system* (see *Chromatography* ⟨621⟩) The liquid chromatograph is equipped with a 254-nm detector, a guard column, and a 5-mm × 10-cm analytical column that contains a 4-µm packing L1. The flow rate is about 3.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for valrubicin related compound A and 1.0 for valrubicin; and the resolution, *R*, between valrubicin related compound A and valrubicin is not less than 2.~~

~~*Procedure*—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Valrubicin taken by the formula:~~

~~$100(r_i/r_u)$~~

~~in which r_i is the peak area for each impurity; and r_u is the sum of the areas of all the peaks. Do not consider any peaks due to solvent or excipients. Not more than 0.3% of any individual impurity with a relative retention time of 0.06, 0.17, 0.27, or 0.52 is found; not more than 0.6% of any impurity with a relative retention time of about 0.14 is found; not more than 0.2% of any other individual impurity is found; not more than 1.0% of total other impurities that are not specified by relative retention time is found; and not more than 2.5% of total impurities that are not less than 0.1% is found.~~

▲*Solution A*—Use *Potassium phosphate buffer* prepared as directed in the *Assay.*

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic System*. Make adjustments if necessary (see *System suitability* under *Chromatography* ⟨621⟩).

Diluent—A mixture of acetonitrile and water (1 : 1).

Resolution solution—Dissolve a suitable amount of USP Valrubicin Resolution Mixture RS in acetonitrile, using about 50% of the final volume, then dilute with water to volume, and mix to obtain a solution containing about 2 mg of the mixture per mL.

Stock standard solution—Dissolve an accurately weighed amount of USP Valrubicin RS in acetonitrile, using about 50% of the final volume, then dilute with water to volume, and mix to obtain a solution having a known concentration of about 2 mg per mL.

Standard solution—Dilute the *Stock standard solution* quantitatively with *Diluent* to obtain a solution having a known concentration of about 0.01 mg per mL.

Test solution—Dissolve an accurately weighed amount of Valrubicin in acetonitrile, using about 50% of the final volume, then dilute with water to volume, and mix to obtain a solution having a nominal concentration of about 2 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, and a 4.6-mm × 5-cm analytical column that contains a 1.8-µm packing L1. The flow rate is about 1.5 mL per minute. Maintain the column at a constant temperature of about 40°. The chromatograph is programmed as follows:

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–45	75→40	25→60	linear gradient
45–55	40	60	isocratic
55–58	40→20	60→80	linear gradient
58–68	20	80	isocratic
68–70	20→75	80→25	linear gradient
70–80	75	25	re-equilibration

[NOTE—Analysis time is 55 minutes. The steps that follow are for column washing and re-equilibration.]

The autosampler temperature is maintained at 4°. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* identify the peaks by the relative retention times listed in *Table 1*; the resolution, *R,* between doxorubicinone and daunorubicin is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all of the peaks. Calculate the percentage of each impurity in the portion of Valrubicin taken by the formula:

$$100(1/\mathrm{F})(C_S/C_U)(r_i/r_s)$$

in which F is the relative response factor as listed in *Table 1*; C_S is the concentration, in mg per mL, of the *Standard solution*; C_U is the nominal concentration, in mg per mL, of the *Test solution*; r_i is the peak area for each impurity obtained from the *Test solution*; and r_s is the response for valrubicin

In-Process Revision

obtained from the *Standard solution*. The limits of the specified and unspecified impurities meet the limits in *Table 1*. Reporting level is 0.05%.

Table 1

Peak Identification	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Doxorubicin	0.05	1.0	0.15
Doxorubicinone[1]	0.10	0.63	0.15
Daunorubicin	0.12	0.86	0.15
Daunorubicin bromoketal[2]	0.42	1.4	0.15
Doxorubicin valerate[3]	0.48	1.3	0.15
Doxorubicinone valerate[4]	0.76	0.80	0.15
Valrubicin	1.0	—	—
Dianhydrovalrubicin[5]	1.2	0.47	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[1] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[2] (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[3] 2-{(2*S*,4*S*)-4-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl}-2-oxoethyl pentanoate.
[4] 2-Oxo-2-[(2*S*,4*S*)-2,4,5,12-tetrahydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl]ethyl pentanoate.
[5] 2-(5,12-Dihydroxy-7-methoxy-6,11-dioxo-6,11-dihydrotetracen-2-yl)-2-oxoethyl pentanoate.▲*USP33*

Change to read:

Assay—

~~*Mobile phase*—Prepare a filtered and degassed mixture of 0.015 M phosphoric acid and acetonitrile (57 : 43). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).~~

~~*Standard preparation*—Dissolve an accurately weighed quantity of USP Valrubicin RS in acetonitrile, and dilute quantitatively with acetonitrile to obtain a solution having a known concentration of about 1 mg per mL.~~

~~*Assay preparation*—Transfer about 25 mg of Valrubicin, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.~~

~~*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a guard column, and a 5-mm × 10-cm analytical column that contains a 4-µm packing L1. The flow rate is about 3.5 mL per minute. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2%.~~

~~*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{34}H_{39}F_3NO_{14}$ in the portion of Valrubicin taken by the formula:~~

~~$$0.25CP(r_U/r_S)$$~~

~~in which C is the concentration, in mg per mL, of USP Valrubicin RS in the *Standard preparation;* P is the specified percentage of valrubicin in USP Valrubicin RS; and r_U and r_S are the valrubicin peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.~~

▲*Potassium phosphate buffer*—Transfer about 3.4 g of monobasic potassium phosphate to a 1000-mL volumetric flask, and dilute with water to volume. Adjust with phosphoric acid to a pH of 3.1.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *potassium phosphate buffer* (60 : 40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Valrubicin RS in acetonitrile, and dilute quantitatively with acetonitrile to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 50 mg of Valrubicin, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, and a 4.6-mm × 5-cm analytical column that contains a 1.8-µm packing L1. The flow rate is about 1.5 mL per minute. Maintain the column at a constant temperature of about 40°. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure

In-Process Revision

the areas for the major peaks. Calculate the percentage of $C_{34}H_{36}F_3NO_{13}$ in the portion of Valrubicin taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Valrubicin RS in the *Standard preparation*; C_U is the nominal concentration of valrubicin in the *Assay preparation*; and r_U and r_S are the valrubicin peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.▲*USP33*

BRIEFING

Vincristine Sulfate Injection, *USP 31* page 3514. On the basis of comments received, it is proposed to add the HPLC retention time agreement as an additional *Identification* test.

(MD-OOD: F. Mao) RTS—C68625

Change to read:

Identification—

▲A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—▲*USP33*

Spray reagent—Dissolve 2.0 g of ceric ammonium sulfate in 100 mL of water with heating and stirring, and slowly add 100 mL of phosphoric acid. Filter if necessary.

Procedure—Transfer a volume of Injection, equivalent to 2 mg of vincristine sulfate, to a small centrifuge tube. For each mL of solution add 1 drop of ammonium hydroxide. Add 0.2 mL of dichloromethane. Place the cap on the tube, shake it vigorously for not less than 1 minute, and centrifuge for 1 minute. Carefully withdraw the dichloromethane layer, and transfer to a small stoppered vial. Proceed as directed for *Procedure* in the test for *Identification* under *Vincristine Sulfate for Injection*, beginning with "Also prepare a 10-mg-per-mL solution of USP Vincristine Sulfate RS."

▲B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the *Standard preparation,* as obtained in the *Assay.*▲*USP33*

BRIEFING

Vincristine Sulfate for Injection, *USP 31* page 3515. On the basis of comments received, it is proposed to add the HPLC retention time agreement as an additional *Identification* test.

(MD-OOD: F. Mao) RTS—C68624

Change to read:

Identification—

▲A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—▲*USP33*

Spray reagent—Dissolve 2.0 g of ceric ammonium sulfate in 100 mL of water with heating and stirring, and slowly add 100 mL of phosphoric acid. Filter if necessary.

Procedure—Dissolve a sufficient quantity in water to obtain a solution containing 25 mg per mL. Further dilute the solution to 10 mg per mL with methanol, and mix. Also prepare a 10-mg-per-mL solution of USP Vincristine Sulfate RS in a mixture of dichloromethane and methanol (3:1), and mix. Use a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩). Develop it in a methanol prewash tank, and dry it, for maximum sensitivity, not more than 2 hours before use. Score it about 15 cm above the points of application. Apply 20 µL of each solution at points about 2.5 cm from the lower edge of the plate, and dry thoroughly (a current of cool air may be used to help dry the spots). Prepare the developing solvent system consisting of a mixture of fresh ether, methanol, and methylamine solution (2 in 5) (95 : 10 : 5) immediately prior to development. Place the plate in the nonequilibrated developing chamber that contains a paper liner around the back and sides and developing solvent to a depth of about 2 cm. Remove the plate when the solvent moves to the scored line (about 80 minutes), and discard the solvent system. Dry the plate in a fume hood at room temperature, heat on a metal plate on a steam bath for about 15 minutes, and spray the plate while still hot with *Spray reagent.* Continue heating the plate for 15 minutes to stabilize the spots: the R_F value and the color of the principal spot obtained from the test specimen correspond to those obtained from the Reference Standard.

▲B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the *Standard preparation,* as obtained in the *Assay.*▲*USP33*

In-Process Revision

DIETARY SUPPLEMENTS—MONOGRAPHS

BRIEFING

***N*-Acetyltyrosine.** Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. Interested parties are encouraged to submit comments.

(DSN: C. Phinney) RTS—C65493

Add the following:

▲*N*-Acetyltyrosine

$C_{11}H_{13}NO_4$ 223.2

N-Acetyl-L-tyrosine.

(2*S*)-2-(Acetylamino)-3-(4-hydroxyphenyl)propanoic acid) [*537-55-3*].

» *N*-Acetyltyrosine contains not less than 98.5 percent and not more than 101.0 percent of $C_{11}H_{13}NO_4$, as *N*-acetyl-L-tyrosine, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—*USP N-Acetyl-L-tyrosine RS. USP L-Tyrosine RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: It meets the requirements for *Specific rotation*.

C: The R_F value of the principal spot in the chromatogram of the *Test solution* in the test for *Chromatographic purity* corresponds to that obtained from *Standard solution 1*.

Specific rotation ⟨781S⟩: between +46.0° and +49.0°, determined at 20°.

Test solution: 10 mg per mL.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—A 0.7-g portion shows no more chloride than corresponds to 0.40 mL of 0.01 N hydrochloric acid: not more than 0.02% is found.

Sulfate ⟨221⟩—A 1.2-g portion shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid: not more than 0.02% is found.

Iron ⟨241⟩: 0.002%.

Heavy metals, *Method I* ⟨231⟩: 0.001%.

Chromatographic purity—

Standard solution 1—Prepare a solution in a mixture of water, glacial acetic acid, and alcohol (3 : 3 : 94) containing 8 mg of USP *N*-Acetyl-L-tyrosine RS per mL. Dilute 0.5 mL of this solution with alcohol to 10 mL to obtain a solution having a known concentration of about 0.4 mg per mL.

Standard solution 2—Dissolve an accurately weighed quantity of USP L-Trosine RS in a mixture of glacial acetic acid and water (1 : 1), and dilute with alcohol to obtain a solution having a known concentration of about 0.8 mg per mL.

Test solution—Transfer 0.8 g of *N*-Acetyltyrosine to a 10-mL volumetric flask, dissolve in 6 mL of a mixture of glacial acetic acid and water (1 : 1), and dilute with alcohol to volume.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having an optimal intensity at 254 nm.

Application volume: 5 µL.

Developing solvent system: a mixture of ammonia and 2-propanol (3 : 7).

Spray reagent—Dissolve 0.2 g of ninhydrin in 100 mL of a mixture of ammonia and 2-propanol (30 : 70).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After air-drying the plate, repeat the development process. After air-drying a second time, spray with *Spray reagent*, and heat between 100° and 105° for about 15 minutes. Examine the plate. Any secondary spot in the chromatogram obtained from the *Test solution* is not larger or more intense than the principal spot in the chromatogram obtained from *Standard solution 1*: not more than 0.5% of any individual impurity is found, and a spot in the chromatogram obtained from the *Test solution* corresponding to tyrosine is not larger or more intense than the principal spot in the chromatogram obtained from *Standard solution 2*: not more than 1.0% of tyrosine is found.

Assay—Dissolve about 180 mg of *N*-Acetyltyrosine in 50 mL of carbon dioxide-free water, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide VS is equivalent to 22.32 mg of $C_{11}H_{13}NO_4$.▲*USP33*

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 26* page 1057, page 3800 of the *Second Supplement,* and page 1511 of *PF* 34(6) [Nov.–Dec. 2008]. It is proposed to add *Chitosan* to the *Coating Agent, Film-Forming Agent, Suspending and/or Viscosity-Increasing Agent*, and *Vehicle (Solid Carrier)* categories; *Polyoxyl 15 Hydroxystearate* to the *Tablet and/or Capsule Lubricant, Vehicle (Oleaginous),* and *Wetting and/or Solubilizing Agent* categories; *Polyvinyl Acetate Dispersion* to the *Coating Agent* category; *Hydrogenated Starch Hydrolysate* to the *Humectant, Sweetening Agent, Tablet Binder*, and *Tablet and Capsule Diluent* categories; and *Starch, Pea* to the *Suspending and/or Viscosity-Increasing Agent, Tablet Binder, Tablet and/or Capsule Diluent, and Tablet Disintegrant* categories, to complement the proposed new monographs for *Chitosan, Polyoxyl 15 Hydroxystearate, Polyvinyl Acetate Dispersion, Hydrogenated Starch Hydrolysate,*and*Pea Starch,* which appear elsewhere in this issue of *PF.*

(EM1; EM2) RTS—C44080; C70200; C66478; C57716; C57641

Change to read:

Antimicrobial Preservative

Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzethonium Chloride
Benzoic Acid
Benzyl Alcohol
Butylparaben

▲Calcium Propionate▲*NF28*

Cetrimonium Bromide
Cetylpyridinium Chloride
Chlorobutanol
Chlorocresol
Cresol
■Dehydroacetic Acid■2S (*NF26*)

▲Erythorbic Acid▲*NF27*

Ethylparaben
Methylparaben
Methylparaben Sodium
Phenol
Phenoxyethanol
Phenylethyl Alcohol
Phenylmercuric Acetate
Phenylmercuric Nitrate
Potassium Benzoate
Potassium Sorbate
Propylparaben
Propylparaben Sodium
Sodium Benzoate
Sodium Dehydroacetate
Sodium Propionate
Sorbic Acid
Thimerosal
Thymol

Change to read:

Antioxidant

Ascorbic Acid
Ascorbyl Palmitate
Butylated Hydroxyanisole
Butylated Hydroxytoluene
■Stannous Chloride■2S (*NF26*)

▲Erythorbic Acid▲*NF27*

Hypophosphorous Acid
Monothioglycerol
Potassium Metabisulfite
Propyl Gallate
Sodium Bisulfite
Sodium Formaldehyde Sulfoxylate
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Sulfur Dioxide
Tocopherol
Tocopherols Excipient

Change to read:

Buffering Agent

Acetic Acid
Adipic Acid
Ammonium Carbonate
Ammonium Phosphate
Boric Acid
Citric Acid, Anhydrous
Citric Acid Monohydrate

■Alpha-Lactalbumin■1S (*NF27*)

Lactic Acid
Phosphoric Acid
Potassium Citrate
Potassium Metaphosphate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Citrate
Sodium Lactate Solution
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Succinic Acid

Change to read:

Bulking Agent for Freeze-Drying

Creatinine

■Alpha-Lactalbumin■1S (*NF27*)

Mannitol
▲Polydextrose▲*NF26*
■Pullulan■2S (*NF26*)

■Trehalose■1S (*NF27*)

Change to read:

Coating Agent

Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carboxymethylcellulose, Sodium

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Cellaburate
Cellacefate (formerly Cellulose Acetate Phthalate)
Cellulose Acetate
Cellulose Acetate Phthalate (see Cellacefate)

▲Chitosan▲*NF28*

Coconut Oil

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone
Corn Syrup Solids
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Ethylcellulose Aqueous Dispersion
Gelatin
Glaze, Pharmaceutical
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hydroxypropyl Methylcellulose Phthalate (see Hypromellose Phthalate)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate
Hypromellose Phthalate (formerly HydroxypropylMethylcellulose Phthalate)

■Alpha-Lactalbumin■1S (*NF27*)

Maltodextrin
Methacrylic Acid Copolymer
Methacrylic Acid Copolymer Dispersion
Methylcellulose
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Glycol

▲Polyvinyl Acetate▲*NF28*

▲Polyvinyl Acetate Dispersion▲*NF28*

Polyvinyl Acetate Phthalate
■Pullulan■2S (*NF26*)
▲Fully Hydrogenated Rapeseed Oil▲*NF26*
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Shellac
Starch, Pregelatinized Modified
Sucrose
Titanium Dioxide
Wax, Carnauba
Wax, Microcrystalline
Zein

Change to read:

Complexing Agent

Edetate Calcium Disodium
Edetate Disodium
Edetic Acid

■Alpha-Lactalbumin■1S (*NF27*)

Oxyquinoline Sulfate

Change to read:

Desiccant

Calcium Chloride
Calcium Sulfate

▲Polyvinyl Acetate▲*NF28*

Silicon Dioxide

Change to read:

Emulsifying and/or Solubilizing Agent

Acacia
Carbomer Copolymer
Carbomer Interpolymer
Cholesterol
■Stannous Chloride■2S (*NF26*)
Coconut Oil

▲Desoxycholic Acid▲*NF28*

Diethanolamine (Adjunct)
Diethylene Glycol Stearates
Ethylene Glycol Stearates
■Gamma Cyclodextrin■2S (*NF26*)
Glyceryl Distearate
Glyceryl Monolinoleate
Glyceryl Monooleate
Glyceryl Monostearate

■Alpha-Lactalbumin■1S (*NF27*)

Lanolin Alcohols
Lecithin
Mono- and Di-glycerides
Monoethanolamine (Adjunct)
Oleic Acid (Adjunct)
Oleyl Alcohol (Stabilizer)
▲Oleyl Oleate▲*NF26*
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

Poloxamer
Polyoxyethylene 50 Stearate
Polyoxyl 10 Oleyl Ether
Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate

In-Process Revision

Polyoxyl Lauryl Ether
Polyoxyl Stearyl Ether
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
■Propylene Glycol Dicaprylate/Dicaprate■2S *(NF26)*
■Propylene Glycol Monocaprylate■1S *(NF26)*
Propylene Glycol Monostearate
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Sodium Cetostearyl Sulfate
Sodium Lauryl Sulfate
Sodium Stearate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Stearic Acid
Trolamine
Wax, Emulsifying

Add the following:

▲Film-Forming Agent

Chitosan▲*NF28*

Change to read:

Humectant

Corn Syrup Solids
Erythritol
Glycerin
Hexylene Glycol
■Inositol■2S *(NF26)*
Maltitol
▲Polydextrose▲*NF26*
Propylene Glycol
Sorbitol
Sorbitol Sorbitan Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Tagatose

Change to read:

Stiffening Agent

Castor Oil, Hydrogenated
Cetostearyl Alcohol
Cetyl Alcohol
Cetyl Esters Wax
Cetyl Palmitate
Hard Fat

■Alpha-Lactalbumin■1S *(NF27)*

Paraffin
Synthetic Paraffin
▲Fully Hydrogenated Rapeseed Oil▲*NF26*
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Stearyl Alcohol
Wax, Emulsifying
Wax, White
Wax, Yellow

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

▲Chitosan▲*NF28*

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

■Alpha-Lactalbumin■1S *(NF27)*

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin
Polyethylene Oxide
Polyvinyl Alcohol
Povidone
Propylene Glycol Alginate
■Pullulan■2S *(NF26)*
■Hydrophobic Colloidal Silica■2S *(NF26)*
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca

Starch, Wheat
Tragacanth
Xanthan Gum

Change to read:

Sweetening Agent

Acesulfame Potassium
Aspartame
Aspartame Acesulfame

▲Corn Syrup▲*NF27*

Corn Syrup Solids
High Fructose Corn Syrup
Dextrates
Dextrose
Dextrose Excipient
Erythritol
Fructose
Galactose
Maltitol
Maltose
Mannitol
Saccharin
Saccharin Calcium
Saccharin Sodium
Sorbitol
Sorbitol Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Sucralose
Sucrose
Sugar, Compressible
Sugar, Confectioner's
Syrup
Tagatose

■Trehalose■1S (*NF27*)

Change to read:

Tablet Binder

Acacia
Alginic Acid
Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Sodium
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrin
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Gelatin
Glucose, Liquid
Guar Gum
Low-Substituted Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate

■Alpha-Lactalbumin■1S (*NF27*)

Maltodextrin
Maltose
Methylcellulose

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Oxide

▲Polyvinyl Acetate▲*NF28*

Povidone
■Pullulan■2S (*NF26*)
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Syrup

■Trehalose■1S (*NF27*)

Change to read:

Tablet and/or Capsule Diluent

Calcium Carbonate
Calcium Phosphate, Dibasic
Calcium Phosphate, Tribasic
Calcium Sulfate
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

Cellulose, Powdered

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrates
Dextrin
Dextrose Excipient
Fructose
Kaolin

■Alpha-Lactalbumin■1S (*NF27*)

In-Process Revision

Lactitol
Lactose, Anhydrous
Lactose, Monohydrate
Maltitol
Maltodextrin
Maltose
Mannitol
■Propylene Glycol Monocaprylate■1S *(NF26)*
■Pullulan■2S *(NF26)*
Sorbitol
Starch
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Sucrose
Sugar, Compressible
Sugar, Confectioner's

■Trehalose■1S *(NF27)*

Change to read:

Tablet Disintegrant

Alginic Acid
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Croscarmellose Sodium
Crospovidone
Low-Substituted Hydroxypropyl Cellulose
Maltose
Polacrilin Potassium
■Pullulan■2S *(NF26)*
Sodium Starch Glycolate
Starch
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat

■Trehalose■1S *(NF27)*

Change to read:

Tablet and/or Capsule Lubricant

■Behenoyl Polyoxylglycerides■2S *(NF27)*

Calcium Stearate
■Hydrogenated Coconut Oil■1S *(NF27)*

Glyceryl Behenate
Magnesium Stearate
Mineral Oil, Light

■Hydrogenated Palm Oil■1S *(NF27)*

Polyethylene Glycol
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Sodium Lauryl Sulfate
Sodium Stearyl Fumarate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Starch
Stearic Acid
Stearic Acid, Purified
Talc
Vegetable Oil, Hydrogenated, Type I
Zinc Stearate

Change to read:

Tonicity Agent

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrose
Glycerin
Mannitol
Potassium Chloride
Sodium Chloride

Change to read:

Vehicle
FLAVORED AND/OR SWEETENED

Aromatic Elixir
Benzaldehyde Elixir, Compound
Corn Syrup Solids
Dextrose

■Ethyl Maltol■2S *(NF27)*

Peppermint Water
Sorbitol Solution
Syrup

■Trehalose■1S *(NF27)*

OLEAGINOUS

Alkyl (C12-15) Benzoate
Almond Oil
Canola Oil
Corn Oil
Cottonseed Oil
Ethyl Oleate
■Hydrogenated Polydecene■1S (*NF26*)
Isopropyl Myristate
Isopropyl Palmitate
Mineral Oil
Mineral Oil, Light
Octyldodecanol
Olive Oil
Peanut Oil

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Safflower Oil
Sesame Oil
Soybean Oil
Squalane

SOLID CARRIER

▲Chitosan▲*NF28*

Corn Syrup Solids

■Alpha-Lactalbumin■1S (*NF27*)

■Propylene Glycol Dicaprylate/Dicaprate■2S (*NF26*)
■Propylene Glycol Monocaprylate■1S (*NF26*)
Sugar Spheres

STERILE

▲rAlbumin Human▲*NF27*

Sodium Chloride Injection, Bacteriostatic
Water for Injection, Bacteriostatic

Change to read:

Wetting and/or Solubilizing Agent

Benzalkonium Chloride
Benzethonium Chloride
Cetylpyridinium Chloride
Docusate Sodium
Nonoxynol 9
Octoxynol 9
Poloxamer
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
■Pullulan■2S (*NF26*)
Sodium Lauryl Sulfate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Tyloxapol

MONOGRAPHS (NF)

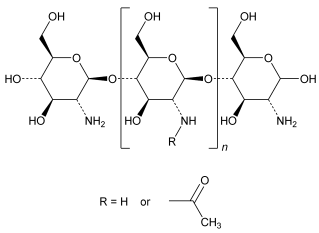
BRIEFING

Chitosan. Because there is no existing monograph for this article, a new monograph, based on validated methods of analysis, is being proposed. The high performance size exclusion chromatography (HPSEC) procedure in the test for *Average molecular weight and molecular weight distribution* is based on analysis performed with the Tosoh Bioscience TSK-GEL PW brand of column containing packing L38.

(EM2: H. Wang; NOM: A. Wilk; MSA: R. Tirumalai) RTS—C44080

Add the following:

▲Chitosan



Poly-β-(1,4)-2-Amino-2-deoxy-D-glucose [*9012-76-4*].

» Chitosan is an unbranched binary polysaccharide consisting of *N*-acetyl-D-glucosamine and D-glucosamine units linked in $\beta(1\rightarrow4)$ manner. Chitosan is obtained by partial deacetylation of chitin which is extracted from the shells of edible shrimps and crabs suitable for human use. Its degree of deacetylation is not less than 70.0 percent.

Packaging and storage—Preserve in light-resistant and well-closed containers in a dry place, and store at a temperature below 30°.

Labeling—Label it to indicate its weight-average molecular weight, M_w, and polydispersity (M_w/M_n). Where Chitosan is intended for use in the manufacture of wound dressings, it is so labeled. Where Chitosan must be subjected to further processing during the preparation of wound dressings, it is so labeled.

USP Reference standards ⟨11⟩—*USP Chitosan RS. USP Endotoxin RS.*

Identification—

A: *Infrared Absorption* ⟨197A⟩.

B: Add 80 g of water to 0.2 g of Chitosan powder, and stir briefly to obtain a dispersion. Separately prepare a glycolic acid solution by dissolving 0.1 g of glycolic acid in 20 g of water. Add the solution so obtained in one step to the dispersion. Stir the mixture gently at room temperature until it becomes a clear solution [NOTE—It takes approximately 30 to 60 minutes to obtain a clear solution]. Add 5 g of a 0.5% sodium lauryl sulfate aqueous solution to the clear solution. A gelatinous mass is formed.

Bacterial endotoxins ⟨85⟩—The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Chitosan is used can be met. Where the label states that Chitosan must be subjected to further processing during the preparation of wound dressing dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Chitosan is used can be met.

Test stock solution—Transfer 0.5 g of Chitosan to a 50-mL volumetric flask, add LAL Reagent Water[1] and 4.6 mL of 1 N hydrochloric acid, dilute with the LAL Reagent Water to volume, and mix well. Incubate this solution in a water-bath at 40° for 48 hours.

Test solution—Dilute the *Test stock solution* to 1 : 50 with LAL Reagent Water, including dilution 1 : 2 with β-glucan blocker.[2]

[1] Sterile Water for Injection or other water that shows no reaction with the specific LAL Reagent with which it is to be used, at the limit of sensitivity of such reagent.
[2] Available from Cambrex Europe s.p.r.l., Verviers, Belgium.

Microbial limits ⟨61⟩—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g. It meets the requirements of the tests for absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Sample preparation—Prepare a solution (1 in 50).

Loss on drying ⟨731⟩—Dry 1.0 g in an oven at 100–105° for 7 hours: it loses not more than 5.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 1.0%.

Heavy metals, *Method III* ⟨231⟩**:** not more than 10 µg per g.

Limit of lead, mercury, chromium, nickel, cadmium, and arsenic—

65% Nitric acid—Use ultratrace nitric acid, 65-70% HNO_3, ACS reagent grade.

Internal standard—Transfer 0.2 mL of a solution containing 1000 ppm of yttrium [NOTE—Yttrium ICP standard solutions are commercially available.[3]] and 0.2 mL of a solution containing 1000 ppm of lutetium [NOTE—Lutetium ICP standard solutions are commercially available.[4]] to a 100-mL volumetric flask, add 1 mL of *65% Nitric acid*, dilute with water to volume, and mix.

Blank standard—Transfer 1.0 mL of *Internal standard* to a 100-mL volumetric flask, add 1 mL of *65% Nitric acid,* dilute with water to volume, and mix.

Standard solutions—Transfer 0.1 mL of a solution containing 10 ppm of each for lead, mercury, chromium, nickel, cadmium, and arsenic [NOTE—Multi-element ICP standard solutions are commercially available.[5]] to a 100-mL volumetric flask, add 1 mL of *Internal standard* and 1 mL of *65% Nitric acid*, dilute with water to volume (*Standard solution 0.01 ppm*). Transfer 0.1 mL of a solution containing 10 ppm of each for lead, mercury, chromium, nickel, cadmium, and arsenic [NOTE—Multi-element ICP standard solutions are commercially available.[5]] to a 1000-mL volumetric flask, add 10 mL of *Internal standard* and 10 mL of *65% Nitric acid*, and dilute with water to volume (*Standard solution 0.001 ppm*).

Test solution—Transfer about 1.0 g of Chitosan, accurately weighed, to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside, and add portions of the same acid mixture, heating after each addition, until a total of 18 mL of the acid mixture has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 mL of nitric acid, and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30 percent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 mL. If the solution is still yellow, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, rinse into a 20-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of digestion solution to a 100-mL volumetric flask, and retain the remaining digestion solution for use in the *Limit of iron*. Add 1 mL of *Internal standard* to the 100-mL volumetric flask, dilute with water to volume, and mix.

Blank solution—Prepare the blank digestion solution following the preparation procedure for the *Test solution* but without using Chitosan. Transfer 5.0 mL of blank digestion solution to a 100-mL volumetric flask, and retain

[3] Suitable yttrium ICP standard is available from LGC Promochem (www.lgcpromochem.com) or from Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany.
[4] Suitable lutetium ICP standard is available from LGC Promochem (www.lgcpromochem.com) or from Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany.
[5] Suitable multi-element ICP standard is available from LGC Promochem (www.lgcpromochem.com) or from Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany.

In-Process Revision

the remaining digestion solution for use in the *Limit of iron*. Add 1 mL of *Internal standard* to the 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure (see *Plasma Spectrochemistry* ⟨730⟩)—The inductively coupled plasma–mass spectrometer (ICP–MS) is equipped with a quadrupole mass spectrometer and an ion detector maintained under vacuum. The instrument should read all isotopes for the following elements shown in the accompanying table, for the yttrium internal standard (89 amu) and the lutetium internal standard (175 and 176 amu), and should report the total element contents using the most naturally abundant isotopes.

Element	Isotope (amu)
Lead	208
Mercury	201
	202
Chromium	53
Nickel	58
	60
Cadmium	114
Arsenic	75

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Perform the evaluation using instrument software such as correction equations for interferences and taking the *Internal standard* into account. Generate the calibration curve using the *Blank standard, Standard solution 0.001 ppm,* and *Standard solution 0.01 ppm*: the linear regression coefficient is not less than 0.999.

Aspirate the *Blank solution* and *Test solution*, respectively, at least in duplicate, and report the average reading as each element content of the sample. Determine the concentration C_B, in µg per mL, of each element in the *Blank solution*, and also determine the concentration C_S, in µg per mL, of each element in the *Test solution* using the calibration curve. Calculate the quantity, in µg per g, of each element in the portion of Chitosan taken by the formula:

$$4(100)(C_S - C_B) / W$$

in which 4 is a dilution factor; 100 is the volume, in mL, of the *Test solution*; and W is the weight, in g, of the Chitosan taken to prepare the *Test solution*: not more than 0.1 µg lead per g is found; not more than 0.1 µg mercury per g is found; not more than 0.5 µg chromium per g is found; not more than 1.0 µg nickel per g is found; not more than 0.2 µg cadmium per g is found; not more than 0.1 µg arsenic per g is found.

Limit of iron—

65% Nitric acid—Use ultratrace nitric acid, 65-70% HNO_3, ACS reagent grade.

Blank standard—Use water.

Standard stock solution 100 ppm—Immediately before use, dilute appropriate amount of iron standard[6] with a solution of *65% Nitric acid* (1 in 100) to prepare an acidic solution containing the equivalent of 100 µg of iron per mL.

Standard solutions—Separately transfer 0.1 and 0.5 mL of *Standard stock solution 100 ppm* to 100-mL volumetric flasks, dilute with a solution of *65% Nitric acid* (1 in 100) to volume, and mix. These solutions contain, respectively, 0.1 and 0.5 µg of iron per mL (*Standard solution 0.1 ppm* and *Standard solution 0.5 ppm*).

Test solution—Transfer 10.0 mL of the digestion solution from the test for *Limit of lead, mercury, chromium, nickel, cadmium, and arsenic* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Blank solution—Transfer 10.0 mL of the blank digestion solution from the test for *Limit of lead, mercury, chromium, nickel, cadmium, and arsenic* to a 100-mL volumetric flask, dilute with water to volume, and mix.

[6] Suitable iron standards are available from LGC Promochem (www.lgcpromochem.com) (Single element standard for ICP, Iron 10,000 µg per mL dilute nitric acid) or from Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany (iron ICP standard, 10000 mg per L in 10% nitric acid).

Procedure (see *Plasma Spectrochemistry* ⟨730⟩)—Iron is determined using an inductively coupled plasma–atomic emission spectrometer (ICP–AES) by measuring the emission at 238.040 nm and 239.562 nm with the settings optimized as directed by the manufacturer.

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Blank standard, Standard solution 0.1 ppm,* and *Standard solution 0.5 ppm*: the linear regression coefficient is not less than 0.999.

Aspirate the *Blank solution* and *Test solution,* respectively, at least in duplicate, and report the average reading as the iron content of the sample. Determine the concentration, C_B, in µg per mL, of iron in the *Blank solution,* and also determine the concentration, C_S, in µg per mL, of iron in the *Test solution* using the calibration curve. Calculate the quantity, in µg, of iron per g in the portion of Chitosan taken by the formula:

$$2(100)(C_S - C_B) / W$$

in which 2 is a dilution factor; 100 is the volume, in mL, of the *Test solution*; and W is the weight, in g, of the Chitosan taken: not more than 10 µg per g is found.

Degree of deacetylation—

[NOTE—If tetramethylsilane is not used as *NMR reference*, a suitable signal of the solvent itself can be used as a reference.]

Solvent—Use deuterated formic acid.

NMR reference—Use tetramethylsilane.

Test solution—Into a 20-mL scintillation vial with screw cap, dissolve 5 mg to 10 mg of Chitosan in deuterated formic acid containing 0.5% to 1.0% of tetramethylsilane to obtain 1 mL of solution. Tightly close the vial, and dissolve Chitosan using a magnetic stirrer. It needs about 48 hours for complete dissolution and the stirring is stopped when a clear solution with a high viscosity is obtained. Break up any clumps formed during the dissolution process with a spatula, if necessary.

Procedure—Transfer 0.5 mL to 1.0 mL of the *Test solution* to a standard 5-mm NMR spinning tube. Proceed as directed for *Relative Method of Quantitation* under *Nuclear Magnetic Resonance* ⟨761⟩, using the *Test solution* volumes specified here, scanning the region from 0 ppm to 7 ppm, and using the calculation formulas specified here. Record as A_1 the average area of the composite band from about 6 ppm to 3 ppm, representing the seven protons with oxygen neighbors in the sugar ring, and record as A_2 the average area of the signals at about 2 ppm, due to the methyl groups of the acetyl units, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the percentage of deacetylation degree, by weight, in the Chitosan, taken by the formula:

$$100[1 - (7A_2 / 3A_1)]$$

The degree of deacetylation is not less than 70.0%.

Limit of protein content—

Control solution A—Prepare a solution of bovine serum albumin in water to obtain a known concentration of about 1.0 mg per mL.

Control solution B—Prepare a solution of bovine serum albumin in water to obtain a known concentration of about 0.1 mg per mL.

Test solution C—Dissolve about 100 mg of Chitosan, accurately weighed, to 4 mL of 100% formic acid, mix, and stir for about 48 hours at room temperature using a magnetic stirrer. This solution contains 25 mg of Chitosan per mL in 100% formic acid.

Test solutions D, E, F—Dilute aliquots of the solution with water so as to obtain the following solutions with concentrations of 2.5 mg of Chitosan per mL in 10% formic acid, 0.5 mg of Chitosan per mL in 2% formic acid, and 0.25 mg of Chitosan per mL in 1% formic acid.

Molecular weight standard preparation G—Use a commercially available preparation of apparent molecular weight protein standards of 10,000 to 190,000 Da dissolved in the loading buffer consisting of 50 mM of tris(hydroxymethyl)aminomethane hydrochloride (pH 6.8), 5 mM of ethylenediaminetetraacetic acid, 10 mM of dithiothreitol [NOTE—A 2-5% solution of beta-mercaptoethanol can be used to replace dithiothreitol.], 1% (w/v) sodium dodecyl sulfate, 10% (w/v) glycerol.[7] [NOTE—A protein ladder containing the following molecular weight standards in 10, 15, 20, 25, 40, 50, 60, 85, 120 and 190 KDa or other appropriate combinations can be used.]

Sample buffer—Transfer 666 mg of tris(hydroxymethyl)aminomethane hydrochloride, 682 mg of tris(hydroxymethyl)aminomethane, 800 mg of lithium dodecyl sulfate, 6 mg of ethylenediaminetetraacetic acid, 4 g of glycerol, 0.75 mL of 1% solution of Coomassie blue G250, and 0.25 mL of 1% solution of phenol red to a 10-mL volumetric flask, add 8 mL of water to the flask, and mix. If necessary, adjust with hydrochloric acid or sodium hydroxide to pH of 7.2. Dilute with water to volume. [NOTE—Store the buffer at 4°. It is stable for 6 months when stored at 4°.] A commercially available buffer can be equivalently used.[8]

Running buffer—Prepare a solution containing 1 M of tris(hydroxymethyl)-aminomethane, 1 M of 2-(4-morpholinyl) ethanesulfonic acid, 20.5 mM ethylenediaminetetraacetic acid, and 69.3 mM dodecyl sodium sulfate in water. If necessary, adjust with hydrochloric acid or sodium hydroxide to pH of 7.3. The appropriate SDS running buffer of commercially available is equivalently used.[9]

Fixing solution—A solution containing 40% ethanol and 10% acetic acid.

Sensitizing solution—Transfer 10 mL of a solution mainly containing 10–20% of 2-(4-morpholinyl) ethanesulfonic acid, 0.1-1.0% of sodium hydroxide, and 7–13% of *N*,*N*-dimethylformamide into a 100-mL volumetric flask, add 30 mL of alcohol, and dilute with water to volume. Alternatively, follow the instructions of a commercially available silver staining kit[10] to prepare the *Sensitizing solution, Staining solution,* and *Developing solution*.

Staining solution—Transfer 1.0 mL of a solution mainly containing 10-30% silver nitrate into a 100-mL volumetric flask, and dilute with water to volume.

Developing solution—Transfer 10 mL of a solution mainly containing 10-30% sodium carbonate into a 100-mL volumetric flask, add 1 drop of a solution containing 30-60% of formaldehyde, and dilute with water to volume.

Stopping solution—A solution containing 10-30% ethylenediaminetetraacetic acid and 10-30% tris(hydroxymethyl)-aminomethane. *Stopping solution* is commercially available and included into a silver staining kit.

Procedure—Mix 75 µL of each of *Test solutions C, D, E, and F* with 25 µL of *Sample buffer,* and incubate at 70° for 10 minutes. In a suitable device for polyacrylamide-gel electrophoresis (see *Electrophoresis* ⟨726⟩ and *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩) add appropriate volumes of the *Running buffer* in the upper and the lower buffer chambers. Attach a 4%-12% gradient Bis-Tris ready-made polyacrylamide gel sandwiched between two glass plates, such that the wells for sample application are exposed to the *Running buffer* in the upper buffer chamber. Separately apply equal volumes (about 20 µL) of each of the treated *Test solutions C, D, E, and F, Control solution A,* and *Control solution B* onto separate lanes; apply equal volumes (about 10 µL) of the *Molecular weight standard preparation G* to both sides of the gel. [NOTE—Do not apply any solution in the outside lanes.] Connect the lower buffer chamber electrode to the positive terminal and the upper buffer chamber electrode to the

[7] A suitable molecular weight standard preparation is available as BenchMark Prestained Protein Ladder from Invitrogen, product number: 10748010.
[8] A suitable sample buffer is available as 4X NuPAGE LDS sample buffer from Invitrogen, product number: NP0007.
[9] A suitable running buffer is available as NuPAGE MES SDS Running Buffer from Invitrogen, product number: NP0002.
[10] A suitable silver staining kit is available as NuPAGE Silver Staining Kit from Invitrogen, product number: LC6070.

negative terminal of a suitable power supply unit, and carry out the electrophoresis at a constant voltage of about 100 V for about 100 minutes. Remove the gel from the gel assembly. [NOTE—Do not touch the gel with bare hands. Use gloves.]

Place the gel in a clean staining tray of appropriate size. Rinse the gel briefly with water. Fix the gel in 100 mL of *Fixing solution* for 20 minutes with gentle rotation. [NOTE—The gel can be stored in the *Fixing solution* overnight.] Decant the *Fixing solution*, and wash the gel in 30% ethanol for 10 minutes. Decant the ethanol, and add 100 mL of *Sensitizing solution* to the washed gel in the staining container. Incubate the gel in the *Sensitizing solution* for about 10 minutes. [NOTE—All incubations should be performed on a rotary shaker rotating at a speed of 1 revolution per second at room temperature.] Decant the *Sensitizing solution*, and wash the gel in 100 mL of 30% ethanol for 10 minutes. Wash the gel in 100 mL of water for 10 minutes. Incubate the gel in 100 mL of *Staining solution* for 15 minutes. After staining is complete, decant the *Staining solution*, and wash the gel with 100 mL of water for 20 to 60 seconds. [NOTE—Washing the gel for more than one minute will remove silver ion from the gel resulting in decreased sensitivity.] Incubate the gel in 100 mL of *Developing solution* for 4 to 8 minutes until bands start to appear and the desired band intensity is reached. Once the appropriate staining intensity is achieved, immediately add 10 mL of *Stopping solution* directly to the gel still immersed in the *Developing solution*. Gently agitate the gel for 10 minutes. The color changes from pink to colorless indicating that the development has stopped. Decant the colorless solution and wash the gel with 100 mL of water for 10 minutes. Use a gel imaging system, ideally with CCD camera to record the results. Not more than 0.2% protein is found.

Average molecular weight and molecular weight distribution—

Mobile phase—Transfer 12.75 g sodium nitrate to a 1000-mL volumetric flask containing 800 mL water, add a suitable amount of formic acid, dilute with water to volume, mix well, and make a concentration of formic acid of 0.5 M. The *Mobile phase* contains 0.15 M sodium nitrate in 0.5 M aqueous formic acid.

System suitability solution—Prepare a solution having a known concentration of 1.0 mg of ethylene glycol per mL in *Mobile phase*.

Standard solutions—Prepare several sets of mixtures, containing ten polyethylene glycol (PEG) standards of different known molecular weight, which are used to cover the molecular weight range from about 200 to 1,100,000 g per mol.[11] [NOTE—These standards could be mixtures of polyethylene glycols and polyethylene oxides.] Prepare each set of PEG molecular weight standards to have a known concentration of about 1.0 mg per mL for each standard in *Mobile phase*. Allow the *Standard solutions* to stand at room temperature for at least 8 hours. Do not filter before use.

Test solution—Prepare a solution containing 1.0 mg per mL of Chitosan in *Mobile phase*. Cap, and mix well. Allow the solution to stand at room temperature for at least 12 hours. Pass the chitosan solution through a 0.45-µm membrane filter, discard an appropriate volume of the initial filtrate, and use the rest of the filtered solution for analysis.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector maintained at a temperature of 35°, a 7.5-mm × 30-cm analytical column that contains 10-µm packing L38, a 7.5-mm × 30-cm analytical column that contains 17-µm packing L38, and a 7.5-mm × 30-cm analytical column that contains 17-µm packing L38. The flow rate is 1.0 mL per minute for the *System suitability solution*. Chromatograph the *System suitability solution,* and determine the plate count as directed for *Procedure*: the plate count, *N,* is not less than 80% of the value which is certified by the column manufacture for new columns. The flow rate is 0.5 mL per minute for *Standard solutions* and *Test solution*. Chromatograph the *Standard solutions,* and calculate the retention

[11] Suitable polyethylene glycol molecular weight standards are available as ReadyCal kits from Polymer Standards Service (PSS), www.polymer.de.

volume, V_p, at the peak maximum of each individual molecular weight standard in the *Standard solutions* as directed for *Procedure*: the resolution, *R,* between the PEG standards is not less than 1.7.

Procedure—Inject a volume (20 µL) of the *System suitability solution* into the chromatogram, record the chromatogram, and determine the plate count for the ethylene glycol peak. Separately inject equal volumes (about 100 µL) of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and determine elution peak maxima and corresponding retention volumes (elution volume), V_p, for the ten PEG standards, corresponding to the specified molecular weight, M_p, at the peak maximum of the standards.

Molecular weight calibration—Analyze each polyethylene glycol standard, and use a suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software or an equivalent data handling system to compute the data and calibration. Plot log M_p for each standard in the *Standard solutions* versus its retention volume, V_p, in mL, at each standard peak maximum, and construct the best polynomial line fitting the ten points.

Data analysis for sample—[NOTE—Based on the PEG molecular weight calibration curve, calculate the molecular weight and molecular weight distribution of Chitosan using the slice by slice method.] Analyze the Chitosan sample by identifying retention volumes V_a and V_b corresponding to the beginning and end of the Chitosan chromatogram. The baseline between V_a and V_b is assumed to be linear. [NOTE—Draw a straight line between V_a and V_b.] Data analysis is based on the suitable GPC/SEC computer software or a real-time data acquisition system with either off-line or on-line data processing that is able to provide a means of determining chromatographic peak heights or integrated area segments as prescribed intervals under the SEC chromatogram and handling and reporting the data. The following describes the data processes which can either be computed by the GPC/SEC software or by an equivalent data processing system. Upon acquisition, handle the data under the Chitosan elution peak in discrete segments A_i , integrated area slices, or as digitized chromatogram heights H_i by recording the vertical displacements between the chromatogram trace and the baseline at elution volume, V_i, over designated intervals. A minimum of 40 area segments or heights are required. Obtain the corresponding value of M_i for Chitosan based on its elution volume, V_i, from the molecular weight calibration curve obtained in *Molecular weight calibration*. Calculate the number-, and weight-average molecular weights, M_n and M_w, in g per mol, respectively, using the following formulas.

$$M_n = \frac{\sum_{i=1}^{N} A_i}{\sum_{i=1}^{N}\left(\frac{A_i}{M_i}\right)}$$

$$M_w = \frac{\sum_{i=1}^{N}\left(A_i \bullet M_i\right)}{\sum_{i=1}^{N} A_i}$$

If the elution volume internal ΔV_i , for instance, $V_2 - V_1 = V_3 - V_2$, *etc,* is constant; parameters A_i and M_i are the chromatographic peak slice area and Chitosan molecular weight associated with the elution volume, V_i; and N is the number of data points obtained from the chromatogram between V_a and V_b ($N \geq 40$). [NOTE—If N is sufficiently large, the use of area segments A_i or peak heights H_i will yield equivalent results.]

Calculate the molecular weight distribution or polydispersity for Chitosan using the following expression:

$$M_w / M_n$$

The values of weight-average molecular weight and polydispersity are not less than 85% and not more than 115% of their respective values stated on the label.▲*NF28*

In-Process Revision

BRIEFING

Cystine. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed. Interested parties are encouraged to submit comments.

(DSN: C. Phinney) RTS—C62408

Add the following:

▲Cystine

$C_6H_{12}N_2O_4S_2$ 240.30

L-Cystine.

3,3′-disulfanediylbis [(2*R*)-2-aminopropanoic acid]

[*56-89-3*].

» Cystine contains not less than 98.5 percent and not more than 101.5 percent of $C_5H_9NO_4$, as L-Cystine calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Arginine Hydrochloride RS. USP Cystine RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: Meets the requirements of the test for *Specific rotation*.

C: The R_F value of the principal spot in the chromatogram of the *Test solution* in the test for *Chromatographic purity* corresponds to that obtained from the *Standard solution*.

Identification, *Infrared Absorption* ⟨197K⟩**.**

Specific rotation ⟨781S⟩: between –215° and –225°, determined at 20°.

Test solution: 20 mg per mL, in 1 N hydrochloric acid.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.2% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—A 0.7-g portion shows no more chloride than corresponds to 0.40 mL of 0.01 N hydrochloric acid: not more than 0.02% is found.

Sulfate ⟨221⟩—A 1.2-g portion shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid: not more than 0.02% is found.

Iron ⟨241⟩: 0.001%.

Heavy metals, *Method I* ⟨231⟩: 0.001%.

Chromatographic purity—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution: Transfer 0.1 g of Cystine to a 10-mL volumetric flask, dissolve in 1 N hydrochloric acid, and dilute with water to volume.

System suitability solution—Dissolve accurately weighed quantities of USP Cystine RS and USP Arginine Hydrochloride RS in 1 mL of 1 N hydrochloric acid, and dilute with water to obtain a solution having a known concentration of about 0.4 mg per mL of each.

Standard solution—Dissolve an accurately weighed quantity of USP Cystine RS in 1 mL of 1 N hydrochloric acid, and dilute with water to obtain a solution having a known concentration of about 0.02 mg per mL.

In-Process Revision

Application volume: 5 µL.

Developing solvent system: a mixture of ammonia and 2-propanol (3 : 7).

Spray reagent—Dissolve 0.2 g of ninhydrin in 100 mL of a mixture of ammonia and 2-propanol (30 : 70).

Procedure—Proceed as directed for *System suitability* under *Chromatography* ⟨621⟩. After air-drying the plate, repeat the development process. After air-drying a second time, spray with *Spray reagent*, and heat between 100° and 105° for about 15 minutes. Examine the plate. The chromatogram obtained from the *System suitability solution* exhibits two clearly separated spots. Any secondary spot in the chromatogram obtained from the *Test solution* is not larger or more intense than the principal spot in the chromatogram obtained from the *Standard solution*: not more than 0.2% of any individual impurity is found, and not more than 2.0% of total impurities is found.

Assay—Transfer about 0.1 g of Cystine, accurately weighed, to a glass-stoppered flask, and dissolve in a mixture of 2 mL of dilute sodium hydroxide (1 in 20) and 10 mL of water. Add 10 mL of potassium bromide solution (200 g/L in water), 50.0 mL of 0.1 N potassium bromate VS, and 15 mL of dilute hydrochloric acid (17 in 100); immediately insert the stopper into the flask, and cool in iced water. Allow to stand protected from light for 10 minutes. Add 1.5 g of potassium iodide, and after 1 minute, titrate with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N potassium bromate VS is equivalent to 2.403 mg of $C_6H_{12}N_2O_4S_2$.▲*NF28*

BRIEFING

Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion, *USP 31* page 1132 and page 1247 of *PF* 33(6) [Nov–Dec 2007]. On the basis of comments and data received, it is proposed to make the following revisions.

1. Cancel the proposed test procedure in the *Viscosity* section, which appeared in 33(6), and replace it with an updated procedure that indicates shear rate conditions. This is based on validation data demonstrating that the test solution behaves as a Newtonian fluid under the test conditions for a shear rate of not less than 1 s^{-1} and not more than 100 s^{-1}.
2. In *Coagulum content*, a single-use woven wire cloth is also included, in addition to a stainless sieve.

(EM2: H. Wang) RTS—C65738

Change to read:

Identification— ~~*Infrared Absorption* ⟨197K⟩.~~
~~*Test specimen*—Dry as specified in the *Loss on drying* test.~~

▲Place 1 drop of Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion on a glass plate,[1] and cover the test substance with a water-resistant crystal disk (silver chloride or KRS-5).[2] Gently press on and then remove the crystal disk. Dry the crystal disk at 80° for approximately 15 minutes: the IR absorption spectrum of Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion exhibits maxima corresponding to the same wavelengths as those of a similar preparation of USP Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion RS treated in the same manner.▲*NF27*

Change to read:

Viscosity ⟨911⟩— ~~Equip a suitable rotational viscosimeter with an adapter system consisting of a measuring cylinder and a spindle. The measuring cylinder has an internal diameter of 2.762 cm and a depth of 13.50 cm; the spindle is 2.515 cm in diameter and 9.074 cm in height, and has a shaft that is 0.40 cm in diameter. Mix the Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion, pipet 16 mL into the measuring cylinder, and adjust the temperature of the Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion and the adapter to 20 ± 0.1°. With the spindle rotating at 30 rpm, immediately observe and record the scale reading. Convert the scale reading to centipoises by multiplying the reading by the constant for the viscosimeter, the adapter system, and the speed employed. The viscosity is not more than 50 centipoises.~~

In-Process Revision

[1] A simple glass microscope slide is suitable.
[2] KRS-5 consists of 42% thallium(I) bromide and 58% thallium(I) iodine by molecular weight. Suitable disks of silver chloride and of KRS-5 are available from www.photonic.saint-gobain.com, www.almazoptics.com, and www.internationalcrystal.net.

▲Equip a suitable rotational viscometer with an adapter comprising a cylindrical spindle rotating within an accurately machined chamber (or tube).[3] Mix the Dispersion, pipet the test specimen in the specified volume, which is recommended by the instrument manufacturer, into the chamber (or tube), and ensure that the temperature of the test specimen is at 20 ± 0.1°. The shear rate under the test condition is not less than 1 s^{-1} and not more than 100 s^{-1}.[4] Measure the apparent viscosity following the instrument manufacturer's directions. The viscosity is between 2 and 20 mPa · s.▲*NF28*

Change to read:

Coagulum content—Accurately weigh a stainless steel sieve having 125-µm openings

▲or a suitable single-woven wire cloth with a mesh width of 125 µm,▲*NF28*
and filter 100 g of Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion through it.

▲[NOTE—Suitable single-woven wire cloth mesh meets the requirements set in ISO 9044.]▲*NF28*
Wash the sieve

▲or the cloth▲*NF28*
with distilled water until a clear filtrate is obtained, and dry the sieve

▲or the cloth▲*NF28*
to constant weight at 105°: the weight of the residue does not exceed 1000 mg (1%).

BRIEFING

Hydroxypropyl Cellulose, *NF 26* page 1149. Based on comments received, it is proposed to add a *Note* to the *Identification* section to indicate that the IR spectrum may contain a peak at about 1719 cm^{-1}, and to allow for either the presence or absence of this peak.

(EM2: K. Moore) RTS—C70498

Change to read:

Identification, *Infrared Absorption* ⟨197K⟩.

▲[NOTE—The spectrum may or may not contain a peak at about 1719 cm^{-1}.]▲*USP33*

BRIEFING

Methacrylic Acid Copolymer Dispersion, *NF* 26 page 1174 and page 1254 of *PF* 33(6) [Nov–Dec 2007]. On the basis of data and comments received, the following revisions are proposed.

1. For *Viscosity* the previous proposal published in *PF* 33(6) is hereby cancelled and replaced with an updated test procedure to indicate the shear rate conditions. This is based on validation data demonstrating that the test solution behaves as a Newtonian fluid under the test conditions for a shear rate of not less than 1 s^{-1} and not more than 100 s^{-1}.
2. In the test for *Limit of monomers*, the *Phosphate buffer* is deleted, the *Mobile phase* is updated, and the calculation formula is clarified.

(EM2: H. Wang) RTS—C65736

Change to read:

Packaging and storage~~—Preserve in tight containers, at a temperature not exceeding 30°.~~

■Preserve in tight containers. Store at controlled room temperature.■1S (*NF27*)
Protect from freezing.

Change to read:

Viscosity ⟨911⟩~~—Equip a suitable rotational viscosimeter with an adapter system consisting of a measuring cylinder and a spindle. The measuring cylinder has an internal diameter of 2.762 cm and a depth of 13.50 cm; the spindle is 2.515 cm in diameter and 9.074 cm in height, and has a shaft that is 0.40 cm in diameter. Mix the Dispersion, pipet 16 mL of it into the measuring cylinder, and adjust the temperature of the dispersion and the adapter to 20 ± 0.1°. With the spindle rotating at 30 rpm, immediately observe and record the scale reading. Convert the scale reading to centipoises by multiplying the reading by the constant for the viscosimeter, the adapter system, and the speed employed. The viscosity is not more than 15 centipoises.~~

▲[3] A commercial device is available from Brookfield as an ultra-low (UL) viscosity adapter. The adapter comprises a 0.4-cm diameter shaft, an accurately machined chamber (or tube) with an internal diameter of 2.8 cm and a depth of 13.5 cm, and a cylindrical spindle 2.5 cm in diameter and 9.1 cm in height.▲*NF28*
▲[4] The cylindrical spindle rotates at 30 rpm.▲*NF28*

In-Process Revision

▲Equip a suitable rotational viscometer with an adapter comprising a cylindrical spindle rotating within an accurately machined chamber (or tube).[1] Mix the Dispersion, pipet the test specimen in the specified volume, which is recommended by the instrument manufacturer, into the chamber (or tube), and ensure that the temperature of the test specimen is at 20 ± 0.1°. The shear rate under the test condition is not less than 1 s^{-1} and not more than 100 s^{-1}.[2] Measure the apparent viscosity following the instrument manufacturer's directions. The viscosity is between 2 and 15 mPa · s.▲*NF28*

Change to read:

Limit of monomers—

~~*pH 2.0 Phosphate buffer, fortieth molar; Mobile phase;* and *Chromatographic system*—Prepare as directed in the test for *Limit of monomers* under *Methacrylic Acid Copolymer*.~~

~~*Standard solution*—Prepare a solution in methanol having a known concentration of about 2 µg per mL each of methacrylic acid and ethyl acrylate. To 50.0 mL of this solution add 25.0 mL of water, and mix.~~

~~*Test solution*—Dissolve 1.0 g of the Dispersion in 50.0 mL of methanol, add 25.0 mL of water, and mix.~~

~~*Procedure*—Proceed as directed in the test for *Limit of monomers* under *Methacrylic Acid Copolymer*. The total amount of monomers found is not more than 0.01%, based on the weight of the Dispersion taken.~~

▲*Sodium perchlorate solution*—Dissolve 3.5 g of sodium perchlorate in 100 mL of water. This solution has a concentration of 0.25 M.

~~*Phosphate buffer*—Prepare an aqueous solution containing 17.8 g of anhydrous dibasic sodium phosphate and 17.0 g of monobasic potassium phosphate per L. Adjust with phosphoric acid to a pH of 2.0. This buffer has a concentration of 0.125 M.~~

Mobile phase—~~Prepare a solution in methanol containing 800 mL of *Phosphate buffer* per L.~~ Add drop wise phosphoric acid to water to obtain a solution having a pH of 2.0. Prepare a mixture of this acidified water and methanol (80 : 20), and degas.

Standard solution—Dissolve about 0.01 g of methacrylic acid and 0.01 g of ethyl acrylate, accurately weighed, in 5 mL butanol, and add methanol to make exactly 100 mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with methanol to volume. Mix 5.0 mL of this solution with 5.0 mL of *Sodium perchlorate solution,* accurately measured. This solution contains about 0.5 µg per mL each of methacrylic acid and ethyl acrylate.

Test solution—Transfer a quantity of the Dispersion, equivalent to 3 g of solids on the dried basis, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Add 5 mL of this solution dropwise while continuously stirring into a beaker that contains 5.0 mL of *Sodium perchlorate solution,* accurately measured. Remove the precipitated polymer by centrifugation. Use the clear supernatant.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 202-nm detector and a 4.0-mm × 12.5-cm column that contains 7-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and identify the components on the basis of the following relative retention times: about 1.0 and 2.6 for methacrylic acid and ethyl acrylate, respectively. Record the peak responses as directed for *Procedure:* the resolution, *R,* between methacrylic acid and ethyl acrylate is not less than 2.0; and the relative standard deviation for replicate injections determined from each analyte is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of each monomer in the solid portion of the Dispersion taken by the formula:

~~$100.0(10^{-4})(C/W)(r_U/r_S)$~~

$$100(10)(10)(10^{-6})(C/W)(r_U/r_S)$$

▲[1] A commercial device is available from Brookfield as an ultra-low (UL) viscosity adapter. The adapter comprises a 0.4-cm diameter shaft, an accurately machined chamber (or tube) with an internal diameter of 2.8 cm and a depth of 13.5 cm, and a cylindrical spindle 2.5 cm in diameter and 9.1 cm in height.▲*NF28*

▲[2] The cylindrical spindle rotates at 30 rpm.▲*NF28*

In-Process Revision

in which ~~100.0 is the dilution factor for preparation of the *Test solution;* 10^{-4} is the outcome of multiplying the percentage by the unit conversion factor;~~10 is the dilution factor for preparation of the *Test solution*; 10 is the final volume, in mL, of the *Test solution*; 10^{-6} is a factor converting µg to g; *C* is the concentration, in µg per mL, of the monomer in the *Standard solution; W* is the solid weight calculated on the dried basis, in g, of the Dispersion taken to prepare the *Test solution;* and r_U and r_S are the peak responses for the monomer obtained from the *Test solution* and the *Standard solution,* respectively. The total amount of monomers found is not more than 0.01%, based on the weight of the solid of the Dispersion.▲*NF28*

Change to read:

Coagulum content—Accurately weigh a stainless steel sieve having 90-µm openings

■or a suitable single-woven wire cloth with a mesh width of 90 µm,■1S (*NF27*)
and filter 100 g of the Dispersion through it.

■[NOTE—Suitable single-woven wire cloth mesh meets the requirements set in ISO 9044.]■1S (*NF27*)
Wash the sieve

■or the cloth■1S (*NF27*)
with distilled water until a clear filtrate is obtained, and dry the sieve

■or the cloth■1S (*NF27*)
to constant weight at 110°: the weight of the residue does not exceed 1000 mg (1%).

BRIEFING

Olive Oil, *NF* 26 page 1184. On the basis of comments and data received, and to align with the *Refined Olive Oil* monograph in the *European Pharmacopoeia, 6th Edition*, it is proposed to make the following revisions:

1. Add CAS number.
2. Revise the *Definition*.
3. Revise the *Packaging and storage* section.
4. Add an *Identification* test citing a test for *Fatty acid composition*, and add a *Fatty acid composition* test.
5. Delete the test for *Specific gravity* and move the information to the *Description and Solubility* section (see this issue of *PF*).
6. Delete the tests for *Cottonseed oil*, *Peanut oil*, and *Teaseed oil*; the tests are now unnecessary because of the addition of tests for *Fatty acid composition* and *Sterol composition*.
7. To prevent adulteration with sesame oil, revise the test for *Sesame oil* from a test for sesamolin detection to one for sesamin detection. Both sesamolin and sesamin are specific constitutents of sesame unsaponifiable matter. It is reported that sesamolin is present in crude sesame oil but not in refined sesame oil; however, sesamin is naturally present in both crude and refined sesame oils. In addition, change the title to *Absence of sesame oil*.
8. Replace the test for *Free fatty acid* with a test for *Acid value*.
9. Add a test for *Peroxide value*.
10. Add a test for *Unsaponifiable matter*.
11. Add a test for *Specific absorbance*. References to two chapters in this test will become official in a future Supplement or Book (one is a title change and the other is a new chapter).
12. Delete the tests for *Solidification range of fatty acids*, *Iodine value*, and *Saponification value*; they are now unecessary because of the addition of the *Fatty acid composition* test.
13. Add a test for *Water*.
14. Add a test for *Alkaline impurities*.
15. Add a test for *Sterol composition*.

Other changes are editorial in nature.

(EM2: H. Wang) RTS—C70200

Add the following:

▲[*8001-25-0*].▲*NF28*

Change to read:

» Olive Oil is the

▲refined▲*NF28*
fixed oil obtained from the ripe fruit of *Olea europaea* Linné (Fam. Oleaceae). It may contain suitable antioxidants.

Change to read:

Packaging and storage—Preserve in tight,

▲light-resistant, well-filled▲*NF28*
containers, and prevent exposure to excessive heat.

Add the following:

▲**Identification**—It meets the requirements of the test for *Fatty acid composition*.▲*NF28*

Add the following:

▲**Fatty acid composition**—Olive Oil exhibits the following composition profile of fatty acids, as determined under *Fats and Fixed Oils* ⟨401⟩, the section *Fatty Acid Composition*.

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
<16	0	≤0.1
16	0	7.5–20.0
16	1	≤3.5
18	0	0.5–5.0
18	1	56.0–85.0

In-Process Revision

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
18	2	3.5–20.0
18	3	≤1.2
20	0	≤0.7
20	1	≤0.4
22	0	≤0.2
24	0	≤0.2

▲*NF28*

Delete the following:

▲~~**Specific gravity** ⟨841⟩: between 0.910 and 0.915.~~▲*NF28*

Delete the following:

▲~~**Cottonseed oil**—Mix 5 mL in a test tube with 5 mL of a mixture of equal volumes of amyl alcohol and a 1 in 100 solution of sulfur in carbon disulfide, warm the mixture carefully to expel the carbon disulfide, and immerse the test tube to one third of its length in a boiling, saturated solution of sodium chloride for 2 hours: the mixture develops no reddish color.~~▲*NF28*

Delete the following:

▲~~**Peanut oil**—Saponify 10 g by heating for 1 hour under a reflux condenser with 80 mL of alcoholic potassium hydroxide TS. Add phenolphthalein TS, neutralize with 1 N acetic acid, and wash the solution into 120 mL of boiling lead acetate TS contained in a conical flask. Boil the mixture for 1 minute, and cool by immersing the flask in cold water, rotating the contents occasionally to cause the precipitate to adhere to the walls of the flask. Decant the liquid, wash the precipitate with cold water to remove the excess lead acetate, and then wash with 90 percent (by volume) alcohol. Add 100 mL of ether, stopper the flask, and allow to stand until the precipitate is disintegrated. Connect the flask to a reflux condenser, boil for 5 minutes, cool to about 15°, and allow to stand overnight. Filter, and thoroughly wash the precipitate with ether. With the use of a jet of ether, transfer the precipitate to a 500-mL separator, alternating the jet of ether with 3 N hydrochloric acid at the end if any of the precipitate adheres to the filter paper. Add sufficient 3 N hydrochloric acid to make the total acid layer measure about 100 mL, and add sufficient ether to make the ether layer measure about 100 mL. Shake the mixture vigorously for several minutes, allow the layers to separate, draw off the acid layer, and wash the ether once by shaking with 50 mL of 3 N hydrochloric acid and finally with several portions of water until the last washing is not acid to methyl orange TS. Transfer the ether solution to a dry flask, evaporate the ether, add a small amount of dehydrated alcohol, and evaporate on a steam bath to dryness. Dissolve the residue of dry fatty acids by warming with 60 mL of 90 percent (by volume) alcohol, slowly cool the solution to 15° while shaking frequently, and allow the solution to stand at 15° for 30 minutes: no crystals separate from the solution.~~▲*NF28*

Delete the following:

▲~~**Sesame oil**—Mix 10 mL with 10 mL of hydrochloric acid, add 0.1 mL of a 1 in 50 solution of furfural in alcohol, and shake the mixture vigorously for 15 seconds: no pink to crimson color appears in the acid layer when the emulsion breaks. If any color appears in the acid layer, add 10 mL of water, and again shake the mixture vigorously: in the absence of sesame oil any pink color is evanescent.~~▲*NF28*

Delete the following:

▲~~**Teaseed oil**—In a dry, 18- × 150-mm test tube place 0.8 mL of acetic anhydride, 1.5 mL of chloroform, and 0.2 mL of sulfuric acid, mix, and cool in a water bath to 25°. Add about 200 mg of Olive Oil (about 7 drops), mix, and cool to 25°. If the solution is cloudy, add acetic anhydride, dropwise, shaking after each addition, until the solution suddenly clears. Allow the mixture to remain in the water bath for 5 minutes: it shows a green color by both reflected and transmitted light. Add 10 mL of absolute ether, and mix by inverting the tube: the initial green color fades to a brownish gray. (Before the dilution with ether, the presence of *teaseed oil* will cause a brown color to appear by transmitted light, and after the dilution, a transient red color.) [NOTE—A pink color is regarded as negative, because some olive oils yield this color.]~~▲*NF28*

Add the following:

▲**Absence of sesame oil**—Mix 10 mL of Olive Oil with a mixture of 0.5 mL of a 0.35% (v/v) solution of furfural in acetic anhydride and 4.5 mL of acetic anhydride, and shake the mixture for about 1 minute. Pass through a filter paper previously wetted with acetic anhydride. Add 0.2 mL of sulfuric acid to the filtrate: no bluish-green color develops. ▲*NF28*

Delete the following:

▲~~**Solidification range of fatty acids** ⟨401⟩—The dry, mixed fatty acids of it solidify between 17° and 26°.~~▲*NF28*

Delete the following:

▲~~**Free fatty acids** ⟨401⟩—The free fatty acids in 10 g require for neutralization not more than 5.0 mL of 0.10 N sodium hydroxide.~~▲*NF28*

Add the following:

▲**Acid value** ⟨401⟩**:** not more than 0.3.▲*NF28*

Add the following:

▲**Peroxide value** ⟨401⟩**:** not more than 10.0.▲*NF28*

Add the following:

▲**Unsaponifiable matter** ⟨401⟩**:** not more than 1.5%.▲*NF28*

Add the following:

▲**Specific absorbance**—Dissolve 1.0 g of Olive Oil in cyclohexane, and dilute with cyclohexane to 100 mL. Determine the UV-Vis absorbance using a suitable spectro-

photomer (see *Spectroscopy and Light-Scattering* ⟨851⟩ and *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at a wavelength of 270 nm: the absorbance is not more than 1.20.▲*NF28*

Delete the following:

▲~~**Iodine value** ⟨401⟩: between 79 and 88.~~▲*NF28*

Delete the following:

▲~~**Saponification value** ⟨401⟩: between 190 and 195.~~▲*NF28*

Add the following:

▲**Water,** *Method Ic* ⟨921⟩**:** Not more than 0.1%.▲*NF28*

Add the following:

▲**Alkaline impurities**—Mix 10 mL of acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Neutralize the solution to a green color if necessary with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. Add 10 mL of Olive Oil, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow: not more than 0.1 mL of 0.01 N hydrochloric acid is required.▲*NF28*

Add the following:

▲**Sterol composition**—Proceed as directed under *Fats and Fixed Oils* ⟨401⟩, the section *Sterol Composition*: Olive Oil exhibits the following composition profiles of sterols.

Component	Percentage (%)
Cholesterol	≤0.5
Campesterol	≤4.0
Δ7-Stigmasterol	≤0.5
Sum of the contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol, and Δ5,24-stigmastadienol	≥93.0

The content of stigmasterol is not greater than that of campesterol.▲*NF28*

BRIEFING

Polyoxyl 15 Hydroxystearate. Because there is no existing *NF* monograph for this excipient, a new monograph, based on validated methods, as well as data and comments received, is being proposed. The liquid chromatographic procedure in the test for *Free polyethylene glycols* is based on analysis performed with the Waters Ultrahydrogel 120 brand of column that contains 6-µm packing L25. The typical retention time for polyethylene glycol 1000 is about 7 minutes.

(EM2: H. Wang; NOM: A. Wilk) RTS—C66478

Add the following:

▲Polyoxyl 15 Hydroxystearate

12-Hydroxyoctadecanoic acid polymer with α-hydro-ω-hydroxypoly(oxy-1,2-ethanediyl).

Polyethylene glycol 15 hydroxystearate [*70142-34-6*].

» Polyoxyl 15 Hydroxystearate results from the reaction of about 15 moles of ethylene oxide with 1 mole of 12-hydroxystearic acid. The product consists mainly of 12-hydroxystearic acid polyethoxylated at both the carboxyl and the hydroxyl groups with polyethylene glycol. It contains free polyethylene glycols.

Packaging and storage—Preserve in tight containers at a temperature below 25°.

USP Reference standards ⟨11⟩—*USP 12-Hydroxystearic Acid RS. USP Polyethylene Glycol 1000 RS. USP Polyoxyl 15 Hydroxystearate RS.*

Identification—

A: *Infrared Absorption* ⟨197F⟩—If the sample is solid or too viscous for thin film formation, the sample should be gently warmed to achieve a mobile liquid, which may then be used to prepare the thin film.

In-Process Revision

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution—To 1.0 g of Polyoxyl 15 Hydroxystearate add 100 mL of a 100 mg per mL solution of potassium hydroxide, and boil under a reflux condenser for 30 minutes. Acidify the warm solution with 20 mL of hydrochloric acid, and cool to room temperature. Shake the mixture with 50 mL of ether, and allow to stand until a separation of the layers is visible. Separate the clear upper layer, add 5 g of anhydrous sodium sulfate, wait for 30 minutes, filter, and evaporate to dryness on a water bath. Dissolve 50 mg of the residue in 25 mL of ether.

Standard solution—Dissolve 50 mg of USP 12-Hydroxystearic Acid RS in 25 mL of methylene chloride.

Plate: Octadecylsilyl silica gel for chromatography as the coating substance.

Application volume: 2 µL.

Developing solvent system: acetone–glacial acetic acid–methylene chloride (50 : 40 : 10 v/v/v).

Spray reagent—Prepare a solution of 80 mg per mL of phosphomolybdic acid in 2-propanol.

Procedure—Proceed as directed in the chapter. Develop over two-thirds of the plate, and dry in a current of cold air. Then spray the plate with *Spray reagent*, heat the plate at 120° for 1 to 2 minutes, and locate the spots on the plate: the R_F value and color of the principal spot in the chromatogram obtained from the *Test solution* correspond to those obtained from the *Standard solution*.

C: It meets the requirements of the test for *Free polyethylene glycols*.

Acid value ⟨401⟩**:** not more than 1.0, determined on 2.0 g.

Hydroxyl value ⟨401⟩**:** between 90 and 110.

Iodine value, *Method I* ⟨401⟩**:** not more than 2.0.

Peroxide value ⟨401⟩**:** not more than 5.0.

Saponification value ⟨401⟩**:** between 53 and 63.

Total ash ⟨561⟩**:** not more than 0.3%, determined on 1.0 g.

Water, *Method Ia* ⟨921⟩**:** not more than 1.0%, determined on 2.0 g.

Limit of nickel—

Caution—When using closed high-pressure digestion vessels and microwave laboratory equipment, the safety precautions and operating instructions given by the manufacturer must be followed.

[NOTE—If an alternative apparatus is used, adjustment of the instrument parameters may be necessary.]

Nickel standard stock solution—Dilute nickel standard solution TS two-fold with water. This solution contains the equivalent of 5 µg of nickel per mL.

Standard solutions—Transfer 25 µL, 50 µL, 75 µL, and 100 µL of *Nickel standard stock solution* to four identical 25-mL volumetric flasks. To each flask, add 0.5 mL of a 10 mg per mL solution of magnesium nitrate, 0.5 mL of a 100 mg per mL solution of monobasic ammonium phosphate and 6.0 mL of nickel-free nitric acid, dilute with water to volume, and mix well. [NOTE—Content of nickel in the nickel-free nitric acid is not more than 0.005 ppm.] The *Standard solutions* contain, respectively, 0.005 µg per mL, 0.01 µg per mL, 0.015 µg per mL, and 0.02 µg per mL of nickel.

Test solution—Transfer about 250 mg of Polyoxyl 15 Hydroxystearate, accurately weighed, to a suitable high-pressure-resistant digestion vessel (fluoropolymer or quartz glass), and add 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide. Place the closed vessel in a laboratory microwave oven, and digest using an appropriate program, e.g., 1000 W for 40 minutes. Allow the digestion vessel to cool down before opening. Add 2.0 mL of 30% hydrogen peroxide, and repeat the digestion step. Allow the

digestion vessel to cool down before opening. Quantitatively transfer to a 25-mL volumetric flask, add 0.5 mL of a 10 mg per mL solution of magnesium nitrate and 0.5 mL of a 100 mg per mL solution of monobasic ammonium phosphate, dilute with water to volume, and mix well.

Blank solution—Place 6.0 mL of nickel-free nitric acid and 2.0 mL of 30% hydrogen peroxide in a suitable high-pressure-resistant digestion vessel. Proceed as directed under *Test solution*, beginning with "Place the closed vessel in a laboratory microwave oven, and digest using an appropriate program, e.g., 1000 W for 40 minutes".

Zero solution—In a 50-mL volumetric flask, introduce 1.0 mL of a 10 mg per mL solution of magnesium nitrate, 1.0 mL of a 100 mg per mL solution of monobasic ammonium phosphate, and 12.0 mL of nickel-free nitric acid. Dilute with water to volume, and mix well.

Procedure—Concomitantly determine the absorbances of the *Blank solution, Standard solutions,* and the *Test solution* at the nickel emission line of 232.0 nm, using a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩), equipped with a background compensation system, a coated tube resistant to pyrolysis, and a nickel hollow-cathode lamp. Maintain the drying temperature of the furnace at 120° for 35 seconds after a 5-second ramp; maintain the ashing temperature at 1100° for 10 seconds after a 30-second ramp; maintain the cooling temperature at 800° for 5 seconds after a 5-second decrease; and maintain the atomization temperature at 2600° for 7 seconds. [NOTE—The temperature program may be modified to obtain optimum furnace temperatures.] Use the *Zero solution* to set the instrument to zero. Plot the absorbances of the *Standard solutions* versus concentration, in µg per mL, of nickel, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, C_T, in µg per mL, of nickel in the *Test solution*, and determine the concentration, C_B, in µg per mL, of nickel in the *Blank solution*. If necessary, dilute with the *Zero solution* to obtain a reading within the calibrated absorbance range. Calculate the quantity, in µg, of nickel in each g of Polyoxyl 15 Hydroxystearate taken by the formula:

$$25(C_T - C_B) / W$$

in which 25 is the volume, in mL, of the *Test solution* and the *Blank solution*; and W is the weight, in g, of Polyoxyl 15 Hydroxystearate taken to prepare the *Test solution*: not more than 1 µg of nickel per g is found.

Limit of free ethylene oxide and dioxane—

Caution—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate at a temperature between 4° and 8°.

[NOTE—Before using the polyethylene glycol 200 in this test, remove any volatile components from it by placing 500 mL of polyethylene glycol 200 in a 1000-mL round-bottom flask, attaching the flask to a rotary evaporator maintained at a temperature of 60° and under a vacuum of 10–20 mm Hg for 6 hours.]

Acetaldehyde solution—Prepare a solution of acetaldehyde in water, containing a known concentration of about 10 µg per mL. [NOTE—Prepare the *Acetaldehyde solution* immediately prior to use.]

Ethylene oxide stock solution—Fill a chilled pressure bottle with liquid ethylene oxide, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Tare a glass-stoppered conical flask, add about 50 mL of polyethylene glycol 200, and reweigh the flask. Transfer about 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in a mixture of sodium chloride and ice (1 : 3). Using a gas-tight syringe that has been previously cooled to –10°, transfer about 300 µL (corresponding to about 250 mg) of liquid ethylene oxide to the polyethylene glycol 200, and swirl gently to mix.

Replace the stopper, reweigh the flask, and determine the amount of ethylene oxide absorbed by weight difference. Adjust the weight of the mixture with polyethylene glycol 200 to 100.0 g, replace the stopper, and swirl gently to mix. This stock solution contains about 2.5 mg of ethylene oxide per g. [NOTE—Prepare this stock solution immediately prior to use, and store in a refrigerator.]

Ethylene oxide solution—Tare a glass-stoppered conical flask, and chill it in a refrigerator. Add about 35 mL of polyethylene glycol 200, and reweigh the flask. Using a gas-tight gas chromatographic syringe that has been chilled in a refrigerator, transfer about 1 g of the chilled *Ethylene oxide stock solution,* accurately weighed, to the tared, conical flask. Adjust the weight of the solution with polyethylene glycol 200 to 50.0 g, replace the stopper, and swirl gently to mix. Transfer about 10 g of this solution, accurately weighed, to a 50-mL volumetric flask. Add 30 mL of water, and mix. Dilute with water to volume, and mix to obtain a solution containing about 10 µg of ethylene oxide per mL. [NOTE—Prepare this solution immediately prior to use, and use directly after preparation.]

Dioxane solution—Dissolve about 1.0 g of dioxane, accurately weighed, in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 500 µg of dioxane per mL.

Standard solution A—Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial. [NOTE—Other sizes may be used depending on the operating conditions, however, the same size must be used for *Standard solution A*, *Standard solution B,* and the *Test solution*.] Add 0.1 mL of *Acetaldehyde solution* and 0.1 mL of *Dioxane solution,* seal the vial, and mix.

Standard solution B—Transfer about 1.0 g of Polyoxyl 15 Hydroxystearate, accurately weighed, to another 10-mL pressure headspace vial, add 0.1 mL of *Ethylene oxide solution,* 0.1 mL of *Dioxane solution,* and 1.0 mL of *N,N*-dimethylacetamide. Seal the vial, and mix.

Test solution—Transfer about 1.0 g of Polyoxyl 15 Hydroxystearate, accurately weighed, to a 10-mL pressure headspace vial, add 1.0 mL of *N,N*-dimethylacetamide and 0.2 mL of water, seal the vial, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—[NOTE—The use of a headspace apparatus that automatically transfers a measured amount of headspace is allowed.] The gas chromatograph is equipped with a flame-ionization detector, maintained at about 250°, and a 0.32-mm × 30-m glass or quartz capillary column bonded with a 1.0-µm layer of phase G1. The injection port is equipped with a split injection port with a split ratio of 20 : 1, and the temperature is maintained at about 150°. The column temperature is maintained at 50° for 5 minutes after injection, then programmed to increase at the rate of 5° per minute to 180°, then at the rate of 30° per minute to 230°, and then maintained at 230° for 5 minutes. Each vial is heated at a temperature of 90° for 45 minutes, before a suitable portion of its headspace is injected. The carrier gas is helium flowing with a linear velocity of about 20 cm per second. [NOTE—If the headspace apparatus is used, then an injection time of 12 seconds and a transfer line temperature of 150° are recommended.] Chromatograph the gaseous phase of *Standard solution A,* and record the peak responses as directed for *Procedure*: the relative retention times are about 0.94 for acetaldehyde and 1.0 for ethylene oxide; the resolution, *R,* between acetaldehyde and ethylene oxide is not less than 2.0; the dioxane peak is detected with a signal-to-noise ratio of not less than 5; and the relative standard deviation for replicate injections is not more than 15%.

Procedure—Using a heated, gas-tight, gas chromatographic syringe, separately inject equal volumes (about 1 mL) of the gaseous headspace of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks: the mean areas of the ethylene oxide and dioxane peaks in the

chromatogram obtained from the *Test solution* are not greater than half the mean areas of the corresponding peaks in the chromatogram obtained from *Standard solution B*.

Calculate the concentration of ethylene oxide, in µg per g, in the portion of Polyoxyl 15 Hydroxystearate taken by the formula:

$$A_E\, r_U / [(r_S W_U) - (r_U / W_S)]$$

in which A_E is the quantity, in µg, of ethylene oxide added to *Standard solution B;* r_U and r_S are the ethylene oxide peak responses obtained from the *Test solution* and *Standard solution B,* respectively; and W_U and W_S are the weights, in g, of the substance under test taken to prepare the *Test solution* and *Standard solution B,* respectively: not more than 1 µg per g is found.

Calculate the concentration of dioxane, in µg per g, in the portion of Polyoxyl 15 Hydroxystearate taken by the formula:

$$A_D\, r_U / [(r_S W_U) - (r_U / W_S)]$$

in which A_D is the amount, in µg, of dioxane added to *Standard solution B;* r_U and r_S are the dioxane peak responses obtained from the *Test solution* and *Standard solution B,* respectively; and W_U and W_S are as defined above: not more than 50 µg per g is found.

Free polyethylene glycols—

Mobile phase: A mixture of methanol and water (8 : 2 v/v).

Standard solution A—Prepare a solution of USP Polyethylene Glycol 1000 RS in *Mobile phase,* containing a known concentration of about 1.6 mg per mL.

Standard solution B—Dilute 50.0 mL of *Standard solution A* with *Mobile phase* to 100.0 mL. The solution contains a known concentration of about 0.8 mg per mL.

Test solution—Transfer about 1.2 g of Polyoxyl 15 Hydroxystearate, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix well.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector, two 4-mm × 12.5-cm precolumns that contain 5-µm packing L1 and a 10-nm pore size, and a 7.8-mm × 30-cm analytical column that contains 6-µm packing L25 and a 12-nm pore size. The flow rate is about 1.1 mL per minute. The column and detector temperatures are maintained at room temperature. Connect both precolumns to the analytical column using a 3-way valve, and switch the *Mobile phase* flow according to the following program. [NOTE—Shown in *Figure 1*, the analysis is started with precolumn 2 and an analytical column in series. After about 114 seconds, the valves, controlled by the detector program, switch over such that the eluent flows past precolumn 2, and direct to precolumn 1 and the analytical column. The columns are switched when the components to be determined, but not the interfering matrix, are ready to reach the analytical column. Simultaneously, precolumn 2 is washed out in the reverse direction by a second pump to remove the unwanted matrix components.]

Time (seconds)	Program
0–114	Precolumn 2 and analytical column
115–end	Precolumn 1 and analytical column
115–420	Reverse flow of precolumn 2

Chromatograph *Standard solution A,* and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Inject a volume (about 50 µL) of *Standard solution A*, *Standard solution B,* and the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of polyethylene glycols in the portion of Polyoxyl 15 Hydroxystearate taken by the formula:

$$100(V)(2C_S / W)[r_U / (r_{S1} + 2r_{S2})]$$

In-Process Revision

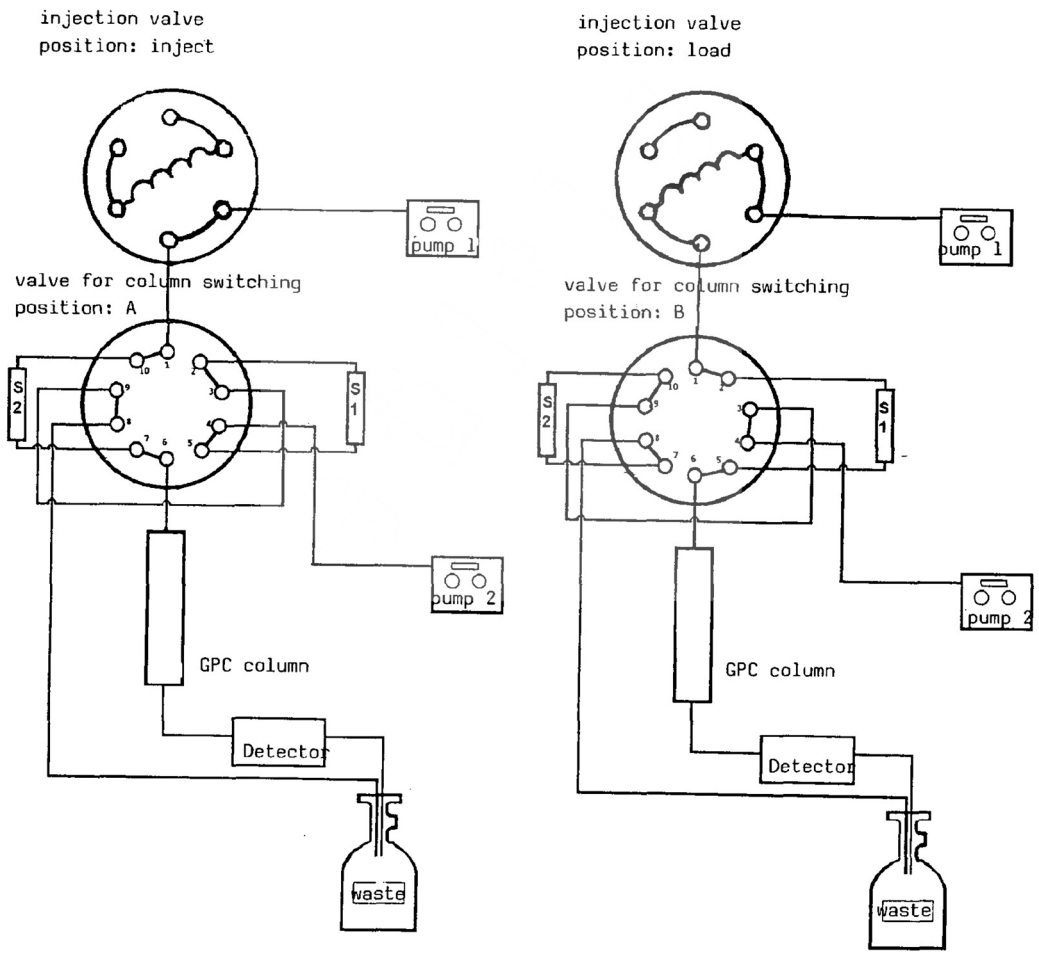


Figure 1. Apparatus

in which V is the volume, in mL, of the *Test solution;* C_S is the concentration, in mg per mL, of USP Polyethylene Glycol 1000 RS in *Standard solution A;* W is the weight, in mg, of Polyoxyl 15 Hydroxystearate taken to prepare the *Test solution*; r_U is the polyethylene glycol peak response obtained from the *Test solution;* and r_{S1} and r_{S2} are the polyethylene glycol 1000 peak responses obtained from *Standard solution A* and *Standard solution B,* respectively: between 27.0% and 39.0% of free polyethylene glycols is found.▲*NF28*

In-Process Revision

BRIEFING

Polyvinyl Acetate Dispersion. On the basis of data and comments received, a new *NF* monograph is being proposed based on the validated methods. The liquid chromatographic procedure in the test for *Limit of vinyl acetate* employs the Thermo Aquasil brand of column that contains 5-µm packing L1. The typical retention times for vinyl acetate and 1-vinylpyrrolidin-2-one are 23.0 and 28.5 minutes, respectively. The liquid chromatographic procedure in the test for *Limit of acetic acid/acetate* is based on analysis performed with the Thermo Aquasil brand of column that contains 5-µm packing L1. The typical retention times for acetic acid and citric acid are 6.0 and 7.4 minutes, respectively.

(EM2: H. Wang; NOM: A. Wilk; MSA: R. Tirumalai) RTS—C57716

Add the following:

▲Polyvinyl Acetate Dispersion

» Dispersion of polyvinyl acetate in water. It contains 25.0 percent to 30.0 percent of polyvinyl acetate. It may contain suitable surface active agents and stabilizers.

Packaging and storage—Preserve in tight containers at a temperature below 25°. Protect from freezing.

Labeling—Label it to indicate the names and quantities of any added surface active agents and stabilizers.

USP Reference standards ⟨11⟩—*USP Polyvinyl Acetate Dispersion RS.*

Identification—

A: Place one drop of sample on a glass plate and allow to dry. A clear and homogeneous film is formed.

B: Place one drop of the Dispersion on a glass plate, and cover the test substance with a water-resistant crystal disk (silver chloride or KRS-5[1]). Gently press on, and then remove the crystal disk. Dry the crystal disk in a drying chamber until a homogeneous film is formed: the IR absorption spectrum of the film so formed exhibits maxima corresponding to the same wavelengths as those of a similar preparation of USP Polyvinyl Acetate Dispersion RS treated in the same manner.

Microbial limits ⟨61⟩—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g.

pH ⟨791⟩**:** between 3.0 and 5.5.

Loss on drying ⟨731⟩—Dry 1.0 g of the Dispersion at 110° for 5 hours: it loses between 68.5% and 71.5% of its weight.

Residue on ignition ⟨281⟩—not more than 0.5%, determined on 1.0 g. Heat a silica crucible to redness for 30 minutes, allow to cool in a desiccator, and weigh. Evenly distribute 1.0 g of Polyvinyl Acetate Dispersion in the crucible and weigh. Dry the crucible at 100° to 105° for 1 hour and ignite in a muffle furnace at 600 ± 25°, until the test substance is thoroughly charred. Continue the experiment as directed under *Residue on Ignition* ⟨281⟩ on the residue obtained, beginning with "Moisten the sample with a small amount (usually 1 mL) of sulfuric acid . . ."

Coagulum content—Accurately weigh a stainless steel sieve having 45-µm openings or a suitable single-woven wire cloth with a mesh width of 45 µm, and filter 100 g of the Dispersion through it. [NOTE—Suitable single-woven wire cloth mesh meets the requirements set in ISO 9044.] Wash the sieve or the cloth with distilled water until a clear filtrate is obtained, and dry the sieve or the cloth to constant weight at 100° to 105°: the weight of the residue does not exceed 500 mg (0.5%).

Stabilizers or surface active agents—

POVIDONE—Perform this test only if the dispersion contains this component.

Perform nitrogen determination by sulfuric acid digestion on 0.25 g as directed under *Nitrogen Determination* ⟨461⟩*, Method II.* Calculate the percentage content of povidone using the following formula:

$$N / 0.126$$

[1] KRS-5 consists of 42% thallium(I) bromide and 58% thallium(I) iodine by molecular weight. Suitable disks of silver chloride and of KRS-5 are available from www.photonic.saint-gobain.com, www.almazoptics.com, and www.internationalcrystal.net.

In-Process Revision

in which *N* is the percentage content of nitrogen, and 0.126 is the percentage content, expressed as a decimal number, of nitrogen in vinylpyrrolidone. The content of povidone is not more than 4.0%.

Limit of vinyl acetate—

Mobile phase A: A mixture of water, acetonitrile, methanol (90 : 5 : 5 v/v/v).

Mobile phase B: A mixture of water, acetonitrile, methanol (50 : 45 : 5 v/v/v).

Standard solution—Transfer 50 mg of vinyl acetate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix well. Dilute 5.0 mL of the solution with *Mobile phase A* to 100 mL. Dilute 10.0 mL of this solution with *Mobile phase A* to 100 mL. The *Standard solution* contains about 2.5 µg of vinyl acetate per mL. [NOTE—This solution should be analyzed within 1 hour when store at room temperature.]

System suitability solution—Transfer 50 mg of vinyl acetate and 50 mg of 1-vinylpyrrolidin-2-one to a 50-mL volumetric flask, add 10 mL of methanol, sonicate or gently shake the flask to dissolve the materials. Dilute with *Mobile phase A* to volume. Dilute 10 mL of this solution with *Mobile phase A* to 100 mL. Dilute 5 mL of this solution with *Mobile phase A* to 100 mL. The *System suitability solution* contains about 5 µg per mL each of vinyl acetate and 1-vinylpyrrolidin-2-one.

Test solution—Transfer about 250 mg of the Dispersion, accurately weighed, to a 10-mL volumetric flask, add about 4 mL of methanol, and sonicate. After cooling to ambient temperature, dilute with water to volume, and mix. Centrifuge at 4000 *g* for 10 minutes, and pass through a 0.2-mm membrane filter. [NOTE—This solution should be analyzed within 1 hour when store at room temperature.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector, and a 4.0-mm × 25-cm analytical column that contains 5-µm packing L1. The column is maintained at a constant temperature of about 30°. A 4.0-mm × 3-cm pre-column that contains 5-µm packing L1 may be used if a matrix effect is observed. [NOTE—The matrix effect may result in poor reproducibility of the retention times and of the peak shapes.] The flow rate is about 1.0 mL per minute. The gradient elution is programmed as follows.

Time (minutes)	*Mobile phase A* (% v/v)	*Mobile phase B* (% v/v)
0–2	100	0
2–40	100→85	0→15
40–42	85→0	15→100
42–48	0	100
48–51	0→100	100→0

Chromatograph the *System suitability solution,* and identify the components based on their relative retention times, which are 1.0 and 1.2 for vinyl acetate and 1-vinylpyrrolidin-2-one, respectively. Record the peak responses as directed for *Procedure*: the resolution, *R,* between vinyl acetate and 1-vinylpyrrolidin-2-one is not less than 5.0; and the relative standard deviation for replicate injections determined from the 1-vinylpyrrolidin-2-one peak is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution,* the *System suitability solution,* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the vinyl acetate peaks. The response of the vinyl acetate peak obtained from the *Test solution* is not more than that of the vinyl acetate peak obtained from the *Standard solution*: not more than 100 ppm is found.

Limit of acetic acid/acetate—

Mobile phase—5 mM sulfuric acid.

Standard solution—Dissolve accurately weighed quantities of acetic acid and citric acid in *Mobile phase* to make a solution having known concentrations of 0.3 mg per mL for each of acetic acid and citric acid.

Test solution—Transfer 200 mg of the Dispersion, accurately weighed, to a 10-mL volumetric flask, add about 8 mL of water, and sonicate for about 10 minutes. Cool to ambient temperature, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm anaytical column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. After each injection, rinse the column with a mixture of equal volumes of *Mobile phase* and acetonitrile. Chromatograph the *Standard solution,* and identify the compounds based on their relative retention times, which are 1.0 and 1.2 for acetic acid and citric acid, respectively. Record the peak responses as directed for *Procedure*: the resolution, *R,* between acetic acid and citric acid is not less than 2.0; and the relative standard deviation for replicate injections determined from the acetic acid peak is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the acetic acid peaks. The response of the acetic acid peak obtained from the *Test solution* is not more than that of the acetic acid peak obtained from the *Standard solution*: not more than 1.5% of acetic acid is found.

Assay—Transfer about 1.5 g of the Dispersion, accurately weighed, to a 250-mL borosilicate glass flask fitted with a reflux condenser. Add 25.0 mL of 0.5 M alcoholic potassium hydroxide and a few glass beads. Attach the condenser and heat under reflux for 30 minutes. Add 1 mL of phenolphthalein TS, and titrate immediately (while still hot) with 0.5 N hydrochloric acid VS. Perform a blank determination under the same conditions (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Calculate the saponification value, I_S, by the formula:

$$[56.11(V_B - V_T)N] / W$$

in which 56.11 is the molecular weight of potassium hydroxide; V_B and V_T are the volumes, in mL, of 0.5 N hydrochloric acid consumed in the blank test and in the actual test, respectively; N is the exact normality of the hydrochloric acid; and W is the weight, in g, of the Dispersion taken for the test.

Calculate the percentage content of polyvinyl acetate using the following formula:

$$100(10^{-3})[(86.09)I_S] / 56.11$$

in which 10^{-3} is a factor converting mg to g; and 86.09 and 56.11 are the molecular weights of vinyl acetate and potassium hydroxide, respectively.▲*NF28*

BRIEFING

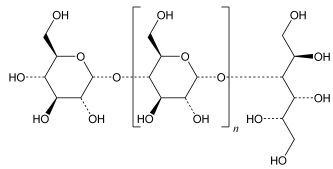
Hydrogenated Starch Hydrolysate. The proposed new monograph for Hydrogenated Starch Hydrolysate, which appeared on page 488 of *PF* 33(3) [May–June 2007], was subsequently canceled. The previous proposal only covered a very limited range of products. On the basis of comments and data received and to replace that monograph, a new monograph for Hydrogenated Starch Hydrolysate is now being proposed. The liquid chromatographic procedures in the tests for *Content of maltitol and sorbitol* and *Hydrogenated polysaccharides* are based on analyses performed with the Polymer Laboratories Hi-Plex brand of L58 column. The typical retention times for maltose, maltitol, dextrose, and sorbitol are 29, 30, 34, and 36 minutes, respectively.

(EM2: H. Wang; MSA: R. Tirumalai) RTS—C57641

Add the following:

▲Hydrogenated Starch Hydrolysate

Hydrogenated Polysaccharides



$C_{12}H_{24}O_{11}(C_6H_{10}O_5)_n$

Polyglucitol.

Polyglycitol syrup [*68425-17-2*].

» Hydrogenated Starch Hydrolysate is a mixture that contains not less than 50% of hydrogenated polysaccharides containing more than 3 D-gluco-

In-Process Revision

pyranosyl units terminated with D-glucityl unit, calculated on the anhydrous basis. Other ingredients can comprise sorbitol, maltitol or other sugar polyols.

Packaging and storage—Preserve in well-closed containers. No storage requirements specified for liquid product; store in a cool and dry place for dried powder product.

Labeling—Label it to indicate *Water* content.

USP Reference standards ⟨11⟩—*USP Dextrose RS. USP Maltitol RS. USP Maltose Monohydrate RS. USP Sorbitol RS.*

Identification—

A: It meets the requirements of the test for *Content of maltitol and sorbitol*.

B: It meets the requirements of the test for *Hydrogenated polysaccharides*.

Microbial limits ⟨61⟩—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*

pH ⟨791⟩**:** between 3.0 and 7.0, in a 20% (w/w) solution in freshly boiled and cooled water.

Water, *Method I* ⟨921⟩**:** not less than 90% and not more than 110% of the value stated on the label.

Residue on ignition ⟨281⟩: not more than 0.15%, ignition of a quantity of Hydrogenated Starch Hydrolysate, equivalent to 1.0 g of solid on the anhydrous basis.

Reducing sugars—Dissolve a quantity of Hydrogenated Starch Hydrolysate, equivalent to 1.0 g on the anhydrous basis, in 6 mL of water with the aid of gentle heat, if necessary. Cool, and add 20.0 mL of cupric citrate TS and a few glass beads. Heat so that boiling begins after 4 minutes, and maintain boiling for 3 minutes. Cool rapidly, and add 40 mL of diluted acetic acid, 60 mL of water, and 20.0 mL of 0.05 N iodine VS. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess of iodine with 0.05 N sodium thiosulfate VS using 2 mL of starch TS as an indicator, added towards the end of the titration. Not less than 12.8 mL of 0.05 N sodium thiosulfate is required, corresponding to not more than 1% of reducing sugars.

Limit of chloride—

Test solution—Transfer a quantity of Hydrogenated Starch Hydrolysate equivalent to 25 g on the anhydrous basis, to a beaker, add 100 mL of water, and stir until the Hydrogenated Starch Hydrolysate is completely dissolved.

Procedure—Add 1.0 mL of potassium chromate indicator solution (1 in 20) into the *Test solution.* Slowly titrate with 0.1 N silver nitrate VS until a reddish-orange color persists. Calculate the quantity, in µg, of chloride in each g of Hydrogenated Starch Hydrolysate taken by the formula:

$$[(10^3)(35.45)NV] / W$$

in which 10^3 is a factor converting mg to µg; 35.45 is the molar mass of chloride; N is the exact normality of the silver nitrate solution; V is the volume, in mL, of the silver nitrate solution consumed in the titration; and W is the weight, in g, of Hydrogenated Starch Hydrolysate taken to prepare the *Test solution:* not more than 50 µg of chloride per g is found.

Limit of sulfate ⟨221⟩—1.0 g of the solid portion of Hydrogenated Starch Hydrolysate shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid: not more than 100 µg of sulfate per g is found.

Limit of nickel—

[NOTE—When water is specified as the diluent, use deionized ultra-filtered water.]

Digester solution (aqua regia)—Add 360 mL of hydrochloric acid and 240 mL of nitric acid to 1200 mL of water.

Blank solution—Add 40 mL of nitric acid to a 2-L volumetric flask, dilute with water to volume, and mix well.

Internal standard solution—Transfer 2.0 mL of commercially prepared yttrium reference standard solution (1000 ppm) to a 1-L volumetric flask, dilute with *Blank solution* to volume, and mix well. The *Internal standard solution* contains 2 µg per mL of ytrrium.

Standard stock solution—[NOTE—Prepare this solution fresh every two months.] Quantitatively dilute an accurately measured volume of a commercially prepared nickel ICP standard (1000 ppm) with *Blank solution* to obtain a solution containing 10 µg of nickel per mL *(Standard stock solution 10 ppm)*.

Standard solutions—[NOTE—Prepare these solutions fresh weekly.] Separately pipet 1.0 mL, 2.0 mL, and 4.0 mL *Standard stock solution,* respectively, into three 200-mL volumetric flasks. Dilute the content in each flask with *Blank solution* to volume, and mix well. These are, respectively, the *Standard nickel solution 50 ppb*, *Standard stock solution 100 ppb,* and *Standard nickel solution 200 ppb*.

Test solution—Transfer a quantity of Hydrogenated Starch Hydrolysate, equivalent to 10.0 g on the anhydrous basis, into a 125-mL conical flask. Add 40 mL of *Digester solution* and place on a hotplate. Heat the solution for about 20 minutes, being careful to prevent the solution from boiling over. The solution will turn a dark caramel color. Transfer into a clean, dry, 50-mL volumetric flask with washings of *Blank solution*. Dilute with *Blank solution* to volume. Filter the sample into a 15-mL centrifuge tube, using a 10-mL BD syringe, fitted with a 0.45-µm syringe filter.

Procedure (see *Plasma Spectrochemistry* ⟨730⟩)—The Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) is configured in an axial optical alignment. Set the ultraviolet detector to scan nickel at 232.005 nm and yttrium at 371.029 nm, and set the sample read time to 10 seconds minimum and 50 seconds maximum. Three replicate scans are taken with the integration set to one point per peak. Set forward power from the RF generator to 1500 watts. The argon plasma feed gas flows at 15 L per minute with the auxiliary gas (shear gas) set to flow at 0.5 L per minute. A gem cone nebulizer is used with a nebulization gas flow rate of 0.55 L per minute. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 1.00 mL per minute. The *Internal standard solution* is added in-line via a mixing block between the sample probe and spray chamber. Samples are flushed through the system for 30 seconds at a rate of 4.0 mL per minute prior to analysis. A 60-second read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples the pumping system is washed by flushing the *Blank solution* for 30 seconds at a rate of 4.0 mL per minute.

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check.

Generate the calibration curve using the *Blank solution, Standard nickel solution 50 ppb, Standard stock solution 100 ppb,* and *Standard nickel solution 200 ppb* as follows. Scan the *Internal standard solution* while running the *Blank solution* to measure the intensity of the yttrium emission. This value is held constant throughout the remainder of the test. Separately scan the *Blank solution, Standard nickel solution 50 ppb, Standard stock solution 100 ppb,* and the *Standard nickel solution 200 ppb* for nickel and yttrium. [NOTE—The *Internal standard solution* is added via an in-line mixing chamber.] Normalize the yttrium intensity to the value of the *Internal standard solution*. Apply this normalization factor to the nickel intensity, which is then referred to as the corrected nickel intensity. A calibration curve is constructed by plotting the corrected nickel intensity versus the known concentration, in ng per mL, of the nickel: the linear regression coefficient is not less than 0.999.

Similarly, analyze the *Test solution* on the ICP. The intensity of the emission of the *Test solution* is plotted on the calibration curve and the concentration, *C*, in ng per mL,

In-Process Revision

is thus obtained. Calculate the content, in µg per g, of nickel in the solid portion of Hydrogenated Starch Hydrolysate taken by the formula:

$$10^{-3}(50)(C/W)$$

in which 10^{-3} is a factor converting ng to µg; 50 is the volume, in mL, of the *Test solution*; *W* is the weight, in g, of Hydrogenated Starch Hydrolysate calculated on anhydrous basis: the nickel content is not more than 1 µg per g.

Content of maltitol and sorbitol—

Mobile phase—Use degassed water.

Standard solution—Dissolve accurately weighed quantities of USP Maltose Monohydrate RS, USP Maltitol RS, USP Dextrose RS, and USP Sorbitol RS in water to obtain a solution having known concentrations of about 1.0 mg per mL for each, calculated on the anhydrous basis.

Test solution—Transfer a quantity of Hydrogenated Starch Hydrolysate, equivalent to 100 mg on the anhydrous basis, to a 100-mL volumetric flask. Dilute with water to volume, and mix. Transfer approximately 10 mL of the solution into a separate container, shake the solution for 30 seconds, and pass through a filter having a 0.45-µm or finer porosity into a suitable autosampler vial, and seal.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector that is maintained at 40° and a 7.7-mm × 30-cm column that contains packing L58. The column temperature is maintained at 80°, controlled within ±2°, and the flow rate is about 0.3 mL per minute. Chromatograph the *Standard solution,* and identify the components based on their relative retention times which are 0.81, 0.84, 0.94, and 1.00 for maltose, maltitol, dextrose, and sorbitol, respectively. Sorbitol is the last peak to elute on the chromatogram. Record the peak responses as directed for *Procedure:* for the maltitol peak, the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of each component, maltose (P_{M1}), maltitol (P_{M2}), dextrose (P_D), and sorbitol (P_S), in the solid portion of Hydrogenated Starch Hydrolysate taken by the formula:

$$100(100)(C/W)/(r_U/r_S)$$

in which 100 is the volume, in mL, of the *Test solution; C* is the concentration, in mg per mL, of the respective component in the *Standard solution; W* is the weight, in mg, of Hydrogenated Starch Hydrolysate calculated on the anhydrous basis, which was taken to prepare the *Test solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively: less than 50% of total maltitol and sorbitol are found and less than 1% of total maltose and dextrose are found, on the anhydrous basis.

Hydrogenated polysaccharides—

Mobile phase, Standard solution, and *Chromatographic system*—Prepare as directed in the test for *Content of maltitol and sorbitol*.

Test solution—Prepare as directed in the test for *Content of maltitol and sorbitol*.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. The elution pattern includes the higher-molecular-weight hydrogenated polysaccharides containing more than 11 D-glucopyranosyl units, followed by some individual peaks representing hydrogenated polysaccharides containing not more than 10 D-glucopyranosyl units, if this hydrogenated species is present. The higher-molecular-weight hydrogenated polysaccharides containing more than 11 D-glucopyranosyl can be intergrated into one peak, the relative retention time is about 0.29 in relative to the peak of

sorbitol. The relative retention times for hydrogenated polysaccharides containing not more than 10 D-glucopyranosyl units are given in *Table 1*.

Table 1. Relative Retention Times for Several Hydrogenated Species

Hydrogenated Species for DP (Degree of Polymerization)	Relative Retention Time
Sorbitol (HDP 1)	1.00
Maltitol (HDP 2)	0.84
Maltotriitol (HDP 3)	0.72
Maltotetraitol (HDP 4)	0.64
Maltopentaitol (HDP 5)	0.58
Maltohexaitol (HDP 6)	0.53
Maltoheptaitol (HDP7)	0.48
Maltooctaitol (HDP8)	0.45
Maltononaitol (HDP9)	0.42
Maltodecaitol (HDP10)	0.40

Calculate the percentage of hydrogenated polysaccharides containing more than 3 D-glucopyranosyl units in the solid portion of Hydrogenated Starch Hydrolysate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the sum of peak areas of hydrogenated polysaccharides containing more than 3 D-glucopyranosyl units obtained from the *Test solution*; and r_s is the sum of all the peak areas obtained from the *Test solution*: not less than 50% of hydrogenated polysaccharides containing more than 3 D-glucopyranosyl units are found, on the anhydrous basis.▲*NF28*

In-Process Revision

BRIEFING

Pea Starch. Because there is no existing *NF* monograph for this excipient, a new monograph, based on validated methods, is proposed.

(EM2: H. Wang; NOM: A. Wilk; MSA: R. Tirumalai) RTS—C68459

Add the following:

▲Pea Starch

» Pea Starch is obtained from the seeds of *Pisum sativum L.*

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

Identification—

A: Examined under a microscope using a mixture of equal volumes of glycerin and water, it presents a majority of large elliptical granules, 25–45 µm in size, sometimes irregular, or reniform. It also presents a minority of small rounded granules, 5–8 µm in size. Granules can present cracks or irregularities. Sometimes, granules show barely visible concentric striations. Exceptionally, granules show a slit along the main axis. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross.

B: Suspend 1 g of it in 50 mL of water, boil for 1 minute, and cool: a thin, cloudy mucilage is formed.

C: To 1 mL of the mucilage obtained in *Identification* test *B*, add 0.05 mL of iodine and potassium iodide TS 2: an orange-red to dark blue color is produced, which disappears on heating.

Microbial limits ⟨61⟩—The total aerobic microbial count does not exceed 1000 cfu per g, the total combined molds and yeasts count does not exceed 100 cfu per g, and it meets the requirements of the test for the absence of *Escherichia coli*.

pH ⟨791⟩—Prepare a slurry by weighing 5.0 g of Pea Starch, transferring to a suitable nonmetallic container, and adding 25.0 mL of freshly boiled and cooled water. Agitate continuously at a moderate rate for 1 minute. Stop the agitation, allow to stand for 15 minutes, and shake again. Determine the pH to the nearest 0.1 unit: the pH, determined potentiometrically, is between 5.0 and 8.0.

Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, at 130° for 90 minutes: it loses not more than 16.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.6%, determined on a 1.0-g test specimen.

Foreign matter—Examine under a microscope, using a mixture of equal volumes of glycerin and water: not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Limit of iron—

Standard iron stock solution—Prepare a solution containing the equivalent of 10 µg of iron per mL, as directed under *Iron* ⟨241⟩.

Diluted standard iron solution—Immediately before use, dilute an accurately measured volume of *Standard iron stock solution* quantitatively with water to obtain a solution containing the equivalent of 1 µg of iron per mL.

Test solution—Shake 1.0 g of Pea Starch with 50 mL of 2 N hydrochloric acid, and filter. Transfer 10 mL of the filtrate to a test tube. Add 2 mL of citric acid solution (2 in 10) and 0.1 mL of thioglycolic acid, and mix. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, dilute with water to 20 mL, and mix.

Procedure—Prepare the *Standard solution* by transferring 10 mL of the *Diluted standard iron solution* to a test tube and proceeding in the same manner as directed for the preparation of the *Test solution*, beginning with "Add 2 mL of citric acid solution (2 in 10)." After 5 minutes, any pink color in the *Test solution* is not more intense than that in the *Standard solution*, corresponding to a limit of 50 µg of iron per g.

Limit of oxidizing substances—Transfer 4.0 g of Pea Starch to a glass-stoppered, 125-mL conical flask, and add 50.0 mL of water. Insert the stopper, and swirl for 5 minutes. Transfer to a glass-stoppered 50-mL centrifuge tube, and centrifuge to clarify. Transfer 30.0 mL of the clear supernatant to a glass-stoppered 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5 to 1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25 to 30 minutes in the dark. Add 1 mL of starch TS, and titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch-iodine color. Perform a blank determination, and make any necessary correction. Each mL of 0.002 N sodium thiosulfate VS is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide. Not more than 1.4 mL of 0.002 N sodium thiosulfate VS is required (20 µg per g, calculated as H_2O_2).

Limit of sulfur dioxide—Not more than 50 µg per g. [NOTE—Perform either *Test 1* or *Test 2*.]

TEST 1—

Reagents—

CARBON DIOXIDE—Use carbon dioxide with a flow regulator that will maintain a flow of 100 ± 10 mL per minute.

HYDROGEN PEROXIDE SOLUTION—Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Neutralize the solution with 0.01 N sodium hydroxide to pH 4.1, determined potentiometrically.

POTASSIUM METABISULFITE SOLUTION—Transfer 0.87 g of potassium metabisulfite ($K_2S_2O_5$) and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Dilute with water to volume before mixing. [NOTE—Edetate disodium is used to protect the sulfite ion from oxidation.]

APPARATUS—In this test, the sulfur dioxide is released from the test specimen in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution where the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali. A suitable apparatus for sulfur dioxide determination is shown in the accompanying diagram (*Figure 1*).

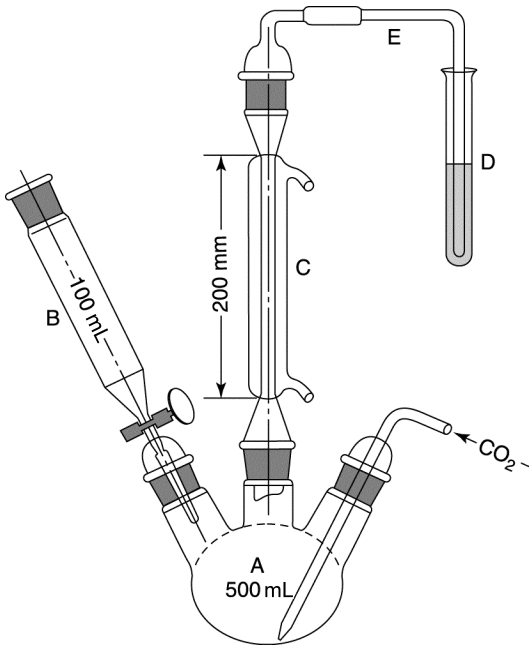


Figure 1

The apparatus consists of a 500-mL three-neck, round-bottom boiling flask, *A;* a separatory funnel, *B,* having a capacity of 100 mL or greater; a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask; a reflux condenser, *C,* having a jacket length of 200 mm; and a delivery tube, *E,* connecting the upper end of the reflux condenser to the bottom of a receiving test tube, *D.* Apply a thin film of stopcock grease to the sealing surfaces of all the joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

System suitability test—

TEST A—Using the *Potassium metabisulfite solution* as the standard, proceed as directed for *Procedure*, except for replacing the 25.0 g of Pea Starch with the 20 mL of the *Potassium metabisulfite solution*. Calculate the content, in µg per mL, of sulfur dioxide in the *Potassium metabisulfite solution* taken by the formula:

$$1000(32.03)VN/V_P$$

in which 1000 is the factor for conversion of mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and V_P is the volume, in mL, of the *Potassium metabisulfite solution* taken for the test.

TEST B—In a 100-mL conical flask, add 20 mL of 0.02 N iodine solution and 5 mL of 2 N hydrochloric acid. Add 1 mL of starch TS, and titrate with *Potassium metabisulfite solution* until the first discoloration is observed. Calculate the content, in µg per mL, of sulfur dioxide in *Potassium metabisulfite solution* by the formula:

$$1000(32.03)V_IN_I/V_P$$

in which 1000 and 32.03 are as defined in *Test A;* V_I is the volume, in mL, of the iodine solution used in the test; N_I is the normality of the iodine solution; and V_P is the volume, in mL, of the *Potassium metabisulfite solution* consumed.

The difference between the sulfur dioxide contents obtained from *Test A* and *Test B* is not more than 5% of their mean value. *Test B* shall be performed within 15 minutes after the completion of *Test A*. [NOTE—This time limit avoids potential variation in the sulfur dioxide content of the *Potassium bisulfite solution* when stored at room temperature.]

Procedure—Add 150 mL of water to the boiling flask (*A*) (see *Figure 1*). Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide through the apparatus at a rate of 100 ± 5 mL per minute. Start the condenser coolant flow. Place 10 mL of *Hydrogen peroxide solution* in the receiving test tube (*D*). After 15 minutes, without interrupting the flow of carbon dioxide, remove the separatory funnel (*B*) from the boiling flask, and transfer 25.0 g of Pea Starch to the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to

flow into the boiling flask, guarding against the escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 hour. Open the stopcock of the funnel, stop the flow of carbon dioxide, discontinue heating the flask, and turn off the cooling water in the condenser. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 minutes, and allow to cool. Titrate the contents with 0.1 N sodium hydroxide VS until the pH reaches 4.1, determined potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the content, in µg per g, of sulfur dioxide in the Pea Starch taken by the formula:

$$1000(32.03)VN/W$$

in which 1000 is the factor for conversion of mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and W is the weight, in g, of the Pea Starch taken.

TEST 2—Determine the content of sulfur dioxide as directed under *Sulfur Dioxide* ⟨525⟩*, Method I*. Use 200 mL of water as a solvent. Then, to 100 mL of the clear filtrate, add 3 mL of starch TS, and titrate with 0.01 N iodine VS to the first permanent blue color.▲*NF28*

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

〈**11**〉 **USP Reference Standards,** *USP 31* page 37, page 3694 of the *Second Supplement,* pages 553 and 1130 of the *Interim Revision Announcement* in *PF* 34(3) [May–June 2008] and in *PF* 34(5) [Sept.–Oct. 2008], respectively, page 2022 of *PF* 29(6) [Nov.–Dec. 2003], page 1674 of *PF* 30(5) [Sept.–Oct. 2004], page 507 of *PF* 31(2) [Mar.–Apr. 2005], page 1154 of *PF* 31(4) [July–Aug. 2005], page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 407 of *PF* 32(2) [Mar.–Apr. 2006], page 1161 of *PF* 32(4) [July–Aug. 2006], page 95 of *PF* 33(1) [Jan.–Feb. 2007], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 1256 of *PF* 33(6) [Nov.–Dec. 2007], page 142 of *PF* 34(1) [Jan.–Feb. 2008], page 332 of *PF* 34(2) [Mar–Apr. 2008], page 680 of *PF* 34(3) [May–June 2008], page 1021 of *PF* 34(4) [July–Aug. 2008], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], and page 1531 of *PF* 34(6) [Nov.–Dec. 2008].

(HDQ) RTS—C44080; C53875; C57716; C59275; C63129; C65734; C66478; C68500; C70696

Add the following:

▲USP Atorvastatin Calcium RS.▲*USP33*

Add the following:

▲USP Atorvastatin Related Compound A RS—

Desfluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt. $C_{66}H_{70}CaN_4O_{10}$ ✧1119.38.▲*USP33*

Add the following:

▲USP Atorvastatin Related Compound B RS

[3*S*,5*R* isomer, or (3*S*,5*R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt] ($C_{66}H_{68}CaF_2N_4O_{10}$ ✧1155.34).▲*USP33*

Add the following:

▲USP Atorvastatin Related Compound C RS—

Difluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt ($C_{66}H_{66}F_4N_4O_{10}$ ✧1191.34).▲*USP33*

Add the following:

▲USP Atorvastatin Related Compound D RS—

Oxirane impurity, or 3-(4-fluorobenzoyl)-2-isobutyryl-3-phenyl-oxirane-2-carboxylic acid phenylamide ($C_{26}H_{22}FNO_4$ ✧431.46).▲*USP33*

Add the following:

▲USP Atorvastatin Related Compound E RS—

3*S*,5*S* enantiomer, or (3*S*,5*S*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt ($C_{66}H_{68}CaF_2N_4O_{10}$ ✧1155.34).▲*USP33*

Add the following:

▲USP Chitosan RS.▲*USP33*

Add the following:

▲USP Diclazuril RS.▲*USP33*

Add the following:

▲USP Diclazuril System Suitability Mixture RS—Contains diclazuril and specified impurities.▲*USP33*

Add the following:

▲USP 12-Hydroxystearic Acid RS.▲*USP33*

In-Process Revision

Delete the following:

▲~~**USP Norethynodrel RS.**~~▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 200 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 300 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 400 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 600 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 1000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 1500 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 3000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 3350 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 4000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 6000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 8000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 10000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 12000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 20000 RS.**▲*USP33*

Add the following:

▲**USP Polyethylene Glycol 35000 RS.**▲*USP33*

Add the following:

▲**USP Polyoxyl 15 Hydroxystearate RS.**▲*USP33*

Add the following:

▲**USP Polyvinyl Acetate Dispersion RS.**▲*NF28*

Delete the following:

▲~~**USP Trenbolone RS.**~~▲*USP33*

Add the following:

▲**USP Trenbolone Acetate System Suitability Mixture RS.**▲*USP33*

Add the following:

▲**USP Valrubicin Resolution Mixture RS**—This Reference Standard is a mixture of the following with their chemical names:

Doxorubicin: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione

Doxorubicin aglycone: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione

Daunorubicin: (8*S*,10*S*)-8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione

Daunorubicin bromoketal: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione

Doxorubicin valerate: 2-{(2*S*,4*S*)-4-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl}-2-oxoethyl pentanoate

Doxorubicin aglycone valerate: 2-oxo-2-[(2*S*,4*S*)-2,4,5,12-tetrahydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl]ethyl pentanoate

Valrubicin: (8*S*,10*S*)-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-10-[[2,3,6-trideoxy-3-(2,2,2-trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione 8^2-valerate

Dianhydrovalrubicin: 2-(5,12-Dihydroxy-7-methoxy-6,11-dioxo-6,11-dihydrotetracen-2-yl)-2-oxoethyl pentanoate ▲*USP33*

Microbiological Tests

BRIEFING

⟨**63**⟩ **Mycoplasma Tests.** This new chapter proposal contains methods to detect Mycoplasma contamination of test articles, tissues and/or cell cultures used to produce test articles, or any other material in which Mycoplasma contamination is suspected.

(MSA: R. Tirumalai) RTS—C69320

Add the following:

▲⟨63⟩ MYCOPLASMA TESTS

INTRODUCTION

The genus Mycoplasma represents a group of minute bacteria which have no cell walls. The genus comprises more than 120 species. They are the smallest self-replicating prokaryotic organisms. The cells vary in size and morphology and cannot be Gram stained, but impressions of colonies on solid agar can be stained with Dienes' stain. Mycoplasma are considered parasites, and many are pathogenic to a variety of animal and plant hosts. In humans, Mycoplasma are usually surface parasites that colonize the epithelial lining of the respiratory and urogenital tracts. Mycoplasma are common and may cause serious contamination in cell and/or tissue cultures used to generate compendial articles. They may also cause contamination of filtered sterilized soybean casein digest broth used in challenge studies to validate industrial sterile equipment for aseptic processing. A cell culture infection may persist for an extended period of time without causing apparent cell damage. Infection of cells in a culture can affect nearly every pathway of cell metabolism, including alteration of the cells' phenotypical characteristics and normal growth. The presence of Mycoplasma species does not always result in turbid growth in cultures or visible alteration of the cells.

Testing for Mycoplasma is a necessary quality control requirement to assure reliably pure biotechnological products and allied materials used to generate these products. This general chapter describes two methods to detect Mycoplasma contamination of test articles, tissues and/or cell cultures used to produce test articles, digest broth, or any other material in which Mycoplasma contamination is suspected. These are: (A) the agar and broth media procedure and (B) the indicator cell culture procedure. These tests require careful aseptic technique and suitable laboratory conditions. In order to ensure correct testing and interpretation of results, personnel should be properly trained and qualified. A validated nucleic acid amplification technique (NAT) or an enzymatic activity based method may be used to detect Mycoplasma, provided such a method is shown to be comparable to both methods (A) and (B). Alternative methods must be suitably validated. Validation requirements for alternate methods will not be addressed in this chapter.

CULTURE METHOD

Choice of Media

The test is carried out using a sufficient number of both solid and liquid media to ensure growth in the chosen incubation conditions of small numbers (approximately 100 colony-forming units, cfu; or 100 color-changing units, ccu) of Mycoplasmas that may be present in the test article/material. Liquid media must contain phenol red. The range of media chosen is shown to have satisfactory nutritive properties for at least the microorganisms shown in *Quality Control Test Strain Organisms* (below). The nutritive properties of each new batch of medium are verified for the appropriate microorganisms in the list. When testing for Mycoplasmas include in each test at least

In-Process Revision

two known Mycoplasma species or strains (listed in *Quality Control Test Strain Organisms*) as positive controls, one of which should be a dextrose fermenter (i.e., *M. pneumoniae* or equivalent species and strain) and one of which should be an arginine hydrolyzer (i.e., *M. orale* or equivalent species and strain). Only when testing insect cell lines should one include a *Spiroplasma* control strain [e.g., *S. citri* ATCC 29747, *S. melliferum* ATCC 29416, or equivalent species and strains]. Additionally, these strains may be a little more fastidious in their nutritional requirements. They require lower incubation temperatures (as do insect cell lines).

Quality Control Test Strain Organisms

Positive control cultures should be not more than 15 passages from isolation. Mycoplasma species or strains suitable for use are listed below:

— *Acholeplasma laidlawii* (vaccines and/or cell-derived materials/cultures for human and veterinary use when an antibiotic has been used during production)

— *M. gallisepticum* (when avian material has been used during production or when the vaccine or cell culture is intended for use in poultry)

— *M. hyorhinis* (nonavian veterinary vaccines or cell cultures)

— *M. orale* (vaccines for human and veterinary use)

— *M. pneumoniae* (vaccines or cell banks for human use) or another suitable species of D-glucose fermenter such as *M. fermentans*

— *M. synoviae* (when avian material has been used during production or when the vaccine or cell bank is intended for use in poultry)

The test strains may be field isolates that have undergone a limited number of subcultures (not more than 15), are stored frozen (–20° or lower) or freeze-dried, and are identified as being of the required species by comparison with type cultures, for example, those shown in *Table 1*.

Incubation Conditions

Incubate liquid media in tightly stoppered containers at 36 ± 1°. Incubate solid media in microaerophilic conditions (nitrogen containing 5%–10% carbon dioxide in nitrogen and/or hydrogen atmosphere containing $< 0.5\%$ oxygen). Sufficient humidity should be available to prevent desiccation of the agar surface) at 36 ± 1°.

Table 1. Type Cultures for Identifying Field Isolates Used as Test Strains

Test Organism	NCTC Number	CIP Number	ATCC Number
A. laidlawii	NCTC 10116	CIP 75.27	ATCC 23206
M. gallisepticum	NCTC 10115	CIP 104967	ATCC 19610
M. fermentans	NCTC 10117	CIP 105680	ATCC 19989
M. hyorhinis	NCTC 10130	CIP 104968	ATCC 17981
M. orale	NCTC 10112	CIP 104969	ATCC 23714
M. pneumoniae	NCTC 10119	CIP 103766	ATCC 15531
M. synoviae	NCTC 10124	CIP 104970	ATCC 25204

Nutritive Properties

Carry out the test for nutritive properties for each new batch of medium. Inoculate the chosen media with the appropriate test microorganisms; use not more than 100 cfu per plate containing at least 9 mL of solid media and per 100-mL container of liquid medium; use a separate plate and container for each species of microorganism. Incubate the media and make subcultures from 0.2 mL of liquid medium to solid medium at the specified intervals (see below under *Test for Mycoplasma in the Test Article/Material*). The solid medium complies with the test if a count within a 0.5 log unit range of the inoculate amount is found for each test microorganism. The liquid medium complies with the test if growth is found on agar plates subcultured from the broth, for at least 1 subculture for each test microorganism. The use of a microscope at 100X or greater may be helpful.

Inhibitory Substances

The test for inhibitory substances is carried out once for a given product and is repeated whenever there is a change in production method that may affect the detection of Mycoplasma. To demonstrate absence of inhibitory substances, carry out the test for nutritive properties in the presence and absence of the test article/material. If growth of a test microorganism occurs more than 1 subculture sooner in the absence of the test article/material than in its presence, inhibitory substances are present. The same is true if plates directly inoculated with the test article/material are not within a 0.5 log unit range of the number of colonies of those inoculated without the test article/material. In both cases, inhibitory substances must be neutralized or their effect otherwise countered, by an appropriate method, for example, by passage in substrates not containing inhibitors or dilution in a larger volume of medium, before the test. If dilution is used, larger medium volumes may be used or the inoculums' volume may be divided among several 100-mL flasks. The effectiveness of the neutralization or other process is checked by repeating the test for inhibitory substances after neutralization.

Test for Mycoplasma in the Test Article/Material

Inoculate no less than 10 mL of the test article/material per 100 mL of each liquid medium. If a significant pH change occurs upon the addition of the test article/material, the liquid medium is restored to its original pH value by the addition of a sterile solution of either sodium hydroxide or hydrochloric acid. Inoculate 0.2 mL of the test article/material on each plate of each solid medium. Incubate liquid media for 20–21 days. Incubate solid media for not less than 14 days, except those plates corresponding to the 20–21 day subculture, which are incubated for 7 days. Concurrently, incubate an uninoculated 100-mL portion of each liquid medium and agar plate, as a negative control. On days 2–4 after inoculation, subculture each liquid medium by inoculating 0.2 mL on at least 1 plate of each solid medium. Repeat the procedure between the 6 and 8 days, again between the 13 and 15 days and again between the 19 and 21 days of the test. Observe the liquid media every 2 or 3 days and if a color change occurs, subculture. If a liquid medium shows bacterial or fungal contamination, the test is invalid. If one or more plate(s) per medium and per inoculation day cannot be read, the test is invalid. Include in the test positive controls prepared by inoculation of not more than 100 cfu of at least 1 test microorganism on agar medium or into broth medium. Where the test for Mycoplasmas is carried out regularly, it is recommended to use the test microorganisms in regular rotation. The test microorganisms used are those listed under *Choice of Media*. Incubate broths and plates in a humidified atmosphere with microaerophilic conditions (5%–10% CO_2).

Interpretation of Results

At the end of the prescribed incubation period, examine all inoculated solid media for the presence of Mycoplasma colonies. The product complies with the test if growth of typical Mycoplasma colonies has not occurred. The product does not comply with the test if growth of typical Mycoplasma colonies has occurred on any of the solid media. The test is invalid if 1 or more of the positive controls do not show growth of Mycoplasmas on at least 1 subculture plate. The test is invalid if 1 or more of the negative controls show growth of Mycoplasmas. If suspect colonies are observed, use a suitable validated method to determine whether they are due to Mycoplasmas.

Recommended Solutions and Media For The Culture Method

NOTE—This section is provided for information.

SOLUTIONS

Beef Heart Infusion Broth

Beef heart (for preparation of the infusion)	500 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	to 1000 mL

Essential Vitamins

Biotin	100 mg
Calcium pantothenate	100 mg
Choline chloride	100 mg
Folic acid	100 mg
i-Inositol	200 mg

Essential Vitamins *(Continued)*

Nicotinamide	100 mg
Pyridoxal hydrochloride	100 mg
Riboflavine	10 mg
Thiamine hydrochloride	100 mg
Distilled water	to 1000 mL

Agar, Purified

A highly refined agar for use in microbiology and immunology, prepared by an ion-exchange procedure that results in a product having superior purity, clarity, and gel strength. It contains the following ingredients:

Water	12.2%
Ash	1.5%
Acid-insoluble ash	0.2%
Chlorine	0
Phosphate (calculated as P_2O_5)	0.3%
Total nitrogen	0.3%
Copper	8 ppm
Iron	170 ppm
Calcium	0.28%
Magnesium	0.32%

Hanks' Balanced Salt Solution (modified)

Sodium chloride	6.4 g
Potassium chloride	0.32 g
Magnesium sulphate heptahydrate	0.08 g
Magnesium chloride hexahydrate	0.08 g
Calcium chloride, anhydrous	0.112 g
Disodium hydrogen phosphate dihydrate	0.0596 g
Potassium dihydrogen phosphate, anhydrous	0.048 g
Distilled water	to 800 mL

Brain Heart Infusion

Calf-brain infusion	200 g
Beef-heart infusion	250 g
Proteose peptone	10 g
Glucose monohydrate	2 g
Sodium chloride	5 g
Disodium hydrogen phosphate, anhydrous	2.5 g
Distilled water	to 1000 mL

PPLO Broth

Beef-heart infusion	50 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	to 1000 mL

MEDIA

The following media are recommended. Other media may be used, provided they meet the criteria given in the sections *Choice of Culture Media, Incubation Conditions, Nutritive Properties,* and *Inhibitory Substances.*

Hayflick Media (Recommended for the General Detection of Mycoplasmas)

Liquid Medium

Beef heart infusion broth	90.0 mL
Horse serum (unheated)	20.0 mL
Yeast extract (250 g/L)	10.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL
Deoxyribonucleic acid (2 g/L solution)	1.2 mL
Adjust to pH 7.8	

Hayflick Media (Recommended for the General Detection of Mycoplasmas) *(Continued)*

Solid Medium

Prepare as described above replacing beef heart infusion broth by beef heart infusion agar containing 15 g/L of agar.

Frey Media

(Recommended for the detection of *M. synoviae*)

Liquid Medium

Beef heart infusion broth	90.0 mL
Essential vitamins	0.025 mL
Glucose monohydrate (500 g/L solution)	2.0 mL
Swine serum (inactivated at 56° for 30 min)	12.0 mL
β-Nicotinamide adenine dinucleotide (10 g/L solution)	1.0 mL
Cysteine hydrochloride (10 g/L solution)	1.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL

Mix the solutions of β-nicotinamide adenine dinucleotide and cysteine hydrochloride and after 10 min add to the other ingredients. AdjuSt to a pH of 7.8.

Solid Medium

Beef heart infusion broth	90.0 mL
Agar, purified	1.4 g
Adjust to pH 7.8, sterilize by autoclaving then add:	
Essential vitamins	0.025 mL
Glucose monohydrate (500 g/L solution)	2.0 mL
Swine serum (unheated)	12.0 mL
β-Nicotinamide adenine dinucleotide (10 g/L solution)	1.0 mL
Cysteine hydrochloride (10 g/L solution)	1.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL

In-Process Revision

Friis Media
(Recommended for the detection of nonavian mycoplasmas)

Liquid Medium

Hanks' balanced salt solution (modified) (4)	800 mL
Distilled water	67 mL
Brain heart infusion (5)	135 mL
PPLO Broth (6)	248 mL
Yeast extract (170 g/L)	60 mL
Bacitracin	250 mg
Meticillin	250 mg
Phenol red (5 g/L)	4.5 mL
Horse serum	165 mL
Swine serum	165 mL

Adjust to a pH of 7.40–7.45

Solid Medium

Hanks' balanced salt solution (modified)	800 mL
DEAE-dextran	200 mg
Agar, purified	15.65 g

Mix well and sterilize by autoclaving. Cool to 100°. Add to 1740 mL of *Liquid Medium* as described above.

INDICATOR CELL CULTURE METHOD

Cell cultures are stained with a fluorescent dye that binds to DNA. Mycoplasmas are detected by their characteristic particulate or filamentous pattern of fluorescence on the cell surface and, if contamination is heavy, in surrounding areas. Mitochondria in the cytoplasm may be stained but are readily distinguished from Mycoplasmas. For viral suspensions, if the interpretation of results is affected by marked cytopathic effects, neutralize the virus using a specific antiserum that has no inhibitory effects on Mycoplasmas, or use a cell culture substrate that does not allow growth of the virus. To demonstrate the absence of inhibitory effects of serum, carry out the positive control tests in the presence and absence of the antiserum.

Verification of the Substrate

Use Vero cells or equivalent cell culture (for example, the production cell line) that is equivalent in effectiveness for detecting Mycoplasmas. Test the effectiveness of the cells to be used by applying the procedure shown below and inoculating not more than 100 cfu or ccu microorganisms of suitable reference strains of *M. hyorhinis* and *M. orale*. The cells are suitable if both reference strains are detected. The indicator cells must be subcultured without an antibiotic before use in the test.

Test Method

NOTE—The following is provided for information.

SOLUTIONS

Phosphate Buffered Saline—

2.0 M Monobasic Potassium Phosphate—Dissolve 13.61 g of anhydrous monobasic potassium phosphate in 50 mL of water.

2.0 M Dibasic Potassium Phosphate—Dissolve 17.42 g of anhydrous dibasic potassium phosphate in 50 mL of water.

Phosphate Buffered Saline Solution (pH 7.4)—Combine 3.6 mL of *2.0 M Monobasic Potassium Phosphate,* 16.4 mL of *2.0 M Dibasic Potassium Phosphate,* 8 g of sodium chloride, and 1 L of water. Mix thoroughly. Adjust the pH if necessary.

Bisbenzimide Stock Solution—Dissolve 5 mg of bisbenzimide in water, and dilute with the same solvent to 100 mL. Store in the dark.

Bisbenzimide Working Solution—Immediately before use, dilute 100 µL of *Bisbenzimide Stock Solution* to 100 mL with *Phosphate Buffered Saline Solution (pH 7.4)*

Phosphate-Citrate Buffer Solution pH 5.5—Mix 56.85 mL of a 28.4 g/L solution of anhydrous disodium hydrogen phosphate and 43.15 mL of a 21 g/L solution of citric acid.

In-Process Revision

METHOD

1. Seed the indicator cell culture at a suitable density (for example, 2×10^4 to 2×10^5 cells/mL, 4×10^3 to 2.5×10^4 cells/cm^2) that will yield confluence after 3 days of growth. Inoculate 1 mL of the product to be examined into the cell culture vessel, and incubate at $36 \pm 1°$.
2. After at least 3 days of incubation, when the cells have grown to confluence, make a subculture on cover slips in suitable containers or on some other surface (for example, chambered slides) suitable for the test procedure. Seed the cells at low density so that they reach 50% confluence after 3–5 days of incubation. Complete confluence impairs visualization of Mycoplasmas after staining and must be avoided.
3. Remove the medium and rinse the indicator cells with phosphate buffered saline, pH 7.4, then add a suitable fixing solution (a freshly prepared mixture of 1 volume of acetic acid, glacial, TS and 3 volumes of methanol, is suitable when bisbenzimide is used for staining).
4. Remove the fixing solution and wash the cells with sterile Purified Water. Dry the slides completely if they are to be stained more than 1 hour later (particular care is needed for staining of slides after drying owing to artifacts that may be produced).
5. Add a suitable DNA stain and allow standing for a suitable time (bisbenzimide working solution and a standing time of 10 minutes are suitable).
6. Remove the stain and rinse the monolayer with Purified Water.
7. Mount each coverslip, where applicable (a mixture of equal volumes of glycerol and *Phosphate-Citrate Buffer Solution pH 5.5* is suitable for mounting). Examine by fluorescence (for bisbenzimide stain a 330 nm/380 nm excitation filter and an LP 440 nm barrier filter are suitable) at 400 × magnification or greater.
8. Compare the microscopic appearance of the test cultures with that of the negative and positive controls, examining for extranuclear fluorescence. Mycoplasmas produce pinpoints or filaments over the indicator cell cytoplasm. They may also produce pinpoints and filaments in the intercellular spaces. Multiple microscopic fields are examined according to the protocol established during validation.

Interpretation of Results

The product to be examined complies with the test if fluorescence typical of Mycoplasmas is not present. The test is invalid if the positive controls do not show fluorescence typical of Mycoplasmas. The test is invalid if the negative controls show fluorescence typical of Mycoplasmas.▲*USP33*

Chemical Tests and Assays

IDENTIFICATION TESTS

BRIEFING

〈**197**〉 **Spectrophotometric Identification Tests,** *USP 31* page 129. The Spectroscopy Advisory Panel recommends that the General Chapters Expert Committee update the general chapter, *Spectrophotometric Identification Tests* 〈197〉. In addition, the advisory panel proposes a title change and revisions to *Spectrophotometry and Light-Scattering* 〈851〉, which also appear in this issue of *Pharmacopeial Forum*. The changes follow an established effort to broaden and update the general chapters already published in USP.

The following changes are proposed for general chapter 〈197〉.

1. A title change is proposed.
2. Technical and editorial revisions are proposed for the *Infrared Absorption* and *Ultraviolet Absorption* sections.
3. A new section, *Identification Methodology*, is proposed to be added.
4. References to alternative USP spectroscopic methods have been added: *Mid-Infrared Spectroscopy* 〈854〉, *Nuclear Magnetic Resonance* 〈761〉, *Spectroscopy and Light-Scattering* 〈851〉 (title change per this issue of *PF*), *X-Ray Diffraction* 〈941〉, *Near-Infrared Spectroscopy* 〈1119〉, *Raman Spectroscopy* 〈1120〉, and *Mass Spectrometry* 〈736〉.

(GC: G. Ritchie) RTS—C65854

In-Process Revision

Change to read:

⟨197⟩~~SPECTROPHOTOMETRIC IDENTIFICATION TESTS~~

▲SPECTROSCOPIC IDENTIFICATION TESTS▲*USP33*

Change to read:

~~Spectroscopic tests contribute meaningfully toward the identification of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb IR and/or UV radiation (see *Spectroscopy and Light-Scattering* ⟨851⟩).~~

~~The IR absorption spectrum of a substance, compared with that obtained concomitantly for the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. The UV absorption spectrum, on the other hand, does not exhibit a high degree of specificity. Conformance with both IR absorption and UV absorption test specifications, as called for in a large proportion of compendial monographs, leaves little doubt, if any, regarding the identity of the specimen under examination.~~▲*USP33*

~~INFRARED ABSORPTION~~

~~Six methods are indicated for the preparation of previously dried test specimens and Reference Standards for analysis. The reference ⟨197K⟩ in a monograph signifies that the substance under examination is mixed intimately with potassium bromide. The reference ⟨197M⟩ in a monograph signifies that the substance under examination is finely ground and dispersed in mineral oil. The reference ⟨197F⟩ in a monograph signifies that the substance under examination is suspended neat between suitable (for example, sodium chloride or potassium bromide) plates. The reference ⟨197S⟩ signifies that a solution of designated concentration is prepared in the solvent specified in the individual monograph, and the solution is examined in 0.1 mm cells unless a different cell path length is specified in the individual monograph. The reference ⟨197A⟩ signifies that the substance under examination is intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis. The reference ⟨197E⟩ signifies that the substance under examination is pressed as a thin sample against a suitable plate for IR microscopic analysis. The ATR ⟨197A⟩ and the ⟨197E⟩ techniques can be used as alternative methods for ⟨197K⟩, ⟨197M⟩, ⟨197F⟩, and ⟨197S⟩ where testing is performed qualitatively and the Reference Standard spectra are similarly obtained.~~

~~Record the spectra of the test specimen and the corresponding USP Reference Standard over the range from about 2.6 µm to 15 µm (3800 cm^{-1} to 650 cm^{-1}) unless otherwise specified in the individual monograph. The IR absorption spectrum of the preparation of the test specimen, previously dried under conditions specified for the corresponding Reference Standard unless otherwise specified, or unless the Reference Standard is to be used without drying, exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard.~~

~~Differences that may be observed in the spectra so obtained sometimes are attributed to the presence of polymorphs, which are not always acceptable (see *Procedure* under *Spectrophotometry and Light-Scattering* ⟨851⟩). Unless otherwise directed in the individual monograph, therefore, continue as follows. If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the Reference Standard in equal volumes of a suitable solvent, evaporate the solution to dryness in similar containers under identical conditions, and repeat the test on the residues.~~

~~ULTRAVIOLET ABSORPTION~~

~~The reference ⟨197U⟩ in a monograph signifies that a test solution and a Standard solution are examined spectrophotometrically, in 1-cm cells, over the spectral range from 200 to 400 nm unless otherwise specified in the individual monograph.~~

~~Dissolve a portion of the substance under examination in the designated *Medium* to obtain a test solution having the concentration specified in the monograph for *Solution*. Similarly prepare a Standard solution containing the corresponding USP Reference Standard.~~

~~Record and compare the spectra concomitantly obtained for the test solution and the Standard solution. Calculate absorptivities and/or absorbance ratios where these criteria are included in an individual monograph. Unless otherwise specified, absorbances indicated for these calculations are those measured at the maximum absorbance at about the wavelength specified in the individual monograph. Where the absorbance is to be measured at about the specified wavelength other than that of maximum absorbance, the abbreviations (min) and (sh) are used to indicate a minimum and shoulder, respectively, in an absorption spectrum. The requirements are met if the UV absorption spectra of the test solution and the Standard solution exhibit maxima and minima at the same wavelengths and absorptivities and/or absorbance ratios are within specified limits.~~

▲INTRODUCTION

Spectroscopic tests contribute meaningfully toward the identification of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb IR and/or UV radiation (see *Spectroscopy and Light-Scattering* ⟨851⟩).

The IR absorption spectrum of a substance, compared with that obtained concomitantly for the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. The UV absorption spectrum, on the other hand, does not exhibit a high degree of specificity. Conformance with both IR absorption and UV absorption test specifications, as called for in a large proportion of compendial monographs, leaves little doubt, if any, regarding the identity of the specimen under examination.

INFRARED ABSORPTION

Several methods are indicated for the preparation of previously dried test specimens and Reference Standards for analysis by infrared spectroscopy (see *Table 1* and *Mid-Infrared Spectroscopy* ⟨854⟩).

The reference ⟨197K⟩ in a monograph signifies that the substance under examination is mixed intimately with potassium bromide and compressed into a transparent pellet. The refer-

In-Process Revision

ence ⟨197M⟩ in a monograph signifies that the substance under examination is finely ground and dispersed in mineral oil. The reference ⟨197F⟩ in a monograph signifies that the substance under examination is a liquid or semisolid that is suspended neat between suitable (e.g. sodium chloride or potassium bromide) plates. The reference ⟨197S⟩ signifies that a solution of designated concentration is prepared in the solvent specified in the individual monograph. The solution is examined in 0.1-mm cells, unless a different cell path length is specified. The reference ⟨197A⟩ signifies that the substance under examination is intimately in contact with an internal reflection element for attenuated total reflection (ATR) analysis. The reference ⟨197E⟩ signifies that the substance under examination is pressed as a thin sample against a suitable plate for IR microscopic analysis.

In each instance, infrared absorption spectra of both the Reference Standard and the sample are obtained concomitantly using the identical preparation technique and measurement parameters. Record the spectra of the test specimen and the corresponding USP Reference Standard over the range from 4000 cm^{-1} to 400 cm^{-1}, unless otherwise specified in the individual monograph, or unless the window or internal reflection element precludes measurement to 400 cm^{-1}. The IR absorption spectrum of the preparation of the test specimen, prepared as directed in the monograph, exhibits maxima only at the same wavenumbers as that of a similar preparation of the corresponding USP Reference Standard. The source of additional bands in the spectrum of the test specimen not present in the spectrum of the Reference Standard should be investigated and explained.

Differences between the USP Reference Standard spectrum and sample spectrum that may be observed are sometimes attributed to differences in the solid-state form of materials, which are not always acceptable. When spectral differences between the sample and USP Reference Standard are observed, recrystallize both the sample and USP Reference Standard under identical conditions to produce the same solid-state form, unless specific procedures are provided in the individual monographs. Dissolve equal portions of the test specimen and the Reference Standard in equal volumes of a suitable solvent, evaporate the solutions to dryness in similar containers under identical conditions, and repeat the identification test on the residues. Other techniques for recrystallizing the sample and USP Reference Standard based on known scientific principles may be utilized.

ULTRAVIOLET ABSORPTION

The reference ⟨197U⟩ in a monograph signifies that a test solution and a Standard solution are examined spectrophotometrically, in 1-cm cells, over the spectral range from 200 to 400 nm, unless otherwise specified in the individual monograph (see *Ultraviolet–Visible Spectroscopy* ⟨857⟩).

Dissolve a portion of the substance under examination in the designated medium to obtain a test solution having the concentration specified in the monograph for the solution. Similarly, prepare a Standard solution containing the corresponding USP Reference Standard.

Record and compare the spectra concomitantly obtained for the test solution and the Standard solution. Calculate the absorptivities and/or absorbance ratios where these criteria are included in an individual monograph. Unless otherwise specified, absorbances indicated for these calculations are those measured at the maximum absorbance at about the wavelength specified in the individual monograph. Where the absorbance is to be measured at about the specified wavelength other than that of maximum absorbance, the abbreviations (min) and (sh) are used to indicate a minimum and shoulder, respectively, in an absorption spectrum. The requirements are met if the UV absorption spectra of the test solution and the Standard solution exhibit maxima and minima at the same wavelengths, and absorptivities and/or absorbance ratios are within specified limits.

Spectroscopic tests may be utilized for the identification of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb, scatter,

or interact with electromagnetic radiation in a manner that can be utilized for chemical identification (see *Spectroscopy and Light-Scattering* ⟨851⟩).

The IR absorption spectrum of a substance, compared with that obtained concomitantly for the corresponding USP Reference Standard, is the most widely utilized methodology for chemical identication in compendial monographs. Under conditions where the IR absorption spectrum lacks specificity for definitive chemical identification, additional spectroscopic information can be used to supplement chemical identification. For example, conformance with both IR absorption and UV absorption test specifications, as called for in a large proportion of compendial monographs, provide complementary information for the definitive identity of the specimen under examination. In these instances, the combined spectroscopic information enables discrimination between compounds similar in structure that would not be possible from either IR absorption or UV absorption alone.

In addition to IR and UV absorption, several other spectroscopic methodologies can be utilized for the identification of the specimen under examination. The methods cited in this chapter (see *Table 1* for examples) may be utilized for identification of materials under conditions where the alternative methodology has been demonstrated to be suitable for the intended application. Suitable identification tests should be able to discriminate between compounds similar in molecular structure that are likely to be present. The choice of such potentially interfering materials should be based on sound scientific judgment, with consideration of interferences that could occur. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this instance, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

IDENTIFICATION METHODOLOGY

Suitable identification methodology may be utilized for the chemical identification of materials against compendial standards. Implementation of these techniques requires demonstration that the alternative identification methodology is suitable for the intended application (see *Validation of Compendial Procedures* ⟨1225⟩). Identification procedures should be able to discriminate between materials similar in molecular structure; lack of specificity of a single technique may be compensated by other supporting analytical procedure(s).

Table 1. Examples of Spectroscopic Identification Methodology for Monographs that Refer to Chapter ⟨197⟩

Cited in Monograph	Description	Alternative Method
⟨197K⟩	Potassium bromide dispersion for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩
⟨197M⟩	Finely ground dispersion in mineral oil for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩
⟨197F⟩	Thin film between suitable transparent infrared plates for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩
⟨197S⟩	Solution of designated concentration prepared in specified solvent for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩
⟨197A⟩	Substance in intimate contact with internal reflection element for attenuated total reflection for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩

Table 1. Examples of Spectroscopic Identification Methodology for Monographs that Refer to Chapter ⟨197⟩ *(Continued)*

Cited in Monograph	Description	Alternative Method
⟨197E⟩	Substance pressed against a suitable plate for microscopic analysis for infrared absorption	⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩
⟨197U⟩	Test solution and standard in 1-cm optical cells examined spectrophotometrically from 200 to 400 nm unless otherwise specified	⟨854⟩, ⟨761⟩, ⟨851⟩, ⟨941⟩, ⟨1119⟩, ⟨1120⟩, ⟨736⟩

For information regarding sample preparation and measurement parameters associated with an alternative identification method, refer to the appropriate general chapter. (see *Mass Spectrometry* ⟨736⟩; *Nuclear Magnetic Resonance* ⟨761⟩; *Spectroscopy and Light-Scattering* ⟨851⟩; *Mid-Infrared Spectroscopy* ⟨854⟩; *X-Ray Diffraction* ⟨941⟩; *Near-Infrared Spectroscopy* ⟨1119⟩; and *Raman Spectroscopy* ⟨1120⟩).

Unless stated otherwise in an individual monograph, the sample spectrum is compared to the USP Reference Standard spectrum obtained concomitantly. Differences between the sample and Reference Standard should be investigated and explained.

As described above, spectral differences between the sample and the USP Reference Standard may be due to differences in solid-state form, and recrystallization of both sample and USP Reference Standard under identical conditions to produce the same solid state form may be required for methods that do not require dissolving the sample prior to analysis.▲*USP33*

LIMIT TESTS

BRIEFING

⟨**223**⟩ **Dimethylaniline**, *USP 31* page 132. Revisions are proposed for the *Chromatographic System* and *Procedure* sections, based on a validated method of analysis, in an effort to modernize this general chapter by changing the packed-column GC method to a capillary GC method. The gas chromatographic procedure is based on analyses performed using the Restek Rtx-35 Amine brand of G## column (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*). The typical retention time for dimethylaniline is about 8.5 minutes.

(MD-ANT: A. Wise) RTS—C61282

Change to read:

Chromatographic System (see *Chromatography* ⟨621⟩)—~~The gas chromatograph is equipped with a flame ionization detector and a 2-mm × 2-m column packed with 3% liquid phase G3 on silanized packing S1A and is maintained at 120°. Nitrogen is used as the carrier gas, flowing at the rate of about 30 mL per minute.~~

▲The gas chromatograph is equipped with a flame-ionization detector, maintained at about 250°, and a 0.53-mm × 30-m fused silica capillary column bonded with a 1.0-µm film of phase G##. The carrier gas is helium, with a linear velocity of about 30 cm per second and a split ratio of 10 : 1. The column temperature is maintained at 110° for the first 4 minutes after an injection is made, then increased from 110° to 200° at 8° per minute, and then held at 200° for 5 minutes. The injection port temperature is maintained at 250°. Chromatograph the *Standard preparation* and record the responses as directed

In-Process Revision

for *Procedure:* identify the dimethylaniline and naphthalene peaks by their relative retention times, which are 1.0 and 1.3, respectively. The signal-to-noise ratio for the dimethylaniline peak is not less than 10.▲*USP33*

Change to read:

Procedure—Inject equal volumes ~~within the range of 2 µL to 20 µL~~

▲(about 1 µL)▲*USP33*
of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The ratio of the response of any dimethylaniline peak to the response of the naphthalene peak obtained from the *Test Preparation* is not greater than that obtained from the *Standard Preparation* (0.002%).

Physical Tests and Determinations

BRIEFING

⟨851⟩ Spectrophotometry and Light-Scattering, *USP 31* page 355. The Spectroscopy Advisory Panel recommends that the General Chapters Expert Committee update this general chapter, ⟨851⟩ *Spectrophotometry and Light-Scattering*, considered by the panel to be the gateway to other spectroscopy chapters. These new spectroscopy chapters, currently being proposed, appear in *PF* 34(5) [Sept.–Oct. 2008]. In addition, the advisory panel proposes revisions to chapter ⟨197⟩ *Spectrophotometric Identification Tests*, which also appears in this issue of *PF*. These changes follow an effort to broaden and update the general chapters already in *USP–NF*.

The following changes are proposed for this chapter:

1. Change the title to ⟨851⟩ *Spectroscopy and Light-Scattering*.
2. Place discussions of ultraviolet-visible, mid-infrared, fluorescence, near-infrared, and Raman spectroscopic techniques in their respective proposed chapters, referred to above.
3. Delete the following sections: *Theory and Terms*; *Apparatus*; and *Procedure*.
4. Make both technical and editorial revisions to the following sections: *Ultraviolet, Visible, Infrared, Atomic Absorption, Fluorescence, Turbidimetry, Nephelometry, and Raman Measurement; Comparative Utility of Spectral Ranges; Use of Reference Standards;* and, in *Light-Scattering Spectroscopy,* the introductory text and the subsection *Visual Comparison*.
5. Add the sections *Atomic Absorption* and *Light-Scattering Spectroscopy.*

(GC: G. Ritchie) RTS—C58890

Change to read:

⟨851⟩ ~~SPECTROPHOTOMETRY AND LIGHT-SCATTERING~~

▲SPECTROSCOPY AND LIGHT-SCATTERING▲*USP33*

~~ULTRAVIOLET, VISIBLE, INFRARED, ATOMIC ABSORPTION, FLUORESCENCE, TURBIDIMETRY, NEPHELOMETRY, AND RAMAN MEASUREMENT~~

~~*Absorption spectrophotometry* is the measurement of an interaction between electromagnetic radiation and the molecules, or atoms, of a chemical substance. Techniques frequently employed in pharmaceutical analysis include UV, visible, IR, and atomic absorption spectroscopy. Spectrophotometric measurement in the visible region was formerly referred to as *colorimetry;* however, it is more precise to use the term "colorimetry" only when considering human perception of color.~~

~~*Fluorescence spectrophotometry* is the measurement of the emission of light from a chemical substance while it is being exposed to UV, visible, or other electromagnetic radiation. In general, the light emitted by a fluorescent solution is of maximum intensity at a wavelength longer than that of the exciting radiation, usually by some 20 to 30 nm.~~

~~*Light Scattering* involves measurement of the light scattered because of submicroscopic optical density inhomogeneities of solutions and is useful in the determination of weight-average molecular weights of polydisperse systems in the molecular weight range from 1000 to several hundred million. Two such techniques utilized in pharmaceutical analysis are *turbidimetry* and *nephelometry.*~~

~~*Raman spectroscopy* (inelastic light scattering) is a light scattering process in which the specimen under examination is irradiated with intense monochromatic light (usually laser light) and the light scattered from the specimen is analyzed for frequency shifts.~~

~~The wavelength range available for these measurements extends from the short wavelengths of the UV through the IR. For convenience of reference, this spectral range is roughly divided into the UV (190 to 380 nm), the visible (380 to 780 nm), the near IR (780 to 3000 nm), and the IR (2.5 to 40 µm or 4000 to 250 cm^{-1}).~~

~~COMPARATIVE UTILITY OF SPECTRAL RANGES~~

~~For many pharmaceutical substances, measurements can be made in the UV and visible regions of the spectrum with greater accuracy and sensitivity than in the near IR and IR. When solutions are observed in 1-cm cells, concentrations of about 10 µg of the specimen per mL often will produce absorbances of 0.2 to 0.8 in the UV or the visible region. In the IR and near IR, concentrations of 1 to 10 mg per mL and up to 100 mg per mL, respectively, may be needed to produce sufficient absorption; for these spectral ranges, cell lengths of from 0.01 mm to upwards of 3 mm are commonly used.~~

~~The UV and visible spectra of substances generally do not have a high degree of specificity. Nevertheless, they are highly suitable for quantitative assays, and for many substances they are useful as additional means of identification.~~

~~There has been increasing interest in the use of near IR spectroscopy in pharmaceutical analysis, especially for rapid identification of large numbers of samples, and also for water determination.~~

~~The near IR region is especially suitable for the determination of –OH and –NH groups, such as water in alcohol, –OH in the presence of amines, alcohols in hydrocarbons, and primary and secondary amines in the presence of tertiary amines.~~

In-Process Revision

The IR spectrum is unique for any given chemical compound with the exception of optical isomers, which have identical spectra. However, polymorphism may occasionally be responsible for a difference in the IR spectrum of a given compound in the solid state. Frequently, small differences in structure result in significant differences in the spectra. Because of the large number of maxima in an IR absorption spectrum, it is sometimes possible to quantitatively measure the individual components of a mixture of known qualitative composition without prior separation.

The Raman spectrum and the IR spectrum provide similar data, although the intensities of the spectra are governed by different molecular properties. Raman and IR spectroscopy exhibit different relative sensitivities for different functional groups, e.g., Raman spectroscopy is particularly sensitive to C–S and C–C multiple bonds, and some aromatic compounds are more easily identified by means of their Raman spectra. Water has a highly intense IR absorption spectrum, but a particularly weak Raman spectrum. Therefore, water has only limited IR "windows" that can be used to examine aqueous solutes, while its Raman spectrum is almost completely transparent and useful for solute identification. The two major limitations of Raman spectroscopy are that the minimum detectable concentration of specimen is typically 10^{-4} M to 10^{-2} M and that the impurities in many substances fluoresce and interfere with the detection of the Raman scattered signal.

Optical reflectance measurements provide spectral information similar to that obtained by transmission measurements. Since reflectance measurements probe only the surface composition of the specimen, difficulties associated with the optical thickness and the light scattering properties of the substance are eliminated. Thus, reflectance measurements are frequently more simple to perform on intensely absorbing materials. A particularly common technique used for IR reflectance measurements is termed attenuated total reflectance (ATR), also known as multiple internal reflectance (MIR). In the ATR technique, the beam of the IR spectrometer is passed through an appropriate IR window material (e.g., KRS-5, a TlBr-TlI eutectic mixture), which is cut at such an angle that the IR beam enters the first (front) surface of the window, but is totally reflected when it impinges on the second (back) surface (i.e., the angle of incidence of the radiation upon the second surface of the window exceeds the critical angle for that material). By appropriate window construction, it is possible to have many internal reflections of the IR beam before it is transmitted out of the window. If a specimen is placed in close contact with the window along the sides that totally reflect the IR beam, the intensity of reflected radiation is reduced at each wavelength (frequency) that the specimen absorbs. Thus, the ATR technique provides a reflectance spectrum that has been increased in intensity, when compared to a simple reflectance measurement, by the number of times that the IR beam is reflected within the window. The ATR technique provides excellent sensitivity, but it yields poor reproducibility, and is not a reliable quantitative technique unless an internal standard is intimately mixed with each test specimen.

Fluorescence spectrophotometry is often more sensitive than absorption spectrophotometry. In absorption measurements, the specimen transmittance is compared to that of a blank; and at low concentrations, both solutions give high signals. Conversely, in fluorescence spectrophotometry, the solvent blank has low rather than high output, so that the background radiation that may interfere with determinations at low concentrations is much less. Whereas few compounds can be determined conveniently at concentrations below 10^{-5} M by light absorption, it is not unusual to employ concentrations of 10^{-7} M to 10^{-6} M in fluorescence spectrophotometry.

THEORY AND TERMS

The power of a radiant beam decreases in relation to the distance that it travels through an absorbing medium. It also decreases in relation to the concentration of absorbing molecules or ions encountered in that medium. These two factors determine the proportion of the total incident energy that emerge. The decrease in power of monochromatic radiation passing through a homogeneous absorbing medium is stated quantitatively by Beer's law, $\log_{10}(1/T) = A = abc$, in which the terms are as defined below.

Absorbance [Symbol: *A*] — The logarithm, to the base 10, of the reciprocal of the transmittance *(T)*. [NOTE—Descriptive terms used formerly include optical density, absorbancy, and extinction.]

Absorptivity [Symbol: *a*] — The quotient of the absorbance *(A)* divided by the product of the concentration of the substance *(c)*, expressed in g per L, and the absorption path length *(b)* in cm. [NOTE—It is not to be confused with absorbancy index; specific extinction; or extinction coefficient.]

Molar Absorptivity [Symbol: *ε*] — The quotient of the absorbance *(A)* divided by the product of the concentration, expressed in *moles* per L, of the substance and the absorption path length in cm. It is also the product of the absorptivity *(a)* and the molecular weight of the substance. [NOTE—Terms formerly used include molar absorbancy index, molar extinction coefficient, and molar absorption coefficient.]

For most systems used in absorption spectrophotometry, the absorptivity of a substance is a constant independent of the intensity of the incident radiation, the internal cell length, and the concentration, with the result that concentration may be determined photometrically.

Beer's law gives no indication of the effect of temperature, wavelength, or the type of solvent. For most analytical work the effects of normal variation in temperature are negligible.

Deviations from Beer's law may be caused by either chemical or instrumental variables. Apparent failure of Beer's law may result from a concentration change in solute molecules because of association between solute molecules or between solute and solvent molecules, or dissociation or ionization. Other deviations might be caused by instrumental effects such as polychromatic radiation, slit-width effects, or stray light.

Even at a fixed temperature in a given solvent, the absorptivity may not be truly constant. However, in the case of specimens having only one absorbing component, it is not necessary that the absorbing system conform to Beer's law for use in quantitative analysis. The concentration of an unknown may be found by comparison with an experimentally determined standard curve.

Although, in the strictest sense, Beer's law does not hold in atomic absorption spectrophotometry because of the lack of quantitative properties of the cell length and the concentration, the absorption processes taking place in the flame under conditions of reproducible aspiration do follow the Beer relationship in principle. Specifically, the negative log of the transmittance, or the absorbance, is directly proportional to the absorption coefficient, and, consequently, is proportional to the number of absorbing atoms. On this basis, calibration curves may be constructed to permit evaluation of unknown absorption values in terms of concentration of the element in solution.

Absorption Spectrum — A graphic representation of absorbance, or any function of absorbance, plotted against wavelength or function of wavelength.

Transmittance [Symbol: *T*] — The quotient of the radiant power transmitted by a specimen divided by the radiant power incident upon the specimen. [NOTE—Terms formerly used include transmittancy and transmission.]

Fluorescence Intensity [Symbol: *I*] — An empirical expression of fluorescence activity, commonly given in terms of arbitrary units proportional to detector response. The *fluorescence emission spectrum* is a graphical presentation of the spectral distribution of radiation emitted by an activated substance, showing intensity of emitted radiation as ordinate, and wavelength as abscissa. The *fluorescence excitation spectrum* is a graphical presentation of the activation spectrum, showing intensity of radiation emitted by an activated substance as ordinate, and wavelength of the incident (activating) radiation as abscissa. As in absorption spectrophotometry, the important regions of the electromagnetic spectrum encompassed by the fluorescence of organic compounds are the UV, visible, and near IR, i.e., the region from 250 to 800 nm. After a molecule has absorbed radiation, the energy can be lost as heat or released in the form of radiation of the same or longer wavelength as the absorbed radiation. Both absorption and emission of radiation are due to the transitions of electrons between different energy levels, or orbitals, of the molecule. There is a time delay between the absorption and emission of light; this interval, the duration of the excited state, has been measured to be about 10^{-9} second to 10^{-8} second for most organic fluorescent solutions. The short lifetime of fluorescence distinguishes this type of luminescence from phosphorescence, which is a long-lived afterglow having a lifetime of 10^{-3} second up to several minutes.

In-Process Revision

Turbidance [Symbol: *S*]—The light-scattering effect of suspended particles. The amount of suspended matter may be measured by observation of either the transmitted light (turbidimetry) or the scattered light (nephelometry).

Turbidity [Symbol: τ]—In light-scattering measurements, the turbidity is the measure of the decrease in incident beam intensity per unit length of a given suspension.

Raman Scattering Activity—The molecular property (in units of cm^4 per g) governing the intensity of an observed Raman band for a randomly oriented specimen. The scattering activity is determined from the derivative of the molecular polarizability with respect to the molecular motion giving rise to the Raman shifted band. In general, the Raman band intensity is linearly proportional to the concentration of the analyte.

USE OF REFERENCE STANDARDS

With few exceptions, the Pharmacopeial spectrophotometric tests and assays call for comparison against a USP Reference Standard. This is to ensure measurement under conditions identical for the test specimen and the reference substance. These conditions include wavelength setting, slit-width adjustment, cell placement and correction, and transmittance levels. It should be noted that cells exhibiting identical transmittance at a given wavelength may differ considerably in transmittance at other wavelengths. Appropriate cell corrections should be established and used where required.

The expressions, "similar preparation" and "similar solution," as used in tests and assays involving spectrophotometry, indicate that the reference specimen, generally a USP Reference Standard, is to be prepared and observed in a manner identical for all practical purposes to that used for the test specimen. Usually in making up the solution of the specified Reference Standard, a solution of about (i.e., within 10%) the desired concentration is prepared and the absorptivity is calculated on the basis of the exact amount weighed out; if a previously dried specimen of the Reference Standard has not been used, the absorptivity is calculated on the anhydrous basis.

The expressions, "concomitantly determine" and "concomitantly measured," as used in tests and assays involving spectrophotometry, indicate that the absorbances of both the solution containing the test specimen and the solution containing the reference specimen, relative to the specified test blank, are to be measured in immediate succession.

APPARATUS

Many types of spectrophotometers are available. Fundamentally, most types, except those used for IR spectrophotometry, provide for passing essentially monochromatic radiant energy through a specimen in suitable form, and measuring the intensity of the fraction that is transmitted. Fourier transform IR spectrophotometers use an interferometric technique whereby polychromatic radiation passes through the analyte and onto a detector on an intensity and time basis. UV, visible, and dispersive IR spectrophotometers comprise an energy source, a dispersing device (e.g., a prism or grating), slits for selecting the wavelength band, a cell or holder for the test specimen, a detector of radiant energy, and associated amplifiers and measuring devices. In *diode array* spectrophotometers, the energy from the source is passed through the test specimen and then dispersed via a grating onto several hundred light-sensitive diodes, each of which in turn develops a signal proportional to the number of photons at its small wavelength interval; these signals then may be computed at rapid chosen intervals to represent a complete spectrum. Fourier transform IR systems utilize an interferometer instead of a dispersing device and a digital computer to process the spectral data. Some instruments are manually operated, whereas others are equipped for automatic and continuous recording. Instruments that are interfaced to a digital computer have the capabilities also of co-adding and storing spectra, performing spectral comparisons, and performing difference spectroscopy (accomplished with the use of a digital absorbance subtraction method).

Instruments are available for use in the visible, in the visible and UV, in the visible, UV, and near-IR, and in the IR regions of the spectrum. Choice of the type of spectrophotometric analysis and of the instrument to be used depends upon factors such as the composition and amount of available test specimen, the degree of accuracy, sensitivity, and selectivity desired, and the manner in which the specimen is handled.

The apparatus used in atomic absorption spectrophotometry has several unique features. For each element to be determined, a specific source that emits the spectral line to be absorbed should be selected. The source is usually a hollow-cathode lamp, the cathode of which is designed to emit the desired radiation when excited. Since the radiation to be absorbed by the test specimen element is usually of the same wavelength as that of its emission line, the element in the hollow-cathode lamp is the same as the element to be determined. The apparatus is equipped with an aspirator for introducing the test specimen into a flame, which is usually provided by air–acetylene, air–hydrogen, or, for refractory cases, nitrous oxide–acetylene. The flame, in effect, is a heated specimen chamber. A detector is used to read the signal from the chamber. Interfering radiation produced by the flame during combustion may be negated by the use of a chopped source lamp signal of a definite frequency. The detector should be tuned to this alternating current frequency so that the direct current signal arising from the flame is ignored. The detecting system, therefore, reads only the change in signal from the hollow-cathode source, which is directly proportional to the number of atoms to be determined in the test specimen. For Pharmacopeial purposes, apparatus that provides the readings directly in absorbance units is usually required. However, instruments providing readings in percent transmission, percent absorption, or concentration may be used if the calculation formulas provided in the individual monographs are revised as necessary to yield the required quantitative results. Percent absorption or percent transmittance may be converted to absorbance, *A*, by the following two equations:

$$A = 2 - \log_{10}(100 - \%\ \text{absorption})$$

or:

$$A = 2 - \log_{10}(\%\ \text{transmittance})$$

Depending upon the type of apparatus used, the readout device may be a meter, digital counter, recorder, or printer. Both single-beam and double-beam instruments are commercially available, and either type is suitable.

Measurement of fluorescence intensity can be made with a simple *filter fluorometer*. Such an instrument consists of a radiation source, a primary filter, a specimen chamber, a secondary filter, and a fluorescence detection system. In most such fluorometers, the detector is placed on an axis at 90° from that of the exciting beam. This right-angle geometry permits the exciting radiation to pass through the test specimen and not contaminate the output signal received by the fluorescence detector. However, the detector unavoidably receives some of the exciting radiation as a result of the inherent scattering properties of the solutions themselves, or if dust or other solids are present. Filters are used to eliminate this residual scatter. The primary filter selects short-wavelength radiation capable of exciting the test specimen, while the secondary filter is normally a sharp cut-off filter that allows the longer-wavelength fluorescence to be transmitted but blocks the scattered excitation.

Most fluorometers use photomultiplier tubes as detectors, many types of which are available, each having special characteristics with respect to spectral region of maximum sensitivity, gain, and electrical noise. The photocurrent is amplified and read out on a meter or recorder.

A *spectrofluorometer* differs from a filter fluorometer in that filters are replaced by monochromators, of either the prism or the grating type. For analytical purposes, the spectrofluorometer is superior to the filter fluorometer in wavelength selectivity, flexibility, and convenience, in the same way in which a spectrophotometer is superior to a filter photometer.

Many radiation sources are available. Mercury lamps are relatively stable and emit energy mainly at discrete wavelengths. Tungsten lamps provide an energy continuum in the visible region. The high-pressure xenon arc lamp is often used in spectrofluorometers because it is a high-intensity source that emits an energy continuum extending from the UV into the IR.

In spectrofluorometers, the monochromators are equipped with slits. A narrow slit provides high resolution and spectral purity, while a large slit sacrifices these for high sensitivity. Choice of slit size is determined by the separation between exciting and emitting wavelengths as well as the degree of sensitivity needed.

Specimen cells used in fluorescence measurements may be round tubes or rectangular cells similar to those used in absorption spectrophotometry, except that they are polished on all four vertical sides. A convenient test specimen size is 2 to 3 mL, but some instruments can be fitted with small cells holding 100 to 300 µL, or with a capillary holder requiring an even smaller amount of specimen.

Light-scattering instruments are available and consist in general of a mercury lamp, with filters for the strong green or blue lines, a shutter, a set of neutral filters with known transmittance, and a sensitive photomultiplier to be mounted on an arm that can be rotated around the solution cell and set at any angle from −135° to 0° to +135° by a dial outside the light-tight housing. Solution cells are of various shapes, such as square for measuring 90° scattering; semioctagonal for 45°, 90°, and 135° scattering; and cylindrical for scattering at all angles. Since the determination of molecular weight requires a precise measure of the difference in refractive index between the solution and solvent, $[(n - n_s)/c]$, a second instrument, a differential refractometer, is needed to measure this small difference.

Raman spectrometers include the following major components: a source of intense monochromatic radiation (invariably a laser); optics to collect the light scattered by the test specimen; a (double) monochromator to disperse the scattered light and reject the intense incident frequency; and a suitable light-detection and amplification system. Raman measurement is simple in that most specimens are examined directly in melting-point capillaries. Because the laser source can be focused sharply, only a few microliters of the specimen is required.

PROCEDURE

Absorption Spectrophotometry

Detailed instructions for operating spectrophotometers are supplied by the manufacturers. To achieve significant and valid results, the operator of a spectrophotometer should be aware of its limitations and of potential sources of error and variation. The instruction manual should be followed closely on such matters as care, cleaning, and calibration of the instrument, and techniques of handling absorption cells, as well as instructions for operation. The following points require special emphasis.

Check the instrument for accuracy of calibration. Where a continuous source of radiant energy is used, attention should be paid to both the wavelength and photometric scales; where a spectral line source is used, only the photometric scale need be checked. A number of sources of radiant energy have spectral lines of suitable intensity, adequately spaced throughout the spectral range selected. The best single source of UV and visible calibration spectra is the quartz mercury arc, of which the lines at 253.7, 302.25, 313.16, 334.15, 365.48, 404.66, and 435.83 nm may be used. The glass mercury arc is equally useful above 300 nm. The 486.13-nm and 656.28-nm lines of a hydrogen discharge lamp may be used also. The wavelength scale may be calibrated also by means of suitable glass filters, which have useful absorption bands through the visible and UV regions. Standard glasses containing didymium (a mixture of praseodymium and neodymium) have been used widely, although glasses containing holmium were found to be superior. Standard holmium oxide solution has superseded the use of holmium glass.[1] The wavelength scales of near-IR and IR spectrophotometers are readily checked by the use of absorption bands provided by polystyrene films, carbon dioxide, water vapor, or ammonia gas.

For checking the photometric scale, a number of standard inorganic glass filters as well as standard solutions of known transmittances such as potassium dichromate are available.[2]

Quantitative absorbance measurements usually are made on solutions of the substance in liquid-holding cells. Since both the solvent and the cell window absorb light, compensation must be made for their contribution to the measured absorbance. Matched cells are available commercially for UV and visible spectrophotometry for which no cell correction is necessary. In IR spectrophotometry, however, corrections for cell differences usually must be made. In such cases, pairs of cells are filled with the selected solvent and the difference in their absorbances at the chosen wavelength is determined. The cell exhibiting the greater absorbance is used for the solution of the test specimen and the measured absorbance is corrected by subtraction of the cell difference.

With the use of a computerized Fourier-transform IR system, this correction need not be made, since the same cell can be used for both the solvent blank and the test solution. However, it must be ascertained that the transmission properties of the cell are constant.

Comparisons of a test specimen with a Reference Standard are best made at a peak of spectral absorption for the compound concerned. Assays prescribing spectrophotometry give the commonly accepted wavelength for peak spectral absorption of the substance in question. It is known that different spectrophotometers may show minor variation in the apparent wavelength of this peak. Good practice demands that comparisons be made at the wavelength at which peak absorption occurs. Should this differ by more than ± 1 nm from the wavelength specified in the individual monograph, recalibration of the instrument may be indicated.

TEST PREPARATION

For determinations utilizing UV or visible spectrophotometry, the specimen generally is dissolved in a solvent. Unless otherwise directed in the monograph, determinations are made at room temperature using a path length of 1 cm. Many solvents are suitable for these ranges, including water, alcohols, chloroform, lower hydrocarbons, ethers, and dilute solutions of strong acids and alkalies. Precautions should be taken to utilize solvents free from contaminants absorbing in the spectral region being used. It is usually advisable to use water-free methanol or alcohol, or alcohol denatured by the addition of methanol but not containing benzene or other interfering impurities, as the solvent. Solvents of special spectrophotometric quality, guaranteed to be free from contaminants, are available commercially from several sources. Some other analytical reagent-grade organic solvents may contain traces of impurities that absorb strongly in the UV region. New lots of these solvents should be checked for their transparency, and care should be taken to use the same lot of solvent for preparation of the test solution and the standard solution and for the blank.

No solvent in appreciable thickness is completely transparent throughout the near-IR and IR spectrum. Carbon tetrachloride (up to 5 mm in thickness) is practically transparent to 6 µm (1666 cm^{-1}). Carbon disulfide (1 mm in thickness) is suitable as a solvent to 40 µm (250 cm^{-1}) with the exception of the 4.2-µm to 5.0-µm (2381 cm^{-1} to 2000 cm^{-1}) and the 5.5-µm to 7.5-µm (1819 cm^{-1} to 1333 cm^{-1}) regions, where it has strong absorption. Other solvents have relatively narrow regions of transparency. For IR spectrophotometry, an additional qualification for a suitable solvent is that it must not affect the material, usually sodium chloride, of which the cell is made. The test specimen may also be prepared by dispersing the finely ground solid specimen in mineral oil or by mixing it intimately with previously dried alkali halide salt (usually potassium bromide). Mixtures with alkali halide salts may be examined directly or as transparent disks or pellets obtained by pressing the mixture in a

[1] National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899: "Spectral Transmittance Characteristics of Holmium Oxide in Perchloric Acid," J. Res. Natl. Bur. Stds. 90, No. 2, 115 (1985). The performance of an uncertified filter should be checked against a certified standard.

[2] For further detail regarding checks on photometric scale of a spectrophotometer, reference may be made to the following NIST publications: J. Res. Natl. Bur. Stds. 76A, 469 (1972) [re: SRM 931, "Liquid Absorbance Standards for Ultraviolet and Visible Spectrophotometry" as well as potassium chromate and potassium dichromate]; NIST Spec. Publ. 260–116 (1994) [re: SRM 930 and SRM 1930, "Glass Filters for Spectrophotometry".

In-Process Revision

die. Typical drying conditions for potassium bromide are 105° in vacuum for 12 hours, although grades are commercially available that require no drying. Infrared microscopy or a mineral oil dispersion is preferable where disproportionation between the alkali halide and the test specimen is encountered. For suitable materials the test specimen may be prepared neat as a thin sample for IR microscopy or suspended neat as a thin film for mineral oil dispersion. For Raman spectrometry, most common solvents are suitable, and ordinary (non-fluorescing) glass specimen cells can be used. The IR region of the electromagnetic spectrum extends from 0.8 to 400 µm. From 800 to 2500 nm (0.8 to 2.5 µm) is generally considered to be the near-IR (NIR) region; from 2.5 to 25 µm (4000 to 400cm^{-1}) is generally considered to be the mid-range (mid-IR) region; and from 25 to 400 µm is generally considered to be the far-IR (FIR) region. Unless otherwise specified in the individual monograph, the region from 3800 to 650 cm^{-1} (2.6 to 15 µm) should be used to ascertain compliance with monograph specifications for IR absorption.

Where values for IR line spectra are given in an individual monograph, the letters *s, m,* and *w* signify strong, medium, and weak absorption, respectively; *sh* signifies a shoulder, *bd* signifies a band, and *v* means very. The values may vary as much as 0.1 µm or 10 cm^{-1}, depending upon the particular instrument used. Polymorphism gives rise to variations in the IR spectra of many compounds in the solid state. Therefore, when conducting IR absorption tests, if a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test substance and the standard in equal volumes of a suitable solvent, evaporate the solutions to dryness in similar containers under identical conditions, and repeat the test on the residues.

In NIR spectroscopy much of the current interest centers around the ease of analysis. Samples can be analyzed in powder form or by means of reflectance techniques, with little or no preparation. Compliance with in-house specifications can be determined by computerized comparison of spectra with spectra previously obtained from reference materials. Many pharmaceutical materials exhibit low absorptivity in this spectral region, which allows incident near-IR radiation to penetrate samples more deeply than UV, visible, or IR radiation. NIR spectrophotometry may be used to observe matrix modifications and, with proper calibration, may be used in quantitative analysis.

In atomic absorption spectrophotometry, the nature of the solvent and the concentration of solids must be given special consideration. An ideal solvent is one that interferes to a minimal extent in the absorption or emission processes and one that produces neutral atoms in the flame. If there is a significant difference between the surface tension or viscosity of the test solution and standard solution, the solutions are aspirated or atomized at a different rate, causing significant differences in the signals generated. The acid concentration of the solutions also affects the absorption processes. Thus, the solvents used in preparing the test specimen and the standard should be the same or as much alike in these respects as possible, and should yield solutions that are easily aspirated via the specimen tube of the burner-aspirator. Since undissolved solids present in the solutions may give rise to matrix or bulk interferences, the total undissolved solids content in all solutions should be kept below 2% wherever possible.

CALCULATIONS

The application of absorption spectrophotometry in an assay or a test generally requires the use of a Reference Standard. Where such a measurement is specified in an assay, a formula is provided in order to permit calculation of the desired result. A numerical constant is frequently included in the formula. The following derivation is provided to introduce a logical approach to the deduction of the constants appearing in formulas in the assays in many monographs.

The Beer's law relationship is valid for the solutions of both the Reference Standard *(S)* and the test specimen *(U)*: in which A_S is the absorbance of the Standard solution of concentration C_S, and A_U is the absorbance of the test specimen solution of concentration C_U. If C_S and C_U are expressed in the same units and the absorbances of both solutions are measured in matching cells having the same dimensions, the absorptivity, *a,* and the cell thickness, *b,* are the same; consequently, the two equations may be combined and rewritten to solve for C_U:

(1) $A_S = abC_S$

(2) $A_U = abC_U$

(3) $C_U = C_S(A_U/A_S)$

Quantities of solid test specimens to be taken for analysis are generally specified in mg. Instructions for dilution are given in the assay and, since dilute solutions are used for absorbance measurements, concentrations are usually expressed for convenience in units of µg per mL. Taking a quantity, in mg, of a test specimen of a drug substance or solid dosage form for analysis, it therefore follows that a volume (V_U), in L, of solution of concentration C_U may be prepared from the amount of test specimen that contains a quantity W_U, in mg, of the drug substance [NOTE—C_U is numerically the same whether expressed as µg per mL or mg per L], such that:

(4) $W_U = V_U C_U$

The form in which the formula appears in the assay in a monograph for a solid article may be derived by substituting C_U of equation (3) into equation (4). In summary, the use of equation (4), with due consideration for any unit conversions necessary to achieve equality in equation (5), permits the calculation of the constant factor (V_U) occurring in the final formula:

(5) $W_U = V_U C_S(A_U/A_S)$

The same derivation is applicable to formulas that appear in monographs for liquid articles that are assayed by absorption spectrophotometry. For liquid dosage forms, results of calculations are generally expressed in terms of the quantity, in mg, of drug substance in each mL of the article. Thus it is necessary to include in the denominator an additional term, the volume *(V)*, in mL, of the test preparation taken.

Assays in the visible region usually call for comparing concomitantly the absorbance produced by the *Assay preparation* with that produced by a *Standard preparation* containing approximately an equal quantity of a USP Reference Standard. In some situations, it is permissible to omit the use of a Reference Standard. This is true where spectrophotometric assays are made with routine frequency, and where a suitable standard curve is available, prepared with the respective USP Reference Standard, and where the substance assayed conforms to Beer's law within the range of about 75% to 125% of the final concentration used in the assay. Under these circumstances, the absorbance found in the assay may be interpolated on the standard curve, and the assay result calculated therefrom.

Such standard curves should be confirmed frequently, and always when a new spectrophotometer or new lots of reagents are put into use.

In spectrophotometric assays that direct the preparation and use of a standard curve, it is permissible and preferable, when the assay is employed infrequently, not to use the standard curve but to make the comparison directly against a quantity of the Reference Standard approximately equal to that taken of the specimen, and similarly treated.

Fluorescence Spectrophotometry

The measurement of fluorescence is a useful analytical technique. *Fluorescence* is light emitted from a substance in an excited state that has been reached by the absorption of radiant energy. A substance is said to be *fluorescent* if it can be made to fluoresce. Many compounds can be assayed by procedures utilizing either their inherent fluorescence or the fluorescence of suitable derivatives.

Test specimens prepared for fluorescence spectrophotometry are usually one-tenth to one-hundredth as concentrated as those used in absorption spectrophotometry, for the following reason. In analytical applications, it is preferable that the fluorescence signal be linearly related to the concentration; but if a test specimen is too concentrated, a significant part of the incoming light is absorbed by the specimen near the cell surface, and the light reaching the center is reduced. That is, the specimen itself acts as an "inner filter." However, fluorescence spectrophotometry is inherently a highly sensitive technique, and

concentrations of 10^{-5} M to 10^{-3} M frequently are used. It is necessary in any analytical procedure to make a working curve of fluorescence intensity versus concentration in order to establish a linear relationship. All readings should be corrected for a solvent blank.

Fluorescence measurements are sensitive to the presence of dust and other solid particles in the test specimen. Such impurities may reduce the intensity of the exciting beam or give misleading high readings because of multiple reflections in the specimen cell. It is, therefore, wise to eliminate solid particles by centrifugation; filtration also may be used, but some filter papers contain fluorescent impurities.

Temperature regulation is often important in fluorescence spectrophotometry. For some substances, fluorescence efficiency may be reduced by as much as 1% to 2% per degree of temperature rise. In such cases, if maximum precision is desired, temperature-controlled specimen cells are useful. For routine analysis, it may be sufficient to make measurements rapidly enough so that the specimen does not heat up appreciably from exposure to the intense light source. Many fluorescent compounds are light-sensitive. Exposed in a fluorometer, they may be photo-degraded into more or less fluorescent products. Such effects may be detected by observing the detector response in relationship to time, and may be reduced by attenuating the light source with filters or screens.

Change of solvent may markedly affect the intensity and spectral distribution of fluorescence. It is inadvisable, therefore, to alter the solvent specified in established methods without careful preliminary investigation. Many compounds are fluorescent in organic solvents but virtually nonfluorescent in water; thus, a number of solvents should be tried before it is decided whether or not a compound is fluorescent. In many organic solvents, the intensity of fluorescence is increased by elimination of dissolved oxygen, which has a strong quenching effect. Oxygen may be removed by bubbling an inert gas such as nitrogen or helium through the test specimen.

A semiquantitative measure of the strength of fluorescence is given by the ratio of the fluorescence intensity of a test specimen and that of a standard obtained with the same instrumental settings. Frequently, a solution of stated concentration of quinine in 0.1 N sulfuric acid or fluorescein in 0.1 N sodium hydroxide is used as a reference standard.

Light Scattering

Turbidity can be measured with a standard photoelectric filter photometer or spectrophotometer, preferably with illumination in the blue portion of the spectrum. Nephelometric measurements require an instrument with a photocell placed so as to receive scattered rather than transmitted light; this geometry applies also to fluorometers, so that, in general, fluorometers can be used as nephelometers, by proper selection of filters. A ratio turbidimeter combines the technology of 90° nephelometry and turbidimetry: it contains photocells that receive and measure scattered light at a 90° angle from the sample as well as receiving and measuring the forward scatter in front of the sample; it also measures light transmitted directly through the sample. Linearity is attained by calculating the ratio of the 90° angle scattered light measurement to the sum of the forward scattered light measurement and the transmitted light measurement. The benefit of using a ratio turbidimetry system is that the measurement of stray light becomes negligible.

In practice, it is advisable to ensure that settling of the particles being measured is negligible. This is usually accomplished by including a protective colloid in the liquid suspending medium. It is important that results be interpreted by comparison of readings with those representing known concentrations of suspended matter, produced under precisely the same conditions.

Turbidimetry or nephelometry may be useful for the measurement of precipitates formed by the interaction of highly dilute solutions of reagents, or other particulate matter, such as suspensions of bacterial cells. In order that consistent results may be achieved, all variables must be carefully controlled. Where such control is possible, extremely dilute suspensions may be measured.

The specimen solute is dissolved in the solvent at several different accurately known concentrations, the choice of concentrations being dependent on the molecular weight of the solute and ranging from 1% for M_w = 10,000 to 0.01% for M_w = 1,000,000. Each solution must be very carefully cleaned before measurement by repeated filtration through fine filters. A dust particle in the solution vitiates the intensity of the scattered light measured. A criterion for a clear solution is that the dissymmetry, 45°/135° scattered intensity ratio, has attained a minimum.

The turbidity and refractive index of the solutions are measured. From the general 90° light scattering equation, a plot of HC/τ versus C is made and extrapolated to infinite dilution, and the weight-average molecular weight, M, is calculated from the intercept, $1/M$.

Visual Comparison

Where a color or a turbidity comparison is directed, color comparison tubes that are matched as closely as possible in internal diameter and in all other respects should be used. For color comparison, the tubes should be viewed downward, against a white background, with the aid of a light source directed from beneath the bottoms of the tubes, while for turbidity comparison the tubes should be viewed horizontally, against a dark background, with the aid of a light source directed from the sides of the tubes.

In conducting limit tests that involve a comparison of colors in two like containers (e.g., matched color comparison tubes), a suitable instrument, rather than the unaided eye, may be used.

▲ULTRAVIOLET, VISIBLE, INFRARED, ATOMIC ABSORPTION, FLUORESCENCE, TURBIDIMETRY, NEPHELOMETRY, AND RAMAN MEASUREMENT

Absorption spectroscopy is the measurement of an interaction between electromagnetic radiation and the molecules, or atoms, of a chemical substance. Spectroscopy techniques frequently employed in pharmaceutical analysis include ultraviolet (UV), visible (see the proposed chapter *Ultraviolet-Visible Spectroscopy* ⟨857⟩), infrared (IR) (see the proposed chapter *Mid-Infrared Spectroscopy* ⟨854⟩), near-infrared (see the official chapter *Near-Infrared Spectrophotometry* ⟨1119⟩), and atomic absorption. Spectrophotometric measurement in the visible region was formerly referred to as *colorimetry*; however, it is more precise to use the term *colorimetry* only when considering human perception of color.

Fluorescence spectroscopy (see the proposed chapter *Fluorescence Spectroscopy* ⟨853⟩) is the measurement of the emission of light from a chemical substance while it is being exposed to UV, visible, or other electromagnetic radiation or during a chemical reaction. In general, the light emitted by a fluorescent solution is of maximum intensity at a wavelength longer than that of the exciting radiation, usually by some 20 to 30 nm.

In-Process Revision

Light-scattering involves measurement of the light scattered because of submicroscopic optical density inhomogeneities of solutions. The technique is useful in the determination of weight-average molecular weights of polydisperse systems in the molecular weight range from 1000 to several hundred million. Two such techniques used in pharmaceutical analysis are *turbidimetry* and *nephelometry.*

Raman spectroscopy (inelastic light-scattering) (see the official chapter *Raman Spectroscopy* ⟨1120⟩) is a light-scattering process in which the specimen under examination is irradiated with intense monochromatic light (usually laser light) and the light scattered from the specimen is analyzed for frequency shifts.

The wavelength range available for these measurements extends from the short wavelengths of UV through near-IR. For convenience of reference, this spectral range is roughly divided into UV (190 to 380 nm), visible (380 to 780 nm), and near-IR (780 to 1400 nm). Raman spectroscopy involves illuminating a sample with a monochromatic (laser) source and measuring the spectrum of the radiation that has been inelastically scattered from the sample. The difference between the wavenumber of the laser and the wavenumbers of the inelastically scattered radiation corresponds to the wavenumber of the vibrational modes of the molecule.

COMPARATIVE UTILITY OF SPECTRAL RANGES

For many pharmaceutical substances, measurements can be made in the UV and visible regions of the spectrum with greater accuracy and sensitivity than in the near-IR and IR regions. When solutions are observed in 1-cm cells, concentrations of about 10 µg of the specimen per mL will often produce absorbances of 0.2 to 0.8 in the UV or the visible region. In the IR and near-IR regions, concentrations of 1 to 10 mg per mL and up to 100 mg per mL, respectively, may be needed to produce sufficient absorption; for these spectral ranges, cell lengths from 0.01 mm to more than 3 mm are commonly used; 0.1 mm is most commonly used for mid-IR measurements.

The UV and visible spectra of substances generally do not have a high degree of specificity. Nevertheless, they are highly suitable for quantitative assays, and for many substances they are useful as additional means of identification.

There has been increasing interest in the use of near-IR spectroscopy in pharmaceutical analysis, especially for rapid identification of large numbers of samples, and also for water determination.

The near-IR region is especially suitable for the determination of –OH and –NH groups, such as water in alcohol, –OH in the presence of amines, alcohols in hydrocarbons, and primary and secondary amines in the presence of tertiary amines.

The IR spectrum is unique for any given chemical compound with the exception of optical isomers, which have identical spectra. However, polymorphism may occasionally be responsible for a difference in the IR spectrum of a given compound in the solid state. Frequently, small differences in structure result in significant differences in the spectra. Because of the large number of maxima in an IR absorption spectrum, it is sometimes possible to quantitatively measure the individual components of a mixture of known qualitative composition without prior separation.

The Raman spectrum and the IR spectrum provide similar data, although the intensities of the spectra are governed by different molecular properties. Raman and IR spectroscopy exhibit different relative sensitivities for different functional groups; for example, Raman spectroscopy is particularly sensitive to C–S as well as C–C multiple bonds, and some aromatic compounds are more easily identified by means of their Raman spectra. Water has a highly intense IR absorption spectrum, but a particularly weak Raman spectrum. Therefore, water has only limited IR ''windows'' that can be used to examine aqueous solutes, whereas its Raman spectrum is almost completely transparent and useful for solute identification. The two major limitations of Raman spectroscopy are that the minimum detectable concentration of specimen is typ-

ically 10^{-1} M to 10^{-2} M and that the impurities in many substances fluoresce and interfere with the detection of the Raman scattered signal.

Optical reflection measurements provide spectral information similar to that obtained by transmission measurements. Because reflection measurements probe only the surface composition of the specimen, difficulties associated with the optical thickness and the light-scattering properties of the substance are eliminated. Thus, reflection measurements are frequently simpler to perform on intensely absorbing materials. A particularly common technique used for IR reflection measurements is termed *attenuated total reflection* (ATR) (see the proposed chapter *Mid-Infrared Spectroscopy* ⟨854⟩*, Attenuated Total Reflection Spectroscopy*). In the ATR technique, the beam of the IR spectrometer is passed through an appropriate IR window material (e.g., zinc selenide, diamond, germanium), which is cut at such an angle that the IR beam enters the first (front) surface of the window, but is totally reflected when it impinges on the second (back) surface (i.e., the angle of incidence of the radiation on the second surface of the window exceeds the critical angle for that material). If a specimen is placed in close contact with the window along the sides that totally reflect the IR beam, the intensity of reflected radiation is reduced at each wavelength (frequency) that the specimen absorbs. By means of appropriate window construction, it is possible to have many internal reflections of the IR beam before it is transmitted out of the window. For accessories with multiple reflections, the ATR technique provides a spectrum in which the absorbance of each band has been increased in intensity, when compared to a single reflection measurement, by the number of times the IR beam is reflected within the window. The ATR technique provides excellent sensitivity, and it can provide good reproducibility, provided that care is taken to ensure good contact between the sample and the internal reflection element.

Fluorescence spectroscopy is often more sensitive than absorption spectroscopy. In absorption measurements, the signal from the radiation that is transmitted through the specimen is compared to the corresponding signal from a blank; and at low concentrations, both solutions give high signals that give rise to high photon shot noise from the detector. Conversely, in fluorescence spectroscopy, the solvent blank has low rather than high output; therefore, the background radiation that may interfere with determinations at low concentrations is much less than in absorption spectroscopy. Whereas few compounds can be determined conveniently at concentrations below 10^{-5} M by light absorption, it is not unusual to employ concentrations of 10^{-7} M to 10^{-8} M in fluorescence spectroscopy.

USE OF REFERENCE STANDARDS

With few exceptions, Pharmacopeial spectrophotometric tests and assays call for comparison against a USP Reference Standard. This requirement is made in order to ensure measurement under conditions that are identical for the test specimen and the reference substance. These conditions include wavelength setting, slit-width adjustment, cell placement and correction, and transmittance levels. It should be noted that cells exhibiting identical transmittance at a given wavelength may differ considerably in transmittance at other wavelengths. Appropriate cell corrections should be established and used where required. The expressions *similar preparation* and *similar solution*, as used in tests and assays involving spectroscopy, indicate that the reference specimen, generally a USP Reference Standard, is to be prepared and observed in a manner identical (for all practical purposes) to that used for the test specimen. Usually in making up the solution of the specified Reference Standard, a solution of about the desired concentration (i.e., within about 10%) is prepared, and the absorptivity is calculated on the basis of the exact amount weighed out; if a previously dried specimen of the Reference Standard has not been used, the absorptivity is calculated on the anhydrous basis.

The expressions *concomitantly determine* and *concomitantly measured*, as used in tests and assays involving spectroscopy, indicate that the absorbances of both the solution containing the test specimen and the solution containing the reference specimen, relative to the specified test blank, are to be measured in immediate succession.

ATOMIC ABSORPTION

The apparatus used in atomic absorption spectroscopy has several unique features. For each element to be determined, a specific source that emits the spectral line to be absorbed should be selected. The source is usually a hollow-cathode lamp, the cathode of which is designed to emit the desired radiation when excited. Since the radiation to be absorbed by the test specimen element is usually of the same wavelength as that of its emission line, the element in the hollow-cathode lamp is the same as the element to be determined. The apparatus is equipped with an aspirator for introducing the test specimen into a flame, which is usually provided by air–acetylene, air–hydrogen, or, for refractory cases, nitrous oxide–acetylene. The flame, in effect, is a heated specimen chamber. A detector is used to read the signal from the chamber. Interfering radiation produced by the flame during combustion may be negated by the use of a chopped source lamp signal of a definite frequency. The detector should be tuned to the modulation frequency so that the direct current signal arising from the flame is ignored. The detecting system, therefore, reads only the change in signal from the hollow-cathode source, which is directly proportional to the number of atoms to be determined in the test specimen. For Pharmacopeial purposes, apparatus that provides the readings directly in absorbance units is usually required. However, instruments providing readings in percent transmission, percent absorption, or concentration may be used if the calculation formulas provided in the individual monographs are revised as necessary to yield the required quantitative results. Percent absorption or percent transmission may be converted to absorbance, A, by the following two equations:

$$A = 2 - \log_{10} (100 - \%\ \text{absorption})$$

or:

$$A = 2 - \log_{10} (\%\ \text{transmission})$$

Depending on the type of apparatus used, the readout device may be a meter, digital counter, recorder, or printer. Both single-beam and double-beam instruments are commercially available, and either type is suitable.

LIGHT-SCATTERING SPECTROSCOPY

Light-scattering instruments consist in general of a mercury lamp, with filters for the strong green or blue lines; a shutter; a set of neutral filters with known transmittance; and a sensitive photocell, to be mounted on an arm that can be rotated around the solution cell and set at any angle from –135° to 0° to +135° by a dial outside the light-tight housing. Solution cells are of various shapes, such as square for measuring 90° scattering; semioctagonal for 45°, 90°, and 135° scattering; and cylindrical for scattering at all angles. The determination of molecular weight requires a precise measure of the difference in refractive index between the solution and solvent, $[(n - n_0)/c]$. Therefore, a second instrument, a differential refractometer, is needed to measure this small difference.

Turbidity can be measured with a standard photoelectric filter photometer or spectrophotometer, preferably with illumination in the blue portion of the spectrum. Nephelometric measurements require an instrument with a photocell placed so as to receive scattered rather than transmitted light. This geometry applies also to fluorometers; therefore, in general, fluorometers can be used as nephelometers by proper selection of

filters. A ratio turbidimeter combines the technology of 90° nephelometry and turbidimetry: it contains photocells that receive and measure scattered light at a 90° angle from the sample as well as receiving and measuring the forward scatter in front of the sample; it also measures light transmitted directly through the sample. Linearity is attained by calculating the ratio of the 90° angle–scattered light measurement to the sum of the forward-scattered light measurement and the transmitted light measurement. The benefit of using a ratio turbidimetry system is that the measurement of stray light becomes negligible.

In practice, it is advisable to ensure that settling of the particles being measured is negligible. This is usually accomplished by including a protective colloid in the liquid suspending medium. It is important that results be interpreted by comparison of readings with those representing known concentrations of suspended matter, produced under precisely the same conditions.

Turbidimetry or nephelometry may be useful for the measurement of precipitates formed by the interaction of highly dilute solutions of reagents or other particulate matter, such as suspensions of bacterial cells. In order that consistent results may be achieved, all variables must be carefully controlled. Where such control is possible, extremely dilute suspensions may be measured.

The specimen solute is dissolved in the solvent at several different accurately known concentrations; the choice of concentrations is dependent on the molecular weight of the solute and ranges from 1% for MW = 10,000 to 0.01% for MW = 1,000,000. Before measurement, each solution must be very carefully cleaned by repeated filtration through fine filters. A dust particle in the solution vitiates the intensity of the scattered light measured. A criterion for a clear solution is that the dissymmetry, 45°/135° scattered intensity ratio, has attained a minimum.

The turbidity (τ) and the refractive index of the solutions are measured. From the general 90° light-scattering equation, a plot of HC/τ versus C, where H is a constant for a given solute–solvent system and C is the concentration of molecules or particles that are dissolved, mixed or suspended in the solutions, is made. It is then extrapolated to infinite dilution; and the weight-average molecular weight, M_W, is calculated from the intercept (on the ordinate, not the abscissa), $1/M_W$.

Visual Comparison

Where a color or a turbidity comparison is directed, color-comparison tubes that match as closely as possible in internal diameter and all other respects should be used. For color comparison, the tubes should be viewed downward, against a white background, with the aid of a light source directed from beneath the bottoms of the tubes; for turbidity comparison, the tubes should be viewed horizontally, against a dark background, with the aid of a light source directed from the sides of the tubes.

In conducting limit tests that involve a comparison of colors in two like containers (e.g., matched color-comparison tubes), a suitable instrument, rather than the unaided eye, may be used.▲*USP33*

In-Process Revision

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1113**⟩ **Microbial Identification.** This new chapter proposal provides general information on available microbial identification methods, selection of appropriate methods for use, and verification of those methods.

(MSA: R. Tirumalai) RTS—C69321

Add the following:

▲⟨1113⟩ MICROBIAL IDENTIFICATION

INTRODUCTION

Microorganisms such as bacteria and fungi are frequently identified in pharmaceutical ingredients, water for pharmaceutical use, the manufacturing environment, intermediates, and finished pharmaceutical products (materials/environment). Microbial Identification is essential when the number of microorganisms exceeds alert and/or action levels for the material or manufacturing environment or when microorganisms are found in sterile products. Microbial identification is also an important component of processes that demonstrate microbial control, exclude objectionable microorganisms from nonsterile pharmaceutical drug products, support aseptic processing, and examine product failures.

Microbiological identification systems are based on different analytical techniques, such as phenotypic, genotypic, and strain typing. Each system has limitations due to method and/or database limitations and shortcomings in terms of accuracy, reproducibility, technical complexity, speed, and cost. A decision must be made as to the appropriate technology to use, considering these limitations and the level of identification required (genus, species, and strain). Routine evaluations for recovered organisms might include characterization by colony morphology, cellular morphology (e.g., rods, cocci, mode of sporulation), Gram reaction or other differential staining technique or diagnostic enzyme activity. This level of information may confirm that the microorganisms found are acceptable for that material or manufacturing environment or indicate no change in the level of environmental control in an aseptic processing area. More definitive identification may be needed to assign a species name to establish a historic baseline, during an investigation, for epidemiological purposes, or to aid in the safety evaluation process.

The need for microbial identification is cited in several USP chapters and in an FDA guidance. USP general chapter *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩ states that microorganisms demonstrating characteristic cellular and colony morphology on selective and/or diagnostic agar media employed in the tests be confirmed by identification tests. USP general chapter *Sterility Tests* ⟨71⟩ allows for invalidation of the test, if after identification of the microorganisms isolated from the test, the growth of this species (or these species) may be unequivocally ascribed to faults with respect to the material and or the technique used in conducting the sterility test procedure. General information chapter *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* ⟨1111⟩ recommends that manufacturers determine what constitutes an objectionable organism among those recovered. Some level of identification will be required to make such a determination. USP general information chapter *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* ⟨1116⟩ recommends that microbial isolates be identified at an appropriate level to support the environmental monitoring program. The 2004 FDA guidance document on aseptic processing[1] recommends that the use of genotypic microbial identification

[1] FDA *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice, 2004.*

methods is preferred because of the higher accuracy and precision of these methods, especially when investigating process or product failures where establishing the relationship of recovered organisms is a critical part of the investigation.

MICROBIAL ISOLATION

The first step in identifying microbes is to isolate a pure culture for analysis. This purification is normally accomplished by subculturing single colonies one or more times on a nonselective solid medium, e.g., soybean–casein digest agar, to ensure purity. This technique also allows full phenotypic expression of stressed microorganisms and growth of sufficient inoculum for the identification. Expression of the microbial phenotype, (cell size and shape, sporulation, cellular composition, antigenicity, biochemical activity, and sensitivity to antimicrobial agents) may depend on the media and growth conditions (see *Table 1*).

In contrast, the microbial genotype is highly conserved and is independent of culture conditions. Therefore, identification may be conducted on uncultured test material, primary enrichments that increase the amount of nucleic acid available for analysis and primary isolation cultures from microbial limit testing or environmental monitoring plates. A recent publication[2] confirmed the stability of repetitive-sequenced PCR patterns of the bacteria *P. aeruginosa, E. coli, P. aeruginosa, E. faecalis, S. epidermidis,* and *A. baumannii* with respect to both the age of the culture and number of subcultures (5–15). *Table 2* lists genotypic characteristics that can be determined.

Table 1. Phenotypic Characteristics Employed in Microbial Taxonomy

Categories	Characteristics
Culture	Colony morphology, colony color, shape and size, pigment production
Morphological	Cellular morphology, cell size, cell shape, flagella type, reserve material, Gram reaction, spore and acid-fast staining, mode of sporulation
Physiological	Oxygen tolerance, pH range, temperature optimum and range, salinity tolerance
Biochemical	Carbon utilization, carbohydrate oxidation or fermentation, enzyme patterns
Inhibition	Bile salt-tolerance, antibiotic susceptibility, dye tolerance
Serological	Agglutination, fluorescent antibody
Chemo-Taxonomic	Fatty acid profile, microbial toxins, whole cell composition
Ecological	Origin of the organism

Table 2. Genotypic/ Phylogenetic Characteristics That Can Be Employed in Microbial Taxonomy

Categories	Characteristics
Genotypic	DNA base ratio (G + C content), restriction fragment patterns, and DNA probes
Phylogenetic	DNA-DNA hybridization, and 16S and 23S rRNA codon sequences

[2] Kang, H.P. and W.M. Dunne. Stability of Repetitive-Sequence PCR Patterns with Respect to Culture Age and Subculture Frequency. *J. Clin. Microbiol. 41 (6) 2694-2603, 2003.*

The gold standard for the taxonomic classification of bacteria is DNA, detection of which is described in the second edition of *Bergey's Manual of Systematic Bacteriology.*[3] When the DNA from an unknown organism is compared to the DNA from a known organism, the degree of relatedness can be determined. Genotypic identification is accomplished through the use of DNA hybridization, restriction fragment pattern comparisons and/or DNA probes. For example, greater than 70% relatedness with DNA-DNA hybridization indicates the organisms are the same species. Phylogenetic analysis of bacteria is typically performed by comparing the 500 base sequence of a portion of the 16S ribosomal RNA gene. PCR is used to amplify this gene sequence, and the amplified region is then isolated and base sequenced using an electrophoretic method. Comparisons can be made using proprietary databases or those that are available online, such as GenBank. When evaluating microbial identification it should be recognized that, side-by-side comparison of two organisms is a more reliable approach to determine relatedness than comparison to a database. Also, it should be noted that the probability that a database would have information on a specific isolate is rather low, unless such an isolate was previously analyzed.

PRELIMINARY SCREENING OF MICROBIAL ISOLATES

Microorganisms isolated from pharmaceutical ingredients and processes will in all likelihood be physiologically stressed. Such microorganisms will go from a metabolic status suitable for slow or no growth for survival under adverse conditions to rapid growth under laboratory culture conditions. This transition can be managed by careful handling of the isolates. Individual representative colonies from the primary isolation media are streaked onto solid media as described above in preparation for identification. The first step is to Gram stain and determine the cellular morphology of the bacteria isolates. This is a critical step for phenotypic identification schemes. Running positive and negative controls with these tests is recommended. Several common preliminary screening tests are described below.

Gram Staining

Gram staining methods employed include the four-step method: crystal violet (primary stain), iodine (mordant), alcohol (decolorizer), safranin (counterstain); or the three-step method in which the decolorization and counterstaining step is combined. When stained correctly, gram-positive organisms retain the crystal violet stain and appear blue; gram-negative organisms lose the crystal violet stain thus containing only the counter-stain safranin and appear red. Common pitfalls in this method include the following: (a) heat fixation may cause gram-positive cells to stain gram-negative, (b) older cultures or sections of a colony containing older organisms may give gram-variable reaction, (c) using too much decolorizer can result in a false gram-negative result, and (d) not using enough decolorizer may yield a false gram-positive result. The bacterial smear on the microscope slide may be fixed with methanol, rather than using heat fixation, which may give more consistent results.The Gram stain reaction is read under a microscope, allowing evaluation of cell and colony morphology (rod or cocci, single cells or chains, or clumps or clusters).

Spore Staining

Spore staining is done by a two-step method: malachite green (primary stain), and safranin (counterstain). The fixed bacterial smear is stained with 7.6% aqueous malachite green solution for 10 minutes, rinsed, and counterstained with a 0.25% aqueous safranin solution for 15 seconds, rinsed, and blotted dry. This procedure stains the spores green, but vegetative bacterial cells are stained red. In many cases, the presence of spores can be determined in organisms with Gram staining only, but when using sporulation as a diagnostic feature, spore staining is recommended.

[3] *Bergey's Manual of Systematic Bacteriology, 2nd Edition,* 2003.

Biochemical Screening

Biochemical screening tests include (1) the oxidase test to separate gram-negative rod-shaped bacteria into nonfermenters (oxidase positive) and enteric (oxidase negative) bacteria, (2) catalase test to separate *Staphylococci* (catalase positive) from *Streptococci* (catalase negative), and (3) the coagulase test to separate *Staphylococci* into coagulase negative (presumptively nonpathogenic) and coagulase positive (more likely pathogenic) *Staphylococci*.

For many types of investigations, these few tests may provide enough information to describe data. However, there are much more definitive bacterial identification schemes or systems.

MICROBIAL IDENTIFICATION BY PHENOTYPIC METHODS

Phenotypic methods utilize expressed gene products to distinguish among different microorganisms. Generally, these require a large number of recently grown cells in pure culture. Disadvantages of culture methods for microbial enumeration and identification include (1) long incubation times, (2) the inability of many environmental microorganisms to grow on artificial media, (3) specific growth requirements of specific microorganisms, (4) the unintentional inhibition of culture methods, and (5) the need to fully express phenotypic properties of recently isolated stressed microorganisms by subculture from primary isolation on selective or diagnostic media prior to microbial identification. Databases of microbiological identification systems include carbon utilization and biochemical reaction patterns, fatty acid profiles, and whole-cell composition for microbial species. All such identification systems rely on inocula that have been cultured using specific media and incubation conditions (e.g., time, temperature, and oxygen level) in order to achieve consistent identification. Despite these limitations, phenotypic microbial identification methods are successfully employed in routine food, water, clinical, and pharmaceutical microbiological testing laboratories.[4]

MICROBIAL IDENTIFICATION BY GENOTYPIC METHODS

Compared to phenotypic procedures, genotypic microbial identification methods based on nucleic acid analyses are less subjective, less dependent on the culture method, and theoretically are more reliable because nucleic acid sequences are highly conserved by microbial species. These methods include DNA-DNA hybridization, polymerase chain reaction (PCR), 16S and 23S rRNA gene sequencing, and analytical ribotyping. Like the Gram staining procedure, 16S rRNA base sequencing has proved to be a powerful diagnostic tool and has become the gold standard for taxonomic species assignment for bacteria. However, without extensive automation of sample handling and reagent standardization these procedures are technically challenging, are expensive in terms of both equipment and testing costs, and often rely on a technology marketed by a single company. Their use may be limited to critical microbiological investigations associated with direct product failure. In such cases identifications are conducted in a pharmaceutical company's specialized research laboratory or are sent to a contract testing laboratory. It is foreseen that these technologies will be applied to routine microbial identification in the pharmaceutical industry. Clearly, the increased accuracy of identification with rRNA base sequencing and the ability to determine the strain of microorganism via ribotyping are useful tools of molecular epidemiology. rRNA base sequencing may allow determination of the origin of microbial contamination.

[4] O'Hara, C.M., M.P. Weinstein, and J.M. Miller. Manual and automated systems for detection and identification of microorganisms. *ASM Manual of Clinical Microbiology, 8th Edition,* 2003.

VERIFICATION OF MICROBIAL IDENTIFICATION METHODS

Microbial identification tests include serological tests, chemical reagents, cellular strains, instrumentation, and kits. The verification of an identification test system may include one of the following three steps: first, consecutive parallel testing of microbial isolates obtained from routine testing using an existing system during a representative period of time. The minimum number of isolates tested is 50, and any discrepancies in identification should be arbitrated using a referee method. The second step involves testing known representative stock cultures of 12–15 different commonly isolated species for a total of 50 tests. The third step is confirmation that results of 20–50 organism identifications including 15–20 different species agree with the results of testing of a split sample by a reference laboratory.[5] In each case the appropriate quality control organisms, as recommended by the supplier, should be included in the verification process.

With identification systems, both verification of the identity of the species evaluated and the level of agreement should be considered. Greater than 90% agreement should be achieved with microorganisms appropriate for the phenotypic identification system. Groups of organisms that are known to be more challenging to identify, including biochemically-unresponsive nonfermenting bacteria, corynebacteria, and coagulase-negative *Staphylococci* should be evaluated, when appropriate, during the verification process.

The hierarchy of microbial identification errors include the following: (1) misidentification to genera, (2) misidentification to species, and (3) no identification using "best fit" criteria at very low confidence levels. When a system does not identify an isolate, it may be due to the organism not being included in the database, the system parameters not being comprehensive enough to identify the organism, the isolate not being reactive in the system, or the species not being taxonomically described. These isolates should be sent to the supplier of the microbial identification system for identification and addition to their database, or identified, preferably using 16S or 23S rRNA base sequencing, and added to the user's in-house database. Misidentification is more difficult to determine, but any microbial identification should be reviewed for reasonableness in terms of the microorganism's cellular morphology, physiological requirements, and source of isolation. Organisms requiring identification only to genus may include nonpathogenic *Bacillus, Staphylococci, Corynebacteria,* and *Micrococci.*

During base sequencing error may result if the original specimen is not a pure culture, the yield of the labeled amplicon is too low, or the capillary array is malfunctioning. These errors should be apparent as unreadable bases. Ultimately the accuracy of 16S rRNA base sequencing identification depends on the accuracy of the sequences in the database, the association of appropriate names and sequences, and the accuracy of the sequence of the isolate. A database search report would list, for example, the top 5 matches with the related percentage match and number of mismatches and would display a phylogenetic tree based on a nearest neighbor algorithm. Current technology in clinical microbiology suggests that $>99\%$ similarity can be used to define species and $>95\%$ can be used to define genus. However, this rule may not hold true for diverse or less well studied genera and species.[6]

The most important verification tests are accuracy and reproducibility. These measurements can be defined as follows:

Accuracy % = (Number of correct results/Total number of results) × 100

Reproducibility % = (Number of correct results in agreement/Total number of results) × 100

Suitable acceptance criteria for accuracy and reproducibility are $>90\%$ and $>95\%$, respectively.

[5] Cumitech 31. *Verification and Validation of Procedures in the Clinical Microbiology Laboratory.* Elder, B. L., S.A. Hansen, J.A. Kellogg, F.J. Marsik and R. J. Zabransky, ASM, February 1997.

[6] J.E. Clarridge III. The Impact of 16S rRNA Gene Sequencing Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases, *Clin. Microbiol. Rev. 17* (2) 840-862, 2004.

Other measurements are sensitivity, specificity, and positive and negative predictive value. These measurements are best illustrated by an example. A clinical microbiology laboratory compared the frequency of isolation of DNA hybridization probe to a culture method for the sexually transmitted bacterium *Neisseria gonorrhaeae*.[5] The frequency of isolation from clinical specimens was historically 10%. The lab ran 100 split samples, and the results are presented in *Table 3*.

Table 3. Comparison of the Distribution of Negative and Positive Results for the DNA Probe and Culture Methods

	Culture Results	
DNA Probe Results	Positive	Negative
Positive	9	2
Negative	1	88

Sensitivity = [9/(9 + 1)] × 100 = 90%

Specificity = [88/(88 + 2)] × 100 = 97.7%

Positive Predictive Value = [9/(9 + 2)] × 100 = 81.8%

Negative Predictive Value = [88/(88 + 1)] × 100 = 98.9%

The mathematical derivation of these functions is outlined in *Table 4*.

Table 4. A Two-Row by Two-Column Contingency Table with Respect to the Reference Culture Method and the Alternate PCR Method (after ISO 5725-1 and 5725-2 2004)*

	PCR		
Culture	Positive	Negative	Sum
Positive	a	b	a + b
	True Positive	False Negative	
Negative	c	d	c + d
	False Positive	True Negative	
Sum	a + c	b + d	

* ISO 5725-1 : 1994 Accuracy (trueness and precision) of measurement methods and results—Part 1: General principles and definitions and ISO 5725-2 : 1994 Accuracy (trueness and precision) of measurement methods and results—Part 2: Basic methods for the determination of repeatability and reproducibility of standard measurement methods.

Inclusivity (%) = [a/(a + b)] × 100

Exclusivity (%) = [d/(c + d)] × 100

Positive Predictivity (%) = [a/(a + c)] × 100

Negative Predictivity (%) = [d/(b + d)] × 100

Analytical Accuracy (%) = [a + d/(a + b + c + d)] × 100

Kappa Index = 2(ad – bc)/(a + c) × (c + d) + (a + b) × (b + d)

Phylogenetic Identification

The second edition of *Bergey's Manual of Systematic Bacteriology* represented a major departure from the first edition, and also from the eighth and ninth editions of the *Manual of Determinative Bacteriology*. The organization of content in *Bergey's Manual* follows a phylogenetic framework, based on analysis of the nucleotide sequence of the ribosomal small subunit 16S RNA, rather than a phenotypic structure. The comparative base sequencing may be performed instrumentally (MicroSeq 500, Applied Biosystems, Foster City, CA). The procedure involves obtaining a pure isolate, lysing the bacterial cells, extracting and concentrating the DNA, amplifying the 500-base section of the 16S rDNA gene, and sequencing the amplicon using fluorescent di-deoxy terminator cycle chemistry. Fragments then are separated by an automated DNA sequencer, and the data are compared to sequences in the database. The MicroSeq database contains approximately 2000 base sequences derived from type strains (the public database GenBank contains approximately 200,000 base sequences). Although the instrumental approach may result in higher quality sequences, the representation of a single species that may contain numerous strains by a single type strain may be inadequate.

[5] Cumitech 31. *Verification and Validation of Procedures in the Clinical Microbiology Laboratory.* Elder, B. L., S.A. Hansen, J.A. Kellogg, F.J. Marsik and R. J. Zabransky, ASM, February 1997.

Phylogenetic trees show the closest genetically related organisms. In general, organisms with relatedness $>1\%$ generally are considered different species and those with $>3\%$ relatedness are considered different genera.

Polyphasic Identification

Differences in genotype and phenotype are relatively common, e.g., same or very similar genotype shared by microorganisms with different phenotypes, similar phenotypes but different genotypes, and microorganisms that are genotypically too distant to be the same species or genus (*Table 5*). The concept of polyphasic taxonomy[7] that refers to assembly and use of many levels of information, e.g., microbial characterization, phenotypic and genotypic data, and origin of the microorganisms, can be most successfully applied to microbial identification. This avoids decisions made solely using genotypic data that make no sense when the microbial characteristics, testing history, and source of isolation are considered.

Table 5. Examples of Discrepant Relationships Between Genotype and Phenotypes

Relationship	First Strain	Second Strain	Number of Base Pair Differences
Same genotype but different phenotypes	Mycobacterium tuberculosis ATCC 27294	M. bovis ATCC 19210	0
	Bacillus anthracis	B. cereus 30.0 and B. thuringenesis	
	E. coli ATCC 11775	Shigella dysenteriae ATCC 13313	3
Similar genotype but different phenotypes	Streptococcus pneumoniae ATCC 33400	S. mitis ATCC 49456	3
Similar phenotypes but different genotypes	Nocardia asteroids ATCC 19247	N. farcinica ATCC 3318	13
Too distant to be the same species	Enterobacter (Pantoea) agglomerans (bg1)	Enterobacter (Pantoea) agglomerans (bg2)	27
Too distant to be the same genus	Clostridium tetani ATCC 19404	Clostridium innocuum ATCC 14501	about 104

[7] Gillis, M., P. Vandamme, P. De Vos, J. Swings and K. Kersters. Polyphasic Taxonomy. *Bergey's Manual of Systematic Bacteriology, 2nd Edition,* 2003.

Pathogen Screening

Nucleic acid-based procedures can be used for screening USP-specified microorganisms. The steps associated with this activity are sample collection, nucleic acid extraction, target amplification, hybridization, and detection. The problem of amplifying DNA from nonviable bacterial cells can be overcome by using reverse transcription to convert transitional rRNA to DNA for PCR amplification. The reaction efficiencies of cell lysis, rRNA extraction, and reverse transcription vary with each bacterium and sample matrix. Therefore, nucleic acid standards must be employed in the construction of a calibration curve to determine the cycle number where the fluorescence in the PCR sample increases above the threshold value. In its *Draft Guidance for Industry: Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens* (2005), FDA addressed the detection of microbial variants, limits of detection, matrix effects, positive cutoff verification, instrument and system carry-over, diagnostic accuracy, and reproducibility.

Objective of a Microbial Identification Program

The objective of a microbial identification program is to provide information that will help exclude microorganisms from products. Knowing the identity of microbial isolates is useful when manufacturers establish a monitoring baseline and investigate sources of microbial contamination. Alert and action levels in water, pharmaceutical ingredients, and the environment, as well as presterile filtration bioburden monitoring, typically initiate microbial identifications. When a pre-set numerical level for microbial count is exceeded, the predominant microbial isolates are identified for trending purposes (alert levels) or as part of the laboratory and manufacturing investigations (action levels). Critical microbial isolations—when all isolates are identified—may follow failures in media fills, sterility tests, microbial limit tests, and marketed product stability testing, or in response to consumer complaints. The identity of the microbial isolate suggests its origin and may suggest effective preventive action.

Because microbial identification can be costly and time-consuming, manufacturers should develop good microbial identification strategies. These help identify the potentially pathogenic microbes from the nonpathogenic and help manufacturers focus on identifications that support investigations most relevant to actual and/or potential product failure. No single microbial identification procedure is ideal in terms of cost, speed, accuracy, and repeatability. Thus manufacturers should develop preliminary screening programs that characterize microbial isolates and facilitate the selection of the most appropriate microbial identification methods. Such screening programs should be optimized for a general class of microorganisms and should reflect the importance of the isolate in terms of product failure (*Table 6*).

Best practices include retention of environmental monitoring plates until product testing is complete and the establishment of an in-house culture library of critical microbial isolates. The logistics of such a program, especially in a large operation, may necessitate a preliminary screening protocol based on colonial morphology and numbers as the plates are initially read to minimize storage issues.

Table 6. Guidelines for the Characterization, Identification, and Stain Typing of Microbial Isolates from Monitoring Programs

Extent of Characterization	Isolate Origin
Gram reaction and cellular morphology only	Environmental monitoring in ISO 7 and 8 classification areas; isolates from excipient, finished product, environmental and water monitoring with number below the alert level.
Identification to genus	Environmental monitoring in ISO 5 and 6 classification areas with number below the alert level.
Identification to species	Environmental monitoring in ISO 5 classification areas; alert and/or action level isolates from all excipient, finished product, environmental and water monitoring.
Strain typing	Significant product failures, e.g., media fill, sterility test and microbial limit test. Significant adverse trends in environmental and water monitoring.

▲*USP33*

REAGENTS, INDICATORS, AND SOLUTIONS

BRIEFING

Reagents, Indicators, and Solutions, Introduction, *USP 31* page 747. It is proposed to add a definition for *Organic-free water* to the definitions under *Water*.

(HDQ: M. Marques) RTS—C70387

Change to read:

This section deals with the reagents and solutions required in conducting the Pharmacopeial and the National Formulary tests and assays.

As is stated in the *General Notices,* listing of reagents, indicators, and solutions in the Pharmacopeia in no way implies that they have therapeutic utility; thus, any reference to the USP in their labeling is to include the term "reagent" or "reagent grade."

Reagents required in the tests and assays for the Pharmacopeial and National Formulary articles are listed in this section, generally with specifications appropriate to their intended uses. Exceptions to the latter include those reagents for which corresponding specifications are presented in the current edition of *Reagent Chemicals,* published by the American Chemical Society, and reagents for which specifications could not be drafted in time for inclusion here. Thus, where it is directed to "Use ACS reagent grade," it is intended that a grade meeting the corresponding specifications of the current edition of ACS *Reagent Chemicals* shall be used. Where no such specifications exist, and where it is directed to "Use a suitable grade," the intent is that a suitable reagent grade available commercially shall be used. Occasionally, additional test(s) augment the designation "suitable grade," as indicated in the text. Listed also are some, but not all, reagents that are required only in determining the quality of other reagents. For those reagents that are not listed, satisfactory specifications are available in standard reference works.

In those instances in which a reagent required in a Pharmacopeial or National Formulary test or assay need not be of analytical reagent quality, it suffices to refer to the monograph for that article appearing in this Pharmacopeia or the National Formulary or the current edition of the Food Chemicals Codex (FCC). In such cases it is to be understood that the specifications are minimum requirements and that any substance meeting more rigid specifications for chemical purity is suitable.

Where the name of a reagent specified in a test or assay is the same as the title of a *USP* or *NF* article, and it does not appear among the following *Reagent Specifications*, a substance meeting the requirements of the *USP* or *NF* monograph is to be used (e.g., *Benzocaine,* USP; or *Propylparaben,* NF). However, reference is specifically made, under *Reagent Specifications*, to a reagent bearing the name of a *USP* or *NF* article: (1) where there are requirements for a reagent in addition to the *USP* or *NF* monograph requirements (e.g., *Sodium Salicylate,* USP; or *Isopropyl Myristate,* NF), (2) where a source other than the *USP* or *NF* monograph is specified (e.g., *Lactose,* ACS reagent; or *Hydrochloric Acid,* ACS reagent), (3) where complete reagent specifications differ from the *USP* or *NF* monograph standards (e.g., *Calcium Lactate;* or *Thymol),* or (4) where a standard material is included among the reagent specifications (e.g., *Calcium Carbonate,* primary standard; or *Sodium Carbonate,* primary standard).

Reagents and solutions should be preserved in tight containers made of resistant glass or other suitable material. Directions for storage in light-resistant containers should be carefully observed.

Stoppers and stopcocks brought into contact with substances capable of attacking or penetrating their surfaces may be given a protective coating of a thin film of a suitable lubricant unless specifically interdicted.

Where a particular brand or source of a material or piece of equipment, or the name and address of a manufacturer, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

Atomic absorption and flame photometry require the use of a number of metal-ion standard solutions. While the individual monographs usually provide directions for preparation of these solutions, use of commercially prepared standardized solutions of the appropriate ions is permissible, provided that the analyst confirms the suitability of the solutions and has data to support their use.

Reagents are substances used either as such or as constituents of solutions.

Indicators are reagents used to determine the specified end-point in a chemical reaction, to measure hydrogen-ion concentration (pH), or to indicate that a desired change in pH has been effected. They are listed together with indicator test papers.

Buffer Solutions are referred to separately.

Colorimetric Solutions, abbreviated "CS," are solutions used in the preparation of colorimetric standards for comparison purposes.

Test Solutions, abbreviated "TS," are solutions of reagents in such solvents and of such definite concentrations as to be suitable for the specified purposes.

Volumetric Solutions, abbreviated "VS" and known also as **Standard Solutions,** are solutions of reagents of known concentration intended primarily for use in quantitative determinations. Concentrations are usually expressed in terms of normality.

Water—As elsewhere in the Pharmacopeia, where "water," without qualification, is mentioned in the tests for reagents or in directions for preparing test solutions, etc., Purified Water (USP monograph) is always to be used. ■*Carbon dioxide-free water*■1S *(USP31)* is Purified Water that has been boiled vigorously for 5 minutes or more and allowed to cool while protected from absorption of carbon dioxide from the atmosphere, or Purified Water that has a resistivity of not less than 18 Mohm-cm. ■*Deaerated water,*■1S *(USP31)* for purposes other than dissolution and drug release testing, is Purified Water that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 minutes and cooling or by the application of ultrasonic vibration. ■*Particle-free water* is water that has been passed through a 0.22-µm filter.■1S *(USP31)*

▲*Organic-free water* is Purified Water that produces no significantly interfering peaks when chromatographed as it is indicated in *Identification, Control, and Quantification of Residual Solvents* under *Residual Solvents* ⟨467⟩.▲*USP33*

Chromatographic Solvents and Carrier Gases—The chromatographic procedures set forth in the Pharmacopeia may require use of solvents and gases that have been especially purified for such use. The purpose may be (a) to exclude certain impurities that interfere with the proper conduct of the test procedure, or (b) to extend the life of a column by reducing the build-up of impurities on the column. Where solvents and gases are called for in chromatographic procedures, it is the responsibility of the analyst to ensure the suitability of the solvent or gas for the specific use. Solvents and gases suitable for specific high-pressure or other chromatographic uses are available as specialty products from various reagent supply houses, although there is no assurance that similar products from different suppliers are of equivalent suitability in any given procedure. The reagent specifications provided herein are for general analytical uses of the solvents and gases and not for chromatographic uses for which the especially purified specialty products may be required.

In-Process Revision

Reagent Specifications

BRIEFING

Alcohol, *USP 31*page 752 and page 442 of *PF* 34(2) [Mar.–Apr. 2008]. It is proposed to add more information regarding the content of this reagent.

(HDQ: M. Marques) RTS—C70300

Change to read:

Alcohol,

▲(*Ethanol, Ethyl Alcohol*),▲*USP33*
C_2H_5OH—**46.07**

▲[*64-17-5*]—▲*USP33*
~~Use *Alcohol* (USP monograph).~~

■Use a suitable grade■1S *(USP32)*

▲with a content of not less than 92.3% and not more than 93.8%, by weight, corresponding to not less than 94.9% and not more than 96% by volume, at 15.56°.▲*USP33*

BRIEFING

Ammonium Molybdate, *USP 31* page 754. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques) RTS—C70301

Change to read:

Ammonium Molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$—**1235.86** ~~[*13106-76-8*]~~

▲[*12054-85-2*]▲*USP33*
—Use ACS reagent grade.

BRIEFING

Chromotropic Acid, *USP 31* page 766. It is proposed to make a clarification about the CAS number.

(HDQ: M. Marques) RTS—C70359

Change to read:

Chromotropic Acid *(4,5-Dihydroxy-2,7-naphthalenedisulfonic Acid),* $C_{10}H_8O_8S_2 \cdot 2H_2O$—**356.33** [*148-25-4*],

▲for the anhydrous form▲*USP33*
—Use a suitable grade.

BRIEFING

Chromotropic Acid Disodium Salt, *USP 31* page 766. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques) RTS—C70360

Change to read:

Chromotropic Acid Disodium Salt *(4,5-Dihydroxy-2,7-naphthalenedisulfonic Acid, Disodium Salt),* $C_{10}H_6O_8Na_2S_2 \cdot 2H_2O$—**400.29** ~~[*129-96-4*]~~

▲[*5808-22-0*]▲*USP33*
—Use ACS reagent grade.

BRIEFING

Methyl Red, *USP 31* page 783. It is proposed to include additional information about this reagent.

(HDQ: M. Marques) RTS—C70303

Change to read:

Methyl Red *(2-[4-Dimethylaminophenylazo]benzoic Acid; C. I. Acid Red 2)* ~~$C_{15}H_{15}N_3O_2$— **269.30,**~~

▲$C_{15}H_{15}N_3O_2$, free acid— **269.30**[*493-52-7*]; $C_{15}H_{14}N_3O_2Na$, sodium salt—**291.28**[*845-10-3*]▲*USP33*
—Use ACS reagent grade.

▲The free acid is recommended for nonaqueous titrations, particularly when an aprotic solvent is used. The sodium salt is recommended for titrations in aqueous media and also for nonaqueous titrations where the medium is an amphiprotic solvent.▲*USP33*

In-Process Revision

BRIEFING

Phosphorous Acid. It is proposed to add this new reagent used in the monograph for *Pamidronate Disodium*.

(HDQ: M. Marques) RTS—C70109

Add the following:

▲**Phosphorous Acid** *(Phosphonic Acid),* H_3O_3P—**82.00** [*13598-36-2*]—Use a suitable grade with a content of not less than 99%.▲*USP33*

BRIEFING

Potassium Metabisulfite. It is proposed to add this new reagent used in the test for *Limit of sulfur dioxide, Test 1* under *Pea Starch*, a new monograph also being proposed in this issue of *PF*.

(HDQ: M. Marques) RTS—C70101

Add the following:

▲**Potassium Metabisulfite** *(Potassium Disulfite; Potassium Pyrosulfite),* $K_2S_2O_5$—**222.32** [*16731-55-8*]—Use a suitable grade with a content of not less than 98%.▲*USP33*

BRIEFING

Potassium Sodium Tartrate, *USP 31* page 792. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques) RTS—C70361

Change to read:

Potassium Sodium Tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$—**282.22** ~~[*304-59-6*]~~

▲[*6381-59-5*]▲*USP33*
—Use ACS reagent grade.

BRIEFING

Sodium Biphenyl, *USP 31* page 797. It is proposed to update the specifications of this reagent to reflect the products currently available on the market.

(HDQ: M. Marques) RTS—C70113

Change to read:

Sodium Biphenyl, $C_{12}H_9Na$—**176.19**~~—Supplied as a solution (10 percent to 30 percent, w/w) in a mixture of dimethoxyethane and toluene or xylene. The solution is a viscous, dark green liquid. [NOTE—The solution deteriorates at a rate of about 10% per month. Use only freshly prepared solution.]~~

▲Available as a solution in 2-ethoxyethyl ether.▲*USP33*

Activity—Place 20 mL of dry toluene in a titration flask equipped with a magnetic stirring bar and a stopper having a hole through which the delivery tip of a weight buret may be inserted. Add a quantity of sodium biphenyl sufficient to produce a blue color in the mixture, and titrate with amyl alcohol, contained in a weight buret, to the disappearance of the blue color. (Disregard the amounts of sodium biphenyl and amyl alcohol used in this adjustment.) Weigh accurately the weight buret containing the amyl alcohol. Transfer the contents of a vial of well-mixed test specimen to the titration flask, and titrate quickly with the amyl alcohol to the disappearance of the blue color. Weigh the buret to determine the weight of amyl alcohol consumed, and calculate the activity, in mEq per vial, by the formula:

$$11.25W$$

in which W is the weight of amyl alcohol consumed. Not less than 10% activity is found.

Iodine content—Add 10 mL to 5 mL of toluene contained in a 125-mL separator fitted with a suitable inert plastic stopcock, and shake vigorously for 2 minutes. Extract gently with three 10-mL portions of dilute phosphoric acid (1 in 3), combining the lower phases in a 125-mL iodine flask. Add sodium hypochlorite TS, dropwise, to the combined extracts until the solution turns brown, then add 0.5 mL in excess. Shake intermittently for 3 minutes, add 5 mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 minute, accurately timed. Add 1 g of potassium iodide, shake for 30 seconds, add 3 mL of starch TS, and titrate with 0.1 N sodium thiosulfate VS: not more than 0.1 mL of 0.1 N sodium thiosulfate is consumed.

▲[NOTE—A suitable grade is available as catalog number 277134 from www.sigma-aldrich.com.]▲*USP33*

In-Process Revision

Test Solutions

BRIEFING

Test Solutions, *USP 31* page 814, page 3784 of the *Second Supplement*, and page 811 of *PF* 34(3) [May–June 2008]. It is proposed to make revisions in *Acetic Acid, Glacial*; and *Cupric Citrate TS 2*. It is also proposed to add *Denaturated Alcoholic TS*, used in the new monograph *Polyvinyl Acetate*, previously published in *PF* 34(6) [Nov.–Dec. 2008].

(HDQ: M. Marques) RTS—C70061; C70111; C70116

Change to read:

Acetic Acid, Glacial, TS—Determine the water content of a specimen of glacial acetic acid by the *Titrimetric Method* (see *Water Determination* ⟨921⟩). If the acid contains more than ~~0.05%~~

▲0.4%▲*USP33*
of water, add a few mL of acetic anhydride, mix, allow to stand overnight, and again determine the water content. If the acid contains less than 0.02% of water, add sufficient water to make the final concentration between 0.02% and ~~0.05%~~

▲0.4%,▲*USP33*
mix, allow to stand overnight, and again determine the water content. Repeat the adjustment with acetic anhydride or water, as necessary, until the resulting solution shows a water content ~~between 0.02% and 0.05%~~

■of not more than 0.4%.■1S (*USP32*)

Add the following:

■**Alcoholic TS**—It contains 95 parts of specially denaturated alcohol 3A with 5 parts of isopropyl alcohol. The final concentrations are approximately 90% alcohol, 5% methanol, and 5% isopropanol.

NOTE—A suitable grade is available as Reagent alcohol, catalog number R8382, available at www.sigma-aldrich.com.■1S (*USP32*)

Add the following:

■**Ammonia TS 2**—Prepare by diluting 13.5 mL of ammonium water, stronger (see *Reagent Specifications* in the section *Reagents*) with water to make 100 mL.■1S (*USP32*)

Change to read:

Cupric Citrate TS 2, Alkaline—With the aid of heat, dissolve about 173 g of sodium citrate dihydrate and 117 g of sodium carbonate monohydrate in about 700 mL of water, and filter. In a second flask, dissolve about 27.06 g of cupric sulfate ~~($Cu_2O_4 \cdot 5H_2O$)~~

▲($CuSO_4.5H_2O$)▲*USP33*
in about 100 mL of water. Slowly combine the two solutions while stirring, and dilute with water to 1000 mL.

Add the following:

▲**Denaturated Alcoholic TS**—A specially denaturated alcohol containing either rubber hydrocarbon solvent or heptane or toluene. [NOTE—A suitable grade is available from www.lyondell.com or from www.sasol.com, as Ethanol SDA 2B HEP 200, or Ethanol SDA 2B TOL 200, or Ethanol SDA 2B TOL 190, or Alcohol SDA 2B-2.]▲*USP33*

Add the following:

■**Iodine and Potassium Iodide TS 3**—Dissolve 0.127 g of iodine and 0.20 g of potassium iodide in water, and dilute with water to 10.0 mL.■1S (*USP32*)

Add the following:

■**Lanthanum Nitrate TS**—Dissolve 5.0 g of lanthanum nitrate hexahydrate in 100 mL of water.■1S (*USP32*)

Add the following:

■**Methyl Red TS 2**—To 1.86 mL of 0.1 M sodium hydroxide and 50 mL of alcohol, add 50 mg of methyl red, and dilute with water to 100 mL.■1S (*USP32*)

Change to read:

Potassium Pyroantimonate TS—Dissolve 2 g of potassium pyroantimonate in 85 mL of hot water. Cool quickly, and add ~~10 mL of a solution of potassium hydroxide (3 in 20). Allow to stand for 24 hours, filter, and dilute with water to 100 mL.~~

■a solution containing 2.5 g of potassium hydroxide in 50 mL of water and 1 mL of sodium hydroxide solution (8.5 in 100). Allow to stand for 24 hours, filter, and dilute with water to 150 mL.■1S (*USP32*)

In-Process Revision

Volumetric Solutions

BRIEFING

Volumetric Solutions, *USP 31* page 821, page 3784 of the *Second Supplement,* and page 1041 of *PF* 34(4) [July–Aug. 2008]. It is proposed to make revisions in *Hydrochloric Acid, Normal (1 N); Hydrochloric Acid, Half-Normal (0.5 N); Hydrochloric Acid, Half-Normal (0.5 N) in Methanol; Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane;* and *Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid.*

(HDQ: M. Marques) RTS—C70099

Change to read:

Bismuth Nitrate, 0.01 ~~mol/L~~

▲M▲*USP32*

$Bi(NO_3)_3 \cdot 5H_2O$, **485.07**

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate

Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add ~~water~~

■0.01 N nitric acid■2S *(USP32)*
to make 1000 mL, and standardize the solution as follows.

Accurately measure 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS, and titrate the solution with ~~0.01 mol/L of disodium dihydrogen ethylenediamine tetraacetate~~

▲0.01 M edetate disodium▲*USP32*
VS until the red color changes to yellow. Calculate the molarity factor.

Change to read:

Hydrochloric Acid, Normal (1 N)
HCl, **36.46**

36.46 g in 1000 mL

Dilute 85 mL of hydrochloric acid with water to 1000 mL. Standardize the solution as follows.

Accurately weigh about 5.0 g of tromethamine, ~~previously dried at 105° for 3 hours~~

▲dried according to the label instructions.▲*USP33*
Dissolve in 50 mL of water, and add 2 drops of bromocresol green TS. Titrate with 1 N hydrochloric acid to a pale yellow endpoint. Each 121.14 mg of tromethamine is equivalent to 1 mL of 1 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

Change to read:

Hydrochloric Acid, Half-Normal (0.5 N)
HCl, **36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of water slowly add 43 mL of hydrochloric acid. Cool, and add water to volume. Standardize the solution as follows.

Accurately weigh about 2.5 g of tromethamine, ~~previously dried at 105° for 3 hours~~

▲dried according to the label instructions.▲*USP33*
Proceed as directed under *Hydrochloric Acid, Normal (1 N),* beginning with "Dissolve in 50 mL of water."

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

Change to read:

Hydrochloric Acid, Half-Normal (0.5 N) in Methanol
HCl, **36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of water slowly add 43 mL of hydrochloric acid. Cool, and add methanol to volume. Standardize the solution as follows.

Accurately weigh about 2.5 g of tromethamine, ~~previously dried at 105° for 3 hours~~

▲dried according to the label instructions.▲*USP33*
Proceed as directed under *Hydrochloric Acid, Normal (1 N),* beginning with "Dissolve in 50 mL of water."

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

Change to read:

Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane

Mix 8.5 mL of perchloric acid with sufficient dioxane to make 1000 mL. Standardize the solution as follows.

Accurately weigh about 700 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 hours, and dissolve in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to bluish green. Carry out a blank determination. Each ~~20.42~~

▲20.423▲*USP33*
mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

$$N = \frac{\text{g } KHC_8H_4O_4}{0.20423 \times \text{mL } HClO_4 \text{ solution (corrected for the blank)}}$$

In-Process Revision

Change to read:

Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid
$HClO_4$, **100.46**

10.05 g in 1000 mL

NOTE—Where called for in the tests and assays, this volumetric solution is specified as "0.1 N perchloric acid." Thus, where 0.1 N or other strength of this volumetric solution is specified, the solution in glacial acetic acid is to be used, unless the words "in dioxane" are stated. [See also *Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane*.]

Mix 8.5 mL of perchloric acid with 500 mL of glacial acetic acid and 21 mL of acetic anhydride, cool, and add glacial acetic acid to make 1000 mL. Alternatively, the solution may be prepared as follows. Mix 11 mL of 60 percent perchloric acid with 500 mL of glacial acetic acid and 30 mL of acetic anhydride, cool, and add glacial acetic acid to make 1000 mL.

Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by *Method I* (see *Water Determination* ⟨921⟩), except to use a test specimen of about 5 g of the 0.1 N perchloric acid that is expected to contain approximately 1 mg of water and the *Reagent* (see *Reagent* under *Method Ia* in *Water Determination* ⟨921⟩) diluted such that 1 mL is equivalent to about 1 to 2 mg of water. If the water content exceeds 0.5%, add more acetic anhydride. If the solution contains no titratable water, add sufficient water to obtain a content of between 0.02% and 0.5% of water. Allow the solution to stand for 1 day, and again titrate the water content. The solution so obtained contains between 0.02% and 0.5% of water, indicating freedom from acetic anhydride.

Standardize the solution as follows.

Accurately weigh about 700 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 hours, and dissolve it in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to blue-green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid. Each ~~20.42~~

▲20.423▲*USP33*
mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

$$N = \frac{\text{g } KHC_8H_4O_4}{0.20423 \times \text{mL } HClO_4 \text{ solution (corrected for the blank)}}$$

Change to read:

Potassium Iodate, Twentieth-Molar (0.05 M)
KIO_3, **214.00**

10.70 g in 1000 mL

Dissolve 10.700 g of potassium iodate, previously dried at 110° to constant weight, in water to make 1000.0 mL.

■Standardize the solution as follows: To 15.0 mL of solution in a 250 mL iodine flask, add 3 g of potassium iodide and 3 mL of hydrochloric acid previously diluted with 10 mL of water. Stopper immediately, and allow to stand in the dark for 5 minutes. Then add 50 mL of cold water, and titrate the liberated iodine with freshly standardized 0.1 N sodium thiosulfate. Add 3 mL of starch indicator solution near the end of the titration, and continue to the absence of the blue-starch-iodine complex.

$$M = \frac{\text{mL} \times \text{N } Na_2S_2O_3}{\text{mL } KIO_3 \times 6}$$

■1S (*USP32*)

Add the following:

■Potassium Thiocyanate, Tenth-Normal (0.1 N)
KSCN, **97.18**

9.72 g in 1000 mL

Weigh exactly 9.72 g of potassium thiocyanate, previously dried for 2 hours at 110°, transfer to a 1-L volumetric flask, dilute with water to volume, and mix well. Standardize as follows: transfer 40.0 mL of freshly standardized 0.1 N silver nitrate VS to a 250-mL Erlenmeyer flask; and add 100 mL of water, 1 mL of nitric acid, and 2 mL of ferric ammonium sulfate TS. Titrate with the potassium thiocyanate solution, with agitation, to a permanent light pinkish-brown color of the supernatant.

$$N = \frac{\text{mL} \times \text{N } AgNO_3}{\text{mL KSCN}}$$

■2S (*USP32*)

Change to read:

Sodium Tetraphenylboron, Fiftieth-Molar (0.02 M)
$NaB(C_6H_5)_4$, **342.22**

6.845 g in 1000 mL

Dissolve an amount of sodium tetraphenylboron, equivalent to 6.845 g of $NaB(C_6H_5)_4$, in water to make 1000 mL, and standardize the solution as follows.

Pipet two 75-mL portions of the solution into separate beakers, and to each add 1 mL of acetic acid and 25 mL of water. To each beaker add, slowly and with constant stirring, 25 mL of potassium biphthalate solution (1 in 20), and allow to stand for 2 hours. Filter one of the mixtures through a filtering crucible, and wash the precipitate with cold water. Transfer the precipitate to a container, add 50 mL of water, shake intermittently for 30 minutes, filter, and use the filtrate as the saturated potassium tetraphenylborate solution in the following standardization procedure. Filter the second mixture

through a tared filtering crucible, and wash the precipitate with three 5-mL portions of saturated potassium tetraphenylborate solution. Dry the precipitate at 105° for 1 hour. Each g of potassium tetraphenylborate (KTPB) is equivalent to 955.1 mg of sodium tetraphenylboron.

▲ ~~$M = \frac{g\ KTPB \times 0.9551 \times 0.075}{342.22}$~~ ▲*USP 31*

■ $M = \frac{g\ KTPB \times 0.9551}{342.22 \times 0.075}$ ■1S (*USP32*)

NOTE—Prepare this solution just before use.

BRIEFING

Chromatographic Reagents, page 3785 of the *Second Supplement* to *USP 31–NF 26*, and pages 1423 and 1559 of *PF* 34(6) [Nov.–Dec. 2008]. It is proposed to change the name of this section to *Chromatographic Columns*, to expand the particle size range in L26 and L48, and to correct the brand name of columns classified as L33, L52, and L59.

(HDQ: M. Marques) RTS—C70085

Change to read:

Chromatographic ~~Reagents~~

▲Columns▲*USP33*

The following list of packings (L), phases (G), and supports (S) is intended to be a convenient reference for the chromatographer. [NOTE—Particle sizes given in this listing are those generally provided. Where other, usually finer, sizes are required, the individual monograph specifies the desired particle size. Within any category of packings or phases listed below, there may be a wide range of columns available. Where it is necessary to define more specifically the chromatographic conditions, the individual monograph so indicates.]

Change to read:

Packings

L1—Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.

L2—Octadecyl silane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50 µm in diameter.

L3—Porous silica particles, ~~3 to 10 µm~~▲*USP31*

▲1.5 µm to 10 µm▲*USP33*

in diameter, ▲or a monolithic silica rod.▲*USP31*

L4—Silica gel of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter.

L5—Alumina of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter.

L6—Strong cation-exchange packing–sulfonated fluorocarbon polymer coated on a solid spherical core, 30 to 50 µm in diameter.

L7—Octylsilane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter, ▲or a monolithic silica rod.▲*USP31*

L8—An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 3 to 10 µm in diameter.

L9—Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.

L10—Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.

L11—Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.

L12—A strong anion-exchange packing made by chemically bonding a quaternary amine to a solid silica spherical core, 30 to 50 µm in diameter.

L13—Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.

L14—Silica gel having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm in diameter.

L15—Hexylsilane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

L16—Dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm in diameter.

L17—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter.

L18—Amino and cyano groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.

L19—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, about 9 µm in diameter.

L20—Dihydroxypropane groups chemically bonded to porous silica particles, ~~5 to 10 µm~~

▲3 µm to 10 µm▲*USP33*
in diameter.

L21—A rigid, spherical styrene-divinylbenzene copolymer, ~~5 to 10 µm~~

▲3 µm to 10 µm▲*USP33*
in diameter.

L22—A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size.

L23—An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, about 10 µm in size.

L24—A semi-rigid hydrophilic gel consisting of vinyl polymers with numerous hydroxyl groups on the matrix surface, 32 to 63 µm in diameter.

[NOTE—Available as YMC-Pack PVA-SIL manufactured by YMC Co., Ltd. and distributed by Waters Corp. (www.waters.com).]

L25—Packing having the capacity to separate compounds with a molecular weight range from 100–5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contained some residual carboxyl functional groups) was found suitable.

L26—Butyl silane chemically bonded to totally porous silica particles, ~~3~~

▲1.5▲*USP33*
to 10 µm in diameter.

L27—Porous silica particles, 30 to 50 µm in diameter.

L28—A multifunctional support, which consists of a high purity, 100 Å, spherical silica substrate that has been bonded with anionic exchanger, amine functionality in addition to a conventional reversed phase C8 functionality.

In-Process Revision

L29—Gamma alumina, reverse-phase, low carbon percentage by weight, alumina-based polybutadiene spherical particles, 5 µm in diameter with a pore volume of 80 Å.

L30—Ethyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

L31—A hydroxide-selective, strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 8.5-µm macroporous particles having a pore size of 2000 Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene.

L32—A chiral ligand-exchange packing–L-proline copper complex covalently bonded to irregularly shaped silica particles, 5 to 10 µm in diameter.

L33—Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability.

[NOTE—Available as ~~TSKgel G4000 SWxl~~

▲TSK-GEL G4000SWxl▲*USP33*
from Tosoh Bioscience (www.tosohbioscience.com).]

L34—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 9 µm in diameter.

L35—A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase having a pore size of 150 Å.

L36—A 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5-µm aminopropyl silica.

L37—Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 Da. It is a polymethacrylate gel.

L38—A methacrylate-based size-exclusion packing for water-soluble samples.

L39—A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.

L40—Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5 to 20 µm in diameter.

L41—Immobilized α_1-acid glycoprotein on spherical silica particles, 5 µm in diameter.

L42—Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5 µm in diameter.

L43—Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 5 to 10 µm in diameter.

L44—A multifunctional support, which consists of a high purity, 60 Å, spherical silica substrate that has been bonded with a cationic exchanger, sulfonic acid functionality in addition to a conventional reversed phase C8 functionality.

L45—Beta cyclodextrin bonded to porous silica particles, 5 to 10 µm in diameter.

L46—Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, about ~~8 µm~~★~~8 µm to 10 µm~~▲*USP33*

▲9 µm to 11 µm▲*USP33*
in diameter.

L47—High-capacity anion-exchange microporous substrate, fully functionalized with trimethlyamine groups, 8 µm in diameter.

[NOTE—Available as CarboPac MA1 and distributed by Dionex Corp. (www.dionex.com).]

L48—Sulfonated, cross-linked polystyrene with an outer layer of submicron, porous, anion-exchange microbeads, ~~15~~

▲10–15▲*USP33*
µm in diameter.

L49—A reversed-phase packing made by coating a thin layer of polybutadiene onto spherical porous zirconia particles, 3 to 10 µm in diameter.

[NOTE—Available as Zirchrom PBD ~~manufactured by ZirChrom Separations, Inc., distributed by Alltech, www.Alltechweb.com~~

▲from www.zirchrom.com.]▲*USP33*

L50—Multifunction resin with reversed-phase retention and strong anion-exchange functionalities. The resin consists of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15 µm in diameter, and a surface area not less than 350 m^2 per g. Substrate is coated with quaternary ammonium functionalized latex particles consisting of styrene cross-linked with divinylbenzene.

[NOTE—Available as OmniPac PAX-500 and distributed by Dionex Corp., (www.dionex.com).]

L51—Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 5 to 10 µm in diameter.

[NOTE—Available as Chiralpak AD from Chiral Technologies, Inc., (www.chiraltech.com).]

L52—A strong cation-exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter.

[NOTE—Available as ■~~TSKgel IC-Cation-SW~~■2S *(USP31)*

▲TSK-GEL IC-Cation-SW▲*USP33*
from Tosoh Bioscience (www.tosohbioscience.com).]

L53—Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15 µm diameter. Substrate is surface grafted with carboxylic acid and/or phosphoric acid functionalized monomers. Capacity not less than 500 µEq/column.

[NOTE—Available as IonPac CS14 distributed by Dionex Corp., (www.dionex.com).]

L54—A size exclusion medium made of covalent bonding of dextran to highly cross-linked porous agarose beads, about 13 µm in diameter.

[NOTE—Available as Superdex Peptide HR 10/30 from Amersham Pharmacia Biotech (www.amershambiosciences.com).]

L55—A strong cation-exchange resin made of porous silica coated with polybutadiene–maleic acid copolymer, about 5 µm in diameter.

[NOTE—Available as IC-Pak C M/D from Waters Corp., (www.waters.com).]

L56—Propyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

[NOTE—Available as Zorbax SB-C3 from Agilent Technologies (www.agilent.com/chem).]

L57—A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5 µm in diameter, with a pore size of 120 Å.

[NOTE—Available as Ultron ES-OVM from Agilent Technologies (www.agilent.com/chem).]

L58—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about ■6 to 30 µm■1S *(USP31)* in diameter.

[NOTE—Available as Aminex HPX-87N from Bio-Rad Laboratories, (2000/01 catalog, #125-0143) www.bio-rad.com.]

L59—Packing having the capacity to separate proteins by molecular weight over the range of ■5 to 7000 kDa.■2S *(USP31)* It is spherical ■(5–10 µm),■2S *(USP31)* silica-based, and processed to provide hydrophilic characteristics and pH stability.

[NOTE—Available as ~~TSKgel G3000SW~~

▲TSK-GEL G3000SWxl▲*USP33*
Column (analytical column) and TSKgel Guard ■Column for 7.5 mm SW■2S *(USP31)* from Tosoh Bioscience ~~(part numbers 05789 and 05371, respectively)~~

▲
▲*USP33*
(www.tosohbioscience.com).]

L60—Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped.

[NOTE—Available as Supelcosil ~~ABZ~~

▲LC-ABZ▲*USP33*
from Supelco (www.sigmaaldrich.com/supelco).]

L61—A hydroxide selective strong anion-exchange resin consisting of a highly cross-linked core of 13 µm microporous particles having a pore size less than 10 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 85 nm diameter microbeads bonded with alkanol quaternary ammonium ions (6%).

[NOTE—Available as Ion Pac AS-11 and AG-11 from Dionex (www.dionex.com).]

L62—C30 silane bonded phase on a fully porous spherical silica, 3 to 15 µm in diameter.

In-Process Revision

L63—■Glycopeptide teicoplanin linked through multiple covalent bonds to a 100-Å units spherical silica.

[NOTE—Available as Chirobiotic T from Astec (www.astecusa.com).]■1S (*USP31*)

■L64—Strongly basic anion-exchange resin consisting of 8% cross-linked styrene-divinylbenzene copolymer with a quartenary ammonium group in the chloride form, 45 to 180 µm in diameter.

[NOTE—A suitable grade is available as AG 1-X8 resin chloride form from www.discover.bio-rad.com.]

L65—Strongly acidic cation-exchange resin consisting of 8% sulfonated cross-linked styrene-divinylbenzene copolymer with a sulfonic acid group in the hydrogen form, 45 to 250 µm in diameter.

[NOTE—A suitable grade is available as AG 50W-X2 resin hydrogen form from www.discover.bio-rad.com.]

L66—A crown ether coated on a 5-µm particle size silica gel substrate. The active site is *(S)*-18-crown-6-ether.

[NOTE—Available as Crownpak CR(+) from Daicel (www.daicel.com).]■2S (*USP31*)

▲L67—Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, ▲2 µm to 10 µm in diameter.▲*USP33*

[NOTE—Available as apHera C18 from Supelco (www.sigma-aldrich.com).]▲*USP32*

•L68—Spherical, porous silica, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped.

[NOTE—Available as SUPELCOSIL SUPLEX pKb-100 from Supelco (www.sigma-aldrich.com).]•6

▲L## (Dalteparin Sodium, anion-exchange Dowex 1X8)—Strongly basic (type I) anion exchange resin in the chloride form.

[NOTE—Available as Dowex 1X8.]

L## (Dalteparin Sodium, cation-exchange Dowex 50WX2)—Strong acidic cation exchange resin in the H^+ form.

[NOTE—Available as Dowex 50WX2.]▲*USP33*

L## (Glucosamine, Shodex NH2P-50)—Polyamine chemically bonded to cross-linked polyvinyl alcohol polymer, 5 µm in diameter.

NOTE—Available as Shodex NH2P-50 from Shodex (www.shodex.com).

~~L## (Trehalose, Sugar KS-801)—Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, 6 to 17 ★30▲*USP34*, µm in diameter.~~

~~[NOTE—Available as Sugar KS-801 from Shodex (www.shodex.com).]~~

▲L## (Ethylhexyl Triazone, Fluofix)—Fluorocarbon chains chemically bonded to 5-µm spherical silica particles.

[NOTE—Available as Wakopak Fluofix from Wako Pure Chemical Ind.]

L## (Nateglinide, Sumichiral OA-3300)—(*R*)-phenylglycine and 3,5-dinitroanaline urea linkage covalently bonded to silica.

[NOTE—Available as Sumichiral OA-3300, distributed by www.phenomenex.com.]

L## (Oxaliplatin and Atorvastatin, Chiralcel OC-H)—Cellulose tris(phenyl carbamate) coated on 5-µm silica.

[NOTE—Available as Chiralcel OC-H from www.chiral tech.com.]

L## (⟨233⟩ *Dimethylaniline*, Rtx-35 Amine)—[To come.]

[NOTE—Available as Rtx-35 Amine from www.restek.com.]

L## (Nateglinide, RSpak DE-613)—A rigid, spherical polymetacrylate, 4 µm to 6 µm in diameter.

[NOTE—Available as RSpak DE-613 from www.shodex.com.]

L## (Polyvinyl Acetate, Jordi-Gel DVB)—A rigid spherical polydivinylbenzene particle, 5 µm to 10 µm in diameter.

[NOTE—Available as Jordi-Gel DBV from www.jordiflp.com.]▲*USP33*

Phases

G1—Dimethylpolysiloxane oil.

G2—Dimethylpolysiloxane gum.

G3—50% Phenyl-50% methylpolysiloxane.

G4—Diethylene glycol succinate polyester.

G5—3-Cyanopropylpolysiloxane.

G6—Trifluoropropylmethylpolysiloxane.

G7—50% 3-Cyanopropyl-50% phenylmethylsilicone.

G8—80% Bis(3-cyanopropyl)-20% 3-cyanopropylphenylpoly-siloxane (percentages refer to molar substitution).

G9—Methylvinylpolysiloxane.

G10—Polyamide formed by reacting a C_{36} dicarboxylic acid with 1,3-di-4-piperidylpropane and piperidine in the respective mole ratios of 1.00 : 0.90 : 0.20.

G11—Bis(2-ethylhexyl) sebacate polyester.

G12—Phenyldiethanolamine succinate polyester.

G13—Sorbitol.

G14—Polyethylene glycol (av. mol. wt. of 950 to 1050).

G15—Polyethylene glycol (av. mol. wt. of 3000 to 3700).

G16—Polyethylene glycol compound (av. mol. wt. about 15,000). A high molecular weight compound of polyethylene glycol with a diepoxide linker.

[NOTE—Available commercially as Polyethylene Glycol Compound 20M, or as Carbowax 20M, from suppliers of chromatographic reagents.]

G17—75% Phenyl-25% methylpolysiloxane.

G18—Polyalkylene glycol.

G19—25% Phenyl-25% cyanopropyl-50% methylsilicone.

G20—Polyethylene glycol (av. mol. wt. of 380 to 420).

G21—Neopentyl glycol succinate.

In-Process Revision

G22—Bis(2-ethylhexyl) phthalate.
G23—Polyethylene glycol adipate.
G24—Diisodecyl phthalate.
G25—Polyethylene glycol compound TPA. A high molecular weight compound of a polyethylene glycol and a diepoxide that is esterified with terephthalic acid.
[NOTE—Available commercially as Carbowax 20M-TPA from suppliers of chromatographic reagents.]
G26—25% 2-Cyanoethyl-75% methylpolysiloxane.
G27—5% Phenyl-95% methylpolysiloxane.
G28—25% Phenyl-75% methylpolysiloxane.
G29—3,3′-Thiodipropionitrile.
G30—Tetraethylene glycol dimethyl ether.
G31—Nonylphenoxypoly(ethyleneoxy)ethanol (av. ethyleneoxy chain length is 30); Nonoxynol 30.
G32—20% Phenylmethyl-80% dimethylpolysiloxane.
G33—20% Carborane-80% methylsilicone.
G34—Diethylene glycol succinate polyester stabilized with phosphoric acid.
G35—A high molecular weight compound of a polyethylene glycol and a diepoxide that is esterified with nitroterephthalic acid.
G36—1% Vinyl-5% phenylmethylpolysiloxane.
G37—Polyimide.
G38—Phase G1 containing a small percentage of a tailing inhibitor.
[NOTE—A suitable grade is available commercially as "SP2100/0.1% Carbowax 1500" from Supelco, Inc. (www.sigmaaldrich.com/supelco).]
G39—Polyethylene glycol (av. mol. wt. about 1500).
G40—Ethylene glycol adipate.
G41—Phenylmethyldimethylsilicone (10% phenyl-substituted).
G42—35% phenyl-65% dimethylpolysiloxane (percentages refer to molar substitution).
G43—6% cyanopropylphenyl-94% dimethylpolysiloxane (percentages refer to molar substitution).
G44—2% low molecular weight petrolatum hydrocarbon grease and 1% solution of potassium hydroxide.
G45—Divinylbenzene-ethylene glycol-dimethylacrylate.
G46—14% Cyanopropylphenyl-86% methylpolysiloxane.
G47—Polyethylene glycol (av. mol. wt. of about 8000).
G48—Highly polar, partially cross-linked cyanopolysiloxane.

Supports

NOTE—Unless otherwise specified, mesh sizes of 80 to 100 or, alternatively, 100 to 120 are intended.
S1A—Siliceous earth for gas chromatography has been flux-calcined by mixing diatomite with Na_2CO_3 flux and calcining above 900°. The siliceous earth is acid-washed, then water-washed until neutral, but not base-washed. The siliceous earth may be silanized by treating with an agent such as dimethyldichlorosilane [NOTE—Unless otherwise specified in the individual monograph, silanized support is intended.] to mask surface silanol groups.
S1AB—The siliceous earth as described above is both acid- and base-washed. [NOTE—Unless otherwise specified in the individual monograph, silanized support is intended.]
S1C—A support prepared from crushed firebrick and calcined or burned with a clay binder above 900° with subsequent acid-wash. It may be silanized.
S1NS—The siliceous earth is untreated.
S2—Styrene-divinylbenzene copolymer having a nominal surface area of less than 50 m^2 per g and an average pore diameter of 0.3 to 0.4 μm.
S3—Copolymer of ethylvinylbenzene and divinylbenzene having a nominal surface area of 500 to 600 m^2 per g and an average pore diameter of 0.0075 μm.
S4—Styrene-divinylbenzene copolymer with aromatic –O and –N groups, having a nominal surface area of 400 to 600 m^2 per g and an average pore diameter of 0.0076 μm.
S5—40- to 60-mesh, high-molecular weight tetrafluorethylene polymer.
S6—Styrene-divinylbenzene copolymer having a nominal surface area of 250 to 350 m^2 per g and an average pore diameter of 0.0091 μm.
S7—Graphitized carbon having a nominal surface area of 12 m^2 per g.
S8—Copolymer of 4-vinyl-pyridine and styrene-divinylbenzene.
S9—A porous polymer based on 2,6-diphenyl-*p*-phenylene oxide.
S10—A highly polar cross-linked copolymer of acrylonitrite and divinylbenzene.
S11—Graphitized carbon having a nominal surface area of 100 m^2 per g modified with small amounts of petrolatum and polyethylene glycol compound.
[NOTE—Commercially available as SP1500 on Carbopack B from Supelco (www.sigmaaldrich.com/supelco).]
S12—Graphitized carbon having a nominal surface area of 100 m^2 per g.

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 31* page 831, page 3789 of the *Second Supplement,* and page 1563 of *PF* 34(6) [Nov.–Dec. 2008].

(HDQ) RTS—C58918

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	T▲*USP33*
Add the following:	
■Amlodipine Besylate Tablets	T, LR■2S (*USP32*)
Add the following:	
▲Arginine Capsules	T, LR▲*USP32*
Add the following:	
▲Arginine Tablets	T, LR▲*USP32*
Add the following:	
■Azithromycin Tablets	T■2S (*USP32*)
Add the following:	
▲Bicalutamide Tablets	T▲*USP32*
Add the following:	
■Cabergoline Tablets	T, LR■1S (*USP32*)
Add the following:	
■Calcium Citrate Tablets	W■1S (*USP32*)
Add the following:	
▲Cefdinir Capsules	T, LR▲*USP32*

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
■Clonazepam Orally Disintegrating Tablets	W, LR■1S (*USP32*)
Add the following:	
▲Curcuminoids Capsules	W, LR▲*USP32*
Add the following:	
▲Curcuminoids Tablets	W, LR▲*USP32*
Change to read:	
Dantrolene Sodium Capsules	T~~, LR~~ ■■2S (*USP32*)
Add the following:	
■Didanosine Tablets for Oral Suspension	T■2S (*USP31*)
Add the following:	
■Doxycycline Hyclate Tablets, Delayed-Release	T, LR■1S (*USP32*)
Add the following:	
▲Fenofibrate Capsules	W▲*USP32*
Add the following:	
▲Flavoxate Hydrochloride Tablets	W, LR▲*USP32*
Add the following:	
■Fluconazole Tablets	W■2S (*USP32*)
Add the following:	
■Granisetron Hydrochloride Tablets	W, LR■2S (*USP32*)
Add the following:	
■Guggul Tablets	W, LR■2S (*USP32*)
Add the following:	
■Ivermectin and Pyrantel Pamoate Tablets	T■1S (*USP32*)
Add the following:	
■Ketoprofen Capsules, Extended-Release	T■1S (*USP32*)

In-Process Revision

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
▲Lisinopril and Hydrochlorothiazide Tablets	W ▲*USP32*
Add the following:	
■Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	LR ■1S (*USP32*)
Add the following:	
■Loratadine Orally Disintegrating Tablets	T ■1S (*USP32*)
Add the following:	
■Losartan Potassium Tablets	T ■2S (*USP32*)
Add the following:	
■Metronidazole Capsules	W, LR ■1S (*USP32*)
Add the following:	
▲Mirtazapine Orally Disintegrating Tablets	LR ▲*USP32*
Add the following:	
■Ondansetron Tablets	T, LR ■2S (*USP32*)
Add the following:	
■Orbifloxacin Tablets	T ■1S (*USP32*)
Add the following:	
■Orphenadrine Citrate Tablets, Extended-Release	T, LR ■1S (*USP32*)

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
▲Oseltamivir Phosphate Capsules	W ▲*USP33*
Add the following:	
▲Oxcarbazepine Tablets	W ▲*USP33*
Add the following:	
▲Pantoprazole Sodium Delayed-Release Tablets	W ▲*USP32*
Add the following:	
■Pilocarpine Hydrochloride Tablets	T ■1S (*USP32*)
Add the following:	
■Potassium Citrate Tablets	W ■1S (*USP32*)
Add the following:	
■Risedronate Sodium Tablets	W ■2S (*USP32*)
Add the following:	
▲Soy Isoflavones Capsules	T, LR ▲*USP32*
Add the following:	
■Tamsulosin Hydrochloride Capsules	T ■2S (*USP32*)
Add the following:	
■Valganciclovir Tablets	T ■1S (*USP32*)
Add the following:	
■Zinc Citrate Tablets	W ■1S (*USP32*)
Add the following:	
▲Zolpidem Tartrate	W ▲*USP33*

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 31* page 840, page 3790 of the *Second Supplement,* page 266 of *PF* 29(1) [Jan.–Feb. 2003], page 591 of *PF* 31(2) [Mar.–Apr. 2005], page 1193 of *PF* 31(4) [July–Aug. 2005], page 188 of *PF* 32(1) [Jan.–Feb. 2006], page 1053 of *PF* 33(5) [Sept.–Oct. 2007], page 1270 of *PF* 33(6) [Nov.–Dec. 2007], page 166 of *PF* 34(1) [Jan.–Feb. 2008], page 450 of *PF* 34(2) [Mar.–Apr. 2008], page 817 of *PF* 34(3) [May–June 2008], page 1046 of *PF* 34(4) [July–Aug. 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], and page 1565 of *PF* 34(6) [Nov.– Dec. 2008].

(HDQ) RTS—C44080; C53875; C57641; C57716; C59275; C64392; C66478; C68459; C68500; C70200

Add the following:

▲**Atorvastatin Calcium:** White to off-white crystalline powder. Freely soluble in methanol; slightly soluble in alcohol; very slightly soluble in distilled water, in pH 7.4 phosphate buffer, and in acetonitrile; and insoluble in aqueous solutions of pH 4 and below.▲*USP33*

Add the following:

▲**Chitosan:** White or almost white powder or granules. Soluble in aqueous solutions of glycolic acid, of formic acid, of acetic acid, of hydrochloric acid, and of lactic acid; and practically insoluble in organic solvents and in water. *NF category:* Coating agent; film forming agent; suspending and/or viscosity increasing agent; vehicle (solid carrier). ▲*NF28*

Add the following:

▲**Diclazuril:** White to yellow powder. Sparingly soluble in dimethylformamide; and practically insoluble in water, in alcohol, and in methylene chloride.▲*USP33*

Change to read:

Mycophenolate Mofetil: White or almost white, crystalline powder.

▲Its melting range is between 94°C and 98°C.▲*USP33*
Freely soluble in acetone; soluble in methanol; sparingly soluble in dehydrated alcohol; and slightly soluble in water.

Delete the following:

▲~~**Norethynodrel:** White or practically white, odorless, crystalline powder. Melts at about 175°, over a range of about 3°. Is stable in air. Very slightly soluble in water and in solvent hexane; freely soluble in chloroform; soluble in acetone; sparingly soluble in alcohol.~~▲*USP33*

Change to read:

Olive Oil: Pale yellow, or light greenish-yellow, oily liquid, having a slight, characteristic odor and taste, with a faintly acrid aftertaste. Slightly soluble in alcohol. Miscible with ether, with chloroform, and with carbon disulfide.

▲*Specific gravity* ⟨841⟩: between 0.910 and 0.915.▲*NF28*
NF category: Vehicle (oleaginous).

Add the following:

▲**Polyoxyl 15 Hydroxystearate:** Yellowish to white waxy mass. Very soluble in water; soluble in alcohol and in 2-propanol; and insoluble in mineral oil. It solidifies at 25°. *NF category*: Tablet and/or capsule lubricant; wetting and/or solubilizing agent; vehicle (oleaginous).▲*NF28*

Add the following:

▲**Polyvinyl Acetate Dispersion:** Opaque, white or off-white, slightly viscous liquid. Miscible with water and with ethanol. It is sensitive to spoilage by microbial contaminants. *NF category:* Coating agent.▲*NF28*

Add the following:

▲**Hydrogenated Starch Hydrolysate:** Concentrated, aqueous solution or spray-dried or dried powder. Very soluble in water; and insoluble in alcohol. *NF category:* Sweetening agent; humectant; tablet binder; tablet and/or capsule diluent.▲*NF28*

Add the following:

▲**Pea Starch:** White or almost white, very fine powder. Practically insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; tablet binder; tablet and/or capsule diluent; tablet disintegrant.▲*NF28*

In-Process Revision

BRIEFING

Atomic Weights, *USP 31* page 895. The following changes to atomic weights are based on new determinations of isotopic abundances and reviews of previous isotopic abundances and atomic masses reported by IUPAC. The table is based on the 2005 table in *Pure Appl. Chem.*, **78**, 2051–2066 (2006) with 2007 changes to the values for lutetium, molybdenum, nickel, ytterbium, and zinc. Temporary names of elements with atomic numbers 110 and 111 have been changed to Darmstadtium and Roentgenium, respectively.

(HDQ) RTS—C68846

ATOMIC WEIGHTS

Change to read:

Standard Atomic Weights of the Elements, Recommended by the Commission on Atomic Weights and Isotopic Abundances of the International Union of Pure and Applied Chemistry ~~(1997) (©1998 IUPAC)~~

▲(2007) (©2008 IUPAC)▲*USP33*

Standard atomic weights ~~1997~~

▲2007▲*USP33*

[In alphabetical order: scaled to A_r(12C) = 12, where ^{12}C is a neutral atom in its nuclear and electronic ground state.]

The atomic weights of many elements are not invariant but depend on the origin and treatment of the material. The standard values of A_r(E) and the uncertainties (in parentheses, following the last significant figure to which they are attributed) apply to elements of natural terrestrial origin. The footnotes to this Table elaborate the types of variation which may occur for individual elements and which may be larger than the listed uncertainties of values of A_r(E). Names of elements with atomic numbers ~~110, 111 and 112,~~

▲112, 113, 114, 115, 116, and 118▲*USP33*
are temporary.

Name	Atomic Symbol	Atomic Number	Atomic Weight	Footnotes
Actinium*	Ac	89		
Aluminum	Al	13	~~26.981538(2)~~	
			▲26.9815386(8)▲*USP33*	
Americium*	Am	95		
Antimony (Stibium)	Sb	51	121.760(1)	g
Argon	Ar	18	39.948(1)	g, r
Arsenic	As	33	74.92160(2)	
Astatine*	At	85		
Barium	Ba	56	137.327(7)	
Berkelium*	Bk	97		
Beryllium	Be	4	9.012182(3)	
Bismuth	Bi	83	~~208.98038(2)~~	
			▲208.98040(1)▲*USP33*	
Bohrium*	Bh	107		
Boron	B	5	10.811(7)	g, m, r
Bromine	Br	35	79.904(1)	
Cadmium	Cd	48	112.411(8)	g
Caesium (Cesium)	Cs	55	~~132.90545(2)~~	
			▲132.9054519(2)▲*USP33*	
Calcium	Ca	20	40.078(4)	g
Californium*	Cf	98		
Carbon	C	6	12.0107(8)	g, r
Cerium	Ce	58	140.116(1)	g
Chlorine	Cl	17	~~35.4527(9)~~	m
			▲35.453(2)▲*USP33*	
Chromium	Cr	24	51.9961(6)	
Cobalt	Co	27	~~58.933200(9)~~	
			▲58.933195(5)▲*USP33*	
Copper	Cu	29	63.546(3)	r
Curium*	Cm	96		

Name	Atomic Symbol	Atomic Number	Atomic Weight	Footnotes
▲Darmstadtium*	Ds	110▲*USP33*		
Dubnium*	Db	105		
Dysprosium	Dy	66	~~162.50(3)~~	g
			▲162.500(1)▲*USP33*	
Einsteinium*	Es	99		
Erbium	Er	68	~~167.26(3)~~	g
			▲167.259(3)▲*USP33*	
Europium	Eu	63	151.964(1)	g
Fermium*	Fm	100		
Fluorine	F	9	18.9984032(5)	
Francium*	Fr	87		
Gadolinium	Gd	64	157.25(3)	g
Gallium	Ga	31	69.723(1)	
Germanium	Ge	32	~~72.61(2)~~	
			▲72.64(1)▲*USP33*	
Gold	Au	79	~~196.96655(2)~~	
			▲196.966569(4)▲*USP33*	
Hafnium	Hf	72	178.49(2)	
Hassium*	Hs	108		
Helium	He	2	4.002602(2)	g, r
Holmium	Ho	67	164.93032(2)	
Hydrogen	H	1	1.00794(7)	g, m, r
Indium	In	49	114.818(3)	
Iodine	I	53	126.90447(3)	
Iridium	Ir	77	192.217(3)	
Iron	Fe	26	55.845(2)	
Krypton	Kr	36	~~83.80(1)~~	g, m
			▲83.798(2)▲*USP33*	
Lanthanum	La	57	~~138.9055(2)~~	g
			▲138.90547(7)▲*USP33*	
Lawrencium*	Lr	103		
Lead	Pb	82	207.2(1)	g, r
Lithium	Li	3	6.941(2)†	g, m, r
Lutetium	Lu	71	~~174.967(1)~~	g
			▲174.9668(1)▲*USP33*	
Magnesium	Mg	12	24.3050(6)	
Manganese	Mn	25	~~54.938049(9)~~	
			▲54.938045(5)▲*USP33*	
Meitnerium*	Mt	109		
Mendelevium*	Md	101		
Mercury	Hg	80	200.59(2)	
Molybdenum	Mo	42	~~95.94(1)~~	g
			▲95.96(2)▲*USP33*	
Neodymium	Nd	60	~~144.24(3)~~	g
			▲144.242(3)▲*USP33*	
Neon	Ne	10	20.1797(6)	g, m
Neptunium*	Np	93		
Nickel	Ni	28	~~58.6934(2)~~	
			▲58.6934(4)▲*USP33*	
Niobium	Nb	41	92.90638(2)	
Nitrogen	N	7	~~14.00674(7)~~	g, r
			▲14.0067(2)▲*USP33*	
Nobelium*	No	102		
Osmium	Os	76	190.23(3)	g
Oxygen	O	8	15.9994(3)	g, r
Palladium	Pd	46	106.42(1)	g
Phosphorus	P	15	~~30.973762(4)~~	
			▲30.973762(2)▲*USP33*	

In-Process Revision

Name	Atomic Symbol	Atomic Number	Atomic Weight	Footnotes
Platinum	Pt	78	~~195.078(2)~~	
			▲195.084(9)▲*USP33*	
Plutonium*	Pu	94		
Polonium*	Po	84		
Potassium (Kalium)	K	19	39.0983(1)	
Praseodymium	Pr	59	140.90765(2)	
Promethium*	Pm	61		
Protactinium*	Pa	91	231.03588(2)	
Radium*	Ra	88		
Radon*	Rn	86		
Rhenium	Re	75	186.207(1)	
Rhodium	Rh	45	102.90550(2)	
▲Roentgenium*	Rg	111▲*USP33*		
Rubidium	Rb	37	85.4678(3)	g
Ruthenium	Ru	44	101.07(2)	g
Rutherfordium*	Rf	104		
Samarium	Sm	62	~~150.36(3)~~	g
			▲150.36(2)▲*USP33*	
Scandium	Sc	21	~~44.955910(8)~~	
			▲44.955912(6)▲*USP33*	
Seaborgium*	Sg	106		
Selenium	Se	34	78.96(3)	
Silicon	Si	14	28.0855(3)	r
Silver	Ag	47	107.8682(2)	g
Sodium (Natrium)	Na	11	~~22.989770(2)~~	
			▲22.98976928(2)▲*USP33*	
Strontium	Sr	38	87.62(1)	g, r
Sulfur	S	16	~~32.066(6)~~	g, r
			▲32.065(5)▲*USP33*	
Tantalum	Ta	73	~~180.9479(1)~~	
			▲180.94788(2)▲*USP33*	
Technetium*	Tc	43		
Tellurium	Te	52	127.60(3)	g
Terbium	Tb	65	~~158.92534(2)~~	
			▲158.92535(2)▲*USP33*	
Thallium	Tl	81	204.3833(2)	
Thorium*	Th	90	~~232.0381(1)~~	g
			▲232.03806(2)▲*USP33*	
Thulium	Tm	69	168.93421(2)	
Tin	Sn	50	118.710(7)	g
Titanium	Ti	22	47.867(1)	
Tungsten (Wolfram)	W	74	183.84(1)	
~~Unnunilium*~~	~~Uun~~	~~110~~		
~~Unununium*~~	~~Uuu~~	~~111~~		
Ununbium*	Uub	112		
▲Ununhexium*	Uuh	116▲*USP33*		
▲Ununoctium*	Uuo	118▲*USP33*		
▲Ununpentium*	Uup	115▲*USP33*		
▲Ununquadium*	Uuq	114▲*USP33*		
▲Ununtrium*	Uut	113▲*USP33*		
Uranium*	U	92	~~238.0289(1)~~	g, m
			▲238.02891(3)▲*USP33*	
Vanadium	V	23	50.9415(1)	
Xenon	Xe	54	~~131.29(2)~~	g, m
			▲131.293(6)▲*USP33*	

In-Process Revision

Name	Atomic Symbol	Atomic Number	Atomic Weight	Footnotes
Ytterbium	Yb	70	~~173.04(3)~~ ▲173.054(5)▲*USP33*	g
Yttrium	Y	39	88.90585(2)	
Zinc	Zn	30	~~65.39(2)~~ ▲65.38(2)▲*USP33*	
Zirconium	Zr	40	91.224(2)	g

[*] Element has no stable nuclides. One or more well-known isotopes are given in the accompanying table with the appropriate relative atomic mass and half-life. However, three such elements (Th, Pa, and U) do have a characteristic terrestrial isotopic composition, and for these an atomic weight is tabulated.

[†] Commercially available Li materials have atomic weights that are known to range between 6.939 and 6.996; if a more accurate value is required, it must be determined for the specific material.

[g] Geological specimens are known in which the element has an isotopic composition outside the limits for normal material. The difference between the atomic weight of the element in such specimens and that given in the *Table* may exceed the stated uncertainty.

[m] Modified isotopic compositions may be found in commercially available material because it has been subjected to an undisclosed or inadvertent isotopic fractionation. Substantial deviations in atomic weight of the element from that given in the *Table* can occur.

[r] Range in isotopic composition of normal terrestrial material prevents a more precise A_r(E) being given; the tabulated A_r(E) value should be applicable to any normal material.

In-Process Revision

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* or an *IRA.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents, Indicators, and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
General Notices (entire *General Notices and Requirements* revised)	34	1	40
USP Monographs			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Albendazole—*Assay*	34	1	69
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Sulfate—*USP Reference standards, Assay*	34	2	242
Albuterol Tablets—*Assay*	31	3	726
Alfuzosin Hydrochloride (new)	34	1	69
Allopurinol—*Related compounds, Limit of hydrazine* (add)	34	1	70
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Aluminum Acetate Topical Solution—*Identification*	34	2	242
Aluminum Subacetate Topical Solution—*Identification*	34	2	242
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Aminophylline—*CAS number*	34	1	72
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	34	5	1137
Amodiaquine Hydrochloride—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	2	243
Amodiaquine Hydrochloride Tablets—*USP Reference standards, Identification, Assay*	34	3	558
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Anastrozole (new)	34	2	244
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Arginine Capsules (new)	33	6	1160
Arginine Tablets (new)	33	6	1161
Aspirin—*Readily carbonizable substances*	34	5	1143
Atovaquone—*Assay*	34	2	247
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam for Injection—*Assay*	34	4	906
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Betamethasone Oral Solution—*Packaging and storage, Thin-layer chromatographic identification test* (delete), *Identification A, B* (add), *Microbial limits* (add), *pH* (add), *Deliverable volume* (add), *Related compounds* (add), *Assay*	34	3	567
Bicalutamide Tablets (new)	34	5	1147
Bisoctrizole (new)	32	2	309
Bisoprolol Fumarate Tablets—*Dissolution*	34	3	570
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Related compounds*	34	4	907
Bupivacaine Hydrochloride—*CAS number*	34	1	75
Bupropion Hydrochloride Extended-Release Tablets—*Related compounds*	34	3	570
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Cabergoline (new)	34	1	75
Cabergoline Tablets (new)	34	3	572
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Cefaclor Capsules—*Identification, Related compounds, Assay*	34	2	248
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefdinir (new)	33	6	1162
Cefdinir for Oral Suspension (new)	34	1	81
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Chlorhexidine Acetate (new)	34	3	582
Chlorhexidine Gluconate Oral Rinse—*Labeling, USP Reference standards*	34	2	250
Chlorhexidine Gluconate Solution—*USP Reference standards, Limit of p-chloroaniline, Assay*	34	2	250
Chlorhexidine Hydrochloride (new)	34	3	585
Chloroquine—*Assay*	34	1	86
Chloroquine Phosphate—*USP Reference standards, Identification, Assay*	34	2	251
Chloroquine Phosphate Tablets—*USP Reference standards, Identification, Assay*	34	3	587
Cilostazol—*Loss on drying*	34	3	589
Cisapride (new)	34	2	253
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Anhydrous Citric Acid *(Harmonization)*—*Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)*—*Sulfate*	31	3	750
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Climbazole (new)	33	5	891
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clonazepam Orally Disintegrating Tablets (new)	34	2	254
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Clozapine Tablets—*Uniformity of dosage units* (add)	34	3	589
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclofenac Potassium—*Identification*	34	1	87
Diclofenac Sodium Delayed-Release Tablets—*Identification*	31	3	751
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine—*USP Reference standards, Related compounds*	34	1	87
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Diethylstilbestrol Diphosphate (delete entire monograph)	33	6	1165
Diethylstilbestrol Diphosphate Injection (delete entire monograph)	33	6	1167
Dimethyl Sulfoxide—*Definition*, *Congealing temperature* (delete), *Substances darkened by potassium hydroxide* (delete), *Limit of dimethyl sulfone* (delete), *Limit of nonvolatile residue, Related compounds* (add), *Assay* (add)	34	1	88
Dipivefrin Hydrochloride—*Assay*	34	1	89
Disopyramide Phosphate—*Assay*	34	1	90
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Doxycycline Hyclate Delayed-Release Tablets (new)	34	3	589
Dronabinol—*Packaging and storage*, *Related compounds, Assay*	34	1	90
Dyclonine Hydrochloride—*Chemical information*	33	6	1167
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Enalaprilat Injection (new)	34	3	593
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Epinephrine—*Assay*	34	1	91
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158
Estradiol—*Chemical information, Labeling* (add)	33	6	1167
Estradiol Tablets—*USP Reference standards, Chromatographic purity* (add)	34	3	596
Estradiol Vaginal Inserts (new)	34	3	597
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Ethinyl Estradiol Tablets—*Dissolution* (add)	31	4	1067
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Eucatropine Hydrochloride (delete entire monograph)	33	6	1168
Eucatropine Hydrochloride Ophthalmic Solution (delete entire monograph)	33	6	1168
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate (new)	31	3	763
Fenofibrate Capsules (new)	34	2	258
Fenoprofen Calcium—*Chromatographic purity*	34	3	601
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets—*Labeling, USP Reference standards, Identification, Dissolution, Related compounds, Assay*	34	6	1445
Flavoxate Hydrochloride (new)	33	6	1172
Flavoxate Hydrochloride Tablets (new)	34	3	607
Fluconazole Injection (new)	34	2	266
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766
Fluticasone Propionate Cream (new)	34	3	609
Fluticasone Propionate Ointment (new)	34	3	611
Fluvestrant (new)	33	5	99
Formoterol Fumarate (new)	33	3	402
Foscarnet Sodium (new)	34	1	97
Fosinopril Sodium—*Related compounds*	34	3	613
Fosphenytoin Sodium—*Related compounds, Assay*	34	2	270
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Labeling* (add), *Dissolution, Related compounds*	34	6	1452
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide Tablets—*Dissolution*	33	4	651
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride (new)	33	6	1176
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163
Haloperidol Decanoate (new)	34	3	614
Heparin Sodium—*Definition, Anti-factor X_a activity, Assay*	33	2	238
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxyzine Pamoate—*Identification, Residue on ignition, Heavy metals, Pamoic acid content* (delete), *Assay*	34	2	271
Hydroxyzine Pamoate Capsules—*Identification, Assay*	34	2	272
Hydroxyzine Pamoate Oral Suspension—*Identification, Assay*	34	2	273
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Iopamidol—*Chemical structure, Reference standards, Identification, Related compounds*	33	6	1179
Iopamidol Injection—*Assay*	33	6	1182
Irbesartan—*Limit of azide*	34	5	1164
Isopropyl Alcohol—*Reference standards*	33	6	1182
Isotretinoin Capsules—*Labeling* (add), *Chromatographic purity, Assay*	34	4	942
Itraconazole (new)	34	4	947
Ivermectin Tablets—*Dissolution* (added)	34	4	948
Ivermectin and Pyrantel Pamoate Tablets (new)	34	2	277
Ketoprofen—*USP Reference standards, Chromatographic purity*	34	3	617
Ketoprofen Extended-Release Capsules (new)	34	4	951
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamotrigine (new)	34	3	617
Levonorgestrel—*USP Reference standards, Chromatographic purity, Assay*	34	3	620
Levorphanol Tartrate—*Assay*	34	2	280
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lipid Injectable Emulsion—*Definition, Limit of free fatty acids*	34	3	621
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium—*Limit of cyclohexane and isopropyl alcohol* (delete)	34	3	626
Losartan Potassium Tablets (new)	34	5	1164

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Mafenide Acetate Cream—*Identification*	34	2	280
Mafenide Acetate for Topical Solution—*Content of acetic acid*	34	3	627
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263
Meclizine Hydrochloride Tablets—*Related compounds, Assay*	33	6	1186
Meclocycline Sulfosalicylate—*Assay*	34	3	627
Meclocycline Sulfosalicylate Cream—*Assay*	34	3	628
Mefenamic Acid—*Heavy metals*	34	2	281
Meradimate—*Assay*	34	1	100
Mesna (new)	34	5	1168
Methacholine Chloride—*Identification, Melting range* (delete)	34	3	629
Methotrexate—*USP Reference standards, Chromatographic purity*	34	3	630
Methoxsalen Capsules—*Assay*	34	1	101
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Methylprednisolone—*Chromatographic purity, Assay*	33	6	1189
Metronidazole—*Packaging and storage, USP Reference standards, Identification, Melting range* (delete), *Non-basic substances* (delete), *Chromatographic purity* (delete), *Related compounds* (add), *Assay*	34	3	631
Metronidazole Capsules (new)	34	3	633
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Mirtazapine Orally Disintegrating Tablets (new)	33	6	1189
Morantel Tartrate—*pH*	32	6	1735
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Calcium—*Identification*, *Related compounds, Assay*	34	1	101
Mupirocin Cream—*Related compounds, Assay*	34	2	281
Mupirocin Nasal Ointment (new)	34	4	966
Naltrexone Hydrochloride—*Related compounds*	34	2	283
Naproxen Delayed-Release Tablets—*Drug release*	33	6	1192
Nateglinide (new)	34	6	1463
Niacinamide—*Readily carbonizable substances*	34	5	1176
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethindrone Acetate and Ethinyl Estradiol Tablets—*Dissolution*	33	3	432
Norethindrone Acetate and Ethinyl Estradiol Tablets—*Identification*	33	6	1194
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine (new)	34	3	641
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Orbifloxacin (new)	34	2	283
Orbifloxacin Tablets (new)	34	2	286
Orlistat Capsules (new)	32	6	1739
Orphenadrine Citrate Extended-Release Tablets (new)	34	3	643
Oseltamivir Phosphate (new)	34	6	1468

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin for Injection (new)	34	6	1473
Oxcarbazepine (new)	34	5	1177
Oxcarbazepine Tablets (new)	34	6	1478
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxytocin—*Definition, USP Reference standards, Identification, Vasopressor activity* (delete), *Acetic acid content* (add)	34	3	647
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Paricalcitol—*Identification, Assay*	33	2	252
Pectin—*Identification*	31	3	783
Penicillamine Capsules—*Dissolution*	31	2	436
Pentazocine and Acetaminophen Tablets—*Title, Assay for pentazocine, Assay for acetaminophen*	33	6	1200
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Pergolide Oral Suspension, Veterinary (new)	34	2	289
Permethrin (new)	32	4	1100
Permethrin Cream (new)	34	1	103
Petrolatum (new)—*Harmonization*	28	2	569
White Petrolatum (new)—*Harmonization*	28	2	570
Phenylephrine Hydrochloride—*Assay*	34	2	291
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride Tablets (new)	34	2	291
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980
Piperazine—*USP Reference standards* (add), *Identification, Primary amines and ammonia* (delete), *Chromatographic purity* (add)	34	1	105
Piperazine Adipate (new)	33	6	1201
Piperazine Citrate—*USP Reference standards* (add) *Identification*, *Primary amines and ammonia* (delete) *Chromatographic purity* (add), *Assay*	34	1	106
Piperazine Dihydrochloride (new)	33	6	1202
Piperazine Phosphate (new)	33	6	1204
Polyethlene Glycol 3350 and Electrolytes for Oral Solution—*Reference standards, Assay for potassium and sodium*	33	6	1205
Polyvinyl Alcohol—*Definition, Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification* (add), *Viscosity, Residue on ignition, Heavy metals* (add), *Acid value* (add), *Water-insoluble substances, Limit of methanol (methyl alcohol) and methyl acetate* (add)	33	6	1206
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Bromide Oral Solution, Veterinary (new)	33	5	936
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180
Pravastatin Sodium—*Chromatographic purity, Assay*	34	2	294

In-Process Revision

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Prednisolone Sodium Phosphate—*Definition*, *Free prednisolone* (delete) *Related compounds* (add), *Assay*	34	1	108
Proguanil Hydrochloride (new)	34	2	296
Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds, Assay*	32	4	1107
Propofol Injectable Emulsion (new)	33	6	1208
Propoxycaine and Procaine Hydrochloride and Norepinephrine Bitartrate Injection—*Assay for norepinephrine*	34	1	110
Pseudoephedrine Hydrochloride—*Definition, USP Reference standards, Ordinary impurities* (delete), *Chromatographic purity* (add), *Assay*	34	2	298
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ramipril—*Definition, Assay*	31	3	787
Ranitidine Hydrochloride—*Chromatograpic purity, Assay*	34	2	299
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Rocuronium Bromide (new)	34	3	648
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Simethicone Emulsion—*Assay*	34	3	652
Simethicone Tablets—*Disintegration*	34	3	652
Sodium Bromide Injection, Veterinary (new)	33	5	949
Sodium Bromide Oral Solution, Veterinary (new)	33	5	950
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Fluoride—*Assay*	34	3	653
Sodium Sulfate—*Assay*	34	5	1192
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Stavudine—*Specific rotation*	34	3	653
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfadoxine and Pyrimethamine Tablets—*Assay*	34	2	301
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfasalazine—*Identification*	34	3	653
Sulfazalazine Tablets—*Identification*	34	3	653
Sulfinpyrazone—*Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	34	6	1483
Tamsulosin Hydrochloride (new)	33	6	1211
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Tazobactam—*Identification, Specific rotation, Related compounds, Organic volatile impurities* (delete), *Assay*	34	2	302
Terbinafine Hydrochloride—*Melting range*	34	5	1197
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride—*Chromatographic purity*	34	2	306
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Tobramycin Inhalation Solution—*Identification* (add), *Osmolarity, Chromatographic purity, Other requirements* (delete), *Assay*	34	2	307
Topiramate Tablets (new)	34	5	1197
Torsemide—*Water*	33	6	1213
Tramadol Hydrochloride (new)	34	5	1200
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Trandolapril (new)	34	2	310
Travoprost (new)	32	4	1115
Travoprost Ophthalmic Solution (new)	32	4	1118
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800
Triamterine Capsules—*USP Reference standards, Related compounds* (add), *Assay*	34	3	654
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Valganciclovir Tablets (new)	33	1	89
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486
Vancomycin Hydrochloride—*Labeling* (add), *USP Reference standards, Chromatographic purity*, *Other requirements* (add)	34	1	111
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Labeling* (add), *Identification* (add), *Water* (add), *pH* (add), *Uniformity of dosage units* (add), *Chromatographic purity*, *Assay*	34	4	992
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Pure Steam (new)	31	2	467
Water for Hemodialysis—*Bacterial endotoxins*	31	2	468
Sterile Water for Inhalation—*pH* (delete), *Ammonia* (delete), *Calcium* (delete), *Carbon dioxide* (delete), *Chloride* (delete), *Sulfate* (delete), *Conductivity* (add), *Oxidizable substances*	31	3	802
Sterile Water for Injection—*Oxidizable substances*	31	3	803
Sterile Water for Irrigation—*Oxidizable substances*	31	3	804
Sterile Purified Water—*Oxidizable substances*	31	3	804
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Zidovudine—*Assay*	34	3	656
Zidovudine Capsules—*Related compounds, Assay*	34	3	657
Zidovudine Injection—*Related compounds, Assay*	34	3	658
Zolpidem Tartrate (new)	34	6	1487
Zonisamide (new)	34	6	1489
<u>*Dietary Supplements Monographs*</u>			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Calcium Citrate Tablets (new)	34	2	312
Curcuminoids (new)	33	6	1215
Curcuminoids Capsules (new)	33	6	1217
Curcuminoids Tablets (new)	33	6	1218
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Asian Ginseng Capsules (new)	30	2	571
Glucosamine Hydrochloride—*Assay*	33	4	691
Glucosamine Sulfate Potassium Chloride—*Assay*	33	4	692
Glucosamine Sulfate Sodium Chloride—*Assay*	33	4	692
Glutamic Acid (new)	34	4	997

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004
Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Powdered Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Omega-3 Acid Triglycerides (new)	34	3	662
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Potassium Citrate Tablets (new)	34	2	313
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Powdered Soy Isoflavones Extract (new)	33	6	1224
Soy Isoflavones Capsules (new)	33	6	1227
Soy Isoflavones Tablets (new)	33	6	1228
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Citrate (new)	34	2	315
Zinc Citrate Tablets (new)	34	2	316
Zinc and Vitamin C Lozenges (new)	34	2	317
USP General Test Chapters			
⟨1⟩ Injections—*Ingredients*	34	4	1020
⟨11⟩ USP Reference Standards—	29	6	2022
	30	5	1674
	31	2	507
	31	6	1680
	32	1	407
	33	1	95
	33	3	497
	33	4	716
	33	5	981
	33	6	1256
	34	1	142
	34	2	332
	34	3	680
	34	4	1021
	34	5	1230
	34	6	1531
⟨41⟩ Weights and Balances—*Introduction, Repeatability* (add), *Verification of Accuracy* (add), *Calibration Check* (add)	34	3	682
⟨85⟩ Bacterial Endotoxins Test—*Harmonization*	33	3	539
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨191⟩ Identification Tests—General—*Introduction*	34	2	333
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨271⟩ Readily Carbonizable Substances Test—*Introduction*	33	6	1258
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨401⟩ Fats and Fixed Oils—*Ester Value, Hydroxyl Value, Iodine Value, Peroxide Value, Saponification Value, Polyunsaturated Fatty Acids Determination and Profile* (add), *Trace Metals* (add), *Sterol Composition* (add)	34	3	736
⟨429⟩ Light Diffraction Measure of Particle Size (new)—*Harmonization*	31	4	1234
⟨467⟩ Organic Volatile Impurities—*Identification, Control, and Quantification of Residual Solvents*	34	3	747
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	34	5	1232
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density—*Harmonization*	31	3	909
⟨621⟩ Chromatography—*System Suitability, Glossary of Symbols*	34	5	1238
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨661⟩ Containers—Plastics—*Introduction, Polyethylene Containers, Polypropylene Containers*	34	2	335
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533

In-Process Revision

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
⟨671⟩ Containers—Performance Testing—*Introduction, Moisture Permeation, Light Transmission Test*	34	2	337
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912
⟨701⟩ Disintegration—*Apparatus*	34	1	155
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	34	2	341
⟨731⟩ Loss on Drying—*Introduction*	34	3	760
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	34	3	761
⟨941⟩ X-Ray Diffraction (new)—*Harmonization*	31	4	1241
General Information Chapters			
⟨1010⟩ Analytical Data—Interpretation and Treatment—*Prerequisite Laboratory Practices and Principles, Measurement Principles and Variation, Comparison of Analytical Methods, Appendixes B, C, D, E, F*	34	3	764
⟨1024⟩ Bovine Serum (new)	34	3	776
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients (entire chapter revised)	34	2	343
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire chapter revised)	31	2	524
⟨1121⟩ Nomenclature—*General Nomenclature Forms*	34	1	159
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients (new)	34	2	375
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation*	30	5	1729
⟨1225⟩ Validation of Compendial Procedures—*Validation*	34	3	794
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1235⟩ Vaccines for Human Use—General Considerations (new)	34	5	1297
⟨1237⟩ Virology Test Methods (new)	34	2	391
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	34	3	798
⟨1788⟩ Particulate Matter Determination in Parenteral and Ophthalmic Products (new)	34	2	421
Reagents, Indicators, and Solutions			
Acetic Acid	33	6	1259
Acetylactone	34	3	808
Alcohol	34	2	442
Alcohol, Denatured (new)	34	3	808
8-Amino-6-methoxyquinoline (new)	34	1	162
p-Aminophenol	34	2	442
α-Amylase	34	1	162
Barium Chloride	34	2	442
Beclomethasone (new)	34	3	808
Bismuth Subnitrate (new)	34	1	162
1-Butanesulfonic Acid Sodium Salt (new)	33	4	766

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Calcium Chloride	34	3	808
Activated Charcoal	33	6	1259
Chloramine T	34	2	442
Diatomaceous Earth (new)	34	3	809
Diethylene Glycol	33	6	1259
2,7-Dihydroxynaphthalene (new)	34	3	809
N,N-Dimethyldecylamine (new)	34	4	1041
Dimethyltin Dibromide (new)	34	2	442
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Ethylenediamine (new)	34	2	442
Ferric Chloride	34	2	443
Ferrous Sulfate	33	6	1260
Hexylamine (new)	33	6	1260
Hydrogen Peroxide, 30 Percent	34	2	443
Hydrogen Peroxide, 30 Percent, Unstabilized (new)	34	3	809
Hydrogen Peroxide, 50 Percent in Water (new)	34	3	809
Lead Acetate	34	2	443
Maltotriose (new)	34	3	809
7-Methoxycoumarin (new)	34	2	443
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Morin (new)	34	2	443
Naphthalene	33	6	1260
4-(*p*-Nitrobenzyl)pyridine	33	6	1260
1-Octanol (new)	32	6	1804
Phloxine B (new)	33	6	1260
Phosphatase Enzyme, Alkaline	34	3	809
Salicylaldehyde	33	6	1260
Silver Nitrate	34	3	810
Sodium Cholate Hydrate (new)	34	3	810
Sodium 1-Decanesulfonate	34	5	1319
Sodium Phosphite Pentahydate (new)	34	1	162
Sorbitol (new)	34	3	810
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Tetrabutylammonium Hydroxide 30-Hydrate (new)	34	3	810
Tetrabutylammonium Hydroxide, 40 Percent in Water (delete)	34	3	810
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled	34	3	810
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled	34	3	811
Tetrahexylammonium Hydrogen Sulfate (new)	34	1	162
Tetrahydro-2-furancarboxylic Acid	33	6	1261
Triethylamine Phosphate (new)	33	6	1261
Triethylenediamine (new)	34	2	443
Trimethyltin Bromide (new)	34	2	444
Test Solutions			
Acetic Acid, Glacial, TS	34	3	811
Alcoholic TS (new)	34	3	811
Ammonia TS 2 (new)	34	2	444
Iodine and Potassium Iodide TS 3 (new)	34	2	444
Lanthanum Nitrate TS (new)	34	2	444
Methyl Red TS 2 (new)	34	2	445
Potasssium Pyroantimonate TS	34	3	812
Volumetric Solutions			
Bismuth Nitrate, 0.01 mol/L	34	4	1041
Potassium Iodate, Twentieth-Molar (0.05 M)	34	3	813
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Sodium Tetraphenylboron, Fiftieth-Molar (0.02 M)	34	2	447
Chromatographc Reagents			
Chromatographic Reagents—*Packings*	34	6	1559
Reference Tables			
Container Specifications for Capsules and Tablets	34	6	1563
Description and Solubility	29	1	266
	31	2	591
	32	1	188
	33	5	1053
	33	6	1270
	34	1	166
	34	2	450

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
Excipients			
USP and *NF* Excipients, Listed by Category	34	6	1511
NF General Notices and Requirements—*Title* (delete), "*Official*" *and* "*Official Articles*" (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
NF Monographs			
Acetone—*USP Reference standards* (add), *Water, Assay*	34	1	120
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
rAlbumin Human (new)	34	1	121
Alfadex—*USP Reference standards, Identification, Heavy metals Reducing sugars, Related compounds, Assay*	34	1	126
Alpha-Lactalbumin (new)	34	3	670
Amino Methacrylate Copolymer—*Definition, Packaging and storage, Viscosity, Limit of monomers*	34	2	326
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Betadex—*Structure* (add), *Packaging and storage, USP Reference standards, Identification, Microbial limits, pH, Heavy metals, Reducing substances, Light-absorbing impurities* (add), *Related compounds* (add), *Assay*	34	1	127
Butylated Hydroxytoluene—*USP Reference standards* (add), *Identification, Related compounds* (add)	34	1	130
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Carbomer 934—*Title, Definition, Packaging and storage, Viscosity*	34	1	131
Carbomer 934P—*Title, Definition, Packaging and storage, Viscosity*	34	1	132
Carbomer 940—*Title, Definition, Packaging and storage, Viscosity*	34	1	133
Carbomer 941—*Title, Definition, Packaging and storage, Viscosity*	34	1	133
Carbomer Copolymer—*Definition, Labeling, Viscosity,Limit of benzene, Limit of acrylic acid*	34	1	134
Carbomer Homopolymer—*Title, Definition, Labeling, Viscosity, Residue on ignition, Limit of benzene, Limit of arylic acid*	34	1	136
Carbomer Interpolymer—*Definition, Labeling,Viscosity, Limit of benzene*, *Limit of acrylic acid*	34	1	138
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Harmonization*	33	3	537
Silicified Microcrystalline Cellulose (new)	34	5	1218
Hydrogenated Coconut Oil (new)	34	2	327
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
High Fructose Corn Syrup—*Total solids, Assay*	34	2	329
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone—*Harmonization*	28	4	1257
Desoxycholic Acid (new)	34	6	1523
Egg Phospholipids (new)	33	4	703

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Erythorbic Acid (new)	33	6	1246
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Identification*	33	6	1247
Ethyl Maltol (new)	34	5	1224
Ethylparaben—*Harmonization*	34	6	1594
Liquid Glucose—*CAS number* (add), *Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification, Assay for reducing sugars (dextrose equivalent)* (add)	33	6	1248
Glyceryl Monooleate—*Chemical name, Reference standards, Identification, Saponification value*	33	6	1248
Hydrogenated Palm Oil (new)	34	2	330
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Low-Substituted Hydroxypropyl Cellulose—*Harmonization*	30	1	338
Anhydrous Lactose—*Harmonization*	32	6	1847
Lecithin—*CAS number* (add), *Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification* (add), *Acid value, Peroxide value* (add), *Hexane-insoluble matter, Lead, Heavy metals, Content of acetone-insoluble matter*	33	6	1249
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methylacrylic Acid Copolymer—*Definition, Packaging and storage, Labeling, Viscosity, Heavy metals, Limit of monomers*	33	6	1251
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Limit of monomers, Coagulum content*	33	6	1254
Methyl Alcohol—*USP Reference standards* (add), *Identification, Readily carbonizable substances, Assay*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Definition, Packaging and storage, Assay*	31	4	1145
Nitrogen 97 Percent—*Definition, Packaging and storage, Assay*	31	4	1146
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete) *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227
Polyethylene Glycol—*Harmonization*	31	3	897
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polyvinyl Acetate (new)	34	6	1526
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Rice Starch (new)—*Harmonization*	30	2	721
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Tagatose (new)	30	5	1672
Tetrafluoroethane (new)	31	6	1672

In-Process Revision

Pending Proposals (*continued*)
(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	*PF Volume, Issue, and Page Numbers of Pending Proposals* *Vol.*	*No.*	*Page(s)*
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	*PF Volume, Issue, and Page Numbers of Canceled Proposals* *Vol.*	*No.*	*Page(s)*
USP Monographs			
†Conjugated Estrogen—*Definition*	30	3	840
†Norethindrone Tablets—*Dissolution* (add)	33	6	1193
USP General Test Chapters			
†<191> Identification Tests—General—*Acetate, Ammonium*	33	4	719
NF Monographs			
†Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
†Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
†Sucralose—*Related compounds*	33	6	1255

† New cancellations in *PF* 35(1).

HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization is composed of several steps **(*Stages*)**.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors.*

INSTRUCTIONS TO AUTHORS

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication.

All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current copy of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current copy of *Pharmacopeial Forum* will offer examples of reference formats.

Copyright—Copyright transfer documents will be sent to authors after manuscripts have been accepted for publication.

Contact Person—USP will designate a Scientific Liaison in the Documentary Standards Division as the corresponding author. This ensures that USP receives all comments generated by the *Stimuli* article. Authors should contact the Scientific Liaison if they would like to receive copies of comments generated by their *Stimuli* articles.

Submission Instructions—Manuscripts must be submitted both as an electronic file and as a printed copy of the electronic file. Submit the text in Microsoft® Word or another current word-processing application. The preferred format for graphics submitted electronically is tagged image file format (TIFF). Photocopies are not acceptable. Manuscripts submitted for publication should be addressed to:

Pharmacopeial Forum
Executive Secretariat, USP
12601 Twinbrook Pkwy.
Rockville, MD 20852

NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–217.*]

MONOGRAPHS

EXCIPIENTS

GENERAL CHAPTERS

REAGENTS, INDICATORS, AND SOLUTIONS

REFERENCE TABLES

GENERAL SUBJECTS

Index

CHROMATOGRAPHIC REAGENTS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Reagents Used in *USP–NF* and *Pharmacopeial Forum*
Jan.–Feb. 2009

AMANTADINE HYDROCHLORIDE CAPSULES (DSD Mgh #2390)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	G1	Rtx-1	Dissolution	Dissolution Test 2. 0.32 mm × 30 m, 0.25 µm. Alternative column in the same dimensions DB-1, manufacturer J&W Scientific. Manufacturer: Restek

ATORVASTATIN CALCIUM (DSD Mgh #6339)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L51	Chiralpak AD	Enantiomeric purity	4.6 mm × 25 cm. Manufacturer: Chiral Technologies, Inc.
35(1)	L7	ZORBAX RX-C8	Related compounds	4.6 mm × 25 cm, 5 µm. Manufacturer: Agilent Technologies

BRINZOLAMIDE OPHTHALMIC SUSPENSION (DSD Mgh #10208)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Prodigy ODS-2	Assay	4.6 mm × 15 cm, 5 µm. Alternative column Symmetry C18, same dimensions, manufacturer Waters. Manufacturer: Phenomenex

CHITOSAN (DSD Mgh #2141)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L38	TSK-GEL G3000 PW	Molecular weight distribution and average molecular weight	7.5 mm × 30 cm, 10 µm. Manufacturer: Tosoh Biosciences
35(1)	L38	TSK-GEL G5000 PW	Molecular weight distribution and average molecular weight	7.5 mm × 30 cm, 17 µm. Manufacturer: Tosoh Biosciences
35(1)	L38	TSK-GEL G6000 PW	Molecular weight distribution and average molecular weight	7.5 mm × 30 cm, 17 µm. Manufacturer: Tosoh Biosciences

DICLAZURIL (DSD Mgh #24944)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L1	Hypersil BDS C-18	Chromatographic purity	4.6 mm × 10 cm, 3 µm. Manufacturer: Thermo Scientific

DILUTED ISOSORBIDE MONONITRATE (DSD Mgh #43610)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	LiChrosphere 100 RP-18	Assay	4 mm × 12.5 cm. Manufacturer: Merck KGaA

ECAMSULE SOLUTION (DSD Mgh #3063)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(5)	L1	Inertsil ODS-2	Related compounds	Test for related compounds A to F. 4.6 mm × 15 cm, 5 µm. Manufacturer: GL Sciences
34(5)	L1	LiChrosorb RP-18	Assay and Related compounds	Test for related compound G, ecamsule exo-2-hydroxyecamsule, ecamsule endo-2-hydroxyecamsule, and unspecified impurities. 4.0 mm × 12.5 cm, 5 µm. Manufacturer: Merck KGaA

GANCICLOVIR (DSD Mgh #34666)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L9	Partisil 10 SCX	Assay and Related compounds	4.6 mm × 25 cm. Manufacturer: Whatman Inc.

GINGER (DSD Mgh #34965)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Hypersil-ODS	Content of	Content of gingerols and gingerdiones. 4.6 mm × 25 cm. Manufacturer: Thermo Scientific

HYDROGENATED STARCH HYDROLYSATE (DSD Mgh #581)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L58	PL Hi-Plex Na	Content of	Content of maltitol and sorbitol. Hydrogenated polysaccharides test. 7.7 mm × 30 cm, 10 µm. Manufacturer: Varian/Polymer Labs

LEVOTHYROXINE SODIUM (DSD Mgh #45000)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L1	YMC-Pack Pro C18	Related compounds	4.0 mm × 15 cm, 3 µm. Manufacturer: YMC Co., Inc.

NARATRIPTAN HYDROCHLORIDE ORAL SUSPENSION (DSD Mgh #1787)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L11	SphereClone	Assay	4.6 mm × 25 cm, 5 µm. Manufacturer: Phenomenex

PANTOPRAZOLE ORAL SUSPENSION (DSD Mgh #3228)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Inertsil ODS-3	Assay	4.6 mm × 150 mm, 5 µm. Manufacturer: GL Sciences

POLYOXYL 15 HYDROXYSTEARATE (DSD Mgh #66720)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L1	LiChrosphere 100 RP-18	Free	Free polyethylene glycols. Pre-column. 4 mm × 12.5 cm, 5 µm. Manufacturer: Merck KGaA
35(1)	L25	Ultrahydrogel DP, + 120	Free	Free polyethylene glycols. 7.8 mm × 30 cm, 6 µm. Manufacturer: Waters Corp

POLYVINYL ACETATE DISPERSION (DSD Mgh #3062)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L1	AQUASIL C18	Limit of	Limit of acetic acid and acetate. 4.6 mm × 25 cm, 5 µm. Manufacturer: Thermo Scientific
35(1)	L1	Nucleosil 120 C18	Limit of	Limit of vinyl acetate. Pre-column. 4.0 mm × 3 cm. Manufacturer: Macherey-Nagel
35(1)	L1	AQUASIL C18	Limit of	Limit of vinyl acetate. Analytical column. 4 mm × 25 cm, 5 µm. Manufacturer: Thermo Scientific

TERBINAFINE ORAL SUSPENSION (DSD Mgh #3232)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Zorbax RX-C18	Assay	4.6 mm × 25 cm, 5 µm. Manufacturer: Phenomenex

TERBUTALINE ORAL SUSPENSION (DSD Mgh #3229)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L11	MicroBondapak Phenyl	Assay	3.9 mm × 30 cm, 10 µm. Manufacturer: Waters Corp

THEOPHYLLINE ORAL SOLUTION (DSD Mgh #82160)

PF	LGS#	Reagent Brand	Type of Test	Comments
25(4)	L1	MicroBondapak C18	Assay	3.9 mm × 30 cm. Alternative columns: 4.6 mm × 25 cm Partisil ODS, manufacturer Whatman; 4.0 mm × 30 cm Chromegabond M C18, manufacturer ES Industries. Manufacturer: Waters Corp.

TIAGABINE HYDROCHLORIDE ORAL SUSPENSION (DSD Mgh #3231)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L10	Zorbax CN Reliance	Assay	3 mm × 150mm, 5 µm. Manufacturer: Agilent Technologies

Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 2 **MAR.–APR. 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *PF* content to Executive Secretariat
USP–NF, 12601 Twinbrook Pkwy.,
Rockville, MD 20852

execsec@usp.org

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

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

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Fax: (301) 816-8148.

STANDARDS DEVELOPMENT

This section presents an overview of the public review and comment process, conducted through *Pharmacopeial Forum* (*PF*), for the development of official pharmaceutical standards.

USP publishes *Pharmacopeial Forum* (*PF*) bimonthly and provides interested parties an opportunity to review and comment on the new or revised standards of the *United States Pharmacopeia* and the *National Formulary* (*USP–NF*).

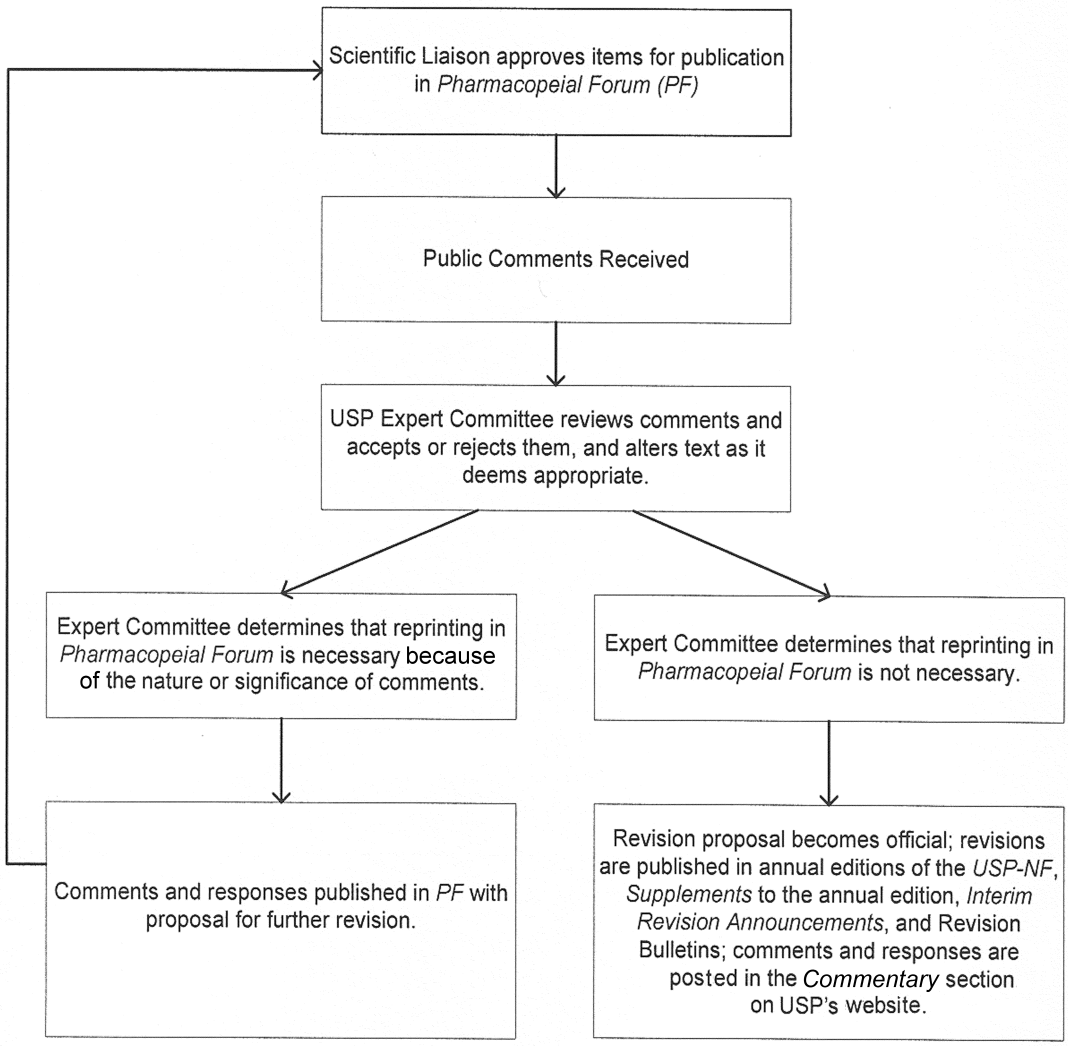
PF includes the following:

1. Potential revisions—entirely new standards, revision ideas, and drafts not yet targeted for official adoption (*Pharmacopeial Previews*)
2. Proposed revisions—new or revised standards targeted for official adoption (*In-Process Revision*)
3. Adopted revisions—new or revised standards that become official and binding before the publication of the next *USP–NF* or *Supplement* (*Interim Revision Announcement*)

USP welcomes comments and data on potential, proposed, or official standards. Comments, along with USP's responses, will be published either in *PF Briefings*, the *Commentary* section of *PF*, the *Commentary* section of *Supplements* to *USP–NF*, or the *Commentary* section of *USP–NF*.

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

This section provides descriptions of the various parts of *PF.* It also includes *Committee Designations* and the *Staff Directory.*

The content of the different sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
In-Process Revision Revisions targeted for adoption	•BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst such as the brand name of the column used in developing the proposed procedure and the USP scientific staff liaison who handled the issue. •New and revised standards that have been approved for publication by the appropriate USP Committee when it is considering whether to advance standards to official status (see *Standards Development*).	Review material and send comments promptly to USP staff liaison (see the *Staff Directory*) identified at the end of the briefing accompanying each item. For general inquiries or in cases where a particular liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-816-8373. Comment deadlines are found at the end of the *Policies and Announcements* section.
Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to harmonize internationally	•BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. The designated stage of harmonization. Two stages are printed in the *Pharmacopeial Forum Harmonization* section: Stage 4 is available for comment, whereas Stage 6 is the final official harmonized standard. The stage determines whether an item appears in the *Harmonization* section. •New or revised text to Stage 6 documents is marked with symbols (▪▪) to specify the tentative earliest date on which the revision would be officially adopted.	Review material and provide comments to the appropriate staff liaison cited in the *Briefing* preceding each *Preview* or *In-Process Revision.* Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopeia, with a copy to USP, for a given article. The addresses for the European and Japanese Pharmacopoeias are as follows: EP Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp

Proposed and Adopted Revisions to the *USP–NF* (*Continued*)

Section	Content	How Readers Can Respond
Interim Revision Announcement Adopted standards	Standards that have been adopted and will become officially binding on the specified date. Effective date is specified in the section's introductory page or within parentheses following a particular item. New or revised text is set off by the symbols ‣•.	Review to see if affected by any of the changes. Note effective date when standards become official and ensure compliance.
Pending Proposals	In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *PF* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF*, its supplements, or an *IRA*. Those items that have not yet been adopted are still pending.	Review items to track pending proposals.
Canceled Proposals	Canceled proposals are items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption into the *USP–NF*.	Review items to track canceled proposals.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including scientific liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Reagents Used in USP–NF and Pharmacopeial Forum
Update of chromatographic reagents based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* (*Continued*)

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Darrell Abernethy, M.D., Ph.D., Chief Science Officer	dra@usp.org	(301) 816-8184	
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT) B&B Vaccines and Virology (BB-VV)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, Senior Scientific Associate	kxb@usp.org	(301) 816-8462	Food Ingredients (FI)
Colleen Brennan, R.Ph. Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	USP–NF Spanish Edition
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D. Scientist	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards
Anthony DeStefano, Ph.D., Vice President, General Chapters	ajd@usp.org	(301) 998-6303	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	Document Disclosure
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE) Radiopharmaceuticals and Medical Imaging Agents Veterinary Drugs (VET)
James Griffiths, Ph.D., Vice President, Food and Dietary Supplement Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	USP Spanish Edition; General Chapters (GC), Pharmaceutical Waters
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products— Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexy Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B: Proteins and Polysaccharides (BB PP)
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Vice President, RSO & Applied Compendial Research	psl@usp.org	(301) 816-8337	Reference Standards
Markus Lipp, Ph.D. Director, Food Ingredients	mxl@usp.org	(301) 816-6366	Food Ingredients
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Marcia D. Mayfield, Project Manager, Monograph Procurement	mxm@usp.org	(301) 816-8358	New Monograph Acquisition
Kate Meringolo, Manager, Publications Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Senior Scientific Associate	jm@usp.org	(301) 816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Scientist	ktm@usp.org	(301) 816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Director, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements Non-Botanicals
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D. Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D. Manager, Pharmacy Compounding EC	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, Director, Excipients	cxs@usp.org	(301) 816-8262	Excipients
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB-VV)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Senior Scientist	dwv@usp.org	(301) 998-6828	Small Molecules Monographs
Mary ''Jeanie'' Waddell Scientist	msw@usp.org	(301) 816-8124	Small Molecules Monographs
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	rstech@usp.org	(301) 816-8129	USP Reference Standards
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section provides general information resources for *USP–NF* standards and processes. Information resources include announcements on scientific and policy issues currently under consideration, schedules for USP publications, and schedules for comment periods to proposed standards.

USP REDESIGNS *PHARMACOPEIAL FORUM*. USP has begun implementing a redesigned *Pharmacopeial Forum (PF)* as part of an initiative that will result in a completely online, freely available *PF* beginning in January 2011. Starting with this issue of *PF*, specific changes will include:

1. *Interim Revision Announcements (IRAs)*and *Errata* will join *Revision Bulletins* on www.usp.org in a ''New Official Text'' section, as well as being printed in *PF*.
2. *In-Process Revision*monographs will appear in the new redesigned monograph format.
3. A new *Proposed IRA* section is now added to distinguish proposed IRAs from regular in-process revisions.
4. The ''*Preview*'' section has been removed.

The following sections will ***not*** be removed from the *PF* at this time, as originally reported in 35(1), and will remain in their original locations:

- The Priority New Monograph Items List (Drug Substances, Drug Products, and Excipients)
- The Reference Standards Abeyance List

General information about the USP *Pharmacopeial Forum* redesign initiative can be found at the PF Redesign FAQ page on www.usp.org. Contact Susan de Mars, J.D., USP Chief Legal Officer (sdm@usp.org) with any questions about the *PF* redesign. If you have any questions about *PF* subscriptions, please contact USP Customer Service [custsvc@usp.org or 301-881-0666, 1-800-227-8772 (U.S. and Canada), or 00-800-4875-5555 (Europe)].

USP IMPLEMENTS GUIDELINE ON THE USE OF ACCELERATED PROCESSES FOR REVISIONS TO THE *USP–NF*. Sections 9.02 and 9.03 of the Rules and Procedures of the 2005–2010 Council of Experts specify various processes (Accelerated Revision Processes) that can be used to make revisions to the *USP–NF* official more quickly than through USP's Standard Revision Process. USP, in collaboration with the Compendial Process Improvement Project Team, has posted the Guideline on the Use of Accelerated Processes for Revision to *USP–NF*, with an official date of January 1, 2009. The Guideline is available at www.usp.org.

USP's Standard Revision Process calls for publication of a proposed revision in the *Pharmacopeial Forum (PF)* for a 90-day notice and comment period and, after the revision is approved by the relevant USP Expert Committee, publication in the next *USP–NF* or Supplement, as applicable. Accelerated Revision Processes, which include *Interim Revision Announcements, Revision Bulletins*, and *Errata* do not always require notice and comment and allow for a revision to become official prior to the next *USP–NF* or *Supplement*.

USP has issued this Guideline to delineate the circumstances under which these Accelerated Revision Processes are utilized. The Guideline includes a Decision Tree that specifies the criteria that are applied by USP in considering whether an Accelerated Revision Process is appropriate rather than USP's Standard Revision Process. This Guideline also addresses the use of delayed official dates for revisions made through the Standard Revision Process, where such revisions have broad industry impact and require additional time to implement.

Contact Susan de Mars, J.D., USP Chief Legal Officer (sdm@usp.org), with any questions.

USP ISSUES CALL FOR CANDIDATES FOR 2010–2015 COUNCIL OF EXPERTS, ITS EXPERT COMMITTEES, AND ITS ADVISORY PANELS. In accordance with the Bylaws of the USP Convention, USP is issuing a *Call for Candidates* for the 2010–2015 Council of Experts. The 2010–2015 Council of Experts includes 20 Expert Committees in the areas of Nomenclature, Small Molecules, Biologics and Biotechnology, Excipients, General Chapters, Reference Standards, Compounding, Food Ingredients, and Dietary Supplements. In the 2010–2015 cycle, USP is expanding the number of Expert Panels (formerly Advisory Panels) that report to Expert Committees. These new Expert Committees and Expert Panels align with the new USP Strategic Plan, which focuses on expanding and enhancing USP's core compendial and standards-setting activities.

The ability to add Expert Panels according to the needs of USP introduces flexibility and scalability into USP's activities. USP plans to continue to attract global base experts, and therefore encourages any qualified individual to apply. Importantly, this approach also enables USP to closely align USP's documentary and reference standards activities for a more efficient standards-setting process.

Specific Expert Committees and Expert Panels for which USP is seeking candidates are listed on the 2010–2015 Council of Experts area of the USP website (www.usp.org). The deadline for applications for the Council of Experts is **December 31, 2009**. The deadline for applications for Expert Committee members is **May 15, 2010**. Recruitment for Advisory Panels members will be continuous.

For further information, contact Angela G. Long, Vice President, Volunteer and Organizational Affairs (agl@usp.org or nominate@usp.org).

USP ANNOUNCES REVISIONS TO GALANTAMINE TABLETS. The Biopharmaceutics Expert Committee (BPC EC) has revised the Galantamine Tablets monograph that was proposed in *Pharmacopeial Forum* volume 34(6). The revisions expand the impurities list, add *Dissolution Test 2*, and revise the Q value. The revised monograph was proposed in *Pharmacopeial Forum* volume 34(6) with an intended official date of April 1, 2009. The official text is presented in this *Pharmacopeial Forum* as an *Interim Revision Announcement*.

Contact Margareth R. C. Marques, M. Sc., Ph.D. with any questions (301-816-8106 or mrm@usp.org).

***PHARMACOPEIAL FORUM* PUBLIC REVIEW AND COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 34(6)	February 15, 2009	*USP 33–NF 28*	November 2009	May 1, 2010
PF 35(1)	April 15, 2009			
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section and incorporated in the upcoming *Supplement* or book. They may also be published as *Revision Bulletins* on www.usp.org in the ''New Official Text'' section. The official publication in which an *IRA* is incorporated will depend upon publication deadlines. See table below. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and, therefore, contains all official text up to and including the contents of the latest *Supplement.* The table below outlines the publications and their release and official dates, and the book or supplement which supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
USP 32–NF 27	November 1, 2008	May 1, 2009	*1st Supplement to USP 32–NF 27*
IRA [*PF* 35(1)]	January 1, 2009	February 1, 2009	*2nd Supplement to USP 32–NF 27*
1st Supplement to USP 32–NF 27	February 1, 2009	August 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*

PRIORITY NEW MONOGRAPH ITEMS. USP is seeking monographs for the following drug substances and drug products that are, or soon will be, off patent and thus are of the highest priority. USP also is seeking monographs for the excipients listed below.

Monographs are marked received upon receipt of monograph proposal. Received monographs are removed from this list upon publication in *PF*. For the most current list, please consult the Priority New Monograph Items List posted at: http://www.usp.org/USPNF/submitMonograph/newMon.html.

Monograph sponsors should consult USP's Guideline for Submitting Requests for Revision to the *USP–NF* at http://www.usp.org/USPNF/submitMonograph/subGuide.html.

For additional information, contact Karen A. Russo, Ph.D., kar@usp.org.

Small Molecules (Drug Substances)—As of December 15, 2008

1. Allopurinol Sodium	2. Aminopropazine Fumarate	3. Aminopterin Sodium
4. Anagrelide Hydrochloride ***(Received)***	5. Arsenic Trioxide	6. Auranfoin
7. Azelaic Acid ***(Received)***	8. Balsalazide Disodium ***(Received)***	9. Bentoquatam
10. Benzphetamine Hydrochloride	11. Bivalirudin ***(Received)***	12. Calcipotriene
13. Calcium Trisodium Pentetate	14. Calfactant	15. Candesartan Cilexetil ***(Received)***
16. Carmustine ***(Received)***	17. Cefditoren Pivoxil ***(Received)***	18. Ceftibuten
19. Cetrorelix	20. Cevimeline	21. Chloroxine
22. Choline Salicylate	23. Cysteamine Bitartrate	24. Dalfopristin
25. Dapirazole Hydrochloride	26. Desirudin	27. Desonide ***(Received)***
28. Dexrazoxane	29. Difenoxin Hydrochloride	30. Entacapone ***(Received)***
31. Epoprostenol Sodium ***(Received)***	32. Erythromycin Phosphate	33. Erythromycin Thiocyanate
34. Esmolol Hydrochloride ***(Received)***	35. Estazolam ***(Received)***	36. Estramustine Phosphate Sodium
37. Ethanolamine Oleate	38. Etomidate ***(Received)***	39. Etoposide Phosphate
40. Exemestane	41. Famciclovir ***(Received)***	42. Felbamate ***(Received)***
43. Fluoromethane F 18	44. Fosfomycin Tromethamine ***(Received)***	45. Gadobenate Dimeglumine
46. Gadopentetic Acid	47. Gallium Nitrate	48. Ganirelix
49. Guanidine Hydrochloride	50. Halobetasol Propionate ***(Received)***	51. Haloperidol Decanoate ***(Received)***
52. Hydrocodone Polistirex	53. Ibandronate Sodium	54. Imipramine Pamoate
55. Imiquimod	56. Isosulfan Blue	57. Latanoprost ***(Received)***
58. Lomustine ***(Received)***	59. Lopinavir ***(Received)***	60. Metipranolol Hydrochloride
61. Miglitol	62. Milrinone Lactate	63. Misoprostol ***(Received)***
64. Moexipril Hydrochloride	65. Nalbuphine Hydrochloride	66. Nedocromil Sodium
67. Nicardipine Hydrochloride	68. Nilutamide	69. Nisoldipine
70. Olsalazine Sodium ***(Received)***	71. Orlistat ***(Received)***	72. Oxcarbazepine ***(Received)***
73. Oxiconazole Nitrate	74. Pemirolast Potassium	75. Pentamidine Isethionate ***(Received)***
76. Pioglitazone Hydrochloride ***(Received)***	77. Piperonyl Butoxide	78. Pirbuterol Acetate ***(Received)***
79. Poractant Alpha	80. Porfimer Sodium	81. Pramiprexole Dihydrochloride ***(Received)***
82. Quetiapine Fumarate ***(Received)***	83. Ranitidine	84. Rivastigmine Tartrate ***(Received)***
85. Ropinirole Hydrochloride ***(Received)***	86. Rose Bengal Disodium	87. Rosiglitazone Maleate
88. Salmeterol Xinafoate ***(Received)***	89. Sertraline Hydrochloride ***(Received)***	90. Sodium Phenylbutyrate

Small Molecules (Drug Substances)—As of December 15, 2008 *(Continued)*

91. Sodium Phosphates	92. Spectinomycin Sulfate	93. Streptozocin
94. Tacrolimus ***(Received)***	95. Tenofovir Disoproxil Fumarate ***(Received)***	96. Tiludronate Disodium
97. Tiopronin	98. Tranylcypromine Sulfate ***(Received)***	99. Trimetrexate Glucuronate
100. Venlafaxine Hydrochloride ***(Received)***	101. Voriconazole ***(Received)***	102. Zaleplon
103. Zinc Tridosium Pentetate	104. Zoledronic Acid	

Small Molecules (Drug Products)—As of December 15, 2008

1. Abacavir Sulfate, Lamivudine, and Zidovudine Tablets	2. Acarbose Tablets	3. Acetaminophen, Butalbital, Caffeine, and Codeine Phosphate Capsules
4. Acetaminophen, Clemastine Fumarate, and Pseudoephedrine Hydrochloride Tablets	5. Acetazolamide Extended-Release Capsules	6. Albuterol and Ipratropium Bromide Inhalation Aerosol
7. Albuterol and Ipratropium Bromide Inhalation Solution	8. Albuterol Extended-Release Tablets	9. Albuterol Inhalation Aerosol
10. Albuterol Sulfate Inhalation Solution	11. Albuterol Sulfate Oral Solution	12. Alendronate Sodium Oral Solution
13. Alfuzosin Extended-Release Tablets	14. Allopurinol for Injection	15. Alprazolam Extended-Release Tablets
16. Alprostadil Urethral Suppository	17. Aminopropazine Fumarate and Neomycin Sulfate Tablets	18. Aminopropazine Fumarate Injection
19. Aminopropazine Fumarate Tablets	20. Aminopterin Sodium Tablets	21. Amiodarone Hydrochloride Injection
22. Amlodipine and Benazepril Hydrochloride Capsules ***(Received)***	23. Amphotericin B Injection	24. Anagrelide Hydrochloride Capsules ***(Received)***
25. Arsenic Trioxide Injection	26. Atovaquone and Proguanil Hydrochloride Tablets	27. Atovaquone Tablets
28. Auranofin Capsules	29. Azatadine Maleate and Pseudoephedrine Sulfate Extended-Release Tablets	30. Azelaic Acid Cream
31. Azithromycin for Injection ***(Received)***	32. Azithromycin Tablets ***(Received)***	33. Baclofen Injection
34. Balsalazide Disodium Capsules ***(Received)***	35. Beclomethasone Dipropionate Inhalation Aerosol	36. Beclomethasone Dipropionate Nasal Suspension
37. Benazepril Hydrochloride and Hydrochlorothiazide Tablets	38. Bentoquatam Topical Suspension	39. Benzocaine and Cetylpyridinium Chloride Lozenges
40. Benzocaine and Menthol Lotion	41. Benzphetamine Hydrochloride Tablets	42. Bivalirudin Injection
43. Brompheniramine Maleate, Dextromethorphan Hydrobromide, and Pseudoephedrine Hydrochloride Oral Solution	44. Budesonide Inhalation Aerosol	45. Bupivacaine and Lidocaine Hydrochlorides Injection
46. Buprenorphine Hydrochloride Injection	47. Butalbital and Acetaminophen Capsules	48. Butalbital and Acetaminophen Tablets
49. Calcipotriene Cream	50. Calcipotriene Ointment	51. Calcipotriene Topical Solution
52. Calcitriol Capsules	53. Calcitriol Oral Solution	54. Calcium Acetate Capsules
55. Calcium Trisodium Pentetate Injection	56. Calfactant Intratracheal Suspension	57. Carbidopa and Levodopa Tablets for Oral Suspension ***(Received)***
58. Carbidopa, Levidopa, and Entacapone Tablets	59. Carmustine For Injection ***(Received)***	60. Carmustine Implant
61. Cefdinir Tablets	62. Cefditoren Pivoxil Tablets	63. Ceftibuten Capsules
64. Ceftibuten for Oral Suspension	65. Ceftiofur Hydrochloride Oral Suspension	66. Cetirizine Hydrochloride Tablets ***(Received)***
67. Cetrorelix Injection	68. Cevimeline Hydrochloride Capsules	69. Chloroxine Cream
70. Chlorpromazine Hydrochloride Extended-Release Capsules	71. Choline and Magnesium Salicylates Oral Solution	72. Choline and Magnesium Salicylates Tablets
73. Choline Salicylate Oral Solution ***(Received)***	74. Ciclopirox Shampoo	75. Ciclopirox Topical Gel
76. Ciclopirox Topical Solution ***(Received)***	77. Cimetidine Oral Solution	78. Ciprofloxacin Extended-Release Tablets
79. Ciprofloxacin Hydrochloride and Hydrocortisone Otic Suspension	80. Ciprofloxacin Otic Solution	81. Cisplatin Injection

Small Molecules (Drug Products)—As of December 15, 2008 *(Continued)*

82. Citalopram Hydrobromide Oral Solution	83. Citric Acid, Gluconolactone, and Magnesium Carbonate Irrigation	84. Cladribine Injection
85. Clemastine Fumarate Syrup	86. Clobetasol Propionate Gel	87. Clorazepate Dipotassium Capsules
88. Clorazepate Dipotassium Extended-Release Tablets	89. Clotrimazole and Betamethasone Dipropionate Lotion	90. Compound Undecylenic Acid Cream
91. Compound Undecylenic Acid Topical Powder	92. Conjugated Estrogens and Medroxyprogesterone Acetate Tablets	93. Cyclosporine Modified Capsules
94. Cyclosporine Modified Oral Solution	95. Cyclosporine Ointment	96. Cyclosporine Topical Solution
97. Cysteamine Bitartrate Capsules	98. Cytarabine Liposome Injection	99. Dalfopristin and Quinupristin Injection
100. Dantrolene Sodium Oral Suspension	101. Dapiprazole for Ophthalmic Solution	102. Desirudin for Injection
103. Desonide Cream	104. Dexrazoxane for Injection	105. Dextroamphetamine Sulfate Extended-Release Capsules
106. Dextromethorphan Polistirex Extended-Release Oral Suspension	107. Diazepam Injectable Emulsion	108. Diclofenac Sodium Ophthalmic Solution
109. Diethylpropion Hydrochloride Extended-Release Tablets	110. Difenoxin Hydrochloride and Atropine Sulfate Tablets	111. Difloxacin Hydrochloride Tablets
112. Dihydroergotamine Mesylate Metered Spray	113. Diltiazem Hydrochloride Injection	114. Dinoprostone Vaginal Suppositories
115. Diphenhydramine Hydrochloride and Acetaminophen Tablets	116. Divalproex Sodium Delayed-Release Capsules	117. Dorzolamide and Timolol Ophthalmic Solution
118. Dorzolamide Ophthalmic Solution	119. Doxepin Hydrochloride Cream	120. Doxycycline Oral Gel
121. Econazole Nitrate Cream	122. Edrophonium Chloride and Atropine Sulfate Injection	123. Enalapril Maleate and Felodipine Extended-Release Tablets
124. Enalaprilat Injection ***(Received)***	125. Entacapone Tablets ***(Received)***	126. Ephedrine Sulfate and Guaifenesin Tablets
127. Epirubicin Hydrochloride for Injection	128. Epirubicin Hydrochloride Injection	129. Epoprostenol for Injection
130. Epoprostenol Injection	131. Esmolol Hydrochloride Injection	132. Esomeprazole Magnesium Capsules
133. Estazolam Tablets ***(Received)***	134. Estramustine Phosphate Sodium Capsules	135. Ethanolamine Oleate Injection
136. Etidronate Disodium Injection Concentrate	137. Etomidate Injection	138. Exemestane Tablets
139. Famotidine Orally Disintegrating Tablets	140. Felbamate Oral Suspension	141. Felbamate Tablets
142. Fentanyl Lozenges	143. Famciclovir Tablets	144. Fentanyl Transdermal System ***(Received)***
145. Ferrous Fumarate and Docusate Sodium Extended-Release Capsules	146. Fluconazole Oral Suspension	147. Flunisolide Inhalation Aerosol
148. Flunisolide Nasal Spray	149. Fluocinolone Acetonide Shampoo	150. Fluorescein Sodium Ophthalmic Solution
151. Fluorometholone Ointment	152. Fluticasone Propionate Cream ***(Received)***	153. Fluticasone Propionate Inhalation Powder ***(Received)***
154. Fluticasone Propionate Ointment ***(Received)***	155. Fluticasone Propionate Pressurized Inhaler	156. Foscarnet Sodium Injection
157. Fosfomycin for Oral Solution	158. Gabapentin Oral Solution	159. Gadobenate Dimeglumine Injection
160. Gallium Nitrate Injection	161. Ganciclovir Capsules	162. Ganirelix Acetate Injection
163. Gatifloxacin Injection	164. Gatifloxacin Tablets	165. Gentamicin Sulfate Oral Solution
166. Gentamicin Sulfate Soluble Powder	167. Glipizide Extended-Release Tablets	168. Guaifenesin and Pseudoephedrine Hydrochloride Extended-Release Tablets
169. Guaifenesin and Salts of Dextromethorphan and Pseudoephedrine Oral Solution	170. Guanidine Hydrochloride Tablets	171. Halobetasol Propionate Cream
172. Halobetasol Propionate Ointment	173. Haloperidol Decanoate Injection	174. Haloperidol Lactate Injection
175. Haloperidol Lactate Oral Concentrate	176. Hydralazine Hydrochloride and Hydrochlorothiazide Capsules	177. Hydrochlorothiazide Capsules
178. Hydrochlorothiazide Oral Solution	179. Hydrocodone Bitartrate and Acetaminophen Capsules	180. Hydrocodone Bitartrate and Acetaminophen Oral Solution
181. Hydrocodone Bitartrate and Aspirin Tablets	182. Hydrocodone Bitartrate and Guaifenesin Oral Solution	183. Hydrocodone Bitartrate and Homatropine Methylbromide Syrup
184. Hydrocortisone Acetate Dental Paste	185. Hydrocortisone Acetate Rectal Foam Aerosol	186. Hydrocortisone Butyrate Lotion

Small Molecules (Drug Products)—As of December 15, 2008 *(Continued)*

187. Hydroflumethiazide and Reserpine Tablets	188. Hydromorphone Hydrochloride Oral Solution ***(Received)***	189. Hydroquinone Lotion
190. Ibandronate Sodium Tablets	191. Ibuprofen Capsules	192. Idarubicin Hydrochloride Injection
193. Imipramine Pamoate Capsules	194. Imiquimod Topical Cream	195. Ipratropium Bromide Inhalation Aerosol
196. Ipratropium Bromide Inhalation Solution	197. Irinotecan Hydrochloride Injection	198. Isosulfan Blue Injection
199. Isradipine Extended-Release Tablets	200. Itraconazole Injection	201. Itraconazole Oral Solution
202. Ketoconazole Cream	203. Ketoconazole Shampoo	204. Ketoprofen Capsules ***(Received)***
205. Ketoprofen Extended-Release Capsules	206. Ketoprofen Tablets	207. Ketotifen Fumarate Ophthalmic Solution
208. Lactic Acid Lotion	209. Lamotrigine Tablets ***(Received)***	210. Latanoprost Ophthalmic Solution
211. Leucovorin Calcium for Injection	212. Levetiracetam Tablets ***(Received)***	213. Levocabastine Ophthalmic Suspension
214. Levofloxacin Solution	215. Lincomycin Hydrochloride and Spectinomycin Sulfate Soluble Powder	216. Liothyronine Injection
217. Lomustine Capsules ***(Received)***	218. Lopinavir and Ritonavir Solution	219. Lopinavir Capsules
220. Lopinavir Solution	221. Loratadine Orally-Disintegrating Tablets ***(Received)***	222. Losartan Potassium Tablets ***(Received)***
223. Mefloquine Hydrochloride Tablets ***(Received)***	224. Melphalan for Injection	225. Mesalamine Suppositories
226. Mesoridazine Besylate Concentrate	227. Metaraminol Bitartrate Injection	228. Methacholine Chloride for Inhalation Solution
229. Methadone Hydrochloride Oral Concentrate	230. Methocarbamol and Aspirin Tablets	231. Methoxsalen Softgels
232. Methyclothiazide and Deserpidine Tablets	233. Methylphenidate Hydochloride Chewable Tablets	234. Metipranolol Ophthalmic Solution
235. Metronidazole Capsules ***(Received)***	236. Metronidazole Cream	237. Metronidazole Extended-Release Tablets
238. Metronidazole Hydrochloride for Injection	239. Metronidazole Lotion	240. Miconazole Nitrate Topical Aerosol
241. Midazolam Injection ***(Received)***	242. Mifepristone Tablets	243. Miglitol Tablets
244. Milrinone Injection	245. Misoprostol Tablets ***(Received)***	246. Moexipril Hydrochloride and Hydrochlorothiazide Tablets
247. Moexipril Hydrochloride Tablets	248. Molindone Hydrochloride Oral Solution	249. Morphine Sulfate for Injection Concentrate
250. Morphine Sulfate Oral Solution	251. Morphine Sulfate Oral Solution Concentrate	252. Morphine Sulfate Tablets
253. Mycophenolate Mofetil Capsules ***(Received)***	254. Mycophenolate Mofetil Oral Solution	255. Mycophenolate Mofetil Tablets ***(Received)***
256. Nalbuphine Hydrochloride Injection	257. Nalmefene Hydrochloride Injection	258. Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution
259. Naproxen Sodium Extended-Release Tablets	260. Nateglinide Tablets ***(Received)***	261. Nedocromil Sodium Inhalation Aerosol
262. Neomycin Sulfate Oral Powder	263. Nicardipine Hydrochloride Capsules	264. Nilutamide Tablets
265. Nimodipine Capsules	266. Nisoldipine Extended-Release Tablets	267. Nitroglycerin Solution In Acrylic Adhesive
268. Nitroglycerin Transdermal System	269. Nizatidine Tablets	270. Ofloxacin in Dextrose Injection
271. Ofloxacin Injection	272. Olsalazine Sodium Capsules	273. Orphenadrine Citrate Extended-Release Tablets ***(Received)***
274. Orphenadrine Citrate, Aspirin, and Caffeine Tablets	275. Oxcarbazepine Suspension	276. Oxiconazole Cream
277. Pamidronate Disodium Injection	278. Pantoprazole Sodium for Injection	279. Pantoprazole Sodium Tablets
280. Paroxetine Hydrochloride Extended-Release Tablets	281. Paroxetine Oral Suspension	282. Pemirolast Potassium Ophthalmic Solution

Small Molecules (Drug Products)—As of December 15, 2008 *(Continued)*

283. Penicillin G Potassium Tablets for Oral Solution	284. Pentamidine Isethionate for Inhalation	285. Pentamidine Isethionate Injection ***(Received)***
286. Pentazocine Hydrochloride and Acetaminophen Tablets	287. Phendimetrazine Tartrate Extended-Release Capsules	288. Phenobarbital Capsules
289. Phentermine Resin Complex Capsules	290. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Capsules	291. Phenylephrine Hydrochloride, Chlorpheniramine Maleate, and Acetaminophen Extended-Release Tablets
292. Pilocarpine Hydrochloride Ophthalmic Gel	293. Pilocarpine Hydrochloride Ophthalmic Ointment	294. Pioglitazone Hydrochloride Tablets ***(Received)***
295. Piperonyl Butoxide and Pyrethrins Aerosol Foam	296. Pirbuterol Acetate Inhalation Aerosol	297. Poractant Alpha Suspension
298. Porfimer Sodium for Injection	299. Povacrylate Solution	300. Povacrylate–Iodine Topical Solution
301. Povidone–Iodine Gauze	302. Povidone–Iodine Swabsticks	303. Povidone–Iodine Topical Aerosol Foam
304. Povidone–Iodine Vaginal Suppositories	305. Pramipexole Dihydrochloride Tablets	306. Prednisolone Sodium Phosphate Oral Solution
307. Prochlorperazine Maleate Extended-Release Capsules	308. Progesterone Capsules	309. Promethazine and Phenylephrine Hydrochlorides and Codeine Phosphate Syrup ***(Received)***
310. Promethazine and Phenylephrine Hydrochlorides Syrup ***(Received)***	311. Promethazine Hydrochloride and Codeine Phosphate Oral Solution ***(Received)***	312. Promethazine Hydrochloride and Dextromethorphan Hydrobromide Syrup ***(Received)***
313. Propafenone Hydrochloride Tablets	314. Pseudoephedrine Hydrochloride and Brompheniramine Maleate Extended-Release Tablets	315. Pseudoephedrine Hydrochloride and Naproxen Sodium Extended-Release Tablets
316. Pseudoephedrine Hydrochloride, Chlorpheniramine Maleate, and Codeine Phosphate Oral Solution	317. Pseudoephedrine Hydrochloride, Guaifenesin, and Codeine Phosphate Oral Solution	318. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Extended-Release Tablets
319. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Oral Solution	320. Pseudoephedrine Sulfate, Dexbrompheniramine Maleate, and Acetaminophen Extended-Release Tablets	321. Pyrilamine Maleate Injection
322. Quinapril Hydrochloride and Hydrochlorothiazide Tablets	323. Quinidine Sulfate Injection	324. Ramipril Capsules ***(Received)***
325. Ranitidine Capsules	326. Rauwolfia Serpentina and Endroflumethiazide Tablets	327. Reserpine and Polythiazide Tablets
328. Rimantadine Hydrochloride Oral Solution	329. Risperidone Oral Solution ***(Received)***	330. Risperidone Orally Disintegrating Tablets
331. Rivastigmine Tartrate Capsules ***(Received)***	332. Rivastigmine Tartrate Oral Solution ***(Received)***	333. Rocuronium Bromide Injection
334. Ropinirole Hydrochloride Tablets	335. Rosiglitazone Maleate Tablets	336. Salicylic Acid and Sulfur Cleansing Lotion
337. Salicylic Acid and Sulfur Lotion	338. Salicylic Acid and Sulfur Shampoo	339. Salicylic Acid Cream
340. Salicylic Acid Ointment	341. Salmeterol Inhalation Aerosol	342. Salmeterol Xinafoate Inhalation Powder
343. Scopolamine Transdermal System	344. Selegiline Hydrochloride Capsules	345. Sertraline Hydrochloride Oral Solution
346. Sibutramine Hydrochloride Capsules	347. Sodium Bicarbonate and Sodium Citrate for Oral Solution	348. Sodium Bicarbonate, Sodium Citrate, and Sodium Tartrate for Oral Suspension
349. Sodium Iodide Injection	350. Sodium Phenylbutyrate Oral Powder	351. Sodium Phenylbutyrate Tablets
352. Sodium Phosphates for Oral Suspension	353. Sodium Phosphates Tablets	354. Sodium Salicylate and Sulfur Shampoo
355. Sterile Talc Aerosol	356. Streptozocin for Injection	357. Sucralfate Oral Suspension
358. Sulconazole Nitrate Cream	359. Sulfacetamide Sodium and Fluorometholone Ophthalmic Suspension	360. Sulfacetamide Sodium and Prednisolone Sodium Phosphate Ophthalmic Solution
361. Sulfasalazine Oral Suspension	362. Sulisobenzone Lotion	363. Sumatriptan Injection
364. Sumatriptan Tablets ***(Received)***	365. Tacrolimus Capsules ***(Received)***	366. Tacrolimus Injection
367. Tacrolimus Ointment	368. Tamsulosin Hydrochloride Capsules ***(Received)***	369. Technetium Tc 99m Teboroxime Injection

Small Molecules (Drug Products)—As of December 15, 2008 *(Continued)*

370. Tenofovir Disoproxil Fumarate Tablets ***(Received)***	371. Terazosin Capsules ***(Received)***	372. Terazosin Tablets ***(Received)***
373. Terbinafine Hydrochloride Cream	374. Terbinafine Tablets ***(Received)***	375. Terbinafine Topical Solution
376. Terconazole Vaginal Cream	377. Terconazole Vaginal Suppositories	378. Testosterone Transdermal Gel
379. Testosterone Transdermal System	380. Tetracycline Hydrochloride Periodontal Fiber	381. Theophylline Extended-Release Tablets
382. Tioconazole Vaginal Ointment	383. Tiopronin Tablets	384. Tolnaftate Topical Aerosol Solution
385. Topiramate Capsules ***(Received)***	386. Topiramate Tablets ***(Received)***	387. Torsemide Injection
388. Torsemide Tablets ***(Received)***	389. Trandolapril and Verapamil Hydrochloride Extended-Release Tablets	390. Trandolapril Tablets
391. Tranexamic Acid Injection	392. Tranylcypromine Sulfate Tablets ***(Received)***	393. Tretinoin Capsules
394. Tretinoin Microsphere Gel	395. Triamcinolone Acetonide Nasal Suspension	396. Trifluridine Ophthalmic Solution
397. Trimetrexate for Injection	398. Trimipramine Maleate Capsules	399. Triprolidine and Pseudoephedrine Hydrochlorides and Codeine Phosphate Syrup
400. Trolamine Salicylate Cream	401. Trolamine Salicylate Gel	402. Trolamine Salicylate Topical Emulsion
403. Undecylenic Acid Topical Foam Aerosol	404. Urea Cream	405. Vecuronium Bromide for Injection
406. Venlafaxine Extended-Release Capsules ***(Received)***	407. Venlafaxine Tablets ***(Received)***	408. Verapamil Hydrochloride Capsules
409. Verapamil Hydrochloride Extended-Release Capsules	410. Voriconazole Injection	411. Voriconazole Oral Suspension
412. Voriconazole Tablets	413. Yttrium Y-90 Chloride Solution	414. Yttrium Y-90 Glass Microspheres
415. Yttrium Y-90 Microspheres Injection	416. Zaleplon Capsules	417. Zidovudine and Lamivudine Tablets ***(Received)***
418. Zinc Acetate Capsules	419. Zinc Tridosium Pentetate Injection	420. Ziprasidone Hydrochloride Capsules
421. Zoledronic Acid for Injection	422. Zonisamide Capsules ***(Received)***	

Excipients—As of December 15, 2008

1. Acetone Sodium Bisulfite	2. Acetylated Monoglycerides	3. Aconitic Acid (Achilleic Acid)
4. Acrylic Acid–Octyl Acrylate Copolymer	5. Albumin Colloidal	6. Aliphatic Polyesters
7. Allantoin–Sodium Pyrrolidone Carboxylate	8. Aluminum Ammonium Sulfate	9. Aluminum Lactate
10. Aluminum Oxide	11. Aluminum Potassium Sulfate	12. Aluminum Silicate
13. Aluminum Sodium Sulfate	14. Aluminum Stearate	15. Ammonium Bicarbonate
16. Ammonium Calcium Alginate	17. Ammonium Phosphate	18. Batylalcohol Monostearate
19. Beeswax, Synthetic	20. Benzododecinium Bromide	21. Benzyl Chloride
22. Benzyl Nicotinate	23. Beta Naphthol	24. Brominated Vegetable Oil
25. Butadiene–Styrene Rubber	26. Butyl Stearate ***(Received)***	27. Butylated Hydromethylphenol
28. Butylene Glycol	29. Butylphthalyl Butylglycolate	30. Calcium Acid Pyrophosphate
31. Calcium Alginate	32. Calcium Alginate and Ammonium Alginate	33. Calcium Bromide
34. Calcium Chloride Solution	35. Calcium Phosphate Monobasic	36. Calcium Propionate
37. Calcium Pyrophosphate	38. Calcium Sorbate	39. Calcium Stearoyl Lactylate
40. Caldiamide Sodium	41. Calteridol Calcium	42. Capric Acid
43. Caprylic/Capric Diglyceryl Succinate	44. Carbon	45. Carboxymethyl Starch
46. Carboxymethylamylopectin Sodium	47. Carboxymethylcellulose Potassium	48. Cetostearyl Isononanoate
49. Chlorodifluoroethane	50. Cholic Acid	51. Cinnamaldehyde
52. Cocamide Diethanolamine	53. Cocamide Oxide	54. Cocoyl Caprylocaprate
55. Crystal Gum	56. Cutina	57. Cystine
58. Dammar Gum	59. Decanoic Acid	60. Decyl Oleate

Excipients—As of December 15, 2008 *(Continued)*

61. Dextrin Palmitate	62. Dextrins Modified	63. Diacetyl Tartaric Acid Esters of Mono- and Digoycerides
64. Dicetyl Phosphate	65. Dichlorofluoromethane	66. Diethyl Sebacate ***(Received)***
67. Difluoroethane	68. Diglycol Stearate	69. Diisobutyl Adipate
70. Diisopropyl Adipate	71. Diisopropylbenzothiazyl-2-Sulfenamide	72. Dilauryl Thiodipropionate
73. Dimethyl Dicarbonate	74. Dimyristoyl Lecithin	75. Dimyristoyl Phosphatidylglycerol
76. Dipropylene Glycol	77. Disodium Edisylate	78. Disodium Guanylate
79. Disodium Inosinate	80. Disodium Monooleamide Sulfasuccinate	81. D-Mannose
82. Docusate Sodium/Sodium Benzoate	83. Erythrosine	84. Ethoxylated Mono- and Diglycerides
85. Ethoxyquin	86. Ethyl Hexanediol	87. Ethyl Linoleate
88. Ethyl Maltol ***(Received)***	89. Ethylene Dichloride	90. Ethylurea
91. Ferric Ammonium Citrate	92. Ferric Citrate	93. Ferric Oxide, Brown
94. Ferric Phosphate	95. Ferric Pyrophosphate	96. Ferrous Citrate
97. Ferrous Glycinate	98. Ferrous Lactate	99. Fluorochlorohydrocarbons
100. Formic Acid	101. Furcelleran	102. Gentistic Acid
103. Geraniol	104. Glutamic Acid Hydrochloride	105. Gluten
106. Glycerol Ester of Gum Rosin (Ester Gum)	107. Glyceryl Laurate	108. Glyceryl Palmitate
109. Glyceryl Ricinoleate	110. Glyceryl Tristearate	111. Glycine Hydrochloride
112. Glycofurol	113. Glycol Stearate	114. Heptafluoropropane
115. Heptylparaben	116. Hexadecyl Isostearate	117. Hexane
118. Hexanetriol(-1,2,6-)	119. Hydrocarbon Gel	120. Hydroxyethylmethylcellulose
121. Hydroxylated Lecithin	122. Indigotine	123. Iron Carbonyl
124. Iron Subcarbonate	125. Isobutylated-Isoprene Copolymer	126. Isooctylacrylate
127. Isopropyl Isostearate	128. Isopropyl Stearate	129. Isostearic Acid
130. Isostearyl Alcohol	131. Lactobionic Acid ***(Received)***	132. Lactose Ferrin, Bovine
133. Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol	134. Lactylic Esters of Fatty Acids	135. Lanolin (Wool Fat), Hydrogenated
136. Lanolin Alcohols, Acetylated	137. Lanolin Hydrous	138. L-Ascorbyl Stearate
139. Lauramine Oxide	140. Lauric Myristic Diethanolamide	141. Lauric Acid
142. Lauric Diethanolamide	143. Lavender Oil	144. L-Cysteine Monohydrochloride
145. Lecithin, Hydroxylated	146. L-Glutamic Acid ***(Received)***	147. Linoleic Acid ***(Received)***
148. L-Leucine	149. Macrogol Sorbitan Tristearate	150. Macrogolglycerol Cocoates
151. Macrogolglycerol Triisostearate	152. Magnesium Aluminum Silicate Hydrate	153. Magnesium Aspartame Dihydrate
154. Magnesium Aspartate	155. Magnesium Phosphate Tribasic	156. Magnesium Phosphate, Diabasic, Trihydrate
157. Magnesium Tartrate	158. Malt Syrup	159. Maltitol Syrup
160. Maltol Isobutyrate	161. Manganese Chloride	162. Manganese Citrate
163. Manganese Glycerophosphate	164. Manganese Hypophosphite	165. Medical Antifoam Emulsion C
166. Medronate Disodium	167. Medronic Acid	168. Methyl Chloride
169. Methylchloroisothiazolinone	170. Methylisothiazolinone	171. Microcrystalline Cellulose, Silicified ***(Received)***
172. Mineral Spirits	173. Monoisostearyl Glyceryl Ester	174. Monopotassium Glutamate Monohydrate
175. Monosodium Citrate	176. Mullein Leaf	177. Myristyl Gamma-Picolinium Chloride
178. Myristyl Lactate	179. *N,N*-Bis(2-Hydroxyethyl)Stearamide	180. *N*-Acetyl-L- Methionine
181. Naphtha	182. *N*-Methylpyrrolidone ***(Received)***	183. Non-Pareil Seeds
184. Nutmeg Oil	185. Octanoic Acid	186. Oxystearin
187. Pentasodium Triphosphate	188. Pentetate Calcium Trisodium	189. Pentetate Pentasodium
190. Phenprobamate	191. Phenylmercuric Acetate	192. Phenylmercuric Nitrate
193. Pine Oil	194. Polacrilin	195. Polyglycerol Esters of Fatty Acids

Excipients—As of December 15, 2008 *(Continued)*

196. Polyglycerol Polyricinoleic Acid	197. Polyoxyethylene Castor Oil (USP has 35)	198. Polyoxyl Stearate (USP has 40)
199. Polypropylene Oleate	200. Polypropylene Stearyl Ether	201. Polysorbate 65
202. Polyvinylacetal Diethylanoacetate	203. Polyvinylpolypyrrolidone	204. Polyvinylpyrrolidone Ethylcellulose
205. Potassium Acid Tartrate	206. Potassium Bromate	207. Potassium Carbonate Solution
208. Potassium Dichloroisocyanurate	209. Potassium Gibberellate	210. Potassium Glycerophospate
211. Potassium Iodate	212. Potassium Nitrite	213. Potassium Phosphate
214. Potassium Phosphate Tribasic	215. Potassium Polymetaphosphate	216. Potassium Pyrophosphate
217. Potassium Stearate	218. Potassium Sulfate	219. Potassium Sulfite
220. Potassium Tripolyphosphate	221. Propyl Propionate	222. Propylene Glycol Diacetate
223. Propylene Glycol Mono- and Diesters	224. Rice Bran Wax	225. Rosin
226. Silicone	227. Sodium Acid Pyrophosphate	228. Sodium Aluminosilicate ***(Received)***
229. Sodium Aluminum Phosphate Acidic	230. Sodium Aluminum Phosphate Basic	231. Sodium Aspartate
232. Sodium Bisulfate	233. Sodium Bisulfite	234. Sodium Carbonate Hydrate
235. Sodium Carboxymethyl Betaglucan	236. Sodium Caseinate	237. Sodium Chlorate
238. Sodium Citrate, Dibasic	239. Sodium Citrate, Monobasic	240. Sodium Dehydroacetate
241. Sodium Diacetate	242. Sodium Erythorbate	243. Sodium Ferric Pyrophosphate
244. Sodium Ferrocyanide	245. Sodium Hypophosphite ***(Received)***	246. Sodium Laureth Sulfate
247. Sodium Lauroyl Sarcosinate	248. Sodium Lauryl Sulfoacetate	249. Sodium Magnesium Aluminosilicate
250. Sodium Magnesium Silicate	251. Sodium Malate	252. Sodium Metaphosphate, Insoluble
253. Sodium Metasilicate	254. Sodium Methylate	255. Sodium Polyphosphates Glassy
256. Sodium Potassium Tripolyphosphate	257. Sodium Pyrophosphate	258. Sodium Pyrrolidone Carboxylate
259. Sodium Sesquicarbonate	260. Sodium Sesquinoleate	261. Sodium Stearoyl Lactylate
262. Sodium Thiomalate	263. Sodium Trimetaphosphate	264. Sodium Trioleate
265. Sodium Tripolyphosphate	266. Soy Polysaccharides	267. Stannous Tartrate
268. Starch, Pregelatinized Corn	269. Starch, Pregelatinized Tapioca	270. Stearalkonium Chloride
271. Stearyl Citrate	272. Stearyl Monoglyceridyl Citrate	273. Succinylated Monoglycerides
274. Sucrose Acetate Isobutyrate	275. Sucrose Fatty Acid Esters	276. Sucrose Stearate ***(Received)***
277. Sugar Fruit Fine	278. Sulfobutyl Ether Beta Cyclodextrin ***(Received)***	279. Tallow
280. Tallow Glycerides	281. Tallow Oil	282. Tetrafluoroethane
283. Thioglycerol	284. Thyme Oil	285. Tribehenin
286. Tricetearet-4 Phosphate	287. Trichloroethylene	288. Trimyristin
289. Trisodium Citrate	290. Trolamine Lauryl Sulfate	291. Vegetable Oil
292. Wheat Flour	293. Wheat Germ Oil	294. Wheat Gluten ***(Received)***
295. Whey		

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:
- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become effective before the effective date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The effective date for these revisions is stated on the next page.)

Readers should review this section to determine if they are affected by any of the changes.

Symbols—Text proposed for deletion or replacement is indicated by a strikethrough the affected text. New text (if any) follows, and is enclosed in symbols and set off from the current official text by a paragraph break and by larger type (print edition only), as shown in the examples below:

— •new text• if slated for an *Interim Revision Announcement;*
— ▲new text▲ if slated for *USP 33–NF 28*; and
— ■new text■ if slated for a *Supplement* to *USP–NF*

Recent revisions that are already official are indicated by the same symbols but do not have the extra paragraph break and there is no increase in type size on the enclosing text. Where the symbols appear together with no enclosed text, such as •• or ■■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (USP32) indicates that the proposed revision is slated for the *Second Supplement* to *USP 32,* and ▲USP33 and ▲NF28 indicate that the revisions are proposed for *USP 33* and *NF 28*, respectively.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements,* that does not accurately reflect the intended official requirements of the Council of Experts. Beginning with *PF* 35(2), Errata will be published both in the *Pharmacopeial Forum* and on the usp.org website. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to *USP 31–NF 26*. The page number indicates where the item is found in *USP–NF.* Errata are updated as necessary in each *Pharmacopeial Forum* issue and monthly on the usp.org website. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF.* The list of Errata has been relocated to www.usp.org, where updates will be posted monthly.

INTERIM REVISION ANNOUNCEMENT to *USP 31* and to *NF 26*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

John W. Mauger, *Chair*
USP Board of Trustees

Roger L. Williams, M.D., *Executive Vice President, CEO,* and *Chairman, USP Council of Experts*

Darrell R. Abernethy, M.D., Ph.D., *Chief Science Officer*
William F. Koch, Ph.D., FACB, *Chief Metrology Officer*

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All inquiries and comments regarding *USP 31* text and *NF 26* text should be addressed to the Executive Secretariat, *USP–NF*, 12601 Twinbrook Parkway, Rockville, MD 20852 (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 31* or *NF 26* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard.

USP 23-Epi-26-deoxyactein RS (January 1, 2009)
USP Actein RS (January 1, 2009)
USP Mibolerone RS (November 1, 2008)
USP Narasin RS (November 1, 2008)
USP Powdered St. John's Wort Extract RS (November 1, 2008)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 31* or *NF 26* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of December 1, 2008. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed online at http://www.uspcatalog.com.

USP *S*-Adenosyl-L-homocysteine RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Fludeoxyglucose Related Compound B RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Alpha Lipoic Acid RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Propylene Glycol Dilaurate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin RS
USP Valrubicin Related Compound A RS
USP Vasopressin RS

MONOGRAPHS (USP)

Galantamine Tablets

Change to read:

Related compounds—

Buffer solution, Solution A, Solution B, Mobile phase, and *Diluent*—Prepare as directed in the *Assay.*

Resolution solution—Prepare a solution of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent* having a concentration of 0.6 mg per mL.

Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph about 20 µL of the *Resolution solution,* and record the responses as directed for *Procedure*. Identify the impurities using the approximate relative retention times given in *Table 1:* the resolution, *R,* between 6β-hexahydrogalantamine and 6β-octahydrogalantamine is not less than 1.5. Chromatograph the *Standard solution,* and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for the galantamine peak.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. [NOTE—Ignore the peak due to bromide near the void volume.] Calculate the percentage of each of the galantamine related compounds in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)(1/F)$$

in which C_S and C_U are the concentrations, in mg per mL, of galantamine in the *Standard solution* and *Test solution,* respectively; r_U is the peak area of each impurity obtained from the *Test solution;* r_S is the peak area of galantamine obtained from the *Standard solution;* and *F* is the *Relative Response Factor* (see *Table 1* for values) for each of the impurities relative to galantamine.

•Table 1

Compound Name	Relative Retention Time (RRT)	Relative Response Factor (*F*)	Limit (%)
N-Desmethylgalantamine[1]	0.41	1.0	0.5
O-Desmethylgalantamine[2]	0.56	1.0	0.5
6β-Hexahydrogalantamime (also known as galantamine *N*-oxide)[3]	0.73	1.1	0.75
6β-Octahydrogalantamine (also known as lycoramine)†[4]	0.86	—	—
Galantamine hydrobromide	1.00	1.0	—
6α-Hexahydrogalantamine (also known as epigalantamine)[5]	1.15	1.0	0.5
Tetrahydrogalantamine†[6]	2.09	—	—
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	1.5

[NOTE—The impurities marked with "†" are not quantified and are intended for system suitability evaluation only.]

[1] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

[2] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-3,6-diol.

[3] [4a*S*-(4aα,6β,8a*R**)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.

[4] [4a*S*-(4aα,6β,8a*R**)]-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

[5] [4a*S*-(4aα,6α,8a*R**)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

[6] [4a*S*-(4a*R**,8a*R**)]-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-4a*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.•$_2$

Interim Revision Announcement

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

USP32–NF27 **Page**	**Title**	**Section**	**Description**
188	⟨561⟩ *Articles of Botanical Origin*	*Test for Pesticides*	Line 7 under *Extraction*: Change ''containing 1.8 g of carbophenothion'' to: containing 1.8 μg of carbophenothion
858	*Reagent Specifications*	*Water, Ammonia-Free, H_2O*	Line 2: Change ''*Chemical Resistance—Glass Containers* under *Containers* ⟨661⟩.'' to: *Chemical Resistance* under *Containers—Glass* ⟨660⟩.
983	*Red Clover*	*Content of isoflavones*	Line 10 under *Procedure:* Change ''50 $F(C/W)$ (r_U/r_S)'' to: $50(1/F)(C/W)(r_U/r_S)$
984	*Powdered Red Clover Extract*	*Content of isoflavones*	Line 4 under *Procedure:* Change ''25 $F(C/W)$ (r_U/r_S)'' to: $25(1/F)(C/W)(r_U/r_S)$
985	*Red Clover Tablets*	*Content of isoflavones*	Line 4 under *Procedure*: Change ''$250FC(r_U/r_S)$'' to: $250(1/F)C(r_U/r_S)$
1104	*Oil- and Water-Soluble Vitamins with Minerals Tablets*	*Assay for cyanocobalamine*	In *Method 2*, line 1 under *Asparagine solution*: Change ''Dissolve 2.0 of L- asparagine'' to: Dissolve 2.0 g of L-asparagine
1723	*Bupropion Hydrochloride Extended-Release Tablets*	*Assay*	Line 2 under *Chromatographic system*: Change ''a 4.6-mm × 10-cm column that contains 3.5-μm packing L1.'' to: a 4.6-mm × 10-cm column that contains 3.5-μm packing L1 and is maintained at 40°.
1830	*Cefdinir for Oral Suspension*	*Uniformity of dosage units* ⟨905⟩	Line 1: Change ''For Oral Suspension packaged'' to: For solid packaged
1830	*Cefdinir for Oral Suspension*	*Deliverable volume* ⟨698⟩	Line 1: Change ''For Oral Suspension packaged'' to: For solid packaged
1935	*Cilostazol Tablets*	*Assay*	Line 6 under *Procedure*: Change the formula ''$100(C_S/C_U)(r_U/r_S)$'' to: $100(C_S/C_U)(R_U/R_S)$ Line 10 under *Procedure*: Change ''and r_U and r_S are the peak responses of cilostazol obtained'' to: and R_U and R_S are the peak area ratios of cilostazol to the internal standard obtained
2172	*Dinoprost Tromethamine Injection*	*Assay*	Line 2 under *Internal standard solution*: Change ''about 0.75 ng of guaifenesin per mL.'' to: about 0.75 mg of guaifenesin per mL.
3834	*Valganciclovir Hydrochloride*	*Diastereomer ratio*	Change the formulas ''$100[r_A(r_A + r_B)]$'' and ''$100[r_B(r_A + r_B)]$'' to: $100[r_A/(r_A + r_B)]$ and $100[r_B/(r_A + r_B)]$
3871	*Sterile Water for Injection*	*Ammonia* and *Chloride*	Line 3 under *Ammonia*, and line 5 under *Chloride*: Change ''under *Containers* ⟨661⟩'' to: under *Containers—Glass* ⟨660⟩

Interim Revision Announcement

PROPOSED INTERIM REVISION ANNOUNCEMENTS

This section includes proposals for *Interim Revision Announcements* (*IRAs*) that will be published as official *USP* or *NF* standards. There is a 60-day comment period for these proposals, beginning on the 15th of the first month of this *Pharmacopeial Forum*. The approved official text will be published in *Pharmacopeial Forum* 35(4) and additionally in the ''New Official Text'' section of USP's web site (www.usp.org). Readers should review material in this section and provide comments to the Scientific Liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status.

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

MONOGRAPHS (USP)

BRIEFING

Heparin Sodium, *USP 31* page 2321 and the *Revision Bulletin* posted on the USP website on June 18, 2008. Because of the suspected serious adverse events associated with the contamination of heparin with oversulfated chondroitin sulfate, USP has revised the USP Heparin Sodium monograph. On the basis of comments received on the published and new methods submitted by the industry, it is proposed to make the following changes:

1. Additional requirements are added to cover overall resonance distribution of the 1H NMR *Identification* test. USP Heparin Sodium System Suitability RS has been removed.
2. The capillary electrophoresis *Identification* test is replaced with a strong ion-exchange HPLC method. The liquid chromatographic procedures are based on analyses performed with the Dionex brand of L61 column, IonPac AS11. Typical retention times are about 20 min for dermatan sulfate, 30 min for heparin, and 50 min for oversulfated chondroitin sulfate.
3. Another *Identification* test is added. The ratio of anti-factor Xa activity against anti-factor IIa potency is NLT 0.9 and NMT 1.1.
4. An impurity limit test to measure percent galactosamine in total hexosamine is added. The intent of this assay is to limit dermatan sulfate and other galactosamine containing impurities in heparin. The liquid chromatographic procedures are based on analyses performed with the Dionex CarboPac PA-20.
5. The sheep plasma-based clotting assay has been replaced with a chromogenic anti-factor IIa assay. USP Heparin potency units will be harmonized with International Units as of August 2009.
6. An impurity test to measure nucleotidic impurities is added.
7. A quantitative test to measure residual proteins is added.
8. A requirement to comply with *Residual Solvents* ⟨467⟩ is added.

(BB BBP: A. Szajek.) RTS—C69239

Change to read:

Heparin Sodium

~~» Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. •The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process must be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* ⟨1050⟩ for general guidance on viral safety evaluation.• (RB 2008 06 18)~~ It is purified to retain a combination of activities against different fractions of the blood clotting sequence. It is composed of polymers of alternating derivatives of •α• (RB 2008 06 18) D-glucosamine (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and uronic acid (•α• (RB 2008 06 18) L-iduronic acid or •β• (RB 2008 06 18) D-glucuronic acid) joined by glycosidic linkages. The component activities of the mixture are in ratios corresponding to those shown by the USP Heparin Sodium Reference Standard. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin III and heparin cofactor II to potentiate the inactivation of thrombin. Other coagulation proteases in the clotting sequence, such as activated factor X, are also inhibited. The potency of Heparin Sodium, calculated on the dried basis, is not less than 140 USP Heparin Units in each mg.

~~NOTE—The USP Heparin Unit is defined by the USP Heparin Sodium Reference Standard and can be independent of International Units. The respective units are not equivalent (see *General Notices*). The Unit for Anti-Factor X_a activity is defined by the USP Heparin Sodium Reference Standard and is equivalent in potency to that Standard.~~

~~**Packaging and storage**—Preserve in tight containers, and store below 40°, preferably at room temperature.~~

~~**Labeling**—Label it to indicate the tissue and the animal species from which it is derived.~~

~~**USP Reference standards**—⟨11⟩—*USP Endotoxin RS*. *USP Heparin Sodium RS*. •*USP Heparin Sodium System Suitability RS*. *USP Heparin Sodium Identification RS*.• (RB 2008 06 18)~~

~~**Identification—**~~

~~•**A:** It meets the requirements under the *Assay*.~~

~~**B:** *^{1}H NMR spectrum* (see *Nuclear Magnetic Resonance* ⟨761⟩)—~~

~~*Standard solution*—Prepare a solution of USP Heparin Sodium Identification RS at not less than 12 mg per mL in deuterium oxide.~~

~~*System suitability solution*—Prepare a solution of USP Heparin Sodium System Suitability RS at not less than 12 mg per mL in deuterium oxide.~~

~~*Test solution*—Prepare a solution containing not less than 12 mg of Heparin Sodium per mL in deuterium oxide.~~

~~*Procedure*—Using a pulsed (Fourier transform) NMR spectrometer operating at not less than 500 MHz for ^{1}H, acquire a free induction decay (FID). Record the ^{1}H NMR spectra of the *Standard solution* and the *System suitability solution* at 25°: the number of transients is adjusted until the signal to noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 200/1 in the region near 2 ppm. The *Standard solution* must be run at least daily when *Test solutions* are being run. For the *Standard solution*, the chemical shift corresponding to *N*-acetyl protons of heparin must be set at 2.04 ppm. The chemical shifts for heparin and over sulfated chondroitin sulfate in the system suitability standard is observed at 2.04 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the ^{1}H NMR spectrum of the *Test solution* at 25°. The *N*-acetyl protons of heparin must show a major signal at 2.04 ± 0.02 ppm. A signal, corresponding to *N*-acetyl protons of dermatan sulfate, may show near 2.08 ppm. •Observation of a resonance at~~

Proposed IRA

2.16 ± 0.03 ppm would indicate the presence of over sulfated chondroitin sulfate. Over sulfated chondroitin sulfate has no other resonances between 2.12 and 3.00 ppm.• (RB 2008 06 18)

C: For heparin sodium of porcine origin (see *Biotechnology Derived Articles—Capillary Electrophoresis* ⟨1053⟩)

Standard solution—Prepare a solution of USP Heparin Sodium Identification RS in water having a concentration of 2 mg per mL.

System suitability solution—Prepare a solution of USP Heparin Sodium System Suitability RS in water having a concentration of 2 mg per mL.

Test solution—Reconstitute an accurately weighed quantity of Heparin Sodium in water to obtain a solution that is 2 mg per mL. Filter the solution if necessary.

Capillary electrophoresis buffer—Transfer 1.0 g of monobasic sodium phosphate monohydrate to a beaker, and add 195 mL of water. Adjust with phosphoric acid to a pH of 3.5. Transfer the solution into a 200-mL volumetric flask, and dilute with water to volume. Filter the buffer with a 0.2-µm membrane filter. Degas the buffer before use if necessary.

Electrophoretic system—•[NOTE—Based on instrument requirements, the field applied across the capillary and the conditions for the sample injection may be varied to achieve system suitability.]• (RB 2008 06 18)—The capillary electropherograph is equipped with a 200-nm detector and a 60- to 65-cm (50- to 56-cm effective length) uncoated fused silica capillary, with an internal diameter of 50 µm, with the temperature controlled at 25°. Apply a field strength of 465.1 V per cm for 15 minutes, using the *Capillary electrophoresis buffer* as the electrolyte in both buffer reservoirs. Electropherograph the USP Heparin Sodium System Suitability RS, using a 30-sec injection at 0.7 psi, and record the peak responses as directed for *Procedure:* the migration time for heparin sodium and over-sulfated chondroitin sulfate differs by not less than 4.0% of the heparin migration time, with over sulfated chondroitin sulfate always migrating faster than heparin sodium. The baseline is stable. Rinse the capillary with 0.1 M phosphoric acid for at least 0.5 minutes at 40 psi, followed by water for at least 2 minutes at 40 psi, and then by the *Capillary electrophoresis buffer* for at least 2 minutes at 40 psi between injections.

Procedure—Electropherograph the *Standard solution* and the *Test solution* using a 30-sec injection at 0.7 psi into the anodic end of the capillary, and record the electropherograms. The electropherogram of the *System suitability solution* is comparable to a typical electropherogram provided with the USP Heparin Sodium System Suitability RS. The electropherogram of the *Test solution* is similar to that of the *Standard solution,* and does not exhibit a sharp distinguishable peak that is less than one minute in front of the main heparin peak.

D:• (RB 2008 06 18) It meets the requirements of the flame test for *Sodium* ⟨191⟩.

Bacterial endotoxins ⟨85⟩—It contains not more than 0.03 USP Endotoxin Unit per USP Heparin Unit.

Sterility ⟨71⟩ (where it is labeled as sterile): meets the requirements.

pH ⟨791⟩: between 5.0 and 7.5, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.

Residue on ignition ⟨281⟩: between 28.0% and 41.0%.

Protein—To 1 mL of a solution (1 in 100) add 5 drops of trichloroacetic acid solution (1 in 5): no precipitate or turbidity forms.

Heavy metals, *Method II* ⟨231⟩: 0.003%.

Anti-factor X_a activity—

pH 8.4 Buffer—Dissolve amounts of tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% of polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust, if necessary, with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Antithrombin III solution—Reconstitute an accurately weighed quantity of antithrombin III (see *Reagent Specifications* under *Reagents, Indicators, and Solutions*) in *pH 8.4 Buffer* to obtain a solution having a concentration of 1.0 Antithrombin III Unit per mL.

Factor X_a solution—Reconstitute an accurately weighed quantity of bovine factor X_a (see Factor X_a in *Reagent Specifications* under *Reagents, Indicators, and Solutions*) in *pH 8.4 Buffer* to obtain a solution that gives an absorbance value between 0.65 and 1.25 at 405 nm when assayed as described below but using 30 µL of *pH 8.4 Buffer* instead of 30 µL of the *Standard solutions* or the *Test solutions*.

NOTE—*Factor X_a solution* contains about 3 nanokatalytic units per mL, but can vary depending upon the manufacturer of factor X_a or the substrate used.

Chromogenic substrate solution—Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagent Specifications* under *Reagents, Indicators, and Solutions*) specific for factor X_a in water to obtain a concentration of about 1 mM.

Stopping solution—Prepare a 20% (v/v) solution of acetic acid in water.

Standard solutions—Dilute an accurately measured volume of USP Heparin Sodium RS with *pH 8.4 Buffer* to obtain at least 5 (out of 7 below) solutions having known activities of about 0.375, 0.3125, 0.25, 0.188, 0.125, 0.0625, and 0.0313 USP Heparin Unit per mL.

Test solutions—Dissolve or dilute an accurately measured quantity of Heparin Sodium in *pH 8.4 Buffer,* and dilute with the same buffer to obtain solutions having activities approximately equal to those of the *Standard solutions*.

Procedure—[NOTE—Perform the test with each *Standard solution* and *Test solution* in duplicate.] To each of a series of suitable plastic tubes placed in a water bath set at 37°, transfer 120 µL of *pH 8.4 Buffer.* Then separately transfer 30 µL of the different dilutions of the *Standard solutions* or the *Test solutions* to the tubes. Add 150 µL of *Antithrombin III solution,* prewarmed at 37° for 15 minutes, to each tube, mix, and incubate for 2 minutes. Add 300 µL of *Factor X_a solution,* prewarmed at 37° for 15 minutes, to each tube, mix, and incubate for 2 minutes. Add 300 µL of *Chromogenic substrate solution,* prewarmed at 37° for 15 minutes, to each tube, mix, and incubate for exactly 2 minutes. Add 150 µL of *Stopping solution* to each tube, and mix. Prepare a blank for zeroing the spectrophotometer by adding the reagents in reverse order, starting with the *Stopping solution* and ending with the addition of 150 µL of *pH 8.4 Buffer,* and excluding the *Standard solutions* or the *Test solutions*. Record the absorbance at 405 nm against the blank.

Calculations—Plot the log of the absorbance values of the *Standard solutions* and the *Test solutions* versus heparin concentrations in USP Units. Construct separate straight lines of best fit using least-squares linear regression analyses for the *Standard solutions* and the *Test solutions,* and determine the slope for each regression line. Calculate the potency of Heparin Sodium by the formula:

$$P(S_u / S_s)$$

in which P is the potency of USP Heparin Sodium RS; and S_u and S_s are the slopes of the lines from the *Test solutions* and the *Standard solutions,* respectively. Express the Anti-factor X_a potency of the *Test solution* as a percentage of the heparin concentration determined in the *Assay*. Calculate the percentage of anti-factor X_a activity against activity by the formula:

100(anti-factor X_a potency / anticoagulant potency).

Not less than 80% and not more than 120% is found.

Nitrogen content, *Method I* ⟨461⟩: between 1.3% and 2.5%, calculated on the dried basis, the procedure for *Nitrates and Nitrates Absent* being used.

Assay—

Standard preparation—Determine by preliminary trial, if necessary, approximately the minimum quantity of USP Heparin Sodium RS which, when added in 0.8 mL of saline TS, maintains fluidity in 1 mL of prepared plasma for 1 hour after the addition of 0.2 mL of calcium chloride solution (1 in

100). This quantity is usually between 1 and 3 USP Heparin Units. On the day of the assay prepare a *Standard preparation* such that it contains, in each 0.8 mL of saline TS, the above-determined quantity of the Reference Standard.

Assay preparation— Dissolve about 25 mg of Heparin Sodium, accurately weighed, in sufficient saline TS to give a concentration of 1 mg per mL, and dilute quantitatively to a concentration estimated to correspond to that of the *Standard preparation.*

Preparation of plasma— Collect blood from sheep directly into a vessel containing 8% sodium citrate solution in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly centrifuge the blood, and pool the separated plasma. To a 1 mL portion of the pooled plasma in a clean test tube add 0.2 mL of calcium chloride solution (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 mL in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.

Procedure— To meticulously clean 13 mm × 100 mm test tubes add graded amounts of the *Standard preparation,* selecting the amounts so that the largest does not exceed 0.8 mL and so that they correspond roughly to a geometric series in which each step is approximately 5% greater than the next lower. To each tube so prepared add sufficient saline TS to make the total volume 0.8 mL. Add 1.0 mL of prepared plasma to each tube. Then add 0.2 mL of calcium chloride solution (1 in 100), note the time, immediately insert a suitable stopper in each tube, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the *Assay preparation,* completing the entire process of preparing and mixing the tubes of both the *Standard preparation* and the *Assay preparation* within 20 minutes after the addition of the prepared plasma. One hour, accurately timed, after the addition of the calcium chloride, determine the extent of clotting in each tube, recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). If the series does not contain 2 tubes graded more than 0.5 and 2 tubes graded less than 0.5, repeat the assay, using appropriately modified *Standard preparation* and *Assay preparation.*

Calculation— Convert to logarithms the volumes of *Standard preparation* used in the successive 5 or 6 tubes that bracket a grade of clotting of 0.5, including at least 2 tubes with a larger and 2 tubes with a smaller grade than 0.5. Number and list the tubes serially, and tabulate for each the grade of clotting observed in each tube. From the log volumes, x_i, and separately from their corresponding grades of clotting, y_i, compute the paired averages x_i and y_i of Tubes 1, 2, and 3, of Tubes 2, 3, and 4, of Tubes 3, 4, and 5, and, where the series consists of 6 tubes, of Tubes 4, 5, and 6, respectively. If for one of these paired averages the average grade, y_u, is exactly 0.50, the corresponding x_i is the median log volume of the *Standard preparation,* x_s. Otherwise, interpolate x_s from the paired values of y_u, x_i, and y_{i-1}, x_{i-1}, that fall immediately below and above grade 0.5 as

$$x_s = x_i + (y_i - 0.5)(x_{i-1} - x_i) / (y_i - y_{i-1}).$$

From the paired data on the tubes of the *Assay preparation,* compute similarly its median log volume x_u.

The log potency of the *Assay preparation* is

$$M = x_s - x_u + \log R,$$

where $R = v_s / v_u$ is the ratio of the USP Heparin Units (v_s) per mL of the *Standard preparation* to the mg (v_u) of Heparin Sodium per mL of the *Assay preparation.*

Repeat the assay independently, and average the two or more values of M to obtain $\overline{M}$. If the second determination of M differs by more than 0.05 from the first determination, continue the assay until the log confidence interval computed as directed under *Confidence Intervals for Individual Assays* in *Design and Analysis of Biological Assays* ⟨111⟩ does not exceed 0.20. The potency of Heparin Sodium in USP Heparin Units per mg is P^u = antilog $\overline{M}$.

•DEFINITION

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process should be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin* ⟨1050⟩ for general guidance on viral safety evaluation. It is composed of polymers of alternating derivatives of α-D-glucosamido (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and *O*-sulfated uronic acid (α-L-iduronic acid or β-D-glucuronic acid). The component activities of the mixture are in ratios corresponding to those shown by the USP Heparin Sodium RS. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin and heparin cofactor II to potentiate the inactivation of thrombin (factor IIa). Other coagulation proteases in the clotting sequence, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity to anti-factor IIa potency is between 0.9 and 1.1. The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

IDENTIFICATION

A. ^{1}H NMR Spectrum

(See *Nuclear Magnetic Resonance* ⟨761⟩.)

Standard solution A: NLT 20 mg/mL of USP Heparin Sodium Identification RS in deuterium oxide with 0.02% (w/v) deuterated trimethylsilylpropionic (TSP) acid sodium salt

Standard solution B: 2 mg/mL of USP Oversulfated Chondroitin Sulfate (OSCS) RS in deuterium oxide

System suitability solution: 1% (v/v) of *Standard solution B* diluted with *Standard solution A*

Sample solution: NLT 20 mg/mL of Heparin Sodium in deuterium oxide with 0.02% (w/v) deuterated TSP

Analysis

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

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for ^{1}H, acquire a free induction decay (FID) using NLT 16 scans using a 90° pulse and 20 s delay. Record the ^{1}H NMR spectra of *Standard solution A* and the *System suitability solution* at 25°. Collect the ^{1}H NMR spectrum with a spectral window of at least 10 to –2 ppm and without spinning. The number of transients should be adjusted until the signal-to-noise of the *N*-acetyl heparin signal in the standard solution is at least 1000/1 in the region near 2 ppm. *Standard solution A* shall be run at least daily when *Sample solutions* are being run. For all samples, the TSP methyl signal should be set to 0.00 ppm. The ppm values for heparin and oversulfated chondroitin sulfate in the *System suitability solution* should be observed at 2.04 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the ^{1}H NMR spectrum of the *Sample solution* at 25°. Draw a baseline from 8.00 ppm to 0.10 ppm. The ppm values for H1 of GlcNAc/GlcNS, 6S (signal 1), H1 of IdoA2S (signal 2), the H2 of GlcNS (signal 3), and the methyl of GlcNAc (signal 4) of heparin are present at 5.42, 5.23, 3.28 (doublet centered at 3.28 ppm), and 2.05 ppm, respectively.[1] The ppm values of these signals do not differ by more than ± 0.03 ppm. Measure the signal heights above the baseline of signal 1 and signal 2 and calculate the mean of these signal heights. Other signals of variable heights and ppm values, attributable to heparin and HOD, may be seen between signal 2 and 4.55 ppm. Residual solvent signals may be observed in the 0.10–3.00 range. Heparin Sodium must meet the requirements stated in *Residual Solvents* ⟨467⟩.

Acceptance criteria: No unidentified signals greater than 4% of the mean of signal height of 1 and 2 are present in the following ranges: 0.10–2.00, 2.10–3.20, and 5.70–8.00 ppm. No signals greater than 200% signal height of the mean of the signal height of 1 and 2 are present in the 3.35–4.55 ppm for porcine heparin.

B. Chromatographic Identity

Solution A: Dissolve 0.8 g of NaH_2PO_4 in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Filter the solution through a 0.45-μm membrane and degas prior to use.

Solution B: Dissolve 0.8 g of NaH_2PO_4 and 280 g of $NaClO_4$ in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Filter the solution through a 0.45-μm membrane and degas prior to use.

Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)	Elution
0	80	20	Equilibration
60	10	90	Linear gradient
61	80	20	Linear gradient
75	80	20	Re-equilibration

[1] GlcNAc, *N*-acetylated glucosamine; GlcNS, *N*-sulfated glucosamine; S, Sulfate; IdoA, iduronic acid; GlcN, glucosamine; GalN, galactosamine.

Dermatan sulfate standard solution: 20 mg/mL of USP Dermatan Sulfate RS in water

Oversulfated chondroitin sulfate standard solution: 20 mg/mL of USP Oversulfated Chondroitin Sulfate RS in water

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in water

System suitability solution: 1% (v/v) solution of each *Dermatan sulfate standard solution* and *Oversulfated chondroitin standard solution* in *Standard solution*

Sample solution: NLT 20 mg/mL of Heparin Sodium in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 202 nm

Column: 2-mm × 25-cm; packing L61

Guard column: 2-mm × 50-mm; packing L61

Flow rate: 0.22 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

NOTE—The retention times for dermatan sulfate, heparin, and oversulfated chondroitin sulfate are about 20, 30, and 50 min, respectively.

Suitability requirements

Resolution: NLT 1.0 between the dermatan sulfate and the heparin peaks, and NLT 1.5 between the heparin and the oversulfated chondroitin sulfate

Relative standard deviation: NMT 2% for the heparin peak determined from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms, and measure the retention times for the major peaks.

Acceptance criteria: The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*.

C. ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO

Anti-factor Xa activity

pH 8.4 buffer: Dissolve amounts of tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% of polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust, if necessary, with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) as directed by the manufacturer and further dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 1.0 Antithrombin IU/mL.

Factor Xa solution: Reconstitute bovine factor Xa as directed by the manufacturer (see *Factor Xa* in *Reagent Specifications* under *Reagents, Indicators, and Solutions*) and further dilute in *pH 8.4 buffer* to obtain a solution that gives an absorbance value between 0.65 and 1.25 at 405 nm when assayed as described below but using 30 µL of *pH 8.4 buffer* instead of 30 µL of the *Standard solutions* or the *Sample solutions*. [NOTE—*Factor Xa solution* contains about 3 nanokatalytic units/mL, but can vary depending upon the manufacturer of factor Xa or the substrate used.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) specific for factor Xa in water to obtain a concentration of 1 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the contents of 1 ampule of USP Heparin Sodium RS with water and dilute with *pH 8.4 buffer* to obtain at least 5 (out of 7 below) solutions having known activities of about 0.375, 0.3125, 0.25, 0.188, 0.125, 0.0625, and 0.0313 USP Heparin Unit/mL.

Sample solutions: Dissolve or dilute an accurately measured quantity of Heparin Sodium in *pH 8.4 buffer*, and dilute with the same buffer to obtain solutions having activities approximately equal to those of the *Standard solutions*.

Analysis

NOTE—Perform the test with each *Standard solution* and *Sample solution* in duplicate.

To each of a series of suitable plastic tubes placed in a water bath set at 37°, transfer 120 µL of *pH 8.4 buffer*. Then separately transfer 30 µL of the different dilutions of the *Standard solutions* or the *Sample solutions* to the tubes. Add 150 µL of *Antithrombin solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Factor Xa solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Chromogenic substrate solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for exactly 2 min. Add 150 µL of *Stopping solution* to each tube, and mix. Prepare a blank for zeroing the spectrophotometer by adding the reagents in reverse order, starting with the *Stopping solution* and ending with the addition of 150 µL of *pH 8.4 buffer*, and excluding the *Standard solutions* or the *Sample solutions*. Record the absorbance at 405 nm against the blank.

Calculations: Plot the log of the absorbance values of the *Standard solutions* and the *Sample solutions* versus heparin concentrations in USP Units. Construct separate straight lines of best fit using least-squares linear regression analyses for the *Standard solutions* and the *Sample solutions*, and determine the slope for each regression line. Calculate the anti-factor Xa activity of Heparin Sodium by the formula:

$$A \times (S_T/S_S)$$

A = the activity of USP Heparin Sodium RS

S_T = slope of the line from the *Sample solutions*

S_S = slope of the line from the *Standard solutions*

Express the anti-factor Xa activity of the *Sample solution* as USP Heparin Units/mg, calculated on the dried basis. Calculate the ratio of anti-factor Xa activity against anti-factor IIa potency (see *Assay* below) by the formula:

anti-factor Xa activity/anti-factor IIa potency

Acceptance criteria: NLT 0.9 and NMT 1.1

D. IDENTIFICATION TESTS—GENERAL, *SODIUM* ⟨191⟩: It meets the requirements of the flame test for sodium.

ASSAY

ANTI-FACTOR IIa POTENCY

pH 8.4 buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the contents of 1 ampule of USP Heparin Sodium RS with water and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add a double volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be pre-warmed to 37° just prior to use.

Two different types of measurements can be recorded:

1. Endpoint Measurement: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). The RSD over the blank readings is less than 10%.
2. Kinetic Measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values as they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

Slope ratio assay: For each series, calculate the log regression of the absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the

potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

Acceptance criteria: The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

INORGANIC IMPURITIES

RESIDUE ON IGNITION ⟨281⟩**:** Between 28.0% and 41.0%

NITROGEN DETERMINATION, *Method I, Nitrates and Nitrites Absent* ⟨461⟩*:* Between 1.3% and 2.5%, calculated on the dried basis

HEAVY METALS, *Method II* ⟨231⟩*:* NMT 30 ppm

ORGANIC IMPURITIES

LIMIT OF GALACTOSAMINE IN TOTAL HEXOSAMINE (a measure of dermatan sulfate and other galactosamine containing impurities)

Mobile phase: 14 mM potassium hydroxide

Glucosamine standard solution: 1.6 mg/mL of USP Glucosamine Hydrochloride RS in 5 N hydrochloric acid

Galactosamine standard solution: 16 μg/mL of USP Galactosamine Hydrochloride RS in 5 N hydrochloric acid

Standard solution: Mix equal volumes of *Glucosamine standard solution* and *Galactosamine standard solution*.

Hydrolyzed standard solution: Transfer 5 mL of the *Standard solution* to a 7-mL screw cap test tube, cap, and heat for 6 h at 100°. Cool to room temperature and quantitatively transfer the solution to a 500-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 12 mg of Heparin Sodium to a 7-mL screw cap test tube, dissolve in 5 mL of 5 N hydrochloric acid, and cap.

Hydrolyzed sample solution: Heat the *Sample solution* for 6 h at 100°. Cool to room temperature and quantitatively transfer the solution to a 500-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*)

Mode: HPIC

Detector: Pulsed amperometric detector, set to the following waveform:

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.1	—
2	0.20	+0.1	Begins
3	0.40	+0.1	Ends
4	0.41	–2.0	—
5	0.42	–2.0	—
6	0.43	+0.6	—
7	0.44	–0.1	—
8	0.50	–0.1	—

Column: 3-mm × 30-mm amino acid trap column in series with 3 × 30-mm guard column and a 3-mm × 15-cm column that contains packing L##

Column temperature: Maintain columns at 30°

Flow rate: 0.5 mL/min

Pre-equilibration: At least 60 min with *Mobile phase*

Injection size: 10 μL

Elution: 10 min with *Mobile phase*

Column cleaning: At least 10 min with 100 mM KOH

Equilibration: At least 10 min with *Mobile phase* before each injection

System suitability

Sample: *Hydrolyzed standard solution*

Suitability requirements

Resolution: NLT 2 between the galactosamine and glucosamine peaks

Column efficiency: NLT 2000 theoretical plates for glucosamine

Tailing factor: Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

Analysis

Samples: *Hydrolyzed standard solution* and *Hydrolyzed sample solution*

Record the chromatograms, and measure the responses for the peaks at the retention time of galactosamine and glucosamine. Calculate the response ratio of galactosamine to glucosamine ($GalN_R$) in the *Hydrolyzed standard solution*:

$$(GalN_B/GalN_W) \times (GlcN_W/GlcN_B)$$

$GalN_B$ = galactosamine peak area from the *Hydrolyzed standard solution*

$GalN_W$ = weight of galactosamine for the *Standard solution*

$GlcN_B$ = glucosamine peak area from the *Hydrolyzed standard solution*

$GluN_W$ = weight of glucosamine for the *Standard solution*

Calculate the percentage of galactosamine in the portion of total hexosamine taken:

$$[(GalN_U/GalN_R)]/[(GalN_U/GalN_R) + GlcN_U] \times 100$$

$GalN_U$ = galactosamine peak area from the *Hydrolyzed sample solution*

$GalN_R$ = galactosamine response ratio

$GlcN_U$ = glucosamine peak area from the *Hydrolyzed sample solution*

Acceptance criteria: The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

NUCLEOTIDIC IMPURITIES: *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩, *Method I* with the following modifications.

Analysis: Dissolve 40 mg of Heparin Sodium in 10 mL of water. Measure the absorbance of this solution at 260 nm using the light scattering correction procedure of *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩, *Method I*.

Acceptance criteria: The absorbance of this solution at 260 nm is NMT 0.20.

ABSENCE OF OVERSULFATED CHONDROITIN SULFATE

A: Proceed as directed in *Identification* test *A*. No features associated with oversulfated chondroitin sulfate are found between 2.12 and 3.00 ppm.

B: Proceed as directed in *Identification* test *B*. No peaks corresponding to oversulfated chondroitin sulfate should be detected eluting after the heparin peak.

PROTEIN IMPURITIES

Standard stock solution: 0.032 mg/mL of bovine serum albumin in water

Standard solutions: Dilute portions of the *Standard stock solution* with water to obtain NLT 5 standard solutions having concentrations between 0.0016 and 0.032 mg/mL of bovine serum albumin, the concentrations being evenly spaced.

Sample solution: 30 mg/mL of Heparin Sodium in water. Prepare in triplicate.

Blank: Water

Lowry reagent A: Prepare a solution of 10 g/L NaOH in water and a solution of 50 g/L Na_2CO_3 in water. Mix equal volumes (2V:2V) of each solution and dilute with water to 5V.

Lowry reagent B: Prepare a solution of 29.8 g/L disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L $CuSO_4 \cdot 5H_2O$ in water. Mix equal volumes of both solutions (2V:2V) and dilute with water to 5V.

Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*.

Diluted Folin-Ciocalteu's Phenol reagent: Dilute *Folin-Ciocalteu's Phenol reagent* 2–4 times with water, as determined by titration.

Analysis

Samples: *Standard solutions, Sample solution,* and *Blank*

To 1 mL of each *Standard solution, Sample solution,* and *Blank*, add 5 mL of *Lowry reagent C*. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin-Ciocalteu's Phenol reagent* to each solution, and mix immediately. Proceed as directed in *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩, *Method 2*.

Calculations: See *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩, *Method 2*.

Acceptance criteria: NMT 1.0% is found.

RESIDUAL SOLVENTS ⟨467⟩**:** It meets the requirements.

SPECIFIC TESTS

pH ⟨791⟩*:* 5.0–7.5, (1 in 100) solution

BACTERIAL ENDOTOXINS TEST ⟨85⟩*:* NMT 0.03 USP Endotoxin Unit/USP Heparin Unit

STERILITY TESTS ⟨71⟩*:* Where it is labeled as sterile, it meets the requirements.

LOSS ON DRYING ⟨731⟩*:* Dry under vacuum at 60° for 3 h. It loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

PACKAGING AND STORAGE*:* Preserve in tight containers, and store below 40°, preferably at room temperature.

LABELING*:* Label it to indicate the tissue and the animal species from which it is derived.

USP REFERENCE STANDARDS ⟨11⟩

USP Oversulfated Chondroitin Sulfate RS

USP Dermatan Sulfate RS

USP Endotoxin RS

USP Galactosamine Hydrochloride RS

USP Glucosamine Hydrochloride RS

USP Heparin Sodium RS

USP Heparin Sodium Identification RS•4

BRIEFING

Heparin Sodium Injection, *USP 31* page 2322 and the *Revision Bulletin* posted on the USP website on June 18, 2008. Because of comments received from the *Revision Bulletin* for the *USP* Heparin Sodium monograph, the final product monograph is revised to include a proposed chromogenic *Anti-Factor IIa Assay.* This assay is intended to replace the currently official sheep plasma-based clotting assay. In addition, *USP* Heparin potency units will be harmonized with International Units as of August 2009.

(BB BBP: A. Szajek.) RTS—C72034

Change to read:

Heparin Sodium Injection

~~» Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits a potency not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label in terms of USP Heparin Units per mL.~~

~~NOTE—The USP Heparin Unit is defined by the USP Heparin Sodium Reference Standard, independent of International Units. The respective units are not equivalent (see *General Notices*).~~

~~**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store at a temperature below 40°, preferably at room temperature.~~

~~**Labeling**—Label it to indicate the volume of the total contents and the potency in terms of USP Heparin Units only per mL, except that single-dose containers may be labeled additionally to indicate the single-unit dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.~~

~~**USP Reference standards** ⟨11⟩—*USP Heparin Sodium RS. USP Endotoxin RS.*~~

~~**Bacterial endotoxins** ⟨85⟩—It contains not more than 0.03 USP Endotoxin Unit per USP Heparin Unit.~~

~~**pH** ⟨791⟩: between 5.0 and 7.5.~~

~~**Particulate matter** ⟨788⟩: meets the requirements for small-volume injections.~~

Proposed IRA

~~**Other requirements**—It meets the requirements under *Injections* ⟨1⟩.~~

~~**Assay**—Proceed as directed in the *Assay* under *Heparin Sodium*, substituting the Injection for the solution of heparin sodium prepared as directed under *Assay preparation*. Under *Calculation*, v_u in the equation for *R* is the volume, in mL, of the Injection per mL of the *Assay preparation*. The potency of Heparin Sodium Injection in USP Heparin Units per mL is $P^* =$ antilog *M*.~~

•DEFINITION

Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits a potency NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

ASSAY

ANTI-FACTOR IIa ACTIVITY

NOTE—Allow alternative platforms.

pH 8.4 Buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagents Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 Buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagents Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 Buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagents Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the contents of 1 ampule of USP Heparin Sodium RS with water and dilute with *pH 8.4 Buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. To each tube add the double volume (100–200 µL), of *Antithrombin solution* and one volume (50–100 µL), of either the *pH 8.4 Buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do

not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be pre-warmed to 37° just prior to use.

Two different types of measurements can be recorded:

1. ENDPOINT MEASUREMENT: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). The RSD over the blank readings is less than 10%.
2. KINETIC MEASUREMENT: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values as they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Slope ratio assay: For each series, calculate the log regression of the absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Acceptance criteria: NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

SPECIFIC TESTS

Bacterial Endotoxins Test ⟨85⟩**:** It contains NMT 0.03 USP Endotoxin Unit/USP Heparin Unit.

Particulate Matter in Injections ⟨788⟩**:** Meets the requirements for small-volume injections

pH ⟨791⟩*:* 5.0–7.5

Other Requirements: It meets the requirements under *Injections* ⟨1⟩.

ADDITONAL REQUIREMENTS

Labeling: Label it to indicate the volume of the total contents and the potency in terms of USP Heparin Units only per mL, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

Packaging and Storage: Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, and store at a temperature below 40°, preferably at room temperature.

USP Reference Standards ⟨11⟩

USP Endotoxin RS

USP Heparin Sodium RS$_{\bullet 4}$

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) items that previously appeared under *Pharmacopeial Previews* and are now formally proposed as revisions, (2) proposed revisions placed directly under *In-Process Revision,* or (3) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

> **Name of Item**, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:
>
> (DSN: L. Evans.) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. New text (if any) follows, and is enclosed in symbols and set off from the current official text by a paragraph break and by larger type (print edition only), as shown in the examples below:

•new text•

if slated for an *Interim Revision Announcement to USP 30–NF 25* (*IRA*);

▲new text▲$_{USP31}$

if slated for *USP 31–NF 26*; and

■new text■

if slated for a *Supplement to USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as •• or ■■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •$_2$ indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S ($_{USP\ 30}$) indicates that the proposed revision is slated for the *Second Supplement to USP 30*, and ▲$_{USP31}$ and ▲$_{NF26}$ indicate that the revisions are proposed for *USP 31* and *NF 26*, respectively.

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

MONOGRAPHS (USP)

BRIEFING

Diclofenac Sodium Delayed-Release Tablets, *USP 32* page 2124. It is proposed to correct the preparation of the *Standard solution* in the *Buffer stage* in the *Dissolution* test.

(BPC: M. Marques.) RTS—C70890

Diclofenac Sodium Delayed-Release Tablets

DEFINITION

Diclofenac Sodium Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION

- **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** Meet the requirements of the flame test

ASSAY

- **PROCEDURE**

Solution A: Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate component to a pH of 2.5 ± 0.2.

Mobile phase: Methanol and *Solution A* (7:3)
[NOTE—Increasing the proportion of buffer increases resolution.]

Diluent: Methanol and water (7:3)

System suitability solution: 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

Standard solution: 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

Sample solution: Transfer 20 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of 0.75 mg/mL of diclofenac sodium is obtained. Add *Diluent* to about 70% of the capacity of the flask, and shake by mechanical means for NLT 30 min to disintegrate the Tablets. Cool to room temperature, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Sample solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7 (end-capped)
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]

Suitability requirements
Resolution: NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks; NLT 6.5 between the diclofenac related compound A and diclofenac peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{10}Cl_2NNaO_2$ in the portion of Tablets taken:

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

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩**:** Proceed as directed for *Procedure*, *Apparatus 1 and Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B* to determine the amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved.

Acid stage
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm, paddles constructed of (or coated with) polytef being used
Time: 2 h
Detector: UV 276 nm
Standard solution: Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with a mixture of 0.1 N hydrochloric acid and 5 N sodium hydroxide (900:20) to volume, and mix. This *Standard solution* contains 13.6 µg/mL of USP Diclofenac Sodium RS.
Sample solution: At the end of 2 h, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessels, and subject them to the test under *Buffer stage*. To the 0.1 N hydrochloric acid remaining in each vessel, add 20.0 mL of 5 N sodium hydroxide, and stir for 5 min.

Buffer stage
Medium: *pH 6.8 phosphate buffer*; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV 276 nm
Solution A: 76 mg/mL of tribasic sodium phosphate
pH 6.8 phosphate buffer: *Solution A* and 0.1 N hydrochloric acid (1:3), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05, if necessary
~~**Standard solution:** Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, and dilute with *Medium* to volume. This *Standard solution* contains about 0.02 mg/mL of USP Diclofenac Sodium.~~
■**Standard solution:** Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask. Add 10.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Buffer stage Medium* to volume, and mix. The final concentration is 0.22 mg/mL of diclofenac sodium.■1S (*USP33*)
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Tolerances: NLT 75% (Q) of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ is dissolved.

In-Process Revision

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Solution A, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Standard solution: 0.8 mg/mL of USP Diclofenac Related Compound A RS in methanol. Prepare 4 µg/mL from this solution with *Diluent*.

Analysis: Measure the peak responses over a period of 40 min.

Calculate the percentage of diclofenac related compound A in relation to the quantity of diclofenac sodium in the portion of Tablets taken:

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

r_U = peak response of diclofenac related compound A from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity, other than diethyl phthalate, if present, in relation to the diclofenac sodium in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for each impurity from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: NMT 0.5% of diclofenac related compound A; NMT 1.0% of any other individual impurity

Total impurities: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Diclofenac Sodium RS

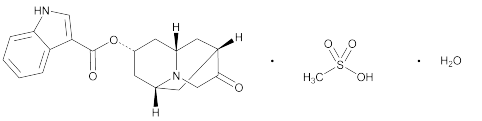
USP Diclofenac Related Compound A RS

BRIEFING

Dolasetron Mesylate, *USP 31* page 2009. On the basis of comments received, it is proposed to establish a reporting level for impurities.

(MD-GRE: E. Gonikberg.) RTS—C69725

Dolasetron Mesylate



$C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ 438.50

1*H*-Indole-3-carboxylic acid, octahydro-3-oxo-2,6-methano-2*H*-quinolizin-8-yl ester, (2α,6α,8α,9a*β*)-, monomethanesulfonate monohydrate;

Indole-3-carboxylic acid, ester with (8*r*)-hexahydro-8-hydroxy-2,6-methano-2*H*-quinolizin-3(4*H*)-one, monomethanesulfonate monohydrate [115956-13-3].

DEFINITION

Dolasetron Mesylate contains NLT 98.0% and NMT 102.0% of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$, calculated on the as-is basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION ⟨197K⟩**

• **B. PROCEDURE**

Sample solution: 1 mg/mL

Analysis: Transfer 5–10 mg of 5,5′-methylenedisalicylic acid to a clean crucible, and heat in an oven at 150° for 5 min. Remove from the oven, and add 10 drops of the *Sample solution*. Return to the oven, and evaporate to dryness.

Acceptance criteria: A red or pink color (presence of methanesulfonic acid) develops in the white residue.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and 1 M ammonium formate (450:440:110), adding 0.19 mL of triethylamine to the acetonitrile portion

Standard solution: 0.04 mg/mL and 0.004 mg/mL, respectively, of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*

Sample solution: 0.04 mg/mL of Dolasetron Mesylate in *Mobile phase*

Chromatographic System

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4 between indole-3-carboxylic acid and dolasetron mesylate

Tailing factor: NMT 1.8

Relative standard deviation: NMT 1.5% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ in the portion of Dolasetron Mesylate taken:

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

r_U = peak area from the *Sample solution*

In-Process Revision

r_S = peak area from the *Standard solution*
C_S = concentration of USP Dolasetron Mesylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.0% and NMT 102.0%

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**

0.01 M Dibasic ammonium phosphate solution: 1.32 mg/mL of dibasic ammonium phosphate. Adjust with 2.0 M phosphoric acid to a pH of 7.0.

Diluent: Acetonitrile and water (1:4)

Solution A: Acetonitrile and *0.01 M Dibasic ammonium phosphate solution* (53:1000), filtered and degassed

Solution B: Acetonitrile and 0.*01 M Dibasic ammonium phosphate solution* (795:295), filtered and degassed

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
28	0	100
38	0	100
40	100	0
50	100	0

System suitability solution: 0.004 mg/mL and 0.03 mg/mL, respectively, of indole and USP Dolasetron Mesylate RS in *Diluent*

Standard solution A: 0.03 mg/mL of USP Dolasetron Mesylate RS in *Diluent*

Standard solution B: 6 mg/mL and 0.0072 mg/mL, respectively, of USP Dolasetron Mesylate RS and USP Dolasetron Mesylate Related Compound A RS in *Diluent*

Sample solution: 6 mg/mL of Dolasetron Mesylate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.5 mL/min

Injection size: 100 µL

System suitability

Suitability requirements

Resolution: NLT 1.5 between the first eluting peak, indole, and the second eluting peak, dolasetron mesylate from the *System suitability solution* [NOTE—If the dolasetron mesylate peak is found to elute before the indole peak, condition the column as follows: fill up the column with *Solution A*, plug the column, and place the column in a convection oven at 105° for about 16 h. Retest the column.]

Relative standard deviation: NMT 5.0% for replicate injections of *Standard solution A*

Analysis

Samples: *Standard solution A, Standard solution B,* and *Sample solution*

Calculate the percentage of dolasetron mesylate related compound A in the portion of Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area for dolasetron mesylate related compound A from the *Sample solution*
r_S = peak area for dolasetron mesylate related compound A from *Standard solution B*
C_S = concentration of USP Dolasetron Mesylate Related Compound A RS in *Standard solution B* (mg/mL)
C_U = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dolasetron mesylate related compound A base, 181.2
M_{r2} = molecular weight of dolasetron mesylate related compound A hydrochloride, 217.8

Acceptance criteria: NMT 0.1%

Calculate the percentage of each impurity (other than dolasetron mesylate related compound A) in the portion of Dolasetron Mesylate taken:

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

r_U = peak area for each impurity from the *Sample solution*
r_S = peak area for dolasetron mesylate from *Standard solution A*
C_S = concentration of USP Dolasetron Mesylate RS in *Standard solution A* (mg/mL)
C_U = nominal concentration of dolasetron mesylate in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.3%

■[NOTE—The reporting level for impurities is 0.05%.]■1S (*USP33*)

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** Between 3.5% and 4.7%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Dolasetron Mesylate RS
USP Dolasetron Mesylate Related Compound A RS

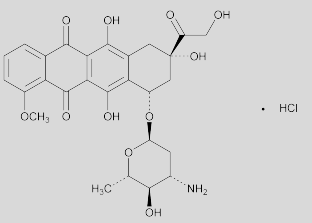
BRIEFING

Epirubicin Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph is proposed based on the current *European Pharmacopoeia* monograph. The liquid chromatographic procedures in the test for *Organic Impurities, Procedure 1* and in the *Assay* are based on analyses performed using a Zorbax TMS brand of L13 column. The typical retention times for doxorubicin and epirubicin are about 7.6 and 9.5 minutes, respectively.

(MD-ANT: A. Wise; . MSA : R. Tirumalai.) RTS—C49304

Add the following:

▪Epirubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$ 579.98

5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-, hydrochloride, (8*S*-*cis*)-; (1*S*,3*S*)-3-Glycoloyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl-3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside hydrochloride. [56390-09-1].

DEFINITION

Epirubicin Hydrochloride contains NLT 97.0% and NMT 102.0% of $C_{27}H_{29}NO_{11} \cdot HCl$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197M⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** A 10 mg/mL solution in a mixture of nitric acid and water (1:1) meets the requirements.

ASSAY

- **PROCEDURE**

[NOTE—Allow the *Standard solution*, *System suitability solution,* and the *Sample solution* to stand for 3 h before use.]

Solution A: Dissolve 3.7 g of sodium lauryl sulfate in 950 mL of water. To the resulting solution, add 28 mL of 10% phosphoric acid, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile, methanol and *Solution A* (29:17:54)

System suitability solution: 0.1 mg/mL each of USP Epirubicin Hydrochloride RS and USP Doxorubicin Hydrochloride RS in *Mobile phase*

Standard solution: 1 mg/mL USP Epirubicin Hydrochloride RS in *Mobile phase*

Sample solution: 1 mg/mL of Epirubicin Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm column; 6-µm packing L13

Temperature: 35°

Flow rate: 2.5 mL/min

Injection size: 10 µL

Run time: About 3.5 times the retention time of the epirubicin peak

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between doxorubicin and epirubicin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{27}H_{29}NO_{11} \cdot HCl$ in the portion of Epirubicin Hydrochloride taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

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

Acceptance criteria: 97.0%–102.0%

IMPURITIES

Organic Impurities

- **PROCEDURE 1**

[NOTE—Allow the *Standard solution*, *System suitability solution,* and *Sample solution* to stand for 3 h before use.]

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*

Peak identification solution: Dissolve 10 mg of USP Doxorubicin Hydrochloride RS in 10 mL of a mixture of water and phosphoric acid (1:1). Allow to stand for 30 min. Adjust with 2 N sodium hydroxide solution to a pH of 2.6. Add 15 mL of acetonitrile and 10 mL of methanol, and mix.

Analysis

Samples: *Peak identification solution, Standard solution,* and *Sample solution*

Calculate the percentage of each impurity in the portion of Epirubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_s) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity peak from the *Sample solution*

r_S = peak response of the epirubicin peak from the *Standard solution*

C_S = concentration of USP Epirubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Epirubicin Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 3.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT %
Doxorubicinone	0.3	1.4	1.0
Daunorubicinone	0.4	1.0	0.5
Doxorubicin	0.8	1.0	1.0
Epirubicin	1.0	—	—

In-Process Revision

Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT %
Dihydrodaunorubicin	1.1	1.0	0.5
Daunorubicin	1.5	1.0	0.5
Epidaunorubicin	1.7	1.0	1.0
Epirubicin dimer	2.1	1.0	1.0
Individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	3.0

- **PROCEDURE 2: LIMIT OF ACETONE**
  Analysis: See *Residual Solvents* ⟨467⟩
  Acceptance criteria: NMT 1.5%

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 4.0%
- **PH** ⟨**791**⟩**:** 4.0–5.5 for a 5 mg/mL solution
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** NMT 1.1 USP Endotoxin Units/mg, where the label states that Epirubicin Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

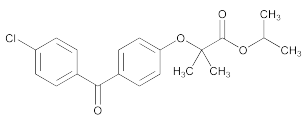
ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store in airtight containers, protected from light, at a temperature between 2° and 8°. If the substance is sterile, store in a sterile, airtight, tamper-proof container.
- **LABELING:** Where applicable, the label states that the substance is free from bacterial endotoxins.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Bacterial Endotoxin RS
  USP Doxorubicin Hydrochloride RS
  USP Epirubicin Hydrochloride RS■1S *(USP33)*

BRIEFING

Fenofibrate, USP 31 page 2145. It is proposed to change the acceptance criteria in the *Definition* from "NLT 98.5% and NMT 101.0%" to "NLT 98.0% and NMT 102.0%", which are typical for chromatographic assay procedures.

(MD-GRE: E. Gonikberg.) RTS—C72070

Fenofibrate



$C_{20}H_{21}ClO_4$ 360.83
Isopropyl 2-[*p*-(*p*-chlorobenzoyl)phenoxy]-2-methylpropanoate [49562-28-9].

DEFINITION

Change to read:

Fenofibrate contains ~~NLT 98.5% and NMT 101.0%~~■NLT 98.0% and NMT 102.0%■1S *(USP33)* of $C_{20}H_{21}ClO_4$, calculated on the dried basis.

IDENTIFICATION
- **INFRARED ABSORPTION** ⟨**197K**⟩

ASSAY

Change to read:

- **PROCEDURE**
  Mobile phase: Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)
  Standard solution: 1 mg/mL of USP Fenofibrate RS in *Mobile phase*
  Sample solution: 1 mg/mL of Fenofibrate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 286 nm
  Column: 4.0-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection size: 5 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 1.0% for six replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{20}H_{21}ClO_4$ in the portion taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)
  C_U = concentration of Fenofibrate in the *Sample solution* (mg/mL)
  Acceptance criteria: ~~98.5%–101.0%~~■98.0%–102.0%■1S *(USP33)*

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%, determined on 1.0 g
- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩
  Sample solution: Add 25 mL of water to 5.0 g of Fenofibrate, and heat at 50° for 10 min. Cool, dilute with water to 50.0 mL, filter, and use the filtrate. [NOTE—Retain the remaining portion of the *Sample solution* for the test for *Sulfate*.]
  Analysis: Use 10 mL of the *Sample solution*.
  Acceptance criteria: It shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid (0.01%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩
  Sample: Use the *Sample solution* prepared in the test for *Chloride*.

In-Process Revision

Analysis: Use 10 mL of the *Sample*.
Acceptance criteria: It shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.01%).

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

• **PROCEDURE**

Mobile phase: Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)
Impurity standard solution: 1 µg/mL each of fenofibrate, fenofibrate related compound A, and fenofibrate related compound B (from USP Fenofibrate RS, USP Fenofibrate Related Compound A RS, and USP Fenofibrate Related Compound B RS), and 2 µg/mL of fenofibrate related compound C (from USP Fenofibrate Related Compound C RS) in *Mobile phase*
Sample solution: 1 mg/mL of Fenofibrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Impurity standard solution*
Suitability requirements
Resolution: NLT 1.5 between fenofibrate related compound A and fenofibrate related compound B
Analysis
Samples: *Impurity standard solution* and *Sample solution*
Identify the fenofibrate peak and the peaks due to the impurities and degradation products listed in *Impurity Table 1*.
Measure the responses for the major peaks, and calculate the percentage of each of fenofibrate related compound A, fenofibrate related compound B, and fenofibrate related compound C in the portion of Fenofibrate taken:

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

r_U = appropriate fenofibrate related compound peak response from the *Sample solution*
r_S = appropriate fenofibrate related compound peak response from the *Impurity standard solution*
C_S = concentration of the appropriate fenofibrate related compound in the *Impurity standard solution* (µg/mL)
C_U = concentration of Fenofibrate in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Fenofibrate taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of Fenofibrate from the *Impurity standard solution*
C_S = concentration of Fenofibrate in the *Impurity standard solution* (µg/mL)
C_U = concentration of Fenofibrate in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
(4-Chlorophenyl)(4-hydroxyphenyl)methanone[a]	0.34	0.1
2-[4-(4-Chlorobenzoyl)-phenoxy]-2-methylpropanoic acid (fenofibric acid)[b]	0.36	0.1
(3*RS*)-3-[4-(4-Chlorobenzoyl)-phenoxy]butan-2-one	0.50	0.1
Methyl 2-[4-(4-chlorobenzoyl)-phenoxy]-2-methyl-propanoate	0.65	0.1
Ethyl 2-[4-(4-chlorobenzoyl)-phenoxy]-2-methyl-propanoate	0.80	0.1
(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanone	0.85	0.1
1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate[c]	1.35	0.2
Any other impurity	—	0.1

[a] Fenofibrate related compound A.
[b] Fenofibrate related compound B.
[c] Fenofibrate related compound C.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨**741**⟩**:** *79°–82°*

• **ACIDITY**
Sample: 1.0 g
Analysis: Dissolve the *Sample* in 50 mL of alcohol previously neutralized to phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS.
Acceptance criteria: NMT 0.2 mL

• **LOSS ON DRYING** ⟨**731**⟩
Analysis: Dry a sample in a vacuum over phosphorus pentoxide at 60° to constant weight.
Acceptance criteria: The sample loses NMT 0.5% of its weight.

• **COLOR AND ACHROMICITY** ⟨**631**⟩
Reference solution: Mix 5 mL of *Matching Fluid G* and 95 mL of 0.3 M hydrochloric acid.
Sample solution: 50 mg/mL of Fenofibrate in acetone
Analysis: Proceed as directed in the chapter.
Acceptance criteria: The *Sample solution* is not more intensely colored than the *Reference solution*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Fenofibrate RS

In-Process Revision

USP Fenofibrate Related Compound A RS
USP Fenofibrate Related Compound B RS
USP Fenofibrate Related Compound C RS

BRIEFING

Lamivudine and Zidovudine Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph based on submitted data is being proposed. The liquid chromatographic procedure in the *Procedure* under *Organic Impurities* and in the *Assay* is based on analysis performed with the Keystone ODS-A brand of column containing 5-µm packing L1. The typical retention times for lamivudine and zidovudine are about 18 and 36 min, respectively.

(MD-AA: L. Santos, B. Davani. BPC: M. Marques.) RTS—C46270

Add the following:

▪Lamivudine and Zidovudine Tablets

DEFINITION
Lamivudine and Zidovudine Tablets contains NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION
- The retention times of the lamivudine and zidovudine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 25 mM of ammonium acetate. Adjust the pH to 4.0 with glacial acetic acid.
Solution B: Methanol
Solution C: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	95	5	0
15.0	95	5	0
30.0	70	30	0
38.0	70	30	0
38.1	0	0	100
45.0	0	0	100
45.1	95	5	0
60.0	95	5	0

Diluent: *Solution A* and *Solution B* (19:1)
System suitability solution: 0.17 mg/mL of USP Lamivudine Resolution Mixture B RS in *Diluent*
Standard solution: 0.15 mg/mL of USP Lamivudine RS and 0.30 mg/mL of USP Zidovudine RS in *Diluent*
Sample stock solution: Transfer a counted number of Tablets, equivalent to 1500 mg of zidovudine and 750 mg of lamivudine, into a 500-mL volumetric flask. Add 250 mL of water, and disintegrate completely by shaking for a minimum of 15 min. Dilute with water to volume, and mix. Pass a portion of the solution through a 0.45-µm filter, discarding the first 2–3 mL. Accurately transfer 5.0 mL of the filtrate into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution: Pass a portion of the solution through a 0.45-µm filter, discarding the first 2–3 mL. Further dilute the filtrate to obtain 0.15 mg/mL of lamivudine and 0.30 mg/mL of zidovudine in *Diluent.*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 3-mm × 25-cm column; packing L1
Flow rate: 0.5 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lamivudine-*trans* and lamivudine are 0.50 and 0.52, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine-*trans* and lamivudine, *System suitability solution*
Relative standard deviation: NMT 2.0% for zidovudine and lamivudine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the portion of Tablets taken:

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

r_U = response of the zidovudine or lamivudine peak from the *Sample solution*
r_S = response of the zidovudine or lamivudine peak from the *Standard solution*
C_S = concentration of USP Zidovudine RS or USP Lamivudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine or lamivudine in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨**711**⟩

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 75 rpm
Time: 15 min
Lamivudine response factor solutions: 0.167 mg/mL of USP Lamivudine RS in *Medium*. [NOTE—Prepare in duplicate.]
Zidovudine response factor solutions: 0.333 mg/mL of USP Zidovudine RS in *Medium*. [NOTE—Prepare in duplicate.]
Sample solution: Pass a portion of the solution under test through a suitable (PTFE, PVDF, or equivalent) 0.45-µm filter.
Detector: UV 240 nm to 300 nm
Blank: *Medium*
Cell length: 0.02-cm flowcell
Analysis: The calculations of the percentages dissolved are done using a multicomponent analysis software.
Tolerances: NLT 85% (Q) of the labeled amount of zidovudine and lamivudine is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements for zidovudine and lamivudine

IMPURITIES
Organic Impurities
- **PROCEDURE**

Solution A, Solution B, Solution C, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_T = sum of the responses of lamivudine and all lamivudine related impurities from the *Sample solution*

Calculate the percentage of each zidovudine related impurity and unidentified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_T = sum of the responses of zidovudine, all zidovudine related impurities, and unidentified impurities from the *Sample solution*

F = relative response factor for each impurity as listed in *Impurity Table 1*

Impurity Table 1

Compound	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Lamivudine-(cytosine)[1]	0.11	1.0	—
Lamivudine-(uracil)[2]	0.14	1.0	—
Lamivudine-(carboxylic acid)[3]	0.17	1.0	0.3
Lamivudine-(*S*-sulfoxide)[4]	0.20	1.0	—
Lamivudine-(*R*-sulfoxide)[5]	0.22	1.0	—
Zidovudine related compound C[6]	0.27	1.7	1.5
Lamivudine-(*trans*)[7]	0.50	1.0	0.2
Lamivudine	0.52	—	—
Zidovudine-(thymidine)[8]	0.60	1.0	—
Lamivudine-(uracil derivative)[9]	0.70	1.0	—
Lamivudine-(salicylic acid)[10]	0.80	1.0	—
Zidovudine	1.00	—	—
Zidovudine Related Compound B[11]	1.10	1.0	—
Individual unidentified impurity	—	1.0	0.1

[1]6-Aminopyrimidin-2(1*H*)-one.
[2]Pyrimidine-2,4(1*H*,3*H*)-dione.
[3](2*R*,5*S*)-5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid (2*R*,5*S*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[4]1-[(2*R*,3*S*,5*SS*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[5]1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[6]5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
[7]1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
[8][1-(2-Deoxy-β-D-ribofuranosyl)]thymine.
[9](2*RS*,5*SR*)1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.
[10]2-Hydroxybenzoic acid.
[11]3′-Chloro-3′-deoxythymidine.

Acceptance criteria

Individual impurities: See *Impurity Table 1*.
Total lamivudine related impurities: NMT 0.6%
Total zidovudine related impurities: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store between 2° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lamivudine RS
  USP Lamivudine Resolution Mixture B RS
  USP Zidovudine RS■1S *(USP33)*

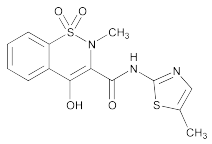
BRIEFING

Meloxicam. *USP 31* page 2607. It is proposed to correct the names of two of the impurites, 4-Hydroxy-2-methyl-*N*-(*N*′-ethyl-5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1, 1-dioxide and 4-Hydroxy-2-methyl-*N*-(*N*′-methyl-5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1, 1-dioxide, listed in *Impurity Table 1* under the *Organic Impurities* section and simultaneously correct their approximate retention times to match the submitted values, on the basis of comments received.

(MD-CCA: C. Anthony.) RTS—C70495

In-Process Revision

Meloxicam



$C_{14}H_{13}N_3O_4S_2$ 351.40
4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*–1,2-benzothiazine-3-carboxamide 1,1-dioxide [71125-38-7].

DEFINITION
Meloxicam contains NLT 99.0% and NMT 100.5% of $C_{14}H_{13}N_3O_4S_2$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B. ULTRAVIOLET ABSORPTION ⟨197U⟩**
  Analytical wavelength: 240–450 nm
  Sample solution: 10 µg/mL in methanol

ASSAY
- **PROCEDURE**
  Solution A: Mixture of a 0.1% (w/v) solution of ammonium acetate adjusted with 10% ammonia solution to a pH of 9.1.
  Mobile phase: Methanol and *Solution A* (21:29)
  System suitability solution: Transfer 4 mg of USP Meloxicam RS and 4 mg of USP Meloxicam Related Compound A RS into a 50-mL volumetric flask, dissolve in 25 mL of methanol and 0.1 mL of 1 N sodium hydroxide, and dilute with water to volume.
  Standard solution: Transfer 20 mg of USP Meloxicam RS into a 100-mL volumetric flask, dissolve in 50 mL of methanol and 0.2 mL of 1 N sodium hydroxide, and dilute with water to volume.
  Sample solution: Transfer 20 mg of Meloxicam into a 100-mL volumetric flask, dissolve in 50 mL of methanol and 0.2 mL of 1 N sodium hydroxide, and dilute with water to volume.
  Chromatographic system
  (See *Chromatography, System Suitability* ⟨621⟩.)
  Mode: LC
  Detector: UV 360 nm
  Column: 4.6-mm × 15-cm; packing L1
  Temperature: 45°
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for meloxicam related compound A and meloxicam are 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between meloxicam related compound A and meloxicam
  Tailing factor: NMT 2.0 for meloxicam peak
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{13}N_3O_4S_2$ in the portion of Meloxicam taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–100.5%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

Change to read:

Organic Impurities
[NOTE—Perform either *Procedure 1* or *Procedure 2* depending on the manufacturing process used.]
- **PROCEDURE 1**
  Solution A: 0.1% (w/v) solution of monobasic potassium phosphate adjusted with 1 N sodium hydroxide to a pH of 6.0
  Solution B: Methanol
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
2	60	40
10	30	70
15	30	70
15.1	60	40
18	60	40

System suitability solution: Transfer 4 mg of USP Meloxicam RS and 4 mg each of USP Meloxicam Related Compound A RS and USP Meloxicam Related Compound B RS into a 50-mL volumetric flask, dissolve in 5 mL of methanol and 0.3 mL of 1 N sodium hydroxide, and dilute with methanol to volume.
Standard stock solution: Transfer 12 mg of USP Meloxicam RS into a 20-mL volumetric flask, dissolve in 5 mL of methanol and 0.3 mL of 1 N sodium hydroxide, and dilute with methanol to volume.
Standard solution: Transfer 2 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Sample solution: Transfer 80 mg of Meloxicam into a 20-mL volumetric flask, dissolve in 5 mL of methanol and 0.3 mL of 1 N sodium hydroxide, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 260 nm and 350 nm (variable wavelength or multi-wavelength detector)
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Temperature: 45°
Flow rate: 1 mL/min
Injection size: 5 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times are listed in *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 3.0 between meloxicam related compound A and meloxicam at 350 nm, *System suitability solution*
NLT 3.0 between meloxicam related compound B and meloxicam at 260 nm, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of meloxicam at 350 nm from the *Standard solution*

C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)

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

F = relative response factor (see *Impurity Table 1*)

[NOTE—For the specified impurities, calculate the percentage content of each impurity, using the *Sample solution* peak responses recorded at the detection wavelength given in *Impurity Table 1*. For an unknown impurity, calculate the percentage content, using peak responses recorded at the wavelength that gives the greater response.]

Acceptance criteria

Individual impurity: See *Impurity Table 1*.

Total impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Times	Wavelength (nm)	Relative Response Factor	Acceptance Criteria, NMT (%)
Meloxicam related compound B[a]	0.4	260	1.0	0.1
Meloxicam	1.0	260/350	—	—
Meloxicam related compound A[b]	1.4	350	0.5	0.1
~~4-Hydroxy-2-methyl-*N*-(*N*′-ethyl-5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide~~■Methylmeloxicam[c]■1S *(USP33)*	1.7	350	1.0	0.05

[a] 2-Amino-5-methyl-thiazole.

[b] 4-Hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylic acid ethyl ester 1,1-dioxide.

■ [c] *N*-(3,5-Dimethylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

■1S *(USP33)*

■ [d] *N*-(3-Ethyl-5-methylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

■1S *(USP33)*

Impurity Table 1 *(continued)*

Name	Relative Retention Times	Wavelength (nm)	Relative Response Factor	Acceptance Criteria, NMT (%)
~~4-Hydroxy-2-methyl-*N*-(*N*′-methyl-5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide~~■Ethylmeloxicam[d]■1S *(USP33)*	1.9	350	1.0	0.05
Individual unknown impurity	—	260/350	1.0	0.1

[a] 2-Amino-5-methyl-thiazole.

[b] 4-Hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylic acid ethyl ester 1,1-dioxide.

■ [c] *N*-(3,5-Dimethylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

■1S *(USP33)*

■ [d] *N*-(3-Ethyl-5-methylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

■1S *(USP33)*

• **PROCEDURE 2:** If an article complies with this test, the labeling indicates that it meets the requirements under *Organic Impurities, Procedure 2*.

Solution A and **Solution B:** Proceed as directed in *Procedure 1*.

Mobile phase: See gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	45	55
25	45	55
30	30	70
40	30	70
45	45	55
50	45	55

Diluent A: *Diluent B* and 0.4 N sodium hydroxide (50:3)

Diluent B: Methanol and water (40:60)

Standard stock solution A: 50 µg/mL of USP Meloxicam RS in *Diluent A*. Dilute 2 mL of this solution with *Diluent B* to 10 mL.

Standard stock solution B: Transfer 5 mg each of USP Meloxicam Related Compound B RS, USP Meloxicam Related Compound C RS, and USP Meloxicam Related Compound D RS into a 100-mL volumetric flask, add 6 mL of 0.4 N sodium hydroxide, and sonicate for 2 min. Add 40 mL of methanol to the resulting solution, sonicate for 2 min, and dilute with water to volume.

Standard solution: Transfer 1 mL each of *Standard stock solution A* and *Standard stock solution B* into a 10-mL volumetric flask, dilute with *Diluent B* to volume, and mix.

System suitability stock solution: 2 mg/mL of USP Meloxicam RS in *Diluent A*
System suitability solution: Transfer 5 mL of *System suitability stock solution* and 1 mL of *Standard stock solution B* into a 10-mL volumetric flask, dilute with *Diluent B* to volume, and mix.
Sample solution: Dissolve 20 mg of Meloxicam in 10 mL of *Diluent A*, and dilute with *Diluent B* to 20 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV variable wavelength or multi-wavelength detector at 260 nm and 350 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Temperature: 45°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Relative retention times are listed in *Impurity Table 2*.]
Suitability requirements
Resolution: NLT 5.0 between meloxicam related compound D and meloxicam at 350 nm, *System suitability solution*
Relative standard deviation: NMT 5.0% for meloxicam related compound C and for meloxicam related compound D at 350 nm; and NMT 5.0% for meloxicam related compound B at 260 nm, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Meloxicam taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding USP Related Compound RS in the *Standard solution* (mg/mL) [NOTE—Use the concentration of the USP Meloxicam RS for unknown impurities.]
C_U = concentration of Meloxicam in the *Sample solution* (mg/mL)

[NOTE—Use the peak response and concentration of USP Meloxicam RS for unknown impurities; for the specified impurities, calculate the percentage content of each impurity using the *Sample solution* peak responses recorded at the detection wavelength given in *Impurity Table 2*. For an unknown impurity, calculate the percentage content using peak responses recorded at the wavelength that gives the greater response.]
Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Total impurities: NMT 0.3%

Impurity Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria NMT (%)
Meloxicam	1.0	260/350	—
Meloxicam related compound B[a]	1.4	260	0.1
Meloxicam related compound C[b]	3.2	350	0.1
Meloxicam related compound D[c]	2.4	350	0.1
Individual unknown impurity	—	260/350	0.1

[a]2-Amino-5-methyl-thiazole.
[b]Isopropyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate-1,1-dioxide.
[c]4-Methoxy-2-methyl-*N*-(5-methyl-1,3-thiazol-2yl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **LABELING:** The labeling states with which *Procedure* under *Organic Impurities* the article complies if a test other than *Procedure 1* is used.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Meloxicam RS
  USP Meloxicam Related Compound A RS
  USP Meloxicam Related Compound B RS
  USP Meloxicam Related Compound C RS
  USP Meloxicam Related Compound D RS

■1S (*USP33*)

BRIEFING

Nateglinide Tablets. Because there is no existing *USP* monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Symmetry 300 C18 brand of L1 column. The typical retention time for the nateglinide peak is about 6 min. The liquid chromatographic procedure in the test for *Dissolution* is also based on analyses performed with the Symmetry 300 C18 brand of L1 column. The typical retention time for the nateglinide peak is about 2 min.

(MDGRE: E. Gonikberg; . BPC: M. Marques.) RTS—C61576

Add the following:

■Nateglinide Tablets

DEFINITION
Nateglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of nateglinide ($C_{19}H_{27}NO_3$).

IDENTIFICATION
- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (11:9)

Mobile phase: Acetonitrile and 0.05% solution of trifluoroacetic acid (23:27)

Standard solution: 0.72 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide RS to a suitable volumetric flask, and add acetonitrile to 40% of the volume of the flask. [NOTE—Sonicate to dissolve.] Add water equivalent to 30% of the final volume, mix, cool the solution to room temperature, and dilute with *Diluent* to volume.

Sample solution: Place 20 Tablets into a 500-mL volumetric flask, and add 60 mL of water to disintegrate the Tablets. [NOTE—Sonicate with cooling, if necessary.] Add 280 mL of acetonitrile, and shake by mechanical means for at least 30 min. Dilute with *Diluent* to volume. Filter a portion using a 0.45-µm glass microfiber filter, discarding the first 10 mL of the filtrate, or use centrifugation to obtain a clear solution. Dilute an aliquot of this solution with *Diluent* to obtain a solution having a concentration of 0.72 mg/mL based on the label claim.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled quantity of $C_{19}H_{27}NO_3$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: 0.01 N hydrochloric acid containing 0.5% (w/v) of sodium lauryl sulfate; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the quantity of $C_{19}H_{27}NO_3$ dissolved by employing the following method.

Solution A: 6.9 mg/mL of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Solution A* (45:55)

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass through a suitable 0.7-µm filter.

Standard stock solution: 0.3 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide RS to a suitable volumetric flask, dissolve in acetonitrile using 5% of the final volume, and dilute with *Medium* to volume.

Standard solution: 0.12 mg/mL USP Nateglinide RS in *Medium*, from *Standard stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 5-cm column; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{27}NO_3$ dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/tablet)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{19}H_{27}NO_3$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Nateglinide RS■1S *(USP33)*

BRIEFING

Olanzapine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic method in the *Procedure* for *Organic Impurities* and in the *Assay* is based on analyses performed with either the Zorbax RX-C8 or Zorbax SB-C8 brand of L7 column manufactured by Agilent Technologies. The typical retention time of the olanzapine peak is about 13 min in the gradient elution method used in the *Procedure* for *Organic Impurities.* The retention time for olanzapine is about 6.7 min in the isocratic method used in the *Assay*. The chromatographic procedure in the test for *Dissolution* is based on analysis performed with a Zorbax SB-CN brand of L10 column. The typical retention time for olanzapine is 2.3 min.

(MD-PP: D. Vicchio, R. Ravichandran. BPC: M. Marques.)
RTS—C66222

In-Process Revision

Add the following:

▪Olanzapine Tablets

DEFINITION
Olanzapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$).

IDENTIFICATION
• **INFRARED ABSORPTION ⟨197S⟩**
Standard solution: 1 mg/mL of USP Olanzapine RS in chloroform
Sample solution: Dissolve a quantity of powdered Tablets, equivalent to 30 mg of olanzapine, in 30 mL of chloroform, and filter. Evaporate completely to dryness with the aid of a current of air. Redissolve the residue in 1 mL of chloroform.

ASSAY
• **PROCEDURE**
Buffer 1: 6.9 g/L of monobasic sodium phosphate. Adjust the pH to 2.5 with phosphoric acid.
Buffer 2: 12 g/L of sodium dodecyl sulfate in *Buffer 1*
Mobile phase: Acetonitrile and *Buffer 2* (1:1)
System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 0.01 mg/mL of USP Olanzapine Related Compound A RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Mobile phase*
Sample solution: Transfer a known quantity of Tablets, equivalent to NLT 25 mg of olanzapine, to a suitable volumetric flask. Dilute with *Mobile phase* to volume, mix, and sonicate for 10 min. Centrifuge a portion of this solution, and dilute the clear supernatant with *Mobile phase* to obtain a solution containing about 0.1 mg/mL of olanzapine. [NOTE—Agitation of the flask may be necessary prior to sonication to prevent Tablets from adhering to the flask, making disintegration and dissolution difficult.]
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for olanzapine related compound A and olanzapine are 0.89 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between olanzapine and olanzapine related compound A, *System suitability solution*
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{20}N_4S$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
C_U = concentration of olanzapine the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION ⟨711⟩**
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Transfer 5.0 mL of the filtrate to a tube and add 2.0 mL of *Mobile phase*.
Standard solution: An amount, in mg, corresponding to the Tablet label claim, of USP Olanzapine RS in 1000 mL of *Medium*. Transfer 5.0 mL of this solution to a tube and add 2.0 mL of *Mobile phase*.
Mobile phase: 10 g/L of ammonium acetate in a mixture of methanol and water (2:3). Adjust the pH to 4.0 with hydrochloric acid.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System suitability*.)
Mode: LC
Detector UV 260 nm
Column: 4.6 mm × 15 cm column, 5-µm packing L10
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Calculate the percentage of olanzapine dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times 900/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
900 = volume of *Medium*
L = label claim (mg/Tablet)
Tolerances: NLT 80% (Q) of the labeled amount of olanzapine is dissolved in 30 min.
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES
Organic Impurities
• **PROCEDURE**
Buffer 1: 3.3 mL/L of phosphoric acid. Adjust the pH to 2.5 with 50% NaOH.
Buffer 2: 8.7 g/L of sodium dodecyl sulfate in *Buffer 1*
Buffer 3: 18.6 mg/L of edetate disodium (EDTA) in *Buffer 2*
Solution A: Acetonitrile: *Buffer 2* (12:13)
Solution B: Acetonitrile: *Buffer 2* (7:3)
Diluent: Acetonitrile: *Buffer 3* (2:3)
System suitability solution: 20 µg/mL of USP Olanzapine RS, and 2 µg/mL each of USP Olanzapine Related Compound B RS and USP Olanzapine Related Compound C RS in *Diluent*
Standard solution: 2 µg/mL of USP Olanzapine RS in *Diluent*
Sensitivity solution: 0.4 µg/mL of USP Olanzapine RS in *Diluent*, from the *Standard solution*
Sample solution: Transfer a known quantity of Tablets to a suitable volumetric flask, and dilute with *Diluent* to volume to obtain a solution containing either 375 or 500 µg/mL of olanzapine (based on the label claim). Centrifuge a portion of this solution and use the supernatant. [NOTE—Immediate agitation of the flask may be necessary to prevent Tablets from adhering to the flask, making dissolution and disintegration difficult. [CAUTION—Do not sonicate.] The *Sample solution* is stable for 12 h at room temperature and 48 h if refrigerated.]

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
27	100	0
35	100	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L7
Temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between olanzapine and olanzapine related compound C, *System suitability solution*
Tailing factor: NMT 1.5 for the olanzapine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (µg/mL)
C_U = concentration of olanzapine in the *Sample solution* (µg/mL)
F = relative response factor for each impurity listed in *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Olanzapine lactam[a]	0.26	1.0	0.50
Olanzapine related compound B[b]	0.30	2.3	0.20
Olanzapine thiolactam[c]	0.34	1.0	0.50
Olanzapine related compound C[d]	0.83	1.0	0.50
Olanzapine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.20

[a](*Z*)-4-(4-Methylpiperazin-1-yl)-3-(2-oxopropylidene)-1*H*-benzo[b][1,4]diazepin-2(3*H*)-one.
[b]2-Methyl-10*H*-thieno-[2,3-*b*][1,5] benzodiazepin-4[5*H*]-one.
[c](*Z*)-1-{4-(4-Methylpiperazin-1-yl)-2-thioxo-1*H*-benzo[b][1,4]diazepin-3(2*H*)-ylidene}propan-2-one.
[d]2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[b]thieno[2,3-e][1,4]diazepine 4′-*N*-oxide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers, and at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Olanzapine RS
  USP Olanzapine Related Compound A RS
  USP Olanzapine Related Compound B RS
  USP Olanzapine Related Compound C RS■1S (*USP33*)

BRIEFING

Oxaliplatin Injection. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedures in the proposed monograph are based on analyses performed with the following columns.
1. *Assay*: Thermo Electron Corporation Hypersil C18 brand of L1 column. The typical retention time reported for oxaliplatin is about 8 min.
2. *Limit of Oxalic Acid*: Thermo Electron Corporation Hypersil BDS C18 brand of L1 column. The typical retention time reported for oxalic acid is about 4.5 min.
3. *Limit of (SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N′]platinum and Unspecified Impurities*: Thermo Electron Corporation Hypersil BDS C18 brand of L1 column. The typical retention time reported for (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum is about 18.5 min.

(MD-OOD: F. Mao; . MSA: R. Tirumalai.) RTS—C64375

Add the following:

■Oxaliplatin Injection

DEFINITION
Oxaliplatin Injection is a sterile solution of Oxaliplatin in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$).

In-Process Revision

IDENTIFICATION

- **A. Ultraviolet Absorption** ⟨197U⟩
  Sample solution: 100 µg/mL
  Medium: Water
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

[Note—All HPLC autosampler vials should be made of polypropylene.]

- **Procedure**
  Acidified water: Adjust pH of water to 3.0 with phosphoric acid.
  Mobile phase: Acetonitrile and *Acidified water* (1:99)
  System suitability solution: 0.1 mg/mL of USP Oxaliplatin RS and 0.1 mg/mL of USP Oxaliplatin System Suitability RS in water. [Note—USP Oxaliplatin System Suitability RS is [*SP*-4-2-(1*R-trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II).]
  Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water
  Sample solution: 0.1 mg/mL of oxaliplatin in water, from the combined contents of NLT three vials of Injection
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm column; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.2 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *System suitability solution*
  [Note—The relative retention times for USP Oxaliplatin System Suitability RS and oxaliplatin are 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between USP Oxaliplatin System Suitability RS and oxaliplatin
  Tailing factor: NMT 2.0, oxaliplatin peak
  Relative standard deviation: NML 1.0%, oxaliplatin peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_8H_{14}N_2O_4Pt$ in the portion of Injection taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of oxaliplatin in the *Standard solution* (mg/mL)
  C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)
  Acceptance criteria: 95.0%–105.0%

IMPURITIES

Organic Impurities

- **Procedure 1: Limit of Oxalic Acid** [Note—All HPLC autosampler vials should be made of polypropylene.]
  Solution A: Dissolve 1.36 g of monobasic potassium phosphate in 10 mL of 10% tetrabutylammonium hydroxide, dilute with water to 1 L, and adjust the pH to 6.0 with phosphoric acid.
  Mobile phase: Acetonitrile and *Solution A* (1:4)
  Standard solution: 35 µg/mL of USP Oxaliplatin Related Compound A RS in water. [Note—USP Oxaliplatin Related Compound A RS is available as dihydrate oxalic acid.]
  System suitability solution: 0.1 mg/mL of succinic acid in *Standard solution*
  Sample solution: Combined contents of NLT three vials of Injection
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm column; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 2 mL/min
  Injection size: 10 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  [Note—The relative retention times for succinic acid and oxalic acid are 0.8 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between succinic acid and oxalic acid, *System suitability solution*
  Tailing factor: Between 0.5 and 2.0, oxalic acid peak, *System suitability solution*
  Relative standard deviation: NML 3.0%, *Standard solution*
  Analysis
  Sample: *Standard solution* and *Sample solution*
  Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = response of oxalic acid from the *Sample solution*
  r_S = response of oxalic acid from the *Standard solution*
  C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)
  C_U = concentration of oxaliplatin in the *Sample solution* (mg/mL)
  M_{r1} = molecular weight of anhydrous oxalic acid, 90.03
  M_{r2} = molecular weight of oxaliplatin related compound A, 126.07
  Acceptance criteria
  Oxalic acid: NMT 0.30%
- **Limit of (*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and Unspecified Impurities**
  [Note—All HPLC autosampler vials should be made of polypropylene.]
  Solution A: Dissolve 1.36 g of monobasic potassium phosphate and 0.55 g of sodium heptanesulphonate in 1 L of water. Adjust to pH 3.0 with phosphoric acid.
  Solution B: Methanol and *Solution A* (19:81)
  Solution C: Methanol and *Solution A* (50.5:49.5)
  Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
45.0	0	100
45.5	100	0
53.0	100	0

  System suitability solution: 2 mg/mL of USP Oxaliplatin RS in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. [Note—The preparation of the *System suitability solution* forms (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and diaquodiaminocyclohexaneplatinum dimer.]
  Standard stock solution: Transfer a weighed quantity of USP Oxaliplatin Related Compound B RS into a suitable volumetric flask, add a volume of methanol equivalent to about 25% of the final volume, and sonicate for approximately 2 min to disperse the solids. Add a volume of 0.01 M nitric acid equivalent to about 65% of the final volume, and sonicate for approximately 30 min to dissolve. Allow to cool if

necessary, and dilute with 0.01 M nitric acid to volume to obtain a solution having a known concentration of about 0.125 mg/mL.

Standard solution: 31.25 µg/mL of USP Oxaliplatin Related Compound B RS in 0.01 M nitric acid, from *Standard stock solution*. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in *Standard solution* preparation.]

Sample solution: Combined contents of NLT three vials of Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 7.5-cm column; 3-µm packing L1

Column temperature: 10°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitabiluty solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between the peaks of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and diaquodiaminocyclohexaneplatinum dimer, *System suitability solution*.

Tailing factor: NMT 2.0, (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum peak, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = response of each impurity from the *Sample solution*

r_S = response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

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

M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30

M_{r2} = molecular weight of oxaliplatin related compound B, 433.28

F = relative response factor for each individual impurity, see *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 2.45%, *Limit of oxalic acid* and *Limit of (SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N′]platinum and Unspecified Impurities*

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
(*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum	1.0	1.0	0.65
Diaquodiaminocyclohexaneplatinum dimer[a]	1.4	2.5	0.50
Any individual unspecified impurity	—	4.0	0.20
Total unspecified impurities	—	—	1.00

[a](*SP*-4-2)-di-µ-Oxobis[(1*R*,2*R*)-cyclohexane-1,2-diamine-k*N*,k*N*′]diplatinum.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 1.0 USP Endotoxin Units/mg of oxaliplatin.
- **STERILITY TESTS ⟨71⟩:** It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to Be Examined*.
- **pH ⟨791⟩:** Between 4.0 and 7.0 using a polymer combination electrode
- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted with water for injection or a 5% dextrose solution. Oxaliplatin Injection must not be diluted in sodium chloride solutions or in chloride-containing solutions.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Endotoxin RS
  USP Oxaliplatin RS
  USP Oxaliplatin Related Compound A RS
  USP Oxaliplatin Related Compound B RS
  USP Oxaliplatin System Suitability RS■1S (*USP33*)

BRIEFING

Oxazepam Capsules, *USP 31* page 2868. On the basis of comments received, it is proposed to revise the *Dissolution* test to include instructions for the preparation of the *Standard solution* and the *Sample solution*. It was reported that oxazepam is not stable in the conditions currently used in the test.

(BPC: M. Marques.) RTS—C71173

Oxazepam Capsules

DEFINITION

Oxazepam Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{15}H_{11}ClN_2O_2$.

IDENTIFICATION

- The solution prepared for measurement of absorbance in the *Assay* exhibits a maximum at 229 ± 2 nm.

ASSAY

- **PROCEDURE**

Standard solution: 4 µg/mL of USP Oxazepam RS in alcohol

Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh. Transfer a portion of the mixed powder, nominally equivalent to 50 mg of oxazepam,

In-Process Revision

to a medium-porosity, sintered-glass funnel that is fitted into a small suction flask. Add 25 mL of alcohol, mix with the aid of a stirring rod, and after about 5 min apply gentle suction to remove the extract. Repeat the extraction with four additional 25-mL portions of alcohol, transfer the extracts to a 250-mL volumetric flask, and dilute with alcohol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with alcohol to volume.

Spectrometric conditions
Cell: 1 cm
Detector: UV 229 nm
Blank: Alcohol

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{11}ClN_2O_2$ in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the solution from the Capsules
A_S = absorbance of the solution from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩
Medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
~~**Standard solution:** USP Oxazepam RS at a known concentration in 0.1 N hydrochloric acid~~
~~[NOTE—A volume of methanol NMT 10% of the final total volume may be used to dissolve the USP Oxazepam RS.]~~
~~**Sample solution:** Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed, and filter.~~
■**Standard solution:** Prepare NMT 30 min before use. Transfer 20 mg of USP Oxazepam RS to a 200-mL volumetric flask, and dilute with methanol to volume. Transfer 10.0 mL to a 100-mL volumetric flask, and dilute with *Medium* to volume. Keep it at about 6° for the *Analysis.* This solution is stable for 72 h if kept refrigerated.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Keep it at about 6° for the *Analysis.*■1S (*USP33*)
Mobile phase: Methanol, water, and glacial acetic acid (60:40:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4-mm × 15-cm; packing L7
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for oxazepam peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{11}ClN_2O_2$ is dissolved in 60 min.
• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Oxazepam RS

BRIEFING

Pectin, *USP 31* page 2923. It is proposed to make the following revisions, on the basis of comments and data received and to align with the Pectins monograph in the *Food Chemicals Codex, 6th Edition*. Interested parties are encouraged to comment on the proposal.

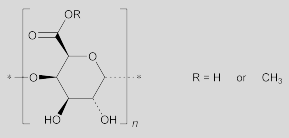
1. A chemical structure for this material is proposed.
2. The *Definition* for Pectin is updated.
3. The empirical *Identification* tests based on precipitations are replaced by a highly specific *Identification* procedure using Pectate Lyase.
4. The *Assay* for *Methoxy Groups* is replaced by the *Assay* for *Degree of Esterification* and subsequently the *Assay* for *Methoxy Groups* is added. In both procedures, a calculation formula is provided.
5. In the *Assay* for *Degree of Esterification*, 0.1 N sodium hydroxide VS is used instead of 0.5 N sodium hydroxide VS, in order to improve the precision for titration using sodium hydroxide.
6. The *Assay* for *Galacturonic Acid* is accordingly updated and a calculation formula is provided.
7. The *Lead* test is replaced by a *Lead* test using ICP, based on the method published in the *Food Chemicals Codex, 6th Edition*.
8. A test for *Sulfur Dioxide* is proposed in the monograph. See the revision to *Sulfur Dioxide* ⟨525⟩, proposed elsewhere in this issue of *PF*.
9. A test for *Methanol, Ethanol, and Isopropanol* is added to the monograph. Thus, USP Methyl Alcohol RS, USP Alcohol RS, USP 2-Propanol RS, and USP 2-Butanol RS are introduced into the monograph. The gas chromatographic procedure is based on analysis performed with the Phenomenex Zebron ZB-624 brand of capillary column or Varian CP-PoraBond Q brand of capillary column. The typical retention times for methanol, ethanol, 2-propanol, and 2-butanol are 2.1, 2.5, 2.8, and 4.2 min, respectively.
10. The *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩ specifications are revised to be consistent with the general requirement for drug substances and excipients recommended by the USP Microbiology and Sterility Assurance Expert Committee.
11. A storage condition is proposed in the *Packaging and Storage* section.
12. The *Labeling* section is updated to align with proposed changes to the *Definition* section.
13. A *USP Reference Standards* section is added.

Other changes are editorial in nature.

(EM2: H. Wang. MSA: R. Tirumalai.) RTS—C40252

Pectin

Change to read:

■


■1S (*USP33*)

Pectin [9000-69-5].

In-Process Revision

DEFINITION

Change to read:

~~Pectin is a purified carbohydrate product obtained from the dilute acid extract of the inner portion of the rind of citrus fruits or from apple pomace. It consists chiefly of partially methoxylated polygalacturonic acids.~~

~~Pectin yields NLT 6.7% of methoxy groups (=OCH_3) and NLT 74.0% of galacturonic acid ($C_6H_{10}O_7$), calculated on the dried basis.~~

~~[NOTE—Commercial pectin for the production of jellied food products is standardized to the convenient "150 jelly grade" by addition of dextrose or other sugars, and sometimes contains sodium citrate or other buffer salts. This monograph refers to the pure pectin to which no such additions have been made.]~~

■Pectin is a purified carbohydrate polymer consisting mainly of a linear backbone of partially methoxylated alpha (1-4) linked D-galacturonic acid. It is obtained from the dilute acid extract of the rind of citrus fruits or from apple pomace. No organic solvents other than methanol, ethanol, and isopropanol are used during its production. Pectin yields NLT 74.0% of galacturonic acid ($C_6H_{10}O_7$), calculated on the dried basis.

[NOTE—Commercial pectin for the production of jellied food products is standardized to the convenient "150 jelly grade" by addition of dextrose or other sugars, and sometimes contains sodium citrate or other buffer salts. This monograph refers to the pectin to which no such additions have been made.]■1S (*USP33*)

IDENTIFICATION

Delete the following:

~~■• **A.** Heat 1 g with 9 mL of water on a steam bath until a solution is formed, replacing water lost by evaporation. it forms a stiff gel on cooling.~~■1S (*USP33*)

Delete the following:

~~■• **B.** To a 1:100 solution add an equal volume of alcohol. a translucent, gelatinous precipitate is formed (distinction from most gums).~~■1S (*USP33*)

Delete the following:

~~■• **C.** To a 1:100 solution add 2 N sodium hydroxide (5:1), and allow to stand at room temperature for 15 min. a gel or semigel forms (distinction from tragacanth).~~■1S (*USP33*)

Delete the following:

~~■• **D.** Acidify the gel from the preceding test with 3 N hydrochloric acid, and shake. a voluminous, colorless, gelatinous precipitate forms, which upon boiling becomes white and flocculent (pectic acid).~~■1S (*USP33*)

Add the following:

■• **PROCEDURE**

Sample stock solution: Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a suitable container, and moisten with 250 µL of 2-propanol. Add 50 mL of water to the container, and mix the solution using a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the pH of the solution to 12, stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 min. Adjust the pH of the solution to 7.0 using 0.5 N hydrochloric acid. Dilute with water to 100 mL.

Tris buffer solution: Transfer 6.055 g of Tris(hydroxylmethyl)aminomethane and 0.147 g of calcium chloride ($CaCl_2 \cdot H_2O$) to a 1000-mL volumetric flask containing 950 mL of water. Adjust the pH of the solution to 7.0 with 1 N hydrochloric acid. Dilute with water to volume.

Enzyme solution: Mix pectate lyase[1] with *Tris buffer solution* to make a solution (1 in 100).

Sample blank: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, and 1.0 mL of water in a quartz cuvette.

Enzyme blank: Mix 0.5 mL of *Tris buffer solution*, 1.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Sample solution: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, 0.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Analysis

Samples: *Enzyme blank*, *Sample blank*, and *Sample solution*

Perform the test with the *Samples* using a suitable ultraviolet/visible spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩) and using water as a blank. Measure the absorbance at 235 nm immediately after mixing the solutions well, and record the value at time 0 for the *Enzyme blank*, $A_{0\text{-}EB}$; for the *Sample blank*, $A_{0\text{-}TB}$; and for the *Sample solution*, $A_{0\text{-}TS}$. After incubation at room temperature for 10 min, determine the absorbance again at 235 nm for the *Enzyme blank*, $A_{10\text{-}EB}$; for the *Sample blank*, $A_{10\text{-}TB}$; and for the *Sample solution*, $A_{10\text{-}TS}$. Calculate the corrected absorbance A_0 at time 0 and the corrected absorbance A_{10} at 10 min:

$$A_0 = A_{0\text{-}TS} - (A_{0\text{-}EB} + A_{0\text{-}TB})$$

$$A_{10} = A_{10\text{-}TS} - (A_{10\text{-}EB} + A_{10\text{-}TB})$$

Calculate the quantity of unsaturated product produced:

$$\text{Result} = (A_{10} - A_0)/(\varepsilon_{235} \times L)$$

ε_{235} = molar extinction coefficient of the reaction product (4600 $M^{-1} \cdot cm^{-1}$)

L = path length of the reaction cuvette, 1 cm

Acceptance criteria: The amount of unsaturated product is NLT 0.5×10^{-5} M.■1S (*USP33*)

ASSAY

Change to read:

• ~~**METHOXY GROUPS**~~■**DEGREE OF ESTERIFICATION**■1S (*USP33*)

Sample: 5.0 g

Analysis: Transfer the *Sample* to a suitable beaker, and stir for 10 min with a mixture of 5 mL of hydrochloric acid and 100 mL of 60% alcohol. Transfer to a sintered-glass filter (30- to 60-mL crucible or Büchner type, coarse), and wash with six 15-mL portions of the hydrochloric acid–60% alcohol mixture, followed by 60% alcohol until the filtrate is free from chlorides. Finally wash with 20 mL of alcohol, dry for 1 h at 105°, cool, and weigh. Transfer exactly one-tenth of the total net weight of the dried *Sample* (representing 500 mg of the original unwashed *Sample*) to a 250-mL conical flask, and moisten with 2 mL of alcohol. Add 100 mL of carbon dioxide-free water, insert the stopper, and swirl occasionally until the Pectin is completely dissolved. ~~Add 5 drops of phenolphthalein TS, titrate with 0.5 N sodium hydroxide VS, and record the results as the initial titer. Add 20.0 mL of 0.5 N sodium hydroxide VS, insert the stopper, shake vigorously, and allow to stand for 15 min. Add 20.0 mL of 0.5 N hydrochloric acid VS, and shake until the pink color disappears. Add phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS to a faint~~

[1] A suitable pure enzyme is available from Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland (www.megazyme.com).

In-Process Revision

~~pink color that persists after vigorous shaking: record this value as the saponification titer. Each mL of 0.5 N sodium hydroxide used in the saponification titer is equivalent to 15.52 mg of $-OCH_3$.~~■Add 5 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Record the results as the initial titer, V_I (mL). Add 20.0 mL of 0.5 N sodium hydroxide VS, insert the stopper, shake vigorously, and allow to stand for 15 min. Add 20.0 mL of 0.5 N hydrochloric acid VS, and shake until the pink color disappears. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a faint pink color that persists after vigorous shaking. Perform a blank determination, and make any necessary correction. Record this value as the saponification titer, V_S (mL). Calculate the degree of esterification:

$$\text{Result} = [V_S/(V_I + V_S)] \times 100$$ ■1S *(USP33)*

Acceptance criteria: ~~NLT 6.7%~~■The value for *Degree of Esterification* is within the range stated on the label.■1S *(USP33)*

Change to read:

• **GALACTURONIC ACID:** ~~Each mL of 0.5 N sodium hydroxide used in the total titration (the initial titer added to the saponification titer) in the *Assay* for *Methoxy Groups* is equivalent to 97.07 mg of $C_6H_{10}O_7$.~~■Each mL of 0.1 N sodium hydroxide used in the total titration (the initial titer added to the saponification titer) in the *Assay* for *Degree of Esterification* is equivalent to 19.41 mg of $C_6H_{10}O_7$. Calculate the percentage of galacturonic acid in the portion of Pectin taken:

$$\text{Result} = 19.41 \times [(V_I + V_S)/W] \times 100$$

W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

■1S *(USP33)*

Acceptance criteria: NLT 74.0%

Add the following:

■• **METHOXY GROUPS:** Each mL of 0.1 N sodium hydroxide used in the saponification titer in the *Assay* for *Degree of Esterification* is equivalent to 3.10 mg of $-OCH_3$. Calculate the percentage of methoxy groups in the portion of Pectin taken:

$$\text{Result} = 3.10 \times (V_S/W) \times 100$$

W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

Acceptance criteria: The percentage of methoxy groups is within the range stated on the label.■1S *(USP33)*

IMPURITIES

Change to read:

Inorganic Impurities

• **ARSENIC,** *Method II* ⟨**211**⟩**:** NMT 3 ppm

• ~~**LEAD** ⟨251⟩~~

~~**Sample:** 2.0 g~~

~~**Analysis:** Add the *Sample* to 20 mL of nitric acid in a 250-mL conical flask, mix, and heat the contents carefully until the Pectin is dissolved. Continue the heating until the volume is reduced to about 7 mL. Cool rapidly to room temperature, transfer to a 100-mL volumetric flask, and dilute with water to volume. Transfer 50 mL and follow the *Procedure* outlined in *Lead* ⟨251⟩ with the following exceptions. Use 15 mL of *Ammonium Citrate Solution*, 3 mL of *Potassium Cyanide Solution*, and 500 µL of *Hydroxylamine Hydrochloride Solution*. After the first dithizone extractions, wash the combined chloroform layers with 5 mL of water, discarding the water layer and continuing in the usual manner by extracting with 20 mL of dilute nitric acid (1 in 100).~~

~~**Acceptance criteria:** NMT 5 µg of lead (corresponding to NMT 5 ppm)~~

■• **LEAD**

Standard stock solution: 1000 µg/mL of lead. [NOTE—Use a commercially available certified solution.]

Standard solution: 2 µg/mL of lead, prepared immediately before use by pipetting 0.10 mL of *Standard stock solution* into a 50-mL volumetric flask containing 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Reference solution: 0.4 µg/mL of lead, prepared by pipetting 5.0 mL of the *Standard solution* into a 25-mL volumetric flask containing 10 mL of water, 2 mL of 20% hydrochloric acid, and 2 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Blank solution: Add 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA into a 50-mL volumetric flask. Dilute with water to volume, and mix.

Sample solution: Transfer 2.0 g of Pectin to a clean, 100-mL glass beaker, add 25 mL of 70% nitric acid, cover with a watch glass, and heat at low to moderate heat on a hot plate in a fume hood for 2 h. Remove the watch glass, and continue to heat until the sample is dry with no visible fumes. Add 0.5 mL of 70% nitric acid, and heat to dryness. Cool to room temperature, and add 2 mL of 20% hydrochloric acid and 2 mL of 0.1 M EDTA. Quantitatively transfer the solution to a 25-mL volumetric flask, dilute with water to volume, and mix.

Analysis: Lead is determined using an inductively coupled plasma–atomic emission spectrometer (ICP–AES) (see *Plasma Spectrochemistry* ⟨730⟩) by measuring the emission at 220.35 nm with the settings optimized as directed by the manufacturer.

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Calibrate the instrument with the *Blank solution* and the *Standard solution*. Then analyze the *Reference solution* and the *Sample solution*.

Acceptance criteria: The concentration in the *Sample solution* is NMT that in the *Reference solution*, corresponding to NMT 5 ppm of lead.■1S *(USP33)*

Change to read:

Organic Impurities

• **PROCEDURE 1: SUGARS AND ORGANIC ACIDS**

Sample: 1 g

Analysis: ~~Moisten the *Sample* with 3–5 mL of alcohol, pour in rapidly 100 mL water, shake, and allow to stand until the solution is complete. To this solution add 100 mL of alcohol containing 0.3 mL of hydrochloric acid, mix, and filter rapidly. Measure 25 mL of the filtrate into a tared dish, evaporate the liquid on a steam bath, and dry the residue in a vacuum oven at 50° for 2 h.~~ ■Place the *Sample* in a 500-mL flask, moisten with 3–5 mL of alcohol, rapidly pour in 100 mL of water, shake, and allow to stand until the solution is complete. To this solution add 100 mL of alcohol containing 0.3 mL of hydrochloric acid, mix, and filter rapidly. Measure 25 mL of the filtrate into a tared dish, evaporate the liquid on a steam bath, and dry the residue in a vacuum oven at 50° for 2 h.■1S *(USP33)*

Acceptance criteria: The weight of the residue does not exceed 20 mg, ■corresponding to NMT 16% of sugars and organic acids.■1S *(USP33)*

In-Process Revision

■• **PROCEDURE 2: SULFUR DIOXIDE,** *Method V* ⟨**525**⟩
Sample: 100 g
Analysis: Suspend the *Sample* in 500 mL of methanol, then transfer this mixture to the flask (C). Prepare a mixture of 20 mL of hydrochloric acid and 10 mL of water, and transfer it to the separatory funnel (B). Add 10 mL of hydrogen peroxide solution to the vessel (G). Perform the refluxing for 2 h before removing the vessel (G).
Acceptance criteria: NMT 50 ppm

• **PROCEDURE 3: METHANOL (METHYL ALCOHOL), ETHANOL (ALCOHOL), AND ISOPROPANOL (2-PROPANOL)**
[NOTE—Residual alcohols are volatile. They should be stored in a cool and dry place. When preparing the *Standard stock solution*, *Standard solutions*, and *Internal standard stock solution* for residual alcohols, mix thoroughly and keep the solutions at 20° when diluting with water to volume.]
Standard stock solution: 5000 µg/mL each for USP Methyl Alcohol RS, USP Alcohol RS, and USP 2-Propanol RS
Internal standard stock solution: 5000 µg/mL of USP 2-Butanol RS. [NOTE—This solution can be stored at 5–8° for 3 months.]
Standard solution: To a 250-mL volumetric flask add 2.5 mL of *Standard stock solution* and 2.5 mL of *Internal standard stock solution*. Dilute with water to volume, and mix. This solution contains 50 µg/mL each for USP Methyl Alcohol RS, USP Alcohol RS, USP 2-Propanol RS, and USP 2-Butanol RS. [NOTE—This solution can be stored at 5–8° for 3 months.] Transfer 1.0 g of this solution to a 10-mL headspace vial.
Sample solution: Transfer 1.0 g of Pectin and 5 g of sucrose to a stoppered 100-mL Erlenmeyer flask containing 90 mL of water, add 1.0 mL of *Internal standard stock solution*, and dilute with water to 100 mL. Mix the solution using a magnetic stirrer. Continue stirring until all Pectin has been completely dissolved; typically it takes about 1–2 h. This solution contains 50 µg/mL for USP 2-Butanol RS. Transfer 1.0 g of this solution to a 10-mL headspace vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: Headspace GC
Detector: Flame ionization
Column: 0.32-mm × 30-m capillary column; 1.8-µm layer of phase G43. [NOTE—An alternative column such as a 0.32-mm × 25-m capillary column bonded with a 5-µm layer of phase S3 can be used as long as the system suitability requirements are met.]
Temperature
Detector: 280°
Column: 70°
Injection port: 200°
Carrier gas: Nitrogen
Flow rate: 1.5 mL/min
Make up gas: Nitrogen
Split flow rate: 30 mL/min
Injection size: 1 mL (the gaseous headspace)
Injection type: Split ratio 20:1
Balanced pressure automatic headspace sampler
Equilibration time: 10 min
Equilibration temperature: 70°
Agitation speed: 500 rpm
Agitation on time: 5 s
Agitation off time: 90 s
Syringe tempertature: 80°
Syringe size: 2.5 mL
Fill speed: 100 µL/s
Pull-up delay: 2.0 s
GC run time: 10.5 min
[NOTE—These GC conditions should be optimized according to the instruments used.]

System suitability
Sample: *Standard solution*
[NOTE—See the relative retention times table below.]

Component	Relative Retention Time (RRT)
Methanol	0.5
Ethanol	0.6
2-Propanol	0.7
2-Butanol	1.0

Suitability requirements
Resolution: NLT 1.5, between each pair of analytes
Relative standard deviation: NMT 10%, determined from each analyte
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methanol, ethanol, and 2-propanol in the portion of Pectin taken:

$$\text{Result} = (R_U/R_S) \times (C/W) \times F \times V \times 100$$

R_U = ratio of the peak area of the respective alcohol to the peak area of the internal standard peak from the *Sample solution*
R_S = ratio of the peak area of the respective alcohol to the peak area of the internal standard peak from the *Standard solution*
C = concentration of the respective residual alcohol (methanol, or ethanol, or 2-propanol) in the *Standard solution* (µg/mL)
W = weight of Pectin taken to prepare the *Sample solution* (g)
F = conversion factor (10^{-6} g/µg)
V = volume of the *Sample solution*, 100 mL
Acceptance criteria: NMT 1% for total methanol, ethanol, and isopropanol■1S *(USP33)*

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩**:** ~~It meets the requirements of the test for absence of *Salmonella* species.~~■The total aerobic microbial count is NMT 1000 cfu/g, and the total combined molds and yeasts count is NMT 100 cfu/g.■1S *(USP33)*

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 3 h: it loses NMT 10.0% of its weight.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ■Store in a cool and dry place.■1S *(USP33)*

Change to read:

• **LABELING:** Label it to indicate whether it is of apple or of citrus origin. ■Label it to indicate the range of *Degree of Esterification* and the range of the percentage of methoxy groups. The

In-Process Revision

labeling also indicates the presence of sulfur dioxide if the residual sulfur dioxide concentration is greater than 10 ppm.■1S *(USP33)*

Add the following:

■• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alcohol RS
USP 2-Butanol RS RS
USP Methyl Alcohol RS
USP 2-Propanol RS■1S *(USP33)*

BRIEFING

Praziquantel Tablets, *USP 32* page 3363. It is proposed to include a *Dissolution* test for veterinary products. The chromatographic procedure in this test was validated using an Ultracarb ODS brand of packing L1.

(BPC: M. Marques.) RTS—C62946

Praziquantel Tablets

DEFINITION
Praziquantel Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{19}H_{24}N_2O_2$.

IDENTIFICATION
• **PROCEDURE**
Sample solution: Equivalent to 30 mg of praziquantel from a quantity of powdered Tablets, in a centrifuge tube. Add 5 mL of methanol, agitate for 5 min, and centrifuge. Use the clear supernatant.
Standard solution: 6 mg/mL of USP Praziquantel RS in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate
Analysis
Samples: *Standard solution* and *Sample solution*
Apply separately the *Sample solution* and *Standard solution*, each as 1-cm wide bands, to a chromatographic plate. Proceed as directed in the general chapter. Develop the chromatogram in an unsaturated chamber, using the *Developing solvent system*, until the solvent front has moved 8 cm. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal band of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (3:2)
Standard solution: 0.18 mg/mL of USP Praziquantel RS in *Mobile phase*
Sample stock solution: Transfer an equivalent to 150 mg of praziquantel, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 70 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, mix, and filter.
Sample solution: Transfer 3.0 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{24}N_2O_2$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Praziquantel RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of praziquantel in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
■For products for human use■1S *(USP33)*
Medium: 0.1 N hydrochloric acid containing 2.0 mg of sodium lauryl sulfate per mL; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
~~**Sample solutions:** Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution.*~~
~~**Standard stock solution:** L/90 mg/mL of USP Praziquantel RS in methanol. [NOTE—L is the labeled quantity of praziquantel in each Tablet, in mg.]~~
~~**Standard solution:** 5.0 mL of *Standard solution* in *Medium*~~
■**Sample solution:** Pass a portion of the solution under test through a suitable 0.45-µm filter.
Standard solution: Prepare a solution of USP Praziquantel RS in methanol containing L/90 mg/mL, where L is the tablet label claim in mg. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Detector: UV 263 nm
Blank: *Medium*■1S *(USP33)*
Tolerances: NLT 75% (Q) of the labeled amount of $C_{19}H_{24}N_2O_2$ is dissolved in 60 min.
■For products for veterinary use
Medium: 0.1 N hydrochloric acid containing 2.0 mg of sodium lauryl sulfate per mL; 900 mL
Apparatus 2: 50 rpm, with peak vessels
Time: 60 min
Mobile phase: Acetonitrile and water (3:2)
Diluent: Mix 600 mL of acetonitrile with 400 mL of pH 7.4 phosphate buffer.
Standard solution: Prepare a solution containing 0.02 mg of USP Praziquantel RS per mL in methanol. Transfer 10.0 mL to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 4.0 mL to a 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, and dilute with *Diluent* to volume.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC
Detector: UV 210 nm
Column: 4.6 mm × 25 cm, 10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis: Percentage of praziquantel dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = tablet label claim (mg)
Tolerances: NLT 75% (Q) of the labeled amount of $C_{19}H_{24}N_2O_2$ is dissolved in 60 min.■1S *(USP33)*

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Praziquantel RS

BRIEFING

Promethazine Hydrochloride and Codeine Phosphate Oral Solution. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which codeine elutes at about 21 min and promethazine elutes at about 39 min. The liquid chromatographic procedure in *Procedure 1* under *Organic Impurities* was validated with a Waters Symmetry brand of L1 column, in which promethazine elutes at about 10 min. The liquid chromatographic procedure in *Procedure 2* under *Organic Impurities* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which promethazine elutes at about 39 min.

(MD-CCA: D. Vicchio, C. Anthony. MSA: R. Tirumalai.)
RTS—C62808

Add the following:

■Promethazine Hydrochloride and Codeine Phosphate Oral Solution

DEFINITION
Promethazine Hydrochloride and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of both promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot {}^1/_2H_2O$). It may contain suitable preservatives.

IDENTIFICATION
• The retention times of the promethazine hydrochloride and codeine phosphate peaks of the sample solutions correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—At all times, protect the sample and standard solutions by using low-actinic glassware.]
Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water, carefully add 50 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.
Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 500 mL of water, carefully add 500 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS and 2.8 mg/mL of USP Codeine Phosphate RS in *Solution A*
Standard stock solution B: Dissolve USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS into a volume of acetonitrile equivalent to 40% of the total volume of the flask. Dilute with *Solution A* to volume, to obtain a solution containing 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS.
Standard solution: Transfer 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.28 mg/mL of USP Codeine Phosphate RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS.
Promethazine hydrochloride sample solution: Equivalent to 0.18 mg/mL, based on the label claim, of promethazine hydrochloride in *Solution A*
Codeine phosphate sample solution: Equivalent to 0.28 mg/mL, based on the label claim, of codeine phosphate in *Solution A*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben
Relative standard deviation: NMT 2.0% for both promethazine and codeine
Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Calculate the quantity, in percent of label claim, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of sample taken:

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

In-Process Revision

r_U = promethazine peak response from the *Promethazine hydrochloride sample solution*
r_S = promethazine peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Codeine phosphate sample solution*

Calculate the quantity, in percent of label claim, of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot {}^1/_2H_2O$ in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (Mr_1/Mr_2) \times 100$$

r_U = codeine peak response from the *Codeine phosphate sample solution*
r_S = codeine peak response from the *Standard solution*
C_S = concentration, on the dried basis, of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Codeine phosphate sample solution* (mg/mL)
Mr_1 = molecular weight of codeine phosphate, 406.37
Mr_2 = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0% for both promethazine hydrochloride and codeine phosphate

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

[NOTE—At all times, protect the sample and standard solutions by using low-actinic glassware.]

Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust the pH to 6.4 with phosphoric acid.

5 N phosphoric acid: Carefully dilute 12 mL of phosphoric acid with water to 100 mL.

Diluent: Methanol, water, and *5 N phosphoric acid* (5:95:1)

Mobile phase: Acetonitrile, methanol, and *Solution C* (3:2:5)

Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*, from the *Standard solution* [NOTE—Prepare at time of use.]

Promethazine hydrochloride sample solution: Equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: 2 times the retention time of promethazine for Standards; 6 times the retention time of promethazine for samples

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

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

Relative standard deviation: NMT 7.5% for the promethazine peak, *Standard solution*

Sensitivity: Signal-to-noise NLT 10 for the promethazine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

[a]10-Methylphenothiazine.

• **PROCEDURE 2**

[NOTE—At all times, protect the sample and standard solutions by using low-actinic glassware.]

Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay.*

Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*

Standard stock solution B: Proceed as directed in the *Assay.*

Standard solution: Transfer 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* into a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS.

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*, from *Standard stock solution A* [NOTE—Prepare at time of use.]

Promethazine hydrochloride sample solution: Proceed as directed in the *Assay.*

[NOTE—Use this solution to determine promethazine specified and unidentified impurities, and any unspecified impurities.]

Codeine phosphate sample solution: Proceed as directed in the *Assay.*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 50 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]

Suitability requirements

Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben, *Standard solution*

Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*

Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Using the data in *Impurity Table 2*, calculate the percentage of promethazine specified and unidentified impurities, and any unspecified impurities, in the portion of sample taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Samples: *Standard solution* and *Codeine phosphate sample solution*

Identify the codeine impurities using the relative retention times given in *Impurity Table 2*. Calculate the percentage of each codeine impurity in the portion of sample taken:

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

r_U = peak response of the impurity from the *Codeine phosphate sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Codeine phosphate sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Acceptance criteria

Individual impurities: See *Impurity Tables 1* and *2*.
Total codeine impurities: NMT 1.0%
Total unspecified impurities: NMT 1.5%
Total specified and unidentified promethazine impurities: NMT 5.0%
Total impurities: NMT 7.5% [NOTE—Sum of total codeine impurities, total promethazine specified and unidentified impurities, and *Total unspecified impurities*.]

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial limit does not exceed 100 cfu/mL. The total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements for absence of *Escherichia coli.*
- **pH** ⟨**791**⟩**:** 4.1–5.1
- **ALCOHOL DETERMINATION** (if present)**,** *Method II* ⟨**611**⟩**:** Between 90.0% and 110.0% of the labeled quantity of C_2H_5OH
- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Codeine Phosphate RS
  USP Methylparaben RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS

Impurity Table 2

Name	Source	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Codeine *N*-oxide[a]	Codeine	0.52	0.83	0.2
Norcodeine[b]	Codeine	0.53	0.83	0.2
Codeine	—	0.54	—	—
Codeinone[c]	Codeine	0.61	0.63	0.3
Codeine methyl ether[d]	Codeine	0.67	—	—
Total codeine impurities	Codeine	—	—	1.0
Phenothiazine sulfoxide[e]	Promethazine	0.71	1.0	0.5
Promethazine sulfoxide[f]	Promethazine	0.72	3.3	2.8
Unidentified impurity 1	Promethazine	0.77	1.0	0.2
Methylphenothiazine sulfoxide[g]	Promethazine	0.81	1.0	0.5
Unidentified impurity 2	Promethazine	0.85	1.0	0.2
Phenothiazinone[h]	Promethazine	0.98	10.0	0.2
Desmethyl promethazine[i]	Promethazine	0.99	1.0	0.2
Promethazine	—	1.0	—	—
Isopromethazine[j]	Promethazine	1.01	—	—
Unidentified impurity 3	Promethazine	1.09	1.0	0.2
Unidentified impurity 4	Promethazine	1.16	1.0	0.2

[a]Morphinan-6-ol, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl,17-oxide-(5α,6α).
[b]Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-(5α,6α).
[c]Morphinan-6-one, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl-(5α).
[d]Cocaine synthetic impurity. Do not quantify.
[e]10*H*-Phenothiazine sulfoxide.
[f]*N*,*N*-Dimethyl-1(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[g]10-Methyl-10*H*-phenothiazine sulfoxide.
[h]1*H*-Phenothiazin-1-one.
[i]*N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[j]Promethazine synthetic impurity. Do not quantify.
[k]Includes phenothiazine and methylphenothiazine from *Procedure 1*.

In-Process Revision

Impurity Table 2 *(continued)*

Total promethazine specified and unidentified impurities	Promethazine	—	—	5.0[k]
Any unspecified impurity	—	—	1.0	0.2
Total unspecified impurities	—	—	—	1.5

[a]Morphinan-6-ol, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl,17-oxide-(5α,6α).
[b]Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-(5α,6α).
[c]Morphinan-6-one, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl-(5α).
[d]Cocaine synthetic impurity. Do not quantify.
[e]10*H*-Phenothiazine sulfoxide.
[f]*N*,*N*-Dimethyl-1(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[g]10-Methyl-10*H*-phenothiazine sulfoxide.
[h]1*H*-Phenothiazin-1-one.
[i]*N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[j]Promethazine synthetic impurity. Do not quantify.
[k]Includes phenothiazine and methylphenothiazine from *Procedure 1*.

■1S (*USP33*)

BRIEFING

Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure for the *Assay* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which dextromethorphan elutes at about 36 min and promethazine elutes at about 39 min. The liquid chromatographic procedure in *Procedure 1* under *Organic Impurities* was validated with a Waters Symmetry brand of L1 column, in which promethazine elutes at about 10 min. The liquid chromatographic procedure in *Procedure 2* under *Organic Impurities* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which promethazine elutes at about 39 min.

(MD-CCA: D. Vicchio, C. Anthony. MSA: R. Tirumalai.) RTS—C62808

Add the following:

■Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution

DEFINITION

Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of both promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) and dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$). It may contain suitable preservatives.

IDENTIFICATION

- The retention times of the promethazine hydrochloride and dextromethorphan hydrobromide peaks of the sample solutions correspond to those of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]

Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water, carefully add 50 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.
Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 500 mL of water, carefully add 500 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS and 4.2 mg/mL of USP Dextromethorphan Hydrobromide RS in *Solution A*
Standard stock solution B: Dissolve USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS into a volume of acetonitrile equivalent to 40% of the total volume of the flask. Dilute with *Solution A* to volume to obtain a solution containing 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS.
Standard solution: Transfer 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.42 mg/mL of anhydrous USP Dextromethorphan Hydrobromide RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS.
Promethazine hydrochloride sample solution: Equivalent to 0.18 mg/mL of promethazine hydrochloride in *Solution A*
Dextromethorphan hydrobromide sample solution: Equivalent to 0.42 mg/mL of dextromethorphan hydrobromide in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]

In-Process Revision

Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben
Relative standard deviation: NMT 2.0% for both promethazine and dextromethorphan
Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Calculate the quantity, in percent of label claim, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of sample taken:

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

r_U = promethazine peak response from the *Promethazine hydrochloride sample solution*
r_S = promethazine peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Dextromethorphan hydrobromide sample solution*
Calculate the quantity, in percent of label claim, of $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (Mr_1/Mr_2) \times 100$$

r_U = dextromethorphan peak response from the *Dextromethorphan hydrobromide sample solution*
r_S = dextromethorphan peak response from the *Standard solution*
C_S = concentration, on the anhydrous basis, of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Dextromethorphan hydrobromide sample solution* (mg/mL)
Mr_1 = molecular weight of dextromethorphan hydrobromide monohydrate, 370.32
Mr_2 = molecular weight of anhydrous dextromethorphan hydrobromide, 352.32

Acceptance criteria: 90.0%–110.0% for both promethazine hydrochloride and dextromethorphan hydrobromide

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]
Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust the pH to 6.4 with phosphoric acid.
5 N phosphoric acid: Carefully dilute 12 mL of phosphoric acid with water to 100 mL.
Diluent: Methanol, water, and *5 N phosphoric acid* (5:95:1)
Mobile phase: Acetonitrile, methanol, and *Solution C* (3:2:5)
Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*, prepared from the *Standard solution.* [NOTE—Prepare at time of use.]
Promethazine hydrochloride sample solution: Equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
Run time: 2 times the retention time of promethazine for Standards; 6 times the retention time of promethazine for samples
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.5 for the promethazine peak, *Standard solution*
Relative standard deviation: NMT 7.5% for the promethazine peak, *Standard solution*
Sensitivity: Signal to noise NLT 10 for the promethazine peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

[a]10-Methylphenothiazine.

• **PROCEDURE 2**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]
Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay.*
Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*
Standard stock solution B: Proceed as directed in the *Assay.*
Standard solution: Transfer 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* into a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS.
Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A.* Prepared from *Standard stock solution A.* [NOTE—Prepare at time of use.]
Promethazine hydrochloride sample solution: Proceed as directed in the *Assay.*
[NOTE—Use this solution to determine promethazine related compounds and specified and unknown impurities.]
Dextromethorphan hydrobromide sample solution: Proceed as directed in the *Assay.*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL

System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben, *Standard solution*
Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*
Signal to noise ratio: NLT 10 for promethazine, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Using the data in *Impurity Table 2*, calculate the percentage of promethazine specified and unidentified impurities, and any unspecified impurities in the portion of sample taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Samples: *Standard solution* and *Dextromethorphan hydrobromide sample solution*
Identify the dextromethorphan impurities using the relative retention times given in *Impurity Table 2*. Calculate the percentage of each dextromethorphan impurity in the portion of sample taken:

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

r_U = peak response of the impurity from the *Dextromethorphan hydrobromide sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Dextromethorphan hydrobromide sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Acceptance criteria
Individual impurities: See *Impurity Tables 1* and *2*.
Total unspecified impurities: NMT 1.0%
Total specified and unidentified promethazine impurities: NMT 5.0%
Total impurities: NMT 6.2% [NOTE—Total includes phenothiazine and methylphenothiazine from *Procedure 1* and dextromethorphanone.]

Impurity Table 2

Name	Source	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Phenothiazine sulfoxide[a]	Promethazine	0.71	1.0	0.5
Promethazine sulfoxide[b]	Promethazine	0.72	3.3	2.8
Unidentified impurity 1	Promethazine	0.77	1.0	0.2
Methylphenothiazine sulfoxide[c]	Promethazine	0.81	1.0	0.5
Unidentified impurity 2	Promethazine	0.85	1.0	0.2
Dextromethorphanone[d]	Dextromethorphan	0.89	5.0	0.2
Dextromethorphan	—	0.91	—	—
3-Methoxymorphinan	Dextromethorphan	0.92	—	—
Phenothiazinone[e]	Promethazine	0.98	10.0	0.2
Desmethyl promethazine[f]	Promethazine	0.99	1.0	0.2
Promethazine	—	1.0	—	—
Isopromethazine[g]	Promethazine	1.01	—	—
Unidentified impurity 3	Promethazine	1.09	1.0	0.2
Unidentified impurity 4	Promethazine	1.16	1.0	0.2
Total specified and unidentified promethazine impurities	Promethazine	—	—	5.0[h]
Any unspecified impurity	—	—	1.0	0.2
Total unspecified impurities	—	—	—	1.0

[a]10*H*-Phenothiazine sulfoxide.
[b]*N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[c]10-Methyl-10*H*-phenothiazine sulfoxide.
[d]3-Methoxy-17-methyl-9α,13α,14α-morphinan-10-one.
[e]1*H*-Phenothiazin-1-one.
[f]*N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[g]Promethazine synthetic impurity. Do not quantify.
[h]Includes phenothiazine and methylphenothiazine from *Procedure 1*.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial limit does not exceed 100 cfu/mL. The total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements for absence of *Escherichia coli.*
- **PH** ⟨**791**⟩**:** 4.1–5.1
- **ALCOHOL DETERMINATION** (if present)**,** *Method II* ⟨**611**⟩**:** Between 90% and 110% of the labeled quantity of C_2H_5OH
- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Dextromethorphan Hydrobromide RS
  USP Methylparaben RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS■1S (*USP33*)

BRIEFING

Promethazine and Phenylephrine Hydrochloride Oral Solution. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which phenylephrine elutes at about 16 min and promethazine elutes at about 39 min. The liquid chromatographic procedure in *Procedure 1* under *Organic Impurities* was validated with a Waters Symmetry brand of L1 column, in which promethazine elutes at about 10 min. The liquid chromatographic procedure in *Procedure 2* under *Organic Impurities* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which promethazine elutes at about 39 min.

(MD-CCA: D. Vicchio, , C. Anthony; . MSA: R. Tirumalai.)
RTS—C62808

Add the following:

■Promethazine and Phenylephrine Hydrochloride Oral Solution

DEFINITION
Promethazine and Phenylephrine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$). It may contain suitable preservatives.

IDENTIFICATION
- The retention times of the promethazine hydrochloride and phenylephrine hydrochloride peaks of the sample solutions correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]

Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water, carefully add 50 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.

Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 500 mL of water, carefully add 500 mL of acetonitrile, and mix. Adjust the apparent pH to 3.0 with phosphoric acid.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS and 1.4 mg/mL of USP Phenylephrine Hydrochloride RS in *Solution A*

Standard stock solution B: Dissolve USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS into a volume of acetonitrile equivalent to 40% of the total volume of the flask. Dilute with *Solution A* to volume, to obtain a solution containing 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS

Standard solution: Transfer 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.14 mg/mL of USP Phenylephrine Hydrochloride RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS.

Promethazine hydrochloride sample solution: Equivalent to 0.18 mg/mL of promethazine hydrochloride in *Solution A*

Phenylephrine hydrochloride sample solution: Equivalent to 0.14 mg/mL of phenylephrine hydrochloride in *Solution A*

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 15 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben
Relative standard deviation: NMT 2.0% for both promethazine and phenylephrine

Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

In-Process Revision

Calculate the quantity, in percent of label claim, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of sample taken:

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

r_U = promethazine peak response from the *Promethazine hydrochloride sample solution*
r_S = promethazine peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*

Calculate the quantity, in percent of label claim, of $C_9H_{13}NO_2 \cdot HCl$ in the portion of sample taken:

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

r_U = phenylephrine peak response from the *Phenylephrine hydrochloride sample solution*
r_S = phenylephrine peak response from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Phenylephrine hydrochloride sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% for both promethazine hydrochloride and phenylephrine hydrochloride

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]

Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust the pH to 6.4 with phosphoric acid.

5 N phosphoric acid: Carefully dilute 12 mL of phosphoric acid with water to 100 mL.

Diluent: Methanol, water, and *5 N phosphoric acid* (5:95:1)

Mobile phase: Acetonitrile, methanol, and *Solution C* (3:2:5)

Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*, from the *Standard solution*. [NOTE—Prepare at time of use.]

Promethazine hydrochloride sample solution: Equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
Run time: 2 times the retention time of promethazine for Standards; 6 times the retention time of promethazine for samples

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.5 for the promethazine peak, *Standard solution*
Relative standard deviation: NMT 7.5% for the promethazine peak, *Standard solution*
Sensitivity: Signal-to-noise NLT 10 for the promethazine peak, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

[a]10-Methylphenothiazine.

• **PROCEDURE 2**

[NOTE—At all times, protect the sample and standard solutions by using low actinic glassware.]

Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay.*

Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*

Standard stock solution B: Proceed as directed in the *Assay.*

Standard solution: Transfer 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* into a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS.

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*, from *Standard stock solution A* [NOTE—Prepare at time of use.]

Promethazine hydrochloride sample solution: Proceed as directed in the *Assay.*
[NOTE—Use this solution to determine promethazine related compounds and specified and unknown impurities.]

Phenylephrine hydrochloride sample solution: Proceed as directed in the *Assay.*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL

System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben, *Standard solution*

Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*
Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*

Identify the phenylephrine impurities using the relative retention times given in *Impurity Table 2*. Calculate the percentage of each phenylephrine impurity in the portion of sample taken:

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

r_U = peak response of the impurity from the *Phenylephrine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Phenylephrine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Using the data in *Impurity Table 3*, calculate the percentage of promethazine specified and unidentified impurities, and any unspecified impurities in the portion of sample taken:

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

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 3*

Acceptance criteria

Individual impurities: See *Impurity Tables 1*, *2*, and *3*.
Total unspecified impurities: NMT 1.0%
Total specified and unidentified phenylephrine impurities: NMT 10.0%
Total specified and unidentified promethazine impurities: NMT 5.0%
Total impurities: NMT 16.0% [NOTE—Sum of total specified and unidentified impurities from *Impurity Table 2*, total specified and unidentified from *Impurity Table 3*, and total unspecified impurities from *Impurity Table 3*.]

Impurity Table 2

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Unidentified impurity 1	0.32	1.4	0.6
Unidentified impurity 2	0.33	1.4	0.5
3-Hydroxyindole dione[a]	0.34	1.4	2.6
Norphenylephrine[b]	0.37	1.4	3.5

[a]3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.
[b](*R*)-3-(2-Amino-1-hydroxyethyl)phenol.
[c]7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione.
[d]4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione, and two 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.
[e](*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
[f]Phenylephrine synthetic impurity. Do not quantify.

Impurity Table 2 *(continued)*

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
7-Hydroxyindole trione[c]	0.38	1.4	3.5
Phenylephrine	0.40	—	—
Unidentified impurity 3	0.42	1.4	0.2
Unidentified impurity 4	0.43	1.4	0.5
4-Hydroxyindole trione and pyrocatecholyl phenylephrine[d]	0.44	1.4	0.6
Unidentified impurity 5	0.45	1.4	0.5
Unidentified impurity 6	0.46	1.4	0.5
Benzyl phenylephrine[e]	0.69	1.1	0.5
Benzyladrianone[f]	0.73	—	—
Promethazine	1.0	—	—
Total phenylephrine specified and unidentified impurities	—	—	10.0

[a]3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.
[b](*R*)-3-(2-Amino-1-hydroxyethyl)phenol.
[c]7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione.
[d]4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione, and two 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.
[e](*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
[f]Phenylephrine synthetic impurity. Do not quantify.

Impurity Table 3

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Phenothiazine sulfoxide[a]	0.71	1.0	0.5
Promethazine sulfoxide[b]	0.72	3.3	2.8
Unidentified impurity 1	0.77	1.0	0.2
Methylphenothiazine sulfoxide[c]	0.81	1.0	0.5
Unidentified impurity 2	0.85	1.0	0.2
Phenothiazinone[d]	0.98	10.0	0.2
Desmethyl promethazine[e]	0.99	1.0	0.2
Promethazine	1.0	—	—
Isopromethazine[f]	1.01	—	—
Unidentified impurity 3	1.09	1.0	0.2
Unidentified impurity 4	1.16	1.0	0.2
Total specified and unidentified impurities	—	—	5.0[g]

[a]10*H*-Phenothiazine sulfoxide.
[b]*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[c]10-Methyl-10*H*-phenothiazine sulfoxide.
[d]1*H*-Phenothiazin-1-one.
[e]*N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[f]Promethazine synthetic impurity. Do not quantify.
[g]Includes phenothiazine and methylphenothiazine from *Procedure 1*.

In-Process Revision

Impurity Table 3 *(continued)*

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Any unspecified impurity	—	1.0	0.2
Total unspecified impurities	—	—	1.0

[a]10*H*-Phenothiazine sulfoxide.
[b]*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[c]10-Methyl-10*H*-phenothiazine sulfoxide.
[d]1*H*-Phenothiazin-1-one.
[e]*N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[f]Promethazine synthetic impurity. Do not quantify.
[g]Includes phenothiazine and methylphenothiazine from *Procedure 1*.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial limit does not exceed 100 cfu/mL. The total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements for absence of *Escherichia coli.*
- **pH** ⟨**791**⟩**:** 3.7–4.7
- **ALCOHOL DETERMINATION** (if present)**,** *Method II* ⟨**611**⟩**:** Between 90.0% and 110.0% of the labeled quantity of C_2H_5OH
- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Methylparaben RS
  USP Phenylephrine Hydrochloride RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS■1S *(USP33)*

BRIEFING

Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which phenylephrine elutes at about 16 min, codeine elutes at about 21 min, and promethazine elutes at about 39 min. The liquid chromatographic procedure under *Organic Impurities*, *Procedure 1*, was validated with a Waters Symmetry brand of L1 column, in which promethazine elutes at about 10 min. The liquid chromatographic procedure under *Organic Impurities*, *Procedure 2*, was validated with a GL Sciences Intersil ODS-3 brand of L1 column, in which promethazine elutes at about 39 min.

(MD-CCA: C. Anthony. MSA: R. Tirumalai.) RTS—C62808

Add the following:

■Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution

DEFINITION

Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$), phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot {}^1/_2H_2O$). It may contain suitable preservatives.

IDENTIFICATION

- The retention times of the promethazine hydrochloride, phenylephrine hydrochloride, and codeine phosphate peaks from the *Sample solutions* correspond to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE** [NOTE—At all times, protect the *Sample solution* and the *Standard solution* by using low-actinic glassware.]
  Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptanesulfonic acid sodium salt monohydrate in 950 mL of water, carefully add 50 mL of acetonitrile, and mix. Adjust the apparent pH with phosphoric acid to 3.0.
  Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptanesulfonic acid sodium salt monohydrate in 500 mL of water, carefully add 500 mL of acetonitrile, and mix. Adjust the apparent pH with phosphoric acid to 3.0.
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS, 1.4 mg/mL of USP Phenylephrine Hydrochloride RS, and 2.8 mg/mL USP Codeine Phosphate RS in *Solution A*
Standard stock solution B: Dissolve USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS in a volume of acetonitrile equivalent to 40% of the total volume of the flask. Dilute with *Solution A* to volume to obtain a solution containing 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS.
Standard solution: Transfer 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.14 mg/mL of USP Phenylephrine Hydrochloride RS, 0.28

mg/mL of USP Codeine Phosphate RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS.

Promethazine hydrochloride sample solution: Equivalent to 0.18 mg/mL, based on the label claim, of promethazine hydrochloride in *Solution A*

Phenylephrine hydrochloride sample solution: Equivalent to 0.14 mg/mL, based on the label claim, of phenylephrine hydrochloride in *Solution A*

Codeine phosphate sample solution: Equivalent to 0.28 mg/mL, based on the label claim, of codeine phosphate in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]

Suitability requirements

Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben

Relative standard deviation: NMT 2.0% for promethazine, phenylephrine, and codeine

Analysis

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Calculate the percentage of the label claim of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of sample taken:

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

r_U = promethazine peak response from the *Promethazine hydrochloride sample solution*

r_S = promethazine peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*

Calculate the percentage of the label claim of $C_9H_{13}NO_2 \cdot HCl$ in the portion of sample taken:

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

r_U = phenylephrine peak response from the *Phenylephrine hydrochloride sample solution*

r_S = phenylephrine peak response from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Phenylephrine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Codeine phosphate sample solution*

Calculate the percentage of the label claim of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot {}^1/_2H_2O$ in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = codeine peak response from the *Codeine phosphate sample solution*

r_S = codeine peak response from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS, on the dried basis, in the *Standard solution* (mg/mL)

C_U = concentration of the *Codeine phosphate sample solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0% for promethazine hydrochloride, phenylephrine hydrochloride, and codeine phosphate

IMPURITIES

Organic Impurities

• **PROCEDURE 1** [NOTE—At all times, protect the sample and the standard solutions by using low-actinic glassware.]

Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust the pH with phosphoric acid to 6.4.

5 N phosphoric acid: Carefully dilute 12 mL of phosphoric acid with water to 100 mL.

Diluent: Methanol, water, and *5 N phosphoric acid* (5:95:1)

Mobile phase: Acetonitrile, methanol, and *Solution C* (3:2:5)

Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*, prepared from the *Standard solution* [NOTE—Prepare at time of use.]

Sample solution: Equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: *Standard solution*, 2 times the retention time of promethazine; *Sample solution*, 6 times the retention time of promethazine

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for promethazine, *Standard solution*

Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*

Sensitivity: Signal-to-noise ratio, NLT 10 for promethazine, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

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

r_U = impurity peak response from the *Sample solution*

r_S = promethazine hydrochloride peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)

F = relative response factor for each impurity shown in *Impurity Table 1*

In-Process Revision

Acceptance criteria
Individual impurities: See *Impurity Table 1* below.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

[a] 10-Methylphenothiazine.

• **PROCEDURE 2** [NOTE—At all times, protect the sample and standard solutions by using low-actinic glassware.]
Solution A, Solution B, Mobile phase, Standard stock solution B, Codeine phosphate sample solution, and **Phenylephrine hydrochloride sample solution:** Proceed as directed in the *Assay*.
Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*
Standard solution: Transfer 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS.
Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*, prepared from *Standard stock solution A* [NOTE—Prepare at time of use.]
Promethazine hydrochloride sample solution: Proceed as directed in the *Assay*. [NOTE—Use this solution to determine promethazine related compounds and specified and unknown impurities.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben, and NLT 1.5 between promethazine and propylparaben, *Standard solution*
Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*
Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*
Identify the phenylephrine impurities using the relative retention times given in *Impurity Table 2* below. Calculate the percentage of each phenylephrine impurity in the portion of sample taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Phenylephrine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 2*

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Using the data in *Impurity Table 3* below, calculate the percentage of each promethazine impurity, unspecified impurities, and unidentified impurities in the portion of sample taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity shown in *Impurity Table 3*

Samples: *Standard solution* and *Codeine phosphate sample solution*
Identify the codeine impurities using the relative retention times given in *Impurity Table 3*. Calculate the percentage of each codeine impurity in the portion of sample taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Codeine phosphate sample solution* (µg/mL)
F = relative response factor for each codeine impurity shown in *Impurity Table 3*

Acceptance criteria
Individual impurities: See *Impurity Tables 2* and *3*.
Total codeine impurities: NMT 1.0%
Total phenylephrine specified and unidentified impurities: NMT 10.0%
Total promethazine specified and unidentified impurities: NMT 5.0%
Total impurities: NMT 17.0%
[NOTE—Sum of total codeine impurities, total promethazine specified and unidentified impurities, total phenylephrine specified and unidentified impurities, and total unspecified impurities]

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Unidentified impurity 1	0.32	1.4	0.6
Unidentified impurity 2	0.33	1.4	0.5
3-Hydroxyindole dione[a]	0.34	1.4	2.6

[a] 3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.
[b] (*R*)-3-(2-Amino-1-hydroxyethyl)phenol.
[c] 7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2H)-trione.
[d] 4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2H)-trione, and two 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.
[e] (*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
[f] Phenylephrine synthetic impurity. Do not quantify.

Impurity Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Norphenylephrine[b]	0.37	1.4	3.5
7-Hydroxyindole trione[c]	0.38	1.4	3.5
Phenylephrine	0.40	—	—
Unidentified impurity 3	0.42	1.4	0.2
Unidentified impurity 4	0.43	1.4	0.5
4-Hydroxyindole trione and pyrocatecholyl phenylephrine[d]	0.44	1.4	0.6
Unidentified impurity 5	0.45	1.4	0.5
Unidentified impurity 6	0.46	1.4	0.5
Benzyl phenylephrine[e]	0.69	1.1	0.5
Benzyladrianone[f]	0.73	—	—
Promethazine	1.0	—	—
Total phenylephrine specified and unidentified impurities	—	—	10.0

[a] 3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.
[b] (*R*)-3-(2-Amino-1-hydroxyethyl)phenol.
[c] 7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2H)-trione.
[d] 4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2H)-trione, and two 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.
[e] (*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
[f] Phenylephrine synthetic impurity. Do not quantify.

Impurity Table 3

Name	Source	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Codeine *N*-oxide[a]	Codeine	0.52	0.83	0.2
Norcodeine[b]	Codeine	0.53	0.83	0.2
Codeine	—	0.54	—	—
Codeinone[c]	Codeine	0.61	0.63	0.3
Codeine methyl ether[d]	Codeine	0.67	—	—
Total codeine impurities	Codeine	—	—	1.0
Phenothiazine sulfoxide[e]	Promethazine	0.71	1.0	0.5
Promethazine sulfoxide[f]	Promethazine	0.72	3.3	2.8
Unidentified impurity 1	Promethazine	0.77	1.0	0.2
Methylphenothiazine sulfoxide[g]	Promethazine	0.81	1.0	0.5
Unidentified impurity 2	Promethazine	0.85	1.0	0.2
Phenothiazinone[h]	Promethazine	0.98	10.0	0.2

[a] Morphinan-6-ol, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl,17-oxide-(5α,6α).
[b] Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-(5α,6α).
[c] Morphinan-6-one, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl-(5α).
[d] Codeine synthetic impurity. Do not quantitate.
[e] 10*H*-Phenothiazine sulfoxide.
[f] *N*,*N*-Dimethyl-1(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[g] 10-Methyl-10*H*-phenothiazine sulfoxide.
[h] 1*H*-Phenothiazin-1-one.
[i] *N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[j] Promethazine synthetic impurity. Do not quantify.
[k] Includes phenothiazine and methylphenothiazine from *Procedure 1*.

Impurity Table 3 *(continued)*

Name	Source	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Desmethyl promethazine[i]	Promethazine	0.99	1.0	0.2
Promethazine	Promethazine	1.0	—	—
Isopromethazine [j]	Promethazine	1.01	—	—
Unidentified impurity 3	Promethazine	1.09	1.0	0.2
Unidentified impurity 4	Promethazine	1.16	1.0	0.2
Total promethazine specified and unidentified impurities	Promethazine	—	—	5.0[k]
Any unspecified impurity	—	—	1.0	0.2
Total unspecified impurities	—	—	—	1.0

[a] Morphinan-6-ol, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl,17-oxide-(5α,6α).
[b] Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-(5α,6α).
[c] Morphinan-6-one, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl-(5α).
[d] Codeine synthetic impurity. Do not quantitate.
[e] 10*H*-Phenothiazine sulfoxide.
[f] *N*,*N*-Dimethyl-1(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[g] 10-Methyl-10*H*-phenothiazine sulfoxide.
[h] 1*H*-Phenothiazin-1-one.
[i] *N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[j] Promethazine synthetic impurity. Do not quantify.
[k] Includes phenothiazine and methylphenothiazine from *Procedure 1*.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 100 cfu/mL. The total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.
- **pH** ⟨**791**⟩**:** 3.7–4.7
- **ALCOHOL DETERMINATION,** *Method II* ⟨**611**⟩ (if present)**:** Between 90.0% and 110.0% of the labeled amount of C_2H_5OH
- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Codeine Phosphate RS
  USP Methylparaben RS
  USP Phenylephrine Hydrochloride RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS■1S *(USP33)*

BRIEFING

Psyllium Husk, *USP 31* page 3131. It is proposed to add limits for pesticides and heavy metals, on the basis of comments received.

(DSB: M. Sharaf.) RTS—C72071

In-Process Revision

Psyllium Husk

DEFINITION

Psyllium Husk is the cleaned, dried seed coat (epidermis) separated by winnowing and thrashing from the seeds of *Plantago ovata* Forssk., known in commerce as Blond Psyllium, Indian Psyllium, or Ispaghula, or from *Plantago arenaria* Waldst. & Kit. (*Plantago psyllium* L.) known in commerce as Spanish or French Psyllium (Fam. *Plantaginaceae*), in whole or in powdered form.

IDENTIFICATION

- **A. Mounted in Cresol:** Cells, viewed microscopically, are composed of polygonal prismatic cells having 4–6 straight or slightly wavy walls.
- **B. Mounted in Alcohol and Irrigated with Water:** Viewed microscopically, the mucilage in the outer part of the epidermal cells swells rapidly and goes into solution.

IMPURITIES

Change to read:

Inorganic Impurities

- **Articles of Botanical Origin,** *Acid-Insoluble Ash* ⟨**561**⟩**:** NMT 1.0%
- **Heavy Metals,** *Method II* ⟨**231**⟩**:** 2 ppm, use 10 g of Psyllium Husk■1S (*USP33*)

Change to read:

Organic Impurities

- **Procedure 1: Light Extraneous Matter**

  [NOTE—Perform this test in a well-ventilated hood.]

  Sample: 99–101 g of Psyllium Husk, weighed to 0.1-g accuracy

  Analysis: Transfer the *Sample* to a 1000-mL tall-form beaker. Add 800 mL of trichloroethylene, previously adjusted to a temperature of 24°–26°, and maintain this temperature throughout the test. Stir the husk for 5 s, and allow it to settle while protecting the surface from drafts in the hood. Remove the floating material with a spoon made of 50-mesh screen, and transfer the material to a piece of filter paper in a dish. Stir the husk mixture again, allow it to settle, remove the floating material again, and combine it with the material on the paper. Repeat this procedure until no more material appears on the surface. Dry the removed material with the paper in a hood and then in an oven at 40° for 3 h. Cool to room temperature. Weigh the filter paper with the material. Brush the material off the paper, weigh the paper, and then calculate the percentage of light extraneous matter.

  Acceptance criteria: NMT 5%

- **Procedure 2: Insect Infestation**

  Sample: 25 g

  Analysis: Transfer the *Sample* to a 250-mL beaker, add sufficient solvent hexane to saturate, add an additional 75–100 mL of solvent hexane, and allow to stand for 10 min, stirring occasionally with a stirring rod. Wet a sheet of filter paper with alcohol, and filter the mixture with the aid of vacuum. Discard the filtrate. Transfer the residue to the original beaker with the aid of alcohol. Add alcohol to bring the volume to 150 mL above the level of the transferred residue. Boil for 10 min. Filter through alcohol-wetted paper as above. Prepare a trap flask consisting of a 2000-mL graduated, narrow-mouth conical flask into which is inserted a rubber disk supported on a stiff metal rod 4 mm in diameter and longer than the height of the flask, the rod being threaded at the lower end and furnished with nuts and washers to hold the disk in place, and the disk being of the proper shape and size to prevent liquid in the body of the flask from spilling when it is pressed up against the neck from the inside. Transfer the residue to the trap flask, completing the transfer with the aid of hot water. Add sufficient hot water to bring the volume to 1000 mL. Add 20 mL of hydrochloric acid. Raise the rod, and support it so that the rubber disk is held above the liquid level. Rinse the rubber disk with hot water. Spray the inside of the neck of the flask with an antifoam spray. Boil for 30 min, and cool to room temperature. Add 40 mL of solvent hexane, and agitate for 1 min by tilting the flask and moving the rod vertically with wrist action. Allow to stand for 5 min. Add water to bring the level of liquid to the neck of the flask, and allow to stand for 20 min. Simultaneously rotate the disk to free it from settled material, and raise it as far as possible into the neck of the flask. Prepare a sheet of ruled filter paper with lines approximately 5 mm apart for filtration by moistening it with water and placing it on a vacuum funnel. Transfer the material trapped in the neck of the flask to the filter with the aid of water. If necessary, wash the paper with alcohol to remove traces of hexane. Place the paper on a 100-mm petri dish that has been wetted with a solution containing equal volumes of glycerin and alcohol. Add 35 mL of solvent hexane to the flask, and gently stir with the trapping rod. Add water to bring the liquid level into the neck of the flask. Allow to stand for 15 min. Using the same technique as before, transfer the trapped material onto a separate paper. Examine the papers at 30× magnification.

  Acceptance criteria

  Powdered Psyllium Husk: NMT 400 insect fragments, including mites and psocids

  Whole Psyllium Husk: NMT 100 insect fragments, including mites and psocids

- **Procedure 3: Articles of Botanical Origin,** *Pesticide Residues* ⟨**561**⟩**:** Meets the requirements■1S (*USP33*)

SPECIFIC TESTS

- **Botanic Characteristics**

  Histology—Husk: The epidermis is composed of large cells having transparent walls filled with mucilage, and the cells swell rapidly in aqueous mounts and appear polygonal to slightly rounded in a surface view when viewed from above (from below they appear elongated to rectangular). The swelling takes place mainly in the radial direction. The mucilage of the epidermal cells stains red with ruthenium red and lead acetate TS. The very occasional starch granules that are present in some of the epidermal cells, and that may be found embedded in the mucilage, are small and simple or compounded with four or more components.

  Powdered psyllium seed husk: It is a pale to medium buff-colored powder having a slight pinkish tinge and a weak characteristic odor. Occasional single and 2- to 4-compound starch granules, the individual grains being spheroidal plano to angular convex from 2–10 µm in diameter, are found embedded in the mucilage. Entire or broken epidermal cells are filled with mucilage. In surface view, the epidermal cells appear polygonal to slightly rounded. Mucilage stains red with ruthenium red TS and lead acetate TS. Some of the elongated and rectangular cells representing the lower part of epidermis and also radially swollen epidermal cells can be found.

- **Microbial Enumeration Tests** ⟨**61**⟩ and **Tests for Specified Microorganisms** ⟨**62**⟩**:** The total combined molds and yeasts count does not exceed 1000 cfu/g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **Articles of Botanical Origin,** *Total Ash* ⟨**561**⟩**:** NMT 4.0%
- **Water Determination,** *Method II* ⟨**921**⟩**:** NMT 12.0%
- **Swell Volume**

  Sample: 3.5 g of Psyllium Husk

  Analysis: Transfer 250 mL of simulated intestinal fluid TS without enzymes to a glass-stoppered 500-mL graduated cylinder. Gradually, with shaking, add the *Sample* until a uniform, smooth suspension is obtained. Dilute with the same fluid to 500 mL. Shake the cylinder for 1 min every 30 min for 8 h. Allow the gel to settle for 16 h (total time is 24 h). Determine the volume of the gel.

Acceptance criteria
Powdered Psyllium Husk: NLT 40 mL/g
Whole Psyllium Husk: NLT 35 mL/g

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, secured against insect attack (see *General Notices—Vegetable and Animal Substances, Preservation*).

BRIEFING

Repaglinide Tablets, *USP 31* page 3166. On the basis of comments received, it is proposed to delete the test for *Loss on Drying* as formulation specific.

(MD-GRE: E. Gonikberg.) RTS—C71505

Repaglinide Tablets

DEFINITION
Repaglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
Sample solution: To 10 mg of repaglinide from powdered Tablets, add 10 mL of a mixture of methanol and methylene chloride (1:1), shake for 15 min, and centrifuge.
Developing solvent system: Toluene, methylene chloride, and methanol (2:2:1)
- **B.** The retention time and UV spectrum of the major peak of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
Solution A: Monobasic ammonium phosphate solution (2 in 1000). Adjust with phosphoric acid to a pH of 4.0.
Solution B: Monobasic ammonium phosphate solution (2 in 1000). Adjust with phosphoric acid to a pH of 2.5.
Diluent: Methanol and *Solution A* (7:3)
Mobile phase: Methanol and *Solution B* (7:3)
Standard solution 1: 800 µg/mL of USP Repaglinide RS in methanol
Standard solution 2: Dilute 5.0 mL of *Standard solution 1* with *Diluent* to 50.0 mL.
System suitability stock solution: 80 µg/mL of USP Repaglinide Related Compound A RS in methanol
System suitability solution: Transfer 1.0 mL of *System suitability stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Standard solution 1*, and dilute with *Diluent* to volume.
Sample solution: 80 µg/mL from dilution of 8 whole Tablets with *Diluent.* [NOTE—Stir the solution for 20 min, and filter a portion of the solution.]
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability.*)
Mode: LC
Detector: 245-nm diode array
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution 2* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between repaglinide and repaglinide related compound A, *System suitability solution*
Capacity factors, k′: For repaglinide and repaglinide related compound A, about 4.9 and 1.2, respectively, *System suitability solution*
Tailing factor: 0.8–2.0, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution 2*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of $C_{27}H_{36}N_2O_4$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 2*
C_S = concentration of USP Repaglinide RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of repaglinide in the *Sample solution* (mg/mL)
Acceptance criteria: 95.0%–105.0%

IMPURITIES
Organic Impurities
- **PROCEDURE**
Solution A, Solution B, Diluent, and **Mobile phase:** Prepare as directed in the *Assay.*
Standard solution 1 and **Standard solution 2:** Proceed as directed in the *Assay.*
Standard solution 3: Dilute 2.5 mL of *Standard solution 2* with *Diluent* to 1000 mL.
System suitability stock solution and **System suitability solution:** Prepare as directed in the *Assay.*
Sample solution: Use the *Sample solution* prepared for the *Assay.*
Chromatographic system
Mode: LC
Detector: 210-nm diode array
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution 3* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between repaglinide and repaglinide related compound A, *System suitability solution*
Capacity factors, k′: For repaglinide and repaglinide related compound A, about 4.9 and 1.2, respectively, *System suitability solution*
Tailing factor: 0.8–2.0, *System suitability solution*
Relative standard deviation: NMT 10.0% for replicate injections, *Sample solution 3*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of repaglinide from *Standard solution 2*
Acceptance criteria: NMT 0.5% of total impurities

PERFORMANCE TESTS
- **DISSOLUTION ⟨711⟩**
Medium: pH 5.0 buffer, prepared by mixing 10.2 g of citric acid monohydrate and 18.16 g of dibasic sodium phosphate dihydrate with 1 L of water; 900 mL

In-Process Revision

Apparatus 2: 75 rpm
Time: 30 min
Solution A: Monobasic potassium phosphate solution (1.5 in 1000), adjusted with phosphoric acid to a pH of 2.3
Mobile phase: Acetonitrile, *Solution A,* and methanol (49:40:11)
Standard stock solution: 11 µg/mL USP Repaglinide RS in methanol
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of methanol, and dilute with *Medium* to volume.
Sample solution: Sample per *Dissolution* ⟨711⟩
Determine the amount of $C_{27}H_{36}N_2O_4$ dissolved by using the following method.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: Fluorometric detector; excitation wavelength of 244 nm and emission wavelength of 348 nm
Column: 4.0-mm × 12.5-cm; 10-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.5 and 2.0
Relative standard deviation: NMT 2.0%
Capacity factor, k′: 1.8
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of $C_{27}H_{36}N_2O_4$ dissolved by comparing the measured peak responses of the *Standard solution* and the *Sample solution.*
Tolerances: NLT 70% (Q) of the labeled amount of $C_{27}H_{36}N_2O_4$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

SPECIFIC TESTS

Delete the following:

■• **~~LOSS ON DRYING~~** ⟨~~731~~⟩**:** ~~Dry 2 g of finely ground Tablets at 105° for 3 h: it loses NMT 6.0% of its weight.~~■1S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Repaglinide RS
USP Repaglinide Related Compound A RS

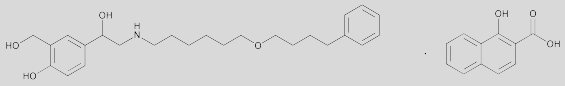
BRIEFING

Salmeterol Xinafoate. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and in the *Procedure* under *Organic Impurities* are based on analyses performed with the Spherisorb ODS-2 brand of L1 column, in which salmeterol elutes at 14 min.

(AER-05: K. Zaidi.) RTS—C64168

Add the following:

■Salmeterol Xinafoate



$C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$ 603.75

1,3-Benzenedimethanol, 4-hydroxy-α¹-[[[6-(4-phenylbutoxy) hexyl]amino]methyl]-, (±)-, 1-hydroxy-2-naphthalenecarboxylate (salt);
(±)-4-Hydroxy-α¹-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-*m*-xylene-α,α′-diol 1-hydroxy-2-naphthoate (salt) [94749-08-3].

DEFINITION
Salmeterol Xinafoate contains NLT 98.0% and NMT 102.0% of $C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$, calculated on the water- and solvent-free basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197M**⟩
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Solution A: 0.1 M of sodium dodecyl sulfate
Solution B: 0.1 M of ammonium acetate
Mobile phase: Acetonitrile, *Solution A*, and *Solution B* (13:6:6). Adjust the pH to 3.8 with glacial acetic acid.
System suitability solution: 0.25 mg/mL of USP Salmeterol Xinafoate RS and 0.017 mg/mL of USP Salmeterol Related Compound B RS in *Mobile phase*; prepare by dilution of *System suitability solution 1* under *Organic Impurities*
Standard solution: 0.25 mg/mL of USP Salmeterol Xinafoate RS in *Mobile phase*
Sample solution: 0.25 mg/mL of Salmeterol Xinafoate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 15-cm column; packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.0 between salmeterol and salmeterol related compound B
Relative standard deviation: NMT 2.0% for salmeterol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$ in the portion of Salmeterol Xinafoate taken:

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

In-Process Revision

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Salmeterol Xinafoate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%

Organic Impurities

• **PROCEDURE**

[NOTE—Prepare test solutions freshly and protect from light.]

Solution A and **Solution B:** Proceed as directed under *Assay*.

Diluent: Acetonitrile and water (1:1)

Solution C: Acetonitrile, *Solution A*, and *Solution B* (13:6:6). Adjust the pH to 3.8 with glacial acetic acid.

Solution D: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution C (%)	Solution D (%)
0	100	0
16.0	100	0
36.0	30	70
45.0	30	70
45.1[a]	100	0

[a]Prior to next injection, run the sytem at the initial condition until equilibration is achieved.

System suitability solution 1: 5.0 mg/mL USP Salmeterol Xinafoate RS and 0.34 mg/mL of USP Salmeterol Related Compound B RS in *Diluent*

System suitability solution 2: 1.0 mg/mL of USP Salmeterol Related Compound A RS in *Diluent*

Sample solution: 5.0 mg/mL of Salmeterol Xinafoate in *Diluent*

Chromatographic system: Proceed as directed under *Assay*.

System suitability

Sample: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: NLT 1.0 between salmeterol and salmeterol related compound B, *System suitability solution 1*

Tailing factor: NMT 2 .5 for salmeterol, *System suitability solution 1*

Relative standard deviation: NMT 2.0% for the salmeterol related compound A peak, *System suitability solution 2*

Analysis

[NOTE—Disregard the peak due to hydroxynaphthoic acid and any peaks from blank injections.]

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Salmeterol Xinafoate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity from the *Sample solution*
r_T = sum of responses for all peaks of the *Sample solution*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.9% area [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

Impurity Table 1

Name	Relative Retention Time (RRT)	Limit (% Area)
Hydroxynaphthoic acid[a]	0.2	—
Salmeterol related compound A[b]	0.3	0.2
Salmeterol-phenylethoxy[c]	0.5	0.1
Salmeterol-phenylpropoxy[d]	0.7	0.1
Salmeterol-*O*-alkyl[e]	0.8	0.3
Salmeterol related compound B[f]	0.9	0.1
Salmeterol xinafoate	1.0	—
Salmeterol-deoxy[g]	1.6	0.2
Salmeterol-*N*-alkyl[h]	2.7	0.2
Any unspecified impurity	—	0.10
Total unspecified impurities	—	0.2

[a]1-Hydroxy-naphthalene-2-carboxylic acid.
[b]4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.
[c]4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.
[d]4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.
[e]4-{1-Hydroxy-2-[4-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenoxy]ethyl}-2-(hydroxymethyl)phenol.
[f]4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.
[g]4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.
[h]4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)-hexylamino]ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl)phenol.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.25%
Sample: 0.5 g

• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –0.5° to +0.5° (t = 20°), calculated on the anhydrous and solvent-free basis
Sample solution: 10 mg/mL in methanol

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature not exceeding 30°.

• **LABELING:** Salmeterol Xinafoate in the form of microcrystals is so labeled.

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Salmeterol Xinafoate RS
USP Salmeterol Related Compound A RS
USP Salmeterol Related Compound B RS■1S (*USP33*)

BRIEFING

Sennosides, *USP 32* page 3551. On the basis of comments received, it is proposed to add a stability-indicating HPLC procedure for the *Content of Sennosides A and B* test. The procedure has been validated using a Hypersil Gold C18 brand of L1 column. The typical retention times for sennoside B and sennoside A are 30.0 and 34.0, respectively.

(DSB: M. Sharaf.) RTS—C70307

In-Process Revision

Sennosides

DEFINITION

Sennosides is a partially purified natural complex of anthraquinone glucosides, isolated from senna leaflets and/or senna pods, *Senna alexandrina* Mill (*Cassia acutifolia* or *C. angustifolia*), as calcium salts. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sennosides. The labeled amount is NLT 60.0% (w/w), calculated on the dried basis.

IDENTIFICATION

• **THIN-LAYER CHROMATOGRAPHY**

Solution A: Ethyl acetate, *n*-propyl alcohol, and water (1:1:1). Shake well and discard the upper layer.

Standard solution: 1 mg/mL of USP Sennosides RS in *Solution A*

Sample solution: 1 mg/mL of Sennosides in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Ethyl acetate, *n*-propyl alcohol, and water (4:4:3)

Analysis: Proceed as directed in the chapter. Apply the solutions, as 1-cm streaks, on a line 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Examine the plate under long-wavelength UV light. Expose the plate to ammonium hydroxide vapor until color develops (about 5 min). Cover the plate with a piece of glass, and heat at 120° for 5 min.

Acceptance criteria: The two most prominent spots from the *Sample solution* correspond in color and mobility to those of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: 9.08 mg/mL of monobasic potassium phosphate

Solution B: 9.46 mg/mL of anhydrous dibasic sodium phosphate

Solution C: Mix *Solution A* and *Solution B* (38.9:61.1). Adjust dropwise, if necessary, with *Solution B* to a pH of 7.0.

Solution D: 37.9 mg/mL of sodium borate

Solution E: 15 mg/mL of sodium dithionite

Standard solution: 1 mg/mL of USP Sennosides RS in *Solution C*

Sample solution: 1 mg/mL of Sennosides in *Solution C*

Fluorometric conditions

Excitation wavelength: 392 nm

Emission wavelength: 505 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 1-mL portions of the *Standard solution* and the *Sample solution* into separate 100-mL volumetric flasks, and dilute with *Solution D* to volume. Transfer 5.0-mL portions of each of the resulting solutions to separate, low-actinic glass, 50-mL volumetric flasks. Add 15.0 mL of *Solution D* and 15.0 mL of *Solution E*. Pass nitrogen through the solutions, seal the flasks with nitrogen-filled balloons, and heat in a water bath for 30 min. Cool the flasks for 15 min in a water bath thermostatically controlled at 20°. Dilute the solutions with *Solution D* to volume. Determine, without delay, the fluorescence intensities of the resulting solutions, the time elapsed between the addition of *Solution E* and the measurement being the same for the two solutions.

Calculate the percentage of sennosides in the portion taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence value observed in the *Sample solution*

I_S = fluorescence value observed in the *Standard solution*

C_S = concentration of USP Sennosides RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Sennosides in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** 5.0%–8.0%, ignited at 800 ± 25°, the use of sulfuric acid being omitted

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 60 ppm

SPECIFIC TESTS

Add the following:

■• **CONTENT OF SENNOSIDES A AND B**

Solvent: Use 1% sodium acetate solution in water.

Buffer solution: Dissolve 3.6 g of dibasic sodium phosphate dodecahydrate in 50 mL of water, add to a solution of 6.2 g sodium dihydrogen phosphate dihydrate in 200 mL of water, mix, and adjust the pH to 5.0. Dilute the final solution 1:10 in water.

Solution A: Use a filtered and degassed mixture of *Buffer solution* and acetonitrile (1:1), containing 0.5% of benzyldimethylstearylammonium chloride.

Solution B: Use filtered and degassed acetonitrile.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Elution
0–35	100	0	isocratic
35–40	100→30	0→70	linear gradient
40–50	30	70	isocratic
50–55	30→100	70→0	linear gradient
55–60	100	0	isocratic

Standard solution 1: 0.3 mg/mL of USP Sennosides RS in *Solvent*. Pass through a membrane filter having a 0.45-µm porosity before injection, discarding the first few mL of the filtrate.

Standard solution 2: 0.1 mg/mL of USP Sennoside A RS in *Solvent*. Pass through a membrane filter having a 0.45-µm porosity before injection, discarding the first few mL of the filtrate.

Standard solution 3: 0.1 mg/mL of USP Sennoside B RS in *Solvent*. Pass through a membrane filter having a 0.45-µm porosity before injection, discarding the first few mL of the filtrate.

Sample solution: 0.3 mg/mL of Sennosides in *Solvent*. Pass through a membrane filter having a 0.45-µm porosity before injection, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: Adjusted so the retention time of the sennoside B peak is 30 min

Column temperature: 40°

Injection size: 10 µL

System suitability

Sample: *Standard solution 1*

Suitability requirements: The chromatogram obtained is similar to the Reference chromatogram provided with the lot of USP Sennosides RS being used; the resolution, R, between the sennoside B peak and the peak before, between the sennoside B and sennoside A peaks, and between the sennoside A peak and the peak after is NLT 1.5; and the relative standard deviation determined for the sum of the areas of the

sennoside A and sennoside B peaks for replicate injections is NMT 2.0%.

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution*

Record the chromatograms. Using the chromatograms of *Standard solution 2* and *Standard solution 3*, identify the peaks corresponding to sennoside A and sennoside B in the *Sample solution* chromatogram. Separately calculate the percentages of sennoside A and sennoside B in the portion of Sennosides taken:

$$\text{Result} = (r_U/r_S)\,(C/W) \times 100 \times F$$

r_U = peak areas for sennoside A or B from the *Sample solution*

r_S = peak areas for sennoside A or B from the corresponding *Standard solution*

C = concentration of USP Sennoside A RS or Sennoside B RS in the corresponding *Standard solution* (mg/mL)

V = final volume of the *Sample solution* (mL)

W = weight of Sennosides, corrected for the loss on drying, taken to prepare the *Sample solution* (mg)

F = conversion factor for the molecular weights of sennoside A or sennoside B to the corresponding calcium salt, 1.044

Calculate the total percentage of sennoside A and sennoside B by adding the individual percentages calculated.

Acceptance criteria: The total percentage of sennoside A and sennoside B is NLT 55% of the labeled amount of sennosides.■1S *(USP33)*

- **pH** ⟨**791**⟩**:** 6.3–7.3, in a solution (1 in 10)
- **Loss on Drying** ⟨**731**⟩**:** Dry a sample in a vacuum at 100° to constant weight: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store protected from light and moisture, at controlled room temperature.

Change to read:

- **USP Reference Standards** ⟨**11**⟩

USP Sennosides RS

■USP Sennoside A RS

USP Sennoside B RS■1S *(USP33)*

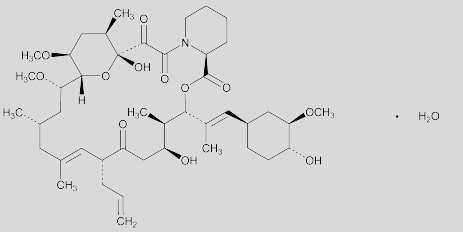
Briefing

Tacrolimus. Because there is no existing *USP* monograph for this drug substance, a new monograph is proposed based on validated methods. The *Assay* is based on analyses performed using a TSKgel ODS-80TM column. The typical retention time for tacrolimus is about 10 min. The *Procedure* for *Organic Impurities* is based on analyses performed using a Supelcosil LC-diol column. The typical retention time for tacrolimus is about 15 min.

(MD-ANT: A. Wise.) RTS—C53605

Add the following:

■Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-;

(–)-(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone, monohydrate [109581-93-3].

DEFINITION

Tacrolimus contains NLT 98.0% and NMT 102.0% of $C_{44}H_{69}NO_{12}$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨**197M**⟩
- **B.** Dissolve 5 mg of Tacrolimus in 1 mL of alcohol, add 1 mL of a solution of 1,3-dinitrobenzene in alcohol (1 in 100) and 1 mL of sodium hydroxide TS and shake: a red-purple color develops.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **Procedure**

[Note—Allow the *Standard solution* and the *Sample solution* to stand for 6 h at 25° before use.]

Mobile phase: Isopropanol, tetrahydrofuran, and water (2:2:5)

Internal standard solution: 0.75 mg/mL solution of heptyl parahydroxybenzoate in dehydrated alcohol

Standard solution: Dissolve an accurately weighed quantity of USP Tacrolimus RS in dehydrated alcohol, using about 30% of the final volume, add 20% of the final volume of *Internal standard solution,* and dilute with water to volume to obtain a solution having a final concentration of about 0.5 mg of tacrolimus/mL.

Sample solution: Dissolve a quantity of Tacrolimus in dehydrated alcohol, using about 30% of the final volume, add

In-Process Revision

20% of the final volume of *Internal standard solution,* and dilute with water to volume to obtain a solution having a nominal concentration of about 0.5 mg of tacrolimus/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Temperature: 50°
Flow rate: 0.8 mL/min [NOTE—Adjust the flow rate so that the retention time of tacrolimus is approximately 10 min. The flow rate may not be adjusted by more than ±10%.]
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{44}H_{69}NO_{12}$ in the portion of Tacrolimus taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of tacrolimus to heptyl parahydroxybenzoate from the *Sample solution*
R_S = peak response ratio of tacrolimus to heptyl parahydroxybenzoate from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩ 10 ppm

Organic Impurities

- **PROCEDURE**

[NOTE—It is suggested that new columns be conditioned with about 500 mL of alcohol before use to meet the resolution criterion.]

Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1)
System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Tacrolimus in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Flow rate: 1.5 mL/min [NOTE—Adjust the flow rate so that the retention time of tacrolimus is approximately 15 min. The flow rate may not be adjusted by more than ±10%.]
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/F_i) \times [1/\Sigma(r_U/F_i)] \times 100$$

r_U = peak response for each peak in the *Sample solution*
F_i = relative response factor for corresponding peak (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Tacrolimus methylacryl aldehyde[1]	0.55	16.7	0.2
Tacrolimus diene[2]	0.79	2.2	0.2
Tacrolimus impurity 1[3]	0.96	1.0	0.2
USP Tacrolimus Related Compound A[4]	0.96	—	—
Tacrolimus	1.0	1.0	—
Tacrolimus 19-epimer[5]	1.1	1.0	1.0
Tacrolimus open ring[6]	1.3	1.0	0.5
Any individual unspecified impurity	—	1.0	0.2
Total impurities[7]	—	—	0.3

[1](*E*)-3-[[(1*R*, 3*R*, 4*R*)-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.
[2](14*E*,18*E*)-17-Allyl-1-hydroxy-12-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0 ^{4,9}] octacosa-14,18-diene-2,3,10,16-tetrone.
[3]Specified unidentified impurity.
[4]For information only; not to be reported.
[5](3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26,26a,hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16,dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
[6](3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.
[7]Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –110° to –115°
  Sample solution: 10 mg/mL in *N,N*-dimethylformamide
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** 1.9%–2.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Tacrolimus RS
  USP Tacrolimus Related Compound A RS■1S (*USP33*)

In-Process Revision

BRIEFING

Tacrolimus Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on validated methods. The liquid chromatographic procedure in the *Assay* is based on analyses performed using a TSKgel ODS-80TM brand of L1 column. The typical retention time for tacrolimus is about 10 min. The liquid chromatographic procedure in the test for *Dissolution* is based on analyses performed using a Nucleosil 100-5C8 brand of L7 column. The typical retention time for tacrolimus is about 14 min. The liquid chromatographic procedure for *Organic Impurities* is based on analyses performed using a Supelcosil LC-diol brand of L20 column. The typical retention time for tacrolimus is about 15 min.

(MD-ANT: A. Wise. BPC: M. Marques.) RTS—C53605

Add the following:

▪Tacrolimus Capsules

DEFINITION

Tacrolimus Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

IDENTIFICATION

- **A. PROCEDURE**

  Analysis: Transfer the contents of Capsules, equivalent to 5 mg of tacrolimus, into a centrifuge tube. Add 2 mL of alcohol, shake for 10 min, and centrifuge. To 1 mL of the supernatant, add 0.5 mL of a solution of 1,3-dinitrobenzene in alcohol (1 in 100) and 0.5 mL of sodium hydroxide TS, shake, and allow to stand for 3 min.

  Acceptance criteria: A light red-purple color develops.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

  [NOTE—Allow the *Standard solution* and *Sample solution* to stand for 6 h at 25° before use.]

  Mobile phase: Isopropanol, tetrahydrofuran, and water (2:2:5)

  Internal standard solution: 0.75 mg/mL solution of heptyl parahydroxybenzoate in dehydrated alcohol

  Standard solution: Dissolve a quantity of USP Tacrolimus RS in dehydrated alcohol, using about 30% of the final volume, add 20% of the final volume of *Internal standard solution*, and dilute with water to volume to obtain a solution having a concentration of about 0.5 mg/mL of tacrolimus.

  Sample solution: Using *Table 1*, transfer a quantity of the contents of Capsules into a suitable centrifuge tube, add dehydrated alcohol and *Internal standard solution*. Shake the centrifuge tube for a few min, and sonicate in an ultrasonic bath for 10 min. Centrifuge this suspension. Pipet 5 mL of the supernatant, and add 5 mL of water.

Table 1

Capsule Strength (mg)	Number of Capsules	Equivalent to Tacrolimus (mg)	Volume of Dehydrated Alcohol (mL)	Volume of Internal Standard (mL)
0.5	50	25	15.0	10.0
1	25	25	15.0	10.0
5	10	50	30.0	20.0

[NOTE—For products with strengths other than those listed in *Table 1*, adjust the number of Capsules and volumes to obtain concentrations equivalent to those in the *Standard solution*.]

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Temperature: 50°

Flow rate: 0.8 mL/min. [NOTE—Adjust the flow rate so that the retention time of tacrolimus is approximately 10 min. The flow rate may not be adjusted by more than ± 10%.]

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{44}H_{69}NO_{12}$ in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of tacrolimus to heptyl parahydroxybenzoate from the *Sample solution*

R_S = peak response ratio of tacrolimus to heptyl parahydroxybenzoate from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

  Medium: Hydroxypropylcellulose in water ($1:2 \times 10^4$) adjusted to a pH of 4.5 with phosphoric acid; 900 mL

  Apparatus 2: 50 rpm with sinker (See *Dissolution* ⟨711⟩, *Figure 2a*.)

  Mobile phase: Acetonitrile, methanol, water, and 6% phosphoric acid (46:18:36:0.1)

  Standard stock solution: L/400 mg/mL in acetonitrile [NOTE—L = label claim (mg/Capsule)]

  Standard solution: To 20.0 mL of the *Standard stock solution* add 50.0 mL of *Medium* and mix to obtain solutions with known concentrations as indicated in *Table 2*. Allow the solution to stand for NLT 6 h at 25° before use.

  Sample solution: Pass 10 mL of the solution under test through a G4 glass filter. To 5.0 mL of the filtrate, add 2.0 mL of acetonitrile and mix. Allow the solution to stand for NLT 1 h at 25° before use.

In-Process Revision

Table 2

Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	0.4
1	0.8
5	4

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperature: 50°
Flow rate: Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.
Injection size: See *Table 3*.

Table 3

Capsule Strength (mg)	Injection Volume (µL)
0.5	800
1	400
5	80

[NOTE—For products with strengths other than those listed in *Table 3*, adjust the injection volume to deliver an equivalent amount of tacrolimus into the column.]
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between tacrolimus 1*S*-epimer and tacrolimus
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of tacrolimus dissolved as a percentage of the labeled amount of $C_{44}H_{69}NO_{12}$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times C_S \times 900 \times (100/L)$$

r_U = peak response for tacrolimus from the *Sample solution*
r_S = peak response for tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{44}H_{69}NO_{12}$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**
[NOTE—It is suggested that new columns be conditioned with about 500 mL of ethanol before use to meet the resolution criterion.]
Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1)
System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*
Sample solution: Transfer the contents of a suitable number of Capsules (equivalent to about 5 mg of tacrolimus for 0.5-mg Capsules or 10 mg of tacrolimus for 1-mg and 5-mg Capsules) into a centrifuge tube. Add 1.5 mL of a mixture of *n*-butyl chloride and acetonitrile (2:1), sonicate in an ultrasonic bath for 2 min, add 3.5 mL of *n*-hexane, and mix. Centrifuge this solution and collect the supernatant or pass the solution through a 0.5-µm membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Flow rate: 1.5 mL/min. [NOTE—Adjust the flow rate so that the retention time of tacrolimus is approximately 15 min. The flow rate may not be adjusted by more than ±10%.]
Injection size: 20 µL
Run time: Three times the retention time of tacrolimus
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/F_i) \times [1/\Sigma(r_U/F_i)] \times 100$$

r_U = peak response of each peak in the *Sample solution*
F_i = relative response factor for corresponding peak (see *Table 4*)

Disregard peaks due to the solvent.
Acceptance criteria
Individual impurities: See *Table 4*.
Total impurities: NMT 0.3%

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus diene[a]	0.79	2.2	0.3
Tacrolimus regioisomer[b]	0.88	1.0	0.5
Tacrolimus impurity 1	0.96	1.0	0.3
Tacrolimus related compound A[c]	0.96	—	—
Tacrolimus	1.0	—	—
Tacrolimus 19-epimer[d]	1.1	1.0	5.0
Tacrolimus open ring[e]	1.3	1.0	3.0

[a](14*E*,18*E*)-17-Allyl-1-hydroxy-12- [(*E*)-2-(-4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0 4,9] octacosa-14,18-diene-2,3,10,16-tetrone.
[b](4*E*,11*E*)-10-Allyl-7,8,10,13,14,15,16,17,18,19,20,21,26,22,28,28a-hexadecahydro-7,21-dihydroxy-3-(4-hydroxy-3-methoxycyclohexyl)-16,18-dimethoxy-4,6,12,14,20-pentamethyl-17,21-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclopentacosine-1,9,22,23(6*H*,25*H*)-tetrone.
[c] For information only. Not to be reported.
[d](3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
[e](3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl] -1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.
[f]Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.2
Total impurities[f]	—	—	1.0

[a](14*E*,18*E*)-17-Allyl-1-hydroxy-12- [(*E*)-2-(-4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0 4,9] octacosa-14,18-diene-2,3,10,16-tetrone.
[b](4*E*,11*E*)-10-Allyl-7,8,10,13,14,15,16,17,18,19,20,21,26,22,28,28a-hexadecahydro-7,21-dihydroxy-3-(4-hydroxy-3-methoxycyclohexyl)-16,18-dimethoxy-4,6,12,14,20-pentamethyl-17,21-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclopentacosine-1,9,22,23(6*H*,25*H*)-tetrone.
[c] For information only. Not to be reported.
[d](3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
[e](3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl] -1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.
[f]Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tacrolimus RS
  USP Tacrolimus Related Compound A RS■1S *(USP33)*

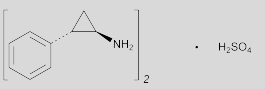
BRIEFING

Tranylcypromine Sulfate. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* was validated with a Phenomenex Synergi Polar RP brand of L11 column, in which tranylcypromine elutes at about 7 min. The liquid chromatographic procedure under *Organic Impurities* was validated with a Waters Atlantis brand of L1 column, in which tranylcypromine elutes at about 7 min.

(MD-PP: D. Vicchio, R. Ravichandran.) RTS—C62528

Add the following:

■Tranylcypromine Sulfate



$(C_9H_{11}N)_2 \cdot H_2SO_4$ 364.46
Cyclopropanamine, 2-phenyl-, *trans*-(±)-, sulfate (2:1); (±)-*trans*-2-Phenylcyclopropylamine sulfate (2:1) [13492-01-8].

DEFINITION

Tranylcypromine Sulfate contains NLT 98.0% and NMT 102.0% of $(C_9H_{11}N)_2 \cdot H_2SO_4$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, prepared as directed in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

ASSAY

- **PROCEDURE**

Buffer: Dissolve 3.4 g of monobasic ammonium phosphate into a 1-L volumetric flask containing about 900 mL of water. Adjust the pH of the solution to 2.2 ± 0.1 with phosphoric acid. Dilute with water to volume.
Mobile phase: Methanol and *Buffer* (3:7)
0.05 N sulfuric acid: Cautiously add 1.3 mL of sulfuric acid into 100 mL of water, cool to room temperature, and dilute to 1000 mL.
Diluent: Methanol, water, and 0.05 N sulfuric acid (1:3:1)
Standard stock solution: Using a sonicator, dissolve USP Tranylcypromine Sulfate RS in *0.05 N sulfuric acid* and methanol (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a 400 µg/mL solution.
Standard solution: 40 µg/mL of USP Tranylcypromine Sulfate RS in *Diluent*, prepared from *Standard stock solution*
Sample stock solution: Using a sonicator, dissolve tranylcypromine sulfate in methanol and *0.05 N sulfuric acid* (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a 400 µg/mL solution.
Sample solution: 40 µg/mL tranylcypromine sulfate in *Diluent*, prepared from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm column; 4-µm packing L11
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $(C_9H_{11}N)_2 \cdot H_2SO_4$ in the portion taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (µg/mL)
C_U = concentration of tranylcypromine sulfate in the *Sample solution* (µg/mL)

In-Process Revision

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS** *Method II*, **⟨231⟩:** NMT 20 ppm

Organic Impurities

- **PROCEDURE**

Buffer: Proceed as directed in the *Assay*.
0.05 N sulfuric acid: Proceed as directed in the *Assay*.
Diluent: Proceed as directed in the *Assay*.
Solution A: Methanol and *Buffer* (3:17)
Solution B: Methanol and *Buffer* (3:7)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
25	0	100
37	0	100
39	100	0
45	100	0

Standard stock solution: 14 µg/mL of USP Tranylcypromine Sulfate RS, and 60 µg/mL each of USP Tranylcypromine Related Compound A RS and USP Tranylcypromine Related Compound B RS in *Diluent*. [NOTE—Sonicate as needed.]
Standard solution: Transfer a portion of *Standard stock solution* to a suitable volumetric flask containing methanol and *0.05 N sulfuric acid* (30% of the final volume of each solvent). Dilute with *Diluent* to volume to obtain a solution containing 0.7 µg/mL of USP Tranylcypromine Sulfate RS and 3.0 µg/mL each of USP Tranylcypromine Related Compound A RS and USP Tranylcypromine Related Compound B RS.
Sample solution: Using a sonicator, dissolve tranylcypromine sulfate in methanol and *0.05 N Sulfuric acid* (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a solution containing 680 µg/mL of tranylcypromine sulfate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 249 for tranylcypromine related compound B; 220 nm for all other specified and unspecified impurities
Column: 4.6-mm × 15-cm column; 3 -µm packing L1
Temperature: 35°
Flow rate: 1.2 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for tranylcypromine, determined at 220 nm
Resolution: NLT 2.0 between tranylcypromine and tranylcypromine related compound A, determined at 220 nm
Relative standard deviation: NMT 6.0% for tranylcypromine and tranylcypromine related compound A, determined at 220 nm. NMT 6.0% for tranylcypromine related compound B, determined at 249 nm
Analysis
Samples: *Sample solution* and *Standard solution*
[NOTE—Disregard the signal for related compound B observed at 220 nm.]
Identify related compound A, *cis*-hydrazide, *trans*-hydrazide, and any unspecified impurity using the data in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of sample taken:

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

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of tranylcypromine sulfate from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (µg/mL)
C_U = concentration of tranylcypromine sulfate in the *Sample solution* (µg/mL)
F = response factor relative to tranylcypromine sulfate

Samples: *Sample solution* and *Standard solution*
Calculate the percentage of tranylcypromine related compound B in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response at 249 nm of related compound B from the *Sample solution*
r_S = peak response at 249 nm of related compound B from the *Standard solution*
C_S = concentration of USP Tranylcypromine Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of tranylcypromine sulfate in the *Sample solution* (µg/mL)
Mr_1 = molecular weight of 3-phenylallylamine free base, 133.19
Mr_2 = molecular weight of 3-phenylallylamine hydrochloride (USP Tranylcypromine Related Compound B RS), 169.66

Acceptance criteria
Individual impurities: See *Impurity Table 1*. [NOTE—Related compound B is determined at 249 nm. All other specified and unspecified impurities are determined at 220 nm.]
Total impurities: NMT 1.0% [NOTE—Sum of related compound A, related compound B, *cis*-hydrazide, *trans*-hydrazide, and any unspecified impurity.]

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Tranylcypromine related compound A[a]	0.82	0.84	0.50
Tranylcypromine sulfate	1.0	1.0	—
Tranylcypromine related compound B[b]	1.2	—	0.50
cis-Hydrazide[c]	1.4	0.86	0.50
trans-Hydrazide[d]	2.5	1.1	0.50
Any unspecified impurity	—	1.0	0.10

[a] (±)-*cis*-2-phenylcyclopropanamine (Ciscypromine).
[b] 3-Phenylallylamine (Cinnamylamine), monitored at 249 nm.
[c] (±)-*cis*-2-Phenylcyclopropanecarbohydrazide.
[d] (±)-*trans*-2-Phenylcyclopropanecarbohydrazide.

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry in vacuum at 60° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Tranylcypromine Sulfate RS
USP Tranylcypromine Related Compound A RS
USP Tranylcypromine Related Compound B RS■1S (*USP33*)

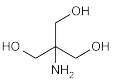
In-Process Revision

BRIEFING

Tromethamine, *USP 31* page 3479. On the basis of comments received, it is proposed to specify the drying conditions under the test for *Melting Range or Temperature*.

(MD-OOD: F. Mao.) RTS—C62475

Tromethamine



$C_4H_{11}NO_3$ 121.14
1,3-Propanediol, 2-amino-2-(hydroxymethyl)-;
2-Amino-2-(hydroxymethyl)-1,3-propanediol [77-86-1].

DEFINITION
Tromethamine contains NLT 99.0% and NMT 101.0% of $C_4H_{11}NO_3$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197M**⟩
- **B. PROCEDURE**
  Sample solution: 1 in 5
  Analysis: To 4.5 mL of a saturated solution of salicylaldehyde, add 0.5 mL of glacial acetic acid, and mix. Add 4.0 mL of the *Sample solution*, and mix.
  Acceptance criteria: A yellow color is produced.
- **C. PROCEDURE**
  Sample solution: 1 in 5
  Analysis: To 0.5 mL of a solution (4 in 10) of ceric ammonium nitrate in 2 N nitric acid, add 3 mL of water and 0.5 mL of the *Sample solution*, and mix.
  Acceptance criteria: The color changes from light yellow to orange.

ASSAY
- **PROCEDURE**
  Sample: 250 mg
  Analysis: Dissolve the *Sample* in 100 mL of water, add bromocresol purple TS, and titrate with 0.1 N hydrochloric acid VS to a yellow endpoint (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N hydrochloric acid is equivalent to 12.11 mg of $C_4H_{11}NO_3$.
  Acceptance criteria: 99.0%–101.0%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS

Change to read:

- **MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** 168°–172° ■Use test specimen previously dried at 105° for 3 h ■1S *(USP33)*
- **pH** ⟨**791**⟩**:** 10.0–11.5, in a solution (1 in 20)
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry it at 105° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Tromethamine RS

BRIEFING

Water for Injection, *USP 31* page 3523. The currently official monograph provides tests for the form of the article that is packaged in bulk for commercial use elsewhere. In this official monograph, the tests for the packaged bulk article are cross-referenced to the tests for Sterile Purified Water, and in the monograph itself there is also a *Bacterial Endotoxins Test*. However, the Sterile Purified Water monograph contains higher conductivity limits than those for bulk Water for Injection. In addition, the Sterile Purified Water monograph contains an *Oxidizable Substances* test, as opposed to the test in Water for Injection for *Total Organic Carbon*. These chemical tests are inconsistent with the specifications for Water for Injection, whether in bulk form (i.e., produced on site for use in manufacturing) or in packaged bulk form.
Therefore the following changes are proposed:
1. Make the specifications consistent for the bulk form (i.e., produced on site for use in manufacturing) and the packaged bulk form.
2. Add requirements for *Sterility, Packaging and Storage,* and *Labeling* to the packaged bulk form.
3. For the bulk form, remove the test for *Total Organic Carbon* and make changes in the specification for *Water Conductivity*.
4. Remove the cross-references to Sterile Purified Water.

The requirements for the bulk article are unchanged.

(PW: A. Hernandez-Cardoso.) RTS—C72119

Water for Injection

DEFINITION

Change to read:

[NOTE—For microbiological guidance, see the general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.]
Water for Injection is water purified by distillation or a purification process that is equivalent or superior to distillation in the removal of chemicals and microorganisms. It is prepared from water complying with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or with the drinking water regulations of the European Union or Japan, or with the World Health Organization's Guidelines for Drinking Water Quality. It contains no added substance.
[NOTE—Water for Injection is intended for use in the preparation of parenteral solutions. Where used for the preparation of parenteral solutions subject to final sterilization, use suitable means to minimize microbial growth, or first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. For parenteral solutions that are prepared under aseptic conditions and are not sterilized by appropriate filtration or in the final container, first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. ~~The tests for *Total Organic Carbon* and *Water Conductivity* apply to Water for Injection produced on site for use in manufacturing.~~ ■■1S *(USP33)* Water for Injection packaged in bulk for commercial use elsewhere meets the ~~requirement of the test for *Bacterial Endotoxins*~~■ additional requirements of the test for *Sterility Tests* in *Specific Tests*, as well as those for *Packaging and Storage* and *Labeling*,■1S *(USP33)* as indicated ~~below and the requirements of all the tests under *Sterile Purified Water*, except *Labeling*~~■ in *Additional Requirements*.■1S *(USP33)*]

In-Process Revision

SPECIFIC TESTS

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** ~~NLT~~ Less than ■1S *(USP33)* 0.25 USP Endotoxin Unit/mL

Change to read:

• **WATER CONDUCTIVITY,** ■*Bulk Water* ■1S *(USP33)*⟨**645**⟩**:** Meets the requirements
• **TOTAL ORGANIC CARBON** ⟨**643**⟩**:** Meets the requirements

Add the following:

■• **STERILITY TESTS** ⟨**71**⟩**:** Where packaged, meets the requirements■1S *(USP33)*

ADDITIONAL REQUIREMENTS

Add the following:

■• **PACKAGING AND STORAGE:** Where packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.■1S *(USP33)*

Add the following:

■• **LABELING:** Where packaged, label the article to state that it contains no antimicrobial or other subtance, and that it is not intended for direct parenteral administration.■1S *(USP33)*

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP 1,4-Benzoquinone RS
USP Endotoxin RS
USP Sucrose RS

BRIEFING

Purified Water, *USP 31* page 3524. This proposal makes the specifications for bulk and packaged Purified Water consistent. Also, it adds packaging, storage, and labeling requirements for the packaged form. Last, the proposal removes the cross references to *Sterile Purified Water*. The requirements for bulk Purified Water are unchanged. The monograph for Purified Water provides for a "packaged in bulk for commercial use elsewhere" form of the article. At present, the *Specific Tests* for this form of the article are cross-referenced to the specification for *Sterile Purified Water*. However, the *Sterile Purified Water* monograph contains higher conductivity limits than for Purified Water. In addition, the *Sterile Purified Water* monograph contains an *Oxidizable Substances Test* as opposed to the *Total Organic Carbon* ⟨643⟩ test for Purified Water. These chemical tests are inconsistent with the specifications for Purified Water, whether the form of the article is in bulk or packaged form.

(PW: A. Hernandez-Cardoso.) RTS—C72119

Purified Water

H_2O 18.02

DEFINITION

Change to read:

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]
Purified Water is water obtained by a suitable process. It is prepared from water complying with the U. S. Environmental Protection Agency National Primary Drinking Water Regulations or with the drinking water regulations of the European Union, Japan, or with the World Health Organization's Guidelines for Drinking Water Quality. It contains no added substance.
[NOTE—Purified Water is intended for use as an ingredient of official preparations and in tests and assays unless otherwise specified (see ~~*Water* in *Ingredients and Processes* and in *Tests and Assays* under~~■ *8.230. Water* under *8. Terms and Definitions* in the■1S *(USP33)* *General Notices and Requirements*). Where used for sterile dosage forms, other than for parenteral administration, process the article to meet the requirements under *Sterility Tests* ⟨71⟩, or first render the Purified Water sterile and thereafter protect it from microbial contamination. Do not use Purified Water in preparations intended for parenteral administration. For such purposes use *Water for Injection*, *Bacteriostatic Water for Injection*, or *Sterile Water for Injection*. ~~The tests for *Total Organic Carbon* and *Conductivity* apply to Purified Water produced on site for use as an ingredient of official preparations and in tests and assays.~~ ■1S *(USP33)* Purified Water packaged in bulk for commercial use elsewhere meets the ■additional■1S *(USP33)* requirements ~~of all of the tests under *Sterile Purified Water*, except *Labeling* and *Sterility* ⟨71⟩~~ ■for *Packaging and Storage* and *Labeling* as indicated under *Additional Requirements*.■1S *(USP33)*]

SPECIFIC TESTS
• **TOTAL ORGANIC CARBON** ⟨**643**⟩**:** Meets the requirements
• **WATER CONDUCTIVITY,** *Bulk Water* ⟨**645**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

Add the following:

■• **PACKAGING AND STORAGE:** Where packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.■1S *(USP33)*

Add the following:

■• **LABELING:** Where packaged, label it to indicate the method of preparation and that it is not intended for parenteral administration.■1S *(USP33)*

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP 1,4-Benzoquinone RS
USP Sucrose RS

In-Process Revision

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 26* page 1057, page 3800 of the *Second Supplement,* and page 109 of *PF* 35(1) [Jan.–Feb. 2009]. It is proposed to add *Ethylene Glycol and Vinyl Alcohol Graft Copolymer* to the *Coating Agent* and *Tablet Binder* categories; *Sucrose Palmitate* to the *Suspending and/or Viscosity-Increasing Agent* category; and *Sucrose Stearate* to the *Emulsifying and/or Solubilizing Agent* and *Tablet and/or Capsule Lubricant* categories to complement the proposed new monographs for *Ethylene Glycol and Vinyl Alcohol Graft Copolymer, Sucrose Palmitate,* and *Sucrose Stearate,* which appear elsewhere in this issue of *PF.*

(EM1; EM2) RTS—C68850; C66403; C66404

Change to read:

Antimicrobial Preservative

Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzethonium Chloride
Benzoic Acid
Benzyl Alcohol
Butylparaben

▲Calcium Propionate▲*NF28*

Cetrimonium Bromide
Cetylpyridinium Chloride
Chlorobutanol
Chlorocresol
Cresol
■Dehydroacetic Acid■2S *(NF26)*

▲Erythorbic Acid▲*NF27*

Ethylparaben
Methylparaben
Methylparaben Sodium
Phenol
Phenoxyethanol
Phenylethyl Alcohol
Phenylmercuric Acetate
Phenylmercuric Nitrate
Potassium Benzoate
Potassium Sorbate
Propylparaben
Propylparaben Sodium
Sodium Benzoate
Sodium Dehydroacetate
Sodium Propionate
Sorbic Acid
Thimerosal
Thymol

Change to read:

Antioxidant

Ascorbic Acid
Ascorbyl Palmitate
Butylated Hydroxyanisole
Butylated Hydroxytoluene
■Stannous Chloride■2S *(NF26)*

▲Erythorbic Acid▲*NF27*

Hypophosphorous Acid
Monothioglycerol
Potassium Metabisulfite
Propyl Gallate
Sodium Bisulfite
Sodium Formaldehyde Sulfoxylate
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Sulfur Dioxide
Tocopherol
Tocopherols Excipient

Change to read:

Buffering Agent

Acetic Acid
Adipic Acid
Ammonium Carbonate
Ammonium Phosphate
Boric Acid
Citric Acid, Anhydrous
Citric Acid Monohydrate

■Alpha-Lactalbumin■1S *(NF27)*

Lactic Acid
Phosphoric Acid
Potassium Citrate
Potassium Metaphosphate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Citrate
Sodium Lactate Solution
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Succinic Acid

Change to read:

Bulking Agent for Freeze-Drying

Creatinine

■Alpha-Lactalbumin■1S *(NF27)*

Mannitol
▲Polydextrose▲*NF26*
■Pullulan■2S *(NF26)*

■Trehalose■1S *(NF27)*

In-Process Revision

Change to read:

Coating Agent

Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carboxymethylcellulose, Sodium

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Cellaburate
Cellacefate (formerly Cellulose Acetate Phthalate)
Cellulose Acetate
Cellulose Acetate Phthalate (see Cellacefate)

▲Chitosan▲*NF28*

Coconut Oil

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone
Corn Syrup Solids
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Ethylcellulose Aqueous Dispersion

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S (*NF28*)

Gelatin
Glaze, Pharmaceutical
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hydroxypropyl Methylcellulose Phthalate (see Hypromellose Phthalate)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate
Hypromellose Phthalate (formerly Hydroxypropyl Methylcellulose Phthalate)

■Alpha-Lactalbumin■1S (*NF27*)

Maltodextrin
Methacrylic Acid Copolymer
Methacrylic Acid Copolymer Dispersion
Methylcellulose
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Glycol

▲Polyvinyl Acetate▲*NF28*

▲Polyvinyl Acetate Dispersion▲*NF28*

Polyvinyl Acetate Phthalate
■Pullulan■2S (*NF26*)
▲Fully Hydrogenated Rapeseed Oil▲*NF26*
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Shellac
Starch, Pregelatinized Modified
Sucrose
Titanium Dioxide
Wax, Carnauba
Wax, Microcrystalline
Zein

Change to read:

Complexing Agent

Edetate Calcium Disodium
Edetate Disodium
Edetic Acid

■Alpha-Lactalbumin■1S (*NF27*)

Oxyquinoline Sulfate

Change to read:

Desiccant

Calcium Chloride
Calcium Sulfate

▲Polyvinyl Acetate▲*NF28*

Silicon Dioxide

Change to read:

Emulsifying and/or Solubilizing Agent

Acacia
Carbomer Copolymer
Carbomer Interpolymer
Cholesterol
■Stannous Chloride■2S (*NF26*)
Coconut Oil

▲Desoxycholic Acid▲*NF28*

Diethanolamine (Adjunct)
Diethylene Glycol Stearates
Ethylene Glycol Stearates
■Gamma Cyclodextrin■2S (*NF26*)
Glyceryl Distearate
Glyceryl Monolinoleate
Glyceryl Monooleate
Glyceryl Monostearate

■Alpha-Lactalbumin■1S (*NF27*)

Lanolin Alcohols
Lecithin
Mono- and Di-glycerides
Monoethanolamine (Adjunct)
Oleic Acid (Adjunct)
Oleyl Alcohol (Stabilizer)
▲Oleyl Oleate▲*NF26*
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

Poloxamer
Polyoxyethylene 50 Stearate
Polyoxyl 10 Oleyl Ether
Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polyoxyl Lauryl Ether
Polyoxyl Stearyl Ether
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
■Propylene Glycol Dicaprylate/Dicaprate■2S *(NF26)*
■Propylene Glycol Monocaprylate■1S *(NF26)*
Propylene Glycol Monostearate
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Sodium Cetostearyl Sulfate
Sodium Lauryl Sulfate
Sodium Stearate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Stearic Acid

■Sucrose Stearate■1S *(NF28)*

Trolamine
Wax, Emulsifying

Add the following:

Film-Forming Agent

▲Chitosan▲*NF28*

Change to read:

Humectant

Corn Syrup Solids
Erythritol
Glycerin
Hexylene Glycol
■Inositol■2S *(NF26)*
Maltitol
▲Polydextrose▲*NF26*
Propylene Glycol
Sorbitol
Sorbitol Sorbitan Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Tagatose

Change to read:

Stiffening Agent

Castor Oil, Hydrogenated
Cetostearyl Alcohol
Cetyl Alcohol
Cetyl Esters Wax
Cetyl Palmitate
Hard Fat

■Alpha-Lactalbumin■1S *(NF27)*

Paraffin
Synthetic Paraffin
▲Fully Hydrogenated Rapeseed Oil▲*NF26*
▲Superglycerinated Fully Hydrogenated Rapeseed Oil▲*NF26*
Stearyl Alcohol
Wax, Emulsifying
Wax, White
Wax, Yellow

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

▲Chitosan▲*NF28*

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

■Alpha-Lactalbumin■1S *(NF27)*

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin
Polyethylene Oxide
Polyvinyl Alcohol

In-Process Revision

Povidone
Propylene Glycol Alginate
■Pullulan■2S (*NF26*)
■Hydrophobic Colloidal Silica■2S (*NF26*)
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca
Starch, Wheat

■Sucrose Palmitate■1S (*NF28*)

Tragacanth
Xanthan Gum

Change to read:

Sweetening Agent

Acesulfame Potassium
Aspartame
Aspartame Acesulfame

▲Corn Syrup▲*NF27*

Corn Syrup Solids
High Fructose Corn Syrup
Dextrates
Dextrose
Dextrose Excipient
Erythritol
Fructose
Galactose
Maltitol
Maltose
Mannitol
Saccharin
Saccharin Calcium
Saccharin Sodium
Sorbitol
Sorbitol Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Sucralose
Sucrose
Sugar, Compressible
Sugar, Confectioner's
Syrup
Tagatose

■Trehalose■1S (*NF27*)

Change to read:

Tablet Binder

Acacia
Alginic Acid
Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Sodium
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrin
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S (*NF28*)

Gelatin
Glucose, Liquid
Guar Gum
Low-Substituted Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate

■Alpha-Lactalbumin■1S (*NF27*)

Maltodextrin
Maltose
Methylcellulose

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Oxide

▲Polyvinyl Acetate▲*NF28*

Povidone
■Pullulan■2S (*NF26*)
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Syrup

■Trehalose■1S (*NF27*)

Change to read:

Tablet and/or Capsule Diluent

Calcium Carbonate
Calcium Phosphate, Dibasic
Calcium Phosphate, Tribasic
Calcium Sulfate
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Cellulose, Powdered

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrates
Dextrin
Dextrose Excipient
Fructose
Kaolin

■Alpha-Lactalbumin■1S *(NF27)*

Lactitol
Lactose, Anhydrous
Lactose, Monohydrate
Maltitol
Maltodextrin
Maltose
Mannitol
■Propylene Glycol Monocaprylate■1S *(NF26)*
■Pullulan■2S *(NF26)*
Sorbitol
Starch
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Sucrose
Sugar, Compressible
Sugar, Confectioner's

■Trehalose■1S *(NF27)*

Change to read:

Tablet Disintegrant

Alginic Acid
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Croscarmellose Sodium
Crospovidone
Low-Substituted Hydroxypropyl Cellulose
Maltose
Polacrilin Potassium
■Pullulan■2S *(NF26)*
Sodium Starch Glycolate
Starch
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat

■Trehalose■1S *(NF27)*

Change to read:

Tablet and/or Capsule Lubricant

■Behenoyl Polyoxylglycerides■2S *(NF27)*

Calcium Stearate

■Hydrogenated Coconut Oil■1S *(NF27)*

Glyceryl Behenate
Magnesium Stearate
Mineral Oil, Light

■Hydrogenated Palm Oil■1S *(NF27)*

Polyethylene Glycol
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Sodium Lauryl Sulfate
Sodium Stearyl Fumarate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Starch
Stearic Acid
Stearic Acid, Purified

■Sucrose Stearate■1S *(NF28)*

Talc
Vegetable Oil, Hydrogenated, Type I
Zinc Stearate

Change to read:

Tonicity Agent

▲Corn Syrup▲*NF27*

Corn Syrup Solids
Dextrose
Glycerin
Mannitol
Potassium Chloride
Sodium Chloride

Change to read:

Vehicle

FLAVORED AND/OR SWEETENED

Aromatic Elixir
Benzaldehyde Elixir, Compound
Corn Syrup Solids
Dextrose

■Ethyl Maltol■2S *(NF27)*

Peppermint Water
Sorbitol Solution
Syrup

■Trehalose■1S *(NF27)*

OLEAGINOUS

Alkyl (C12-15) Benzoate
Almond Oil
Canola Oil
Corn Oil
Cottonseed Oil
Ethyl Oleate
■Hydrogenated Polydecene■1S *(NF26)*
Isopropyl Myristate
Isopropyl Palmitate
Mineral Oil
Mineral Oil, Light
Octyldodecanol
Olive Oil
Peanut Oil

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Safflower Oil
Sesame Oil
Soybean Oil
Squalane

SOLID CARRIER

▲Chitosan▲*NF28*

Corn Syrup Solids

■Alpha-Lactalbumin■1S *(NF27)*

■Propylene Glycol Dicaprylate/Dicaprate■2S *(NF26)*
■Propylene Glycol Monocaprylate■1S *(NF26)*
Sugar Spheres

STERILE

▲rAlbumin Human▲*NF27*

Sodium Chloride Injection, Bacteriostatic
Water for Injection, Bacteriostatic

Change to read:

Wetting and/or Solubilizing Agent

Benzalkonium Chloride
Benzethonium Chloride
Cetylpyridinium Chloride
Docusate Sodium
Nonoxynol 9
Octoxynol 9
Poloxamer
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
■Pullulan■2S *(NF26)*
Sodium Lauryl Sulfate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Tyloxapol

MONOGRAPHS (NF)

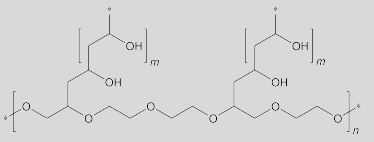
BRIEFING

Ethylene Glycol and Vinyl Alcohol Graft Copolymer. Because there is no existing *NF* monograph for this excipient, a new monograph is being proposed, based on validated methods of analysis. The gas chromatographic procedure for *Procedure 1: Ethylene Oxide and Dioxane* employs a J & W Scientific DB-1 brand of G1 column. The typical retention times for acetaldehyde and ethylene oxide are 5 and 5.4 min, respectively. The liquid chromatographic procedure for *Procedure 2: Vinyl Acetate* employs a Thermo Aquasil brand of column that contains 5-µm packing L1. The typical retention times for vinyl acetate and 1-vinylpyrrolidin-2-one are 19 and 26 min, respectively. The liquid chromatographic procedure for *Procedure 3: Acetic Acid/Acetate* is based on analysis performed with a Thermo Aquasil brand of column that contains 5-µm packing L1. The typical retention times for acetic acid and citric acid are 5 and 7 min, respectively.

(EM2: H. Wang.) RTS—C68850

Add the following:

▪Ethylene Glycol and Vinyl Alcohol Graft Copolymer



Polyethylene glycol-graft-polyvinyl alcohol;
Graft-copoly(ethylene glycol-vinyl alcohol)
[121786-16-1].

DEFINITION

Ethylene Glycol and Vinyl Alcohol Graft Copolymer is a graft copolymer of ethylene glycol and vinyl alcohol. It consists of about 75% of vinyl alcohol (–$CH_2CH(OH)$–) units and 25% of ethylene glycol (–CH_2CH_2O–) units. The Copolymer is produced by using polyethylene glycol and vinyl acetate as starting materials. Polyethylene glycol forms the polymer backbone on which polyvinyl acetate is grafted. Hydrolysis of polyvinyl acetate side chains leads to formation of polyvinyl alcohol grafted chains. Ethylene Glycol and Vinyl Alcohol Graft Copolymer may contain glidant to improve flowability.

IDENTIFICATION

• **INFRARED ABSORPTION**

Sample: 200 mg of Ethylene Glycol and Vinyl Alcohol Graft Copolymer

Analysis: Dissolve the *Sample* in 5–10 mL of water. Spread 1 mL of this solution on a thallium bromoiodide window.[1] Totally moisten the surface area of the window. Dry the window at 110° for approximately 30 min, and allow the window to cool to room temperature.

Acceptance criteria: The IR absorption spectrum of Ethylene Glycol and Vinyl Alcohol Graft Copolymer exhibits maxima corresponding to the same wavelengths as those of a similar preparation of USP Ethylene Glycol and Vinyl Alcohol Graft Copolymer RS treated in the same manner.

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION ⟨281⟩**

Sample: 10.0 g

Acceptance criteria: NMT 3.0%

Organic Impurities

• **PROCEDURE 1: ETHYLENE OXIDE AND DIOXANE**

[**CAUTION**—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate at a temperature between 4° and 8°.]

[NOTE—Before using polyethylene glycol 200 in this test, remove any volatile components from it by placing 500 mL of polyethylene glycol 200 in a 1000-mL round-bottom flask, and attaching the flask to a rotary evaporator maintained at a temperature of 60° and under a vacuum of 10–20 mm Hg for 6 h.]

Acetaldehyde solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare the *Acetaldehyde solution* immediately prior to use.]

Ethylene oxide stock solution: Introduce 300 µL (corresponding to 250 mg) of gaseous ethylene oxide to the polyethylene glycol 200. Determine the absorbed mass of ethylene oxide via the weight of the solution before and after the absorption. Dilute the solution with the polyethylene glyol 200 to 100 g. This stock solution contains 2.5 mg/g of ethylene oxide. [NOTE—Prepare this stock solution immediately prior to use, and store in a refrigerator after preparation.]

Ethylene oxide solution: 100 µg/g of ethylene oxide in polyethylene glycol 200 from *Ethylene oxide stock solution*. Transfer 5 g of 100 µg/g ethylene oxide in polyethylene glycol 200 to a 50-mL volumetric flask filled with 30 mL of water. Dilute with water to volume, and mix to obtain a solution containing 10 µg/mL of ethylene oxide. [NOTE—Prepare this solution immediately prior to use, and use the solution directly after preparation.]

Dioxane solution: 500 µg/mL of dioxane

Standard solution A: Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial. [NOTE—Other sizes such as 22-mL may be used depending on the operating conditions, however, the same size must be used for *Standard solution A*, *Standard solution B*, and the *Sample solution*.] Add 0.1 mL of *Acetaldehyde solution* and 0.1 mL of *Dioxane solution*, seal the vial, and mix.

Standard solution B: Transfer 1.0 g of Ethylene Glycol and Vinyl Alcohol Graft Copolymer to a 10-mL pressure headspace vial, add 0.1 mL of *Ethylene oxide solution*, 0.1 mL of *Dioxane solution*, and 1.0 mL of *N,N*-dimethylacetamide. Seal the vial, and mix.

Sample solution: Transfer 1.0 g of Ethylene Glycol and Vinyl Alcohol Graft Copolymer to a 10-mL pressure headspace vial, add 1.0 mL of *N,N*-dimethylacetamide and 0.2 mL of water, seal the vial, and mix.

[1]Thallium bromoiodide, also known as KRS-5, consists of 42% thallium(I) bromide and 58% thallium(I) iodine by molecular weight. Suitable disks of KRS-5 are available from www.photonic.saint-gobain.com, www.almazoptics.com, and www.internationalcrystal.net.

In-Process Revision

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—Use a headspace apparatus that automatically transfers a measured amount of headspace.]
Mode: GC
Detector: Flame ionization
Detector temperature: 250°
Column: 0.25-mm × 30-m glass or quartz capillary column; 1.0-µm layer of phase G1
Column temperature: See the temperature program table below.

Temperature (°)	Rate (°/min)	Hold Time (min)
50	—	5
50→180	5	—
180→230	30	—
230	—	5

Carrier gas: Helium
Flow rate: 0.8 mL/min
Injection size: 1 mL (the gaseous headspace)
Injection type: Split ratio 20:1
Injection port temperature: 250°
Headspace sampler
Equilibration time: 45 min
Equilibration temperature
Standard solution A: 70°
Standard solution B: 90°
Sample solution: 90°
Transfer line temperature: 150°
Pressurization time: 1 min
Final headspace pressure: 0.7 bar
Injection time: 12 s
System suitability
Sample: *Standard solution A*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.94 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between acetaldehyde and ethylene oxide
Signal-to-noise: NLT 5 determined from the dioxane peak
Relative standard deviation: NMT 15%
Analysis
Samples: *Standard solution B* and *Sample solution*
Calculate the content of ethylene oxide, in ppm, in the portion of Ethylene Glycol and Vinyl Alcohol Graft Copolymer taken:

$$\text{Result} = A_E \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_E = quantity of ethylene oxide added to *Standard solution B* (µg)
r_U = ethylene oxide peak response from the *Sample solution*
r_S = ethylene oxide peak response from the *Standard solution B*
W_U = weight of Ethylene Glycol and Vinyl Alcohol Graft Copolymer taken to prepare the *Sample solution* (g)
W_S = weight of Ethylene Glycol and Vinyl Alcohol Graft Copolymer taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of Ethenylene Glycol and Vinyl Alcohol Graft Copolymer taken:

$$\text{Result} = A_D \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_D = quantity of dioxane added to *Standard solution B* (µg)
r_U = dioxane peak response from the *Sample solution*
r_S = dioxane peak response from *Standard solution B*
W_U = weight of Ethylene Glycol and Vinyl Alcohol Graft Copolymer taken to prepare the *Sample solution* (g)
W_S = weight of Ethylene Glycol and Vinyl Alcohol Graft Copolymer taken to prepare *Standard solution B* (g)

Acceptance criteria: NMT 1 ppm of ethylene oxide; NMT 10 ppm of dioxane

• **PROCEDURE 2: VINYL ACETATE**
Solution A: Acetonitrile, methanol, and water (5:5:90)
Solution B: Acetonitrile, methanol, and water (45:5:50)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0–2	100	0
2–40	100→85	0→15
40–42	85→0	15→100
42–48	0	100
48–51	0→100	100→0

System suitability solution: Transfer 50 mg of vinyl acetate and 50 mg of 1-vinylpyrrolidin-2-one to a 50-mL volumetric flask, add 10 mL of methanol, and sonicate or gently shake the flask to dissolve the materials. Dilute with *Solution A* to volume. Dilute 10 mL of this solution with *Solution A* to 100 mL. Dilute 5 mL of this solution with *Solution A* to 100 mL. The *System suitability solution* contains about 5 µg/mL each of vinyl acetate and 1-vinylpyrrolidin-2-one.
Standard solution: Transfer 50 mg of vinyl acetate to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix well. Dilute 5.0 mL of the solution with *Solution A* to 100 mL. Dilute 10.0 mL of this solution with *Solution A* to 100 mL. The *Standard solution* contains about 2.5 µg/mL of vinyl acetate. [NOTE—This solution should be analyzed within 1 h when stored at room temperature.]
Sample solution: Transfer 250 mg of Ethylene Glycol and Vinyl Alcohol Graft Copolymer to a 10-mL volumetric flask, and add 1–2 mL of methanol, using an ultrasonic bath if necessary. After cooling to ambient temperature, dilute with water to volume, and mix. Pass through a 0.2-mm membrane filter. [NOTE—This solution should be analyzed within 1 h when stored at room temperature.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.0-mm × 25-cm analytical column; 5-µm packing L1. A 4.0-mm × 3-cm pre-column; 5-µm packing L1 may be used if a matrix effect is observed. [NOTE—The matrix effect may result in poor reproducibility of the retention times and of the peak shapes.]
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for vinyl acetate and 1-vinylpyrrolidin-2-one are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 5.0 between vinyl acetate and 1-vinylpyrrolidin-2-one
Relative standard deviation: NMT 5.0% determined from the 1-vinylpyrrolidin-2-one peak
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The response of the vinyl acetate peak from the *Sample solution* is NMT that of the vinyl acetate peak from the *Standard solution*, corresponding to NMT 100 ppm of vinyl acetate.

- **PROCEDURE 3: ACETIC ACID/ACETATE**
  Mobile phase: 5 mM sulfuric acid
  Standard solution: 0.3 mg/mL for each of acetic acid and citric acid in *Mobile phase*
  Sample solution: 20 mg/mL of Ethylene Glycol and Vinyl Alcohol Copolymer
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 205 nm
  Column: 4.6-mm × 25-cm column; 5-µm packing L1
  Column clean: After each injection, rinse the column with a mixture of equal volumes of *Mobile phase* and acetonitrile.
  Column temperature: Ambient
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for acetic acid and citric acid are 1.0 and 1.3, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between acetic acid and citric acid
  Relative standard deviation: NMT 5.0% determined from the acetic acid peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Acceptance criteria: The response of the acetic acid peak from the *Sample solution* is NMT that of the acetic acid peak from the *Standard solution*, corresponding to NMT 1.5% of acetic acid.

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Ester Value* ⟨**401**⟩**:** 10–75
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of Ethylene Glycol and Vinyl Alcohol Graft Copolymer in a vacuum at 140° for 1 h: it loses NMT 5.0% of its weight.
- **pH** ⟨**791**⟩**:** 5.0–8.0, in a solution of 20% (w/w) in carbon dioxide-free water
- **VISCOSITY** ⟨**912**⟩**:** After determining the *Loss on Drying*, weigh a quantity of undried Ethylene Glycol and Vinyl Alcohol Graft Copolymer, equivalent to 100.0 g on the dried basis. Transfer the sample to a short form, 600-mL beaker (internal diameter about 80 mm and height 120 mm), and add water to make the mixture weigh 500 g. Ensure a homogeneous solution by gently stirring at room temperature for 48 h. Afterwards allow the container to stand for 24 h to let the entrapped air dissipate. [NOTE—Ensure that the concentration of this solution is 20% (w/w).] Determine the viscosity of this solution at 23 ± 0.1° using a suitable rotational viscometer with a spindle having a cylinder 4.7 cm in diameter and 0.2 cm high attached to a shaft 0.3 cm in diameter, the distance from the top of the cylinder to the lower tip of the shaft being 2.7 cm, and the immersion depth being 4.9 cm.[2] Operate the viscometer at 100 rpm. Follow the instrument manufacturer's directions to measure the apparent viscosity.
  Acceptance criteria: 25–250 mPa · s

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at a temperature below 25°.
- **LABELING:** Label it to indicate the viscosity, giving the viscosity measurement parameters, concentration of the solution, and the type of equipment used. The labeling also indicates the name and quantity of any added substance.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Ethylene Glycol and Vinyl Alcohol Graft Copolymer RS■1S (*NF28*)

[2] A commercial instrument is available as an RV2 spindle from Brookfield.

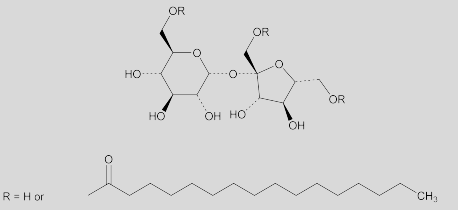
BRIEFING

Sucrose Palmitate. Because there is no existing *NF* monograph for this article, it is proposed to add a new monograph based on the Sucrose Monopalmitate monograph in the *European Pharmacopoeia* 6.2. The proposed liquid chromatographic procedure for *Free Sucrose* under *Organic Impurities* is based on analysis performed using a Waters high-performance carbohydrate cartridge with a Nova-Pak NH2 brand of 5-µm L8 column. The typical retention time is 26 min. The proposed liquid chromatographic procedure for the *Assay* is based on analysis performed using a Phenomenex PLgel brand of 4-µm L21 column.

(EM1: R. Lafaver; NOM: A. Wilk.) RTS—C66403

Add the following:

■Sucrose Palmitate



$C_{28}H_{52}O_{12}$ 580.17
$C_{44}H_{82}O_{13}$ 819.11
$C_{60}H_{112}O_{14}$ 1057.52
Sucrose monopalmitate;
Sucrose hexadecanoate [26446-38-8].

DEFINITION

Sucrose Palmitate is a mixture of sucrose monoesters, mainly sucrose monopalmitate, obtained by transesterification of palmitic acid methyl esters of vegetable origin with sucrose. The manufacture of the fatty acid methyl esters includes a distillation step. It contains variable quantities of mono- and diesters as set forth in the following table:

Content of Monoesters (%)	Content of Diesters (%)	Sum of Triesters and Polyesters (%)
NLT 55.0	NMT 40.0	NMT 20.0

IDENTIFICATION

- **A.** It meets the requirements of the *Fatty Acid Composition* test.
- **B.** It meets the requirements of the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Tetrahydrofuran
  Sample solution: 15 mg/mL of Sucrose Palmitate in tetrahydrofuran

In-Process Revision

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC, size-exclusion
Detector: Differential refractometer
Column: 7-mm × 60-cm column; packing L21, 100Å
[NOTE—Two 7-mm × 30-cm L21 columns may be used in place of one 60-cm column, provided system suitability requirements are met.]
Flow rate: 1.2 mL/min
Injection size: 20 µL
Analysis
Sample: *Sample solution*
[NOTE—The relative retention time with reference to the monoester peak is 0.92 and 0.90 for diesters and for triesters and polyesters, respectively.]
[NOTE—Disregard solvent peaks and peaks having a signal-to-noise ratio less than 10.]
Calculate the percentage of monoesters in the portion of Sucrose Palmitate taken:

$$\text{Result} = A \times (100 - D - S - E)/100$$

A = percentage of monoesters determined by peak normalization
E = percentage of water (below)
S = percentage of free sucrose (below)
D = percentage of free fatty acids, using the following formula:

$$\text{Result} = AV \times 256/561.1$$

where AV = acid value
Calculate the percentage of diesters in the portion of Sucrose Palmitate taken:

$$\text{Result} = B \times (100 - D - S - E)/100$$

B = percentage of diesters determined by peak normalization
E = percentage of water (below)
S = percentage of free sucrose (below)
D = percentage of free fatty acids (below)
Calculate the percentage of triesters and polyesters in the portion of Sucrose Palmitate taken:

$$\text{Result} = C \times (100 - D - S - E)/100$$

C = percentage of triesters and polyesters determined by peak normalization
E = percentage of water (below)
S = percentage of free sucrose (below)
D = percentage of free fatty acids (below)

OTHER COMPONENTS

- **FATTY ACID COMPOSITION:** Sucrose Palmitate exhibits the following composition profiles of fatty acids, as determined under *Fats and Fixed Oils, Fatty Acid Composition* ⟨401⟩.

Fatty Acid	Percentage (%)
Lauric acid	NMT 3.0
Myristic acid	NMT 3.0
Palmitic acid	70.0 to 85.0
Stearic acid	10.0 to 25.0
Sum of the contents of palmitic acid and stearic acid	NLT 90.0

IMPURITIES

Inorganic Impurities

- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 6.0%, determined on a 3-g sample. Use a freshly neutralized mixture of 2-propanol and water (2:1), and gently heat.

Organic Impurities

- **PROCEDURE: FREE SUCROSE**
Solution A: 10 µg/mL of ammonium acetate in acetonitrile
Solution B: 10 µg/mL of ammonium acetate in tetrahydrofuran and water (90:10)
Diluent: Tetrahydrofuran and water (87.5:12.5)
System suitability solution: 10 µg/mL of USP Sucrose RS in *Diluent*
Standard solutions: 0.50 mg/mL, 1.0 mg/mL, 2.0 mg/mL, and 2.5 mg/mL of USP Sucrose RS in *Diluent*
Sample solution: 50 mg/mL of Sucrose Palmitate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: Evaporative light-scattering [NOTE—If the detector has different setting parameters, adjust the detector settings so that they comply with the *System suitability* requirements.]
Carrier gas: Nitrogen
Detector temperature: 45°
Nebulizer temperature: 40°
Column: 4.6-mm × 0.25-m column; packing L8
Injection size: 20 µL
Mobile phase: See gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
1	100	0	1.0
8	0	100	1.0
7	0	100	1.0
0.01	0	100	2.5
15.99	0	100	2.5
1	100	0	2.5
3	100	0	1.0

System suitability
Sample: *System suitability solution*
[NOTE—The retention time is about 26 min for sucrose palmitate.]
Analysis
Samples: *Standard solutions* and *Sample solution*
Prepare a standard curve by plotting the peak response versus concentration of sucrose in the *Standard solutions*. Calculate the amount of free sucrose in Sucrose Palmitate.
Acceptance criteria: NMT 4.0%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** NMT 4.0%, on a 0.20-g sample
- **TOTAL ASH**
Sample: 1.0 g
Analysis: Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator and weigh. Transfer the *Sample* into the crucible. Dry at 100° to 105° for 1 h and ignite to constant weight in a muffle furnace at 600° ± 25°, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, add hot water, filter through an ashless filter paper, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant weight.
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in a well-closed container. Protect from humidity and avoid high temperatures.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Sucrose RS■1S *(NF28)*

In-Process Revision

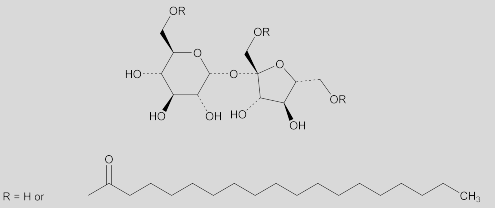
BRIEFING

Sucrose Stearate. Because there is no existing *NF* monograph for this article, it is proposed to add a new monograph based on the Sucrose Stearate monograph in the *European Pharmacopoeia* 6.2. The proposed liquid chromatographic procedures in the test for *Free Sucrose* are based on analysis performed using a Waters high performance analysis carbohydrate cartridge with Nova-Pak NH2 brand of 5-µm, L8 column. The typical retention time is 26 min. The proposed liquid chromatographic procedures in the *Assay* are based on analysis performed using a Phenomenex PLgel brand of 4-µm, L21 column.

(EM1: R. Lafaver; . NOM: A. Wilk.) RTS—C66404

Add the following:

▪Sucrose Stearate



$C_{30}H_{56}O_{12}$ 608.72
$C_{48}H_{90}O_{13}$ 875.22
$C_{66}H_{124}O_{14}$ 1141.68
Sucrose monostearate;
Sucrose octadecanoate [25168-73-4].

DEFINITION
Sucrose Stearate is a mixture of sucrose esters, mainly sucrose stearate, obtained by transesterification of stearic acid methyl esters derived from vegetable origin with sucrose. The manufacture of the fatty acid methyl esters includes a distillation step. The mono- and diesters requirements differ for the two types of sucrose stearate as set forth in the following table.

	Content of Monoesters (%)	Content of Diesters (%)	Sum of Triesters and Polyesters (%)
Type I	NLT 50.0	NMT 40.0	NMT 25.0
Type II	20.0–45.0	30.0–40.0	NMT 30.0

IDENTIFICATION
- **A.** It meets the requirements of the *Fatty Acid Composition* test.
- **B.** It meets the requirements of the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Tetrahydrofuran
Sample solution: 15 mg/mL of Sucrose Stearate in tetrahydrofuran
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC, size-exclusion
Detector: Differential refractometer
Column: 7-mm × 60-cm column; packing L21, 100Å
[NOTE—Two 7-mm × 30-cm L21 columns may be used in place of one 60-cm column, provided system suitability requirements are met.]
Flow rate: 1.2 mL/min
Injection size: 20 µL
Analysis
Sample: *Sample solution*
[NOTE—The relative retention time with reference to the monoester peak is about 0.92 for diesters, and about 0.90 for triesters and polyesters.]
[NOTE—Disregard solvent peaks and peaks having a signal-to-noise ratio less than 10.]
Calculate the percentage of monoesters in the portion of sucrose palmitate taken:

$$\text{Result} = A \times (100 - D - S - E)/100$$

A = percentage of monoesters determined by peak normalization
D = percentage of free fatty acids, using the following formula
S = percentage of free sucrose (above)
E = percentage of water (above)

$$\text{Result} = AV \times 284.5/561.1$$

AV = acid value
Calculate the percentage of diesters in the portion of sucrose palmitate taken:

$$\text{Result} = B \times (100 - D - S - E)/100$$

B = percentage of diesters determined by peak normalization
D = percentage of free fatty acids (above)
S = percentage of free sucrose (above)
E = percentage of water (above)
Calculate the percentage of triesters and polyesters in the portion of sucrose palmitate taken:

$$\text{Result} = C \times (100 - D - S - E)/100$$

C = percentage of triesters and polyesters determined by peak normalization
D = percentage of free fatty acids (above)
S = percentage of free sucrose (above)
E = percentage of water (above)

COMPOSITION
- **FATTY ACID COMPOSITION:** Sucrose Stearate exhibits the following composition profiles of fatty acids, as determined in *Fats and Fixed Oils* ⟨401⟩, *Fatty Acid Composition*.

Fatty Acid	Percentage (%)
Lauric acid	NMT 3.0
Myristic acid	NMT 3.0
Palmitic acid	25.0–40.0
Stearic acid	55.0–75.0
Sum of the contents of palmitic acid and stearic acid	NLT 90.0

In-Process Revision

IMPURITIES

Inorganic Impurities

• **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 6.0%, determined on a 3-g sample. Use a freshly neutralized mixture of 2-propanol and water (2:1), and gently heat.

Organic Impurities

• **PROCEDURE: FREE SUCROSE**

Mobile phase A: 10 µg/mL of ammonium acetate in acetonitrile

Mobile phase B: 10 µg/mL of ammonium acetate in tetrahydrofuran and water (90:10)

Diluent: Tetrahydrofuran and water (87.5:12.5)

System suitability solution: 10 µg/mL of USP Sucrose RS in *Diluent*

Standard solutions: 0.50, 1.0, 2.0, and 2.5 mg/mL of USP Sucrose RS in *Diluent*

Sample solution: 50 mg/mL of Sucrose Stearate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: Evaporative light-scattering [NOTE—If the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability requirements.]

Detector carrier gas: Nitrogen

Detector flow rate: 1 mL/min

Detector temperature: 45°

Nebulizer temperature: 40°

Column: 4.6-mm × 0.25-m column; packing L8

Column flow rate

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow Rate (mL/min)
1	100	0	1.0
8	0	100	1.0
7	0	100	1.0
0.01	0	100	2.5
15.99	0	100	2.5
1	100	0	2.5
3	100	0	1.0

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time for sucrose stearate is about 26 min.]

Suitability requirements

Signal-to-noise ratio: 10:1

Analysis

Samples: *Standard solution* and *Sample solution*

Prepare a standard curve by plotting the peak response versus concentration of sucrose in the *Standard solution*. Calculate the quantity of free sucrose in the Sucrose Stearate taken.

Acceptance criteria: NMT 4.0%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** NMT 4.0%, on a 0.20-g sample

• **TOTAL ASH**

Analysis: Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Transfer a 1.0-g sample into a crucible. Dry at 100°–105° for 1 h and ignite to constant weight in a muffle furnace at 600 ± 25°, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, add hot water, pass through an ashless filter paper, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant weight.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **LABELING:** Label to indicate whether it is Type I or Type II.

• **PACKAGING AND STORAGE:** Preserve in a well-closed container. Protect from humidity and avoid high temperatures.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Sucrose RS■1S *(NF28)*

In-Process Revision

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

〈**11**〉 **USP Reference Standards,** *USP 31* page 37, page 3694 of the *Second Supplement,* pages 553 and 1130 of the *Interim Revision Announcement* in *PF* 34(3) [May–June 2008] and in *PF* 34(5) [Sept.–Oct. 2008], respectively, page 2022 of *PF* 29(6) [Nov.–Dec. 2003], page 1674 of *PF* 30(5) [Sept.–Oct. 2004], page 507 of *PF* 31(2) [Mar.–Apr. 2005], page 1154 of *PF* 31(4) [July–Aug. 2005], page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 181 of *PF* 32(1) [Jan.–Mar. 2006], page 1161 of *PF* 32(4) [July–Aug. 2006], page 95 of *PF* 33(1) [Jan.–Feb. 2007], page 267 of *PF* 33(2) [Mar.–Apr. 2007], page 497 of *PF* 33(3) [May–June 2007], page 716 of *PF* 33(4) [July–Aug. 2007], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 332 of *PF* 34(2) [Mar–Apr. 2008], page 680 of *PF* 34(3) [May–June 2008], page 1021 of *PF* 34(4) [July–Aug. 2008], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], and page 144 of *PF* 35(1) [Jan.–Feb. 2009].

(HDQ) RTS—C49304; C56934; C62528; C64168; C66222; C68850; C71631

Change to read:

USP Cefepime Hydrochloride System Suitability RS—This is a mixture of cefepime hydrochloride related compound A ([6*R*-[6α,7β(*E*)]]-1-[[7-[[(2-amino-4-thiazolyl) (methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, monohydrochloride, monohydrate; ($C_{19}H_{25}ClN_6O_5S_2 \cdot HCl \cdot H_2O$ ✧ 571.50); cefepime related compound B ~~([6*R-trans*]-7-[[[2-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-3-(1-methylpyrrolidinium-1-yl)methyl]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid, inner salt;~~

■([6*R-trans*]-7-[[[2-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-3-(1-methylpyrrolidinium-1-yl)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, inner salt;■1S *(USP33)* ($C_{25}H_{29}N_9O_7S_3$ ✧ 663.75); and cefepime hydrochloride.

Add the following:

■USP Epirubicin Hydrochloride RS.■1S *(USP33)*

Add the following:

■USP Ethylene Glycol and Vinyl Alcohol Graft Copolymer RS.■1S *(USP33)*

Add the following:

■USP Olanzapine Related Compound C RS [(2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[b]thieno[2,3-e][1,4]diazepine 4′-*N*-oxide)**]** ($C_{17}H_{20}N_4OS$ ✧ 328.43).■1S *(USP33)*

Add the following:

■USP Salmeterol Xinafoate RS.■1S *(USP33)*

Add the following:

■USP Salmeterol Related Compound A RS [(4-[1-hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol)**]** ($C_{19}H_{25}NO_3$ ✧ 315.41).■1S *(USP33)*

Add the following:

■USP Salmeterol Related Compound B RS [(4-{1-hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol)**]** ($C_{25}H_{37}NO_4$ ✧ 415.57).■1S *(USP33)*

Add the following:

■USP Tacrolimus RS.■1S *(USP33)*

Add the following:

■USP Tacrolimus Related Compound A RS [(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone**]** ($C_{43}H_{69}NO_{12}$ ✧ 792.01).■1S *(USP33)*

Add the following:

■USP Tranylcypromine Sulfate RS.■1S *(USP33)*

In-Process Revision

Add the following:

▪USP Tranylcypromine Related Compound A RS

[((±)-*cis*-2-phenylcyclopropanamine hydrochloride; *cis*-tranylcypromine hydrochloride)**]** ($C_9H_{11}N \cdot HCl$) ✧169.65).■1S (*USP33*)

Add the following:

▪USP Tranylcypromine Related Compound B RS

[(3-phenylallylamine hydrochloride; cinnamylamine hydrochloride)**]** ($C_9H_{11}N \cdot HCl$ ✧169.65).■1S (*USP33*)

Apparatus for Tests and Assays

BRIEFING

⟨**41**⟩ **Weights and Balances,** *USP 31* page 67 and page 682 of *PF* 34(3) [May–Jun. 2008]. On the basis of comments received, it is proposed to replace the table for *Weights Used for Verification of Accuracy* with a general requirement that these weights have a maximum tolerance that is one third of the limit for the accuracy test.

(GC: H. Pappa) RTS—C68773; C68777; C68911

Change to read:

~~The intent of this section is to bring the requirements for weights into conformity with American National Standard ANSI/ASTM E617, "Laboratory Weights and Precision Mass Standards." This standard is incorporated by reference and should be consulted for full descriptions and information on the tolerances and construction of weights.[1]~~

~~Pharmacopeial tests and assays require balances that vary in capacity, sensitivity, and reproducibility. Unless otherwise specified, when substances are to be "accurately weighed" for *Assay*, the weighing is to be performed with a weighing device whose measurement uncertainty (random plus systematic error) does not exceed 0.1% of the reading. Measurement uncertainty is satisfactory if three times the standard deviation of not less than ten replicate weighings divided by the amount weighed, does not exceed 0.001. Unless otherwise specified, for titrimetric limits tests, the weighing shall be performed to provide the number of significant figures in the weight of the analyte that corresponds to the number of significant figures in the concentration of the titrant.~~

~~The class designations below are in order of increasing tolerances.~~

~~Class 1.1 weights are used for calibration of low capacity, high sensitivity balances. They are available in various denominations from 1 to 500 mg. The tolerance for any denomination in this class is 5 µg. They are recommended for calibration of balances using optical or electrical methods for accurately weighing quantities below 20 mg.~~

~~Class 1 weights are designated as high precision standards for calibration. They may be used for weighing accurately quantities below 20 mg. (For weights of 10 g or less, the requirements of class 1 are met by *USP XXI* class M.)~~

~~Class 2 weights are used as working standards for calibration, built in weights for analytical balances, and laboratory weights for routine analytical work. (The requirements of class 2 are met by *USP XXI* class S.)[2]~~

~~Class 3 and class 4 weights are used with moderate precision laboratory balances. (Class 3 requirements are met by *USP XXI* class S 1; class 4 requirements are met by *USP XXI* class P.)[2]~~

~~A weight class is chosen so that the tolerance of the weights used does not exceed 0.1% of the amount weighed. Generally, class 2 may be used for quantities greater than 20 mg, class 3 for quantities of greater than 50 mg, and class 4 for quantities of greater than 100 mg. Weights should be calibrated periodically, preferably against an absolute standard weight.~~

▪INTRODUCTION

Pharmacopeial ~~tests and assays~~ procedures require balances that vary in capacity, sensitivity, and repeatability. This chapter applies to balances used in Pharmacopeial procedures with a reportable value expressed in at least three significant figures[1] (see §7.10 *Interpretation of Requirements* in §7 *Test Results* under *General Notices and Requirements*), ~~. Analytical methods with less strict requirements need not necessarily follow this standard.~~ when substances are to be "accurately weighed". Measurement uncertainty from the balance is only one contribution to overall weighing errors.~~[1]~~ [2]~~Other~~ Additional contributions include changes in water content of samples during weighing and a static charge on the sample.

~~[1] Copies of ASTM Standard E 617-81 (Reapproved 1985) may be obtained from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.~~

~~[2] Note that the designations S and P no longer designate weight classes but rather weight grades, that is, design limitations such as range of density of materials, surface area, surface finish, corrosion resistance, and hardness.~~

[1] Zeros within a number are always significant. Zeros that do nothing but set the decimal point are not significant. Trailing zeros that are not needed to hold the decimal point are significant. Cases and examples in which this requirement would apply, and examples of these cases follow. (1) Procedures with specification limits (e.g, 90.0% to 110.0%, 98.0% to 102.0%). (2) Procedures in which the result is reported in mg (e.g., mg limits for a 5-mg tablet for which the specification is 90.0% to 110.0% are 4.50 mg to 5.50 mg). (3) Limits expressed with three significant figures (e.g., not more than 1.50%; not more than 0.00100%). Cases and their examples where this requirement would not necessarily apply follow. (1) Procedures with specification limits (e.g., 90% to 110%. (2) Limits expressed with less than three significant figures (e.g., not more than 1.5%; not more than 0.0010%; not more than 150 ppm).

[2] Sources of measurement uncertainties with laboratory balances are (including, but not limited to) readability, repeatability, nonlinearity, sensitivity, temperature, and buoyancy.

This chapter ~~addresses the control of the analytical balance for routine operation.~~ controls the use of an analytical balance for routine operation with ~~Unless otherwise specified, when substances are to be ''accurately weighed'' (for example, in an *Assay* analysis), the weighing is to be performed with a weighing device.~~

~~Assessment of measurement uncertainty is typically done prior to the balance being placed in operation (e.g., during IQ/OQ/PQ) and periodically thereafter. Two steps are performed in measuring uncertainty: (1) measurement of repeatability and (2) verification of accuracy against certified weights. does not exceed 0.1% of the reading. There are three requirements for control of the analytical balance: assessment of measurement uncertainty (represented mainly by the repeatability of the measurement), verification of accuracy, and a calibration check. that meets~~ the following three requirements: repeatability of the measurement, ~~to within 0.1%~~ verification of accuracy, ~~to within 0.1%~~ and a weight check. The first two requirements are typically performed prior to placing the balance in operation ~~(e.g., during IQ/OQ/PQ)~~ and periodically thereafter according to applicable standard operating procedures. The ~~calibration~~ weight check is typically performed each day ~~or prior to each series of weighings.~~ on which the balance is used or at appropriate intervals based on applicable standard operating procedures.

REPEATABILITY

~~Assessment of repeatability may be performed using either *Method A* or *Method B*.~~

~~*Method A*—In this method, repeatability~~ Repeatability is determined ~~at the lower end of the desired operating range (i.e., the range of weights~~ within the range of sample mass for which the balance has been qualified to meet the requirements of this chapter. The measurement of repeatability using this method is satisfactory if two times the standard deviation of not less than 10 replicate weighings divided by the ~~amount weighed~~ nominal mass does not exceed 0.001, as shown in the formula:

$$2s/w \leq 0.001$$

in which s is the standard deviation of not less than 10 replicate weighings; and w is the nominal mass ~~, in mg,~~ of the weight used. Because of display resolution, it is possible to make measurements in which every replicate measurement result is the same value. A true repeatability standard deviation of zero is not statistically possible, although the standard deviation may be less than one display increment d. In this situation, the standard deviation of the balance can be estimated as

$$s = \frac{d}{2\sqrt{3}} = 0.29d$$

~~*Method B Calculation of Minimum Weight*—This method may be used to determine the low end of the operating range (e.g., minimum weight). Minimum weight can be derived from the following formula:[2]~~

~~$(2/U_{rel})s$~~

~~[2] Derived from the expanded uncertainty equation in NISTIR 6919, Recommended Guide for Determining and Reporting Uncertainties for Balances and Scales, January 2002.~~

In-Process Revision

~~in which U_{ur} represents the uncertainty factor of 0.001; and s is the standard deviation from the repeatability measurements, of not less than 10 replicate measurements of a mass near the low end of the operating range in mg. Minimum weight may be used to define the low end of the operating range. Because of scale resolution, it is possible to make measurements in which every one results in the same value. A true scale standard deviation of zero is not statistically possible, although the standard deviation may be less than one display increment d. In this situation, the standard deviation of the scale can be estimated as~~

~~$$s = \frac{d}{\sqrt{3}} = 0.577d$$~~

~~$$s = \frac{d}{2\sqrt{3}} = 0.29d$$~~

VERIFICATION OF ACCURACY

Using ~~multiple~~ weights of suitable ~~accuracy~~ tolerances, ~~Weights Used for Calibration Check of Balances *Weights Used for Verification of Accuracy,*~~ the ~~measured weight is~~ indicated weighing value by the balance must remain within 0.1% of the certified value of the weight over the operating range of the balance. The operating range refers to the range used for performing ~~the assay,~~ analytical procedures under this chapter, and not necessarily to the operating range for other weighing operations. The tolerance of weights used for testing accuracy is considered suitable if the maximum tolerance is one third of the limit for the accuracy test or less.[3]

~~CALIBRATION CHECK~~ WEIGHT CHECK

Analytical balances vary greatly in the features they offer to ensure that the balance is maintained in a calibrated state. A ~~calibration~~ weight check ~~to ensure~~ using an internal or external check weight ensures that the balance is ~~in a calibrated state is performed each day or before each series of weighings. Typically, the calibration check uses internal or external weights to verify that the balance is still in a calibrated state.~~ suitable to use. The weight check is typically performed each day the balance is used or at appropriate intervals based on applicable standard operating procedures, and the measured weight is within 0.1% of its nominal value.

[3] Applicable standards are: ASTM E617 (available from www.astm.org) and OIML R111 (available from www.oiml.org).

~~**Weights Used for Calibration Check of Balances**~~

~~Application~~	~~Appropriate Class of Weight~~	~~Lowest Weight With a Tolerance Within 0.1%*~~
~~Calibration of the weights used for other applications or other specialized applications~~	~~OIML Classes E1, E2, and ASTM Class 0 [NOTE—Special control of humidity and temperature is needed.]~~	~~** OIML E1, 5 mg~~ ~~** OIML E2, 10 mg~~ ~~** ASTM Class 0, 5 mg~~
~~Routine analytical work using microbalances~~	~~ASTM Classes 1, 2~~ ~~**OIML E2~~	~~ASTM Class 1, 10 mg~~ ~~ASTM Class 2, 20 mg~~
~~Routine analytical work using 4–5 place analytical balances~~	~~ASTM Classes 3, 4~~ ~~OIML Classes F1, F2~~	~~ASTM Class 3, 50 mg~~ ~~ASTM Class 4, 100 mg~~ ~~OIML Class F1, 50 mg~~ ~~OIML Class F2, 200 mg~~

~~* ASTM standard E617 may be obtained from ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428. OIML R111 may be obtained from OIML (International Organization of Legal Metrology), 11 Rue Turgot, F-75009, Paris, France.~~
~~** Special control temperature and humidity is needed.~~

■1S (*USP33*)

OTHER TESTS AND ASSAYS

BRIEFING

〈**467**〉 **Residual Solvents,** *USP 31* page 170 and page 1232 of *PF* 34(5) [Sept.–Oct. 2008]. On the basis of information collected at USP laboratories, it is proposed to clarify the preparation of the *Class 1 Standard Solutions* in *Procedure A* for *Water-Soluble Articles*. The *Standard Solution* for *Procedure C* under *Water-Soluble Articles* is renamed *Standard Stock Solution* for consistency with the procedures under *Water-Insoluble Articles*. Also, under *Procedure C* for *Water-Insoluble Articles* the *Note* is modified for consistency with the *Water-Soluble Articles* procedure. On the basis of comments received, it is indicated that in order to use loss on drying to control residual solvents, the limit in the monograph needs to be 0.5% or less.

(GC: H. Pappa) RTS—C69220; C70440

Change to read:

IDENTIFICATION, CONTROL, AND QUANTIFICATION OF RESIDUAL SOLVENTS

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. Because the USP deals with drug products, as well as active ingredients and excipients, it may be acceptable that in cases some of the components of the formulation will not dissolve completely. In those cases, the drug product may first need to be pulverized into a fine powder so that any residual solvent that may be present can be released. This operation should be as fast as possible to prevent the loss of volatile solvents during the procedure.

NOTE—The organic-free water specified in the following procedures produces no significantly interfering peaks when chromatographed.

Class 1 and Class 2 Residual Solvents

The following procedures are useful to identify and quantify residual solvents when the information regarding which solvents are likely to be present in the material is not available. When the information about the presence of specific residual solvents is available, only *Procedure C* is needed to quantify the amount of residual solvents present.

■A flow diagram for the application of the residual solvent limit tests is shown in *Figure 1*.■1S (*USP32*)

WATER-SOLUBLE ARTICLES

Procedure A—
Class 1 Standard Stock Solution—

■[NOTE—When transferring solutions, place the tip of the pipette just below the surface of the liquid. Mix each solution using a magnetic stirrer for 10 to 15 minutes.]■1S (*USP33*)
Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask, ~~add~~

■previously filled with about■1S (*USP33*)
9 mL of dimethyl sulfoxide, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask,

In-Process Revision

■previously filled with about 50 mL of water,■1S (*USP33*) dilute with water to volume, and mix. Transfer ~~1.0 mL of this solution to a 10-mL volumetric flask~~

■10 mL of this solution to a 100-mL volumetric flask, previously filled with about 50 mL of water,■1S (*USP33*) dilute with water to volume, and mix.

Class 1 Standard Solution—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial, ~~add 5.0 mL of water~~

■containing 5.0 mL of water (place the tip of the pipette just below the surface of the liquid for dispensing),■1S (*USP33*) apply the stopper, cap, and mix.

Class 2 Standard Stock Solutions—Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture A RS to a 100-mL volumetric flask, dilute with water to volume, and mix. This is *Class 2 Standard Stock Solution A.* Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture B RS to a 100-mL volumetric flask, dilute with water to volume, and mix. This is *Class 2 Standard Stock Solution B.*

Class 2 Mixture A Standard Solution—Transfer 1.0 mL of *Class 2 Standard Stock Solution A* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 Mixture B Standard Solution—Transfer 5.0 mL of *Class 2 Standard Stock Solution B* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

Test Stock Solution—Transfer about 250 mg of the article under test, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Test Solution—Transfer 5.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

Class 1 System Suitability Solution—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial, add 5.0 mL of *Test Stock Solution,* apply the stopper, cap, and mix.

Chromatographic System (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm × 30-m fused-silica column coated with a 1.8-μm layer of phase G43 or a 0.53-mm × 30-m wide-bore column coated with a 3.0-μm layer of phase G43. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm per second, and a split ratio of 1 : 5. [NOTE—Split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 40° for 20 minutes, then raised at a rate of 10° per minute to 240°, and maintained at 240° for 20 minutes. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution, Class 1 System Suitability Solution,* and *Class 2 Mixture A Standard Solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is not less than 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is not less than 3; and the resolution, *R,* between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is not less than 1.0.

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S (*USP32*) Separately inject (following one of the headspace operating parameter sets described in the table below) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard Solution,* and the *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the *Test Solution* is greater than or equal to a corresponding peak in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions,* or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 Standard Solution,* proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

Table 5. Headspace Operating Parameters

	Headspace Operating Parameter Sets		
	1	2	3
Equilibration temperature (°)	80	105	80
Equilibration time (min.)	60	45	45
Transfer-line temperature (°)	85	110	105
■(if appropriate)■1S (*USP32*)			
■Syringe temperature (°) (if appropriate)■1S (*USP32*)	■80–85■1S (*USP32*)	■80–85■1S (*USP32*)	■80–85■1S (*USP32*)
Carrier gas: nitrogen or helium at an appropriate pressure			
Pressurization time (s)	~~30~~	~~30~~	~~30~~
Injection volume (mL)	■≥60■1S (*USP32*) 1	■≥60■1S (*USP32*) 1	■≥60■1S (*USP32*) 1
■*■1S (*USP32*)			

■

* Or follow the instrument manufacturer's recommendations, as long as the method criteria are met. Injecting less than this amount is allowed as long as adequate sensitivity is achieved.■1S (*USP32*)

Procedure B—

Class 1 Standard Stock Solution, Class 1 Standard Solution, Class 2 Standard Stock Solutions, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard Solution, Test Stock Solution, Test Solution, and *Class 1 System Suitability Solution*—Prepare as directed for *Procedure A.*

Chromatographic System (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm × 30-m fused-silica column coated with a 0.25-μm layer of phase G16, or a 0.53-mm × 30-m wide-bore column coated with a 0.25-μm layer of phase G16. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm per second and a split ratio of 1 : 5. [NOTE—Split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 50° for 20 minutes, then raised at a rate of 6° per minute to 165°, and maintained at 165° for 20 minutes. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution* and the *Class 1 System Suitability Solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio of benzene in the *Class 1 Standard Solution* is not less than 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is not less than 3; and the resolution, *R,* between acetonitrile and *cis*-dichloroethene in the *Class 2 Mixture A Standard Solution* is not less than 1.0.

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S *(USP32)*
Separately inject (following one of the headspace operating parameter sets described in *Table 5*) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution,* the *Class 2 Mixture A Standard Solution,* the *Class 2 Mixture B Standard Solution,* and the *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in the *Test Solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to a corresponding peak(s) in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions,* proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

Procedure C—

Class 1 Standard Stock Solution, Class 1 Standard Solution, Class 2 Standard Stock Solution A, Class 2 Mixture A Standard Solution, Test Stock Solution, Test Solution, and *Class 1 System Suitability Solution*—Prepare as directed for *Procedure A.*

~~*Standard Solution*~~

■*Standard Stock Solution*—■1S *(USP33)*
[NOTE—Prepare a separate ~~*Standard Solution*~~
■*Standard Stock Solution*■1S *(USP33)*
for each peak identified and verified by *Procedures A* and *B.* For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 Standard Stock Solution* in *Procedure A.*] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak identified and verified by *Procedures A* and *B* to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in *Table 1* or *2* (under *Concentration Limit*). ~~Transfer 1.0 mL of this solution to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.~~

■*Standard Solution*—Transfer 1.0 mL of this solution to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.■1S *(USP33)*

Spiked Test Solution—[NOTE—Prepare a separate *Spiked Test Solution* for each peak identified and verified by *Procedures A* and *B.*] Transfer 5.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1.0 mL of the ~~*Standard Solution,*~~

■*Standard Stock Solution,*■1S *(USP33)*
apply the stopper, cap, and mix.

Chromatographic System (see *Chromatography* ⟨621⟩)—[NOTE—If the results of the chromatography from *Procedure A* are found to be inferior to those found with *Procedure B,* the *Chromatographic System* from *Procedure B* may be substituted.] The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm × 30-m fused-silica column coated with a 1.8-μm layer of phase G43 or a 0.53-mm × 30-m wide-bore column coated with a 3.0-μm layer of phase G43. The carrier gas is nitrogen or helium with a linear velocity of about 35 cm per second, and a split ratio of 1 : 5. [NOTE—The split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 40° for 20 minutes, then raised at a rate of 10° per minute to 240°, and maintained at 240° for 20 minutes. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution,* the *Class 1 System Suitability Solution,* and the *Class 2 Mixture A Standard Solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is not less than 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is not less than 3; and the resolution, *R,* between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is not less than 1.0.

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S *(USP32)*
Separately inject (following one of the headspace operating parameters described in *Table 5*) equal volumes of headspace (about 1.0 mL) of the *Standard Solution,* the *Test Solution,* and the *Spiked Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in ppm, of each residual solvent found in the article under test by the formula:

$$5(C/W)[r_U/(r_{ST} - r_U)]$$

in which *C* is the concentration, in μg per mL, of the appropriate USP Reference Standard in the ~~*Standard Solution;*~~

■*Standard Stock Solution;* ■1S *(USP32)*
W is the weight, in g, of the article under test taken to prepare the *Test Stock Solution;* and r_U and r_{ST} are the peak responses of each residual solvent obtained from the *Test Solution* and the *Spiked Test Solution,* respectively.

In-Process Revision

WATER-INSOLUBLE ARTICLES

Procedure A—[NOTE—Dimethyl sulfoxide may be substituted as an alternative solvent to dimethylformamide.]

Class 1 Standard Stock Solution—Transfer 1.0 mL of USP Class 1 Residual Solvents Mixture RS to a 100-mL volumetric flask previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix (reserve a portion of this solution for the *Class 1 System Suitability Solution*). Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Class 1 Standard Solution—Transfer 1.0 mL of *Class 1 Standard Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 Standard Stock Solutions—Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture A RS to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. This is *Class 2 Standard Stock Solution A.* Transfer 0.5 mL of USP Residual Solvents Class 2—Mixture B RS to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix. This is *Class 2 Standard Stock Solution B.*

Class 2 Mixture A Standard Solution—Transfer 1.0 mL of *Class 2 Standard Stock Solution A* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 2 Mixture B Standard Solution—Transfer 1.0 mL of *Class 2 Standard Stock Solution B* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Test Stock Solution—Transfer about 500 mg of the article under test, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix.

Test Solution—Transfer 1.0 mL of *Test Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Class 1 System Suitability Solution—Mix 5 mL of *Test Stock Solution* with 0.5 mL of the intermediate dilution reserved from *Class 1 Standard Stock Solution.* Transfer 1.0 mL of this solution to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Chromatographic System (see *Chromatography* ⟨621⟩—The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m wide-bore column coated with a 3.0-μm layer of phase G43. The carrier gas is helium with a linear velocity of about 35 cm per second, and a split ratio of 1 : 3 [NOTE—Split ratio can be modified in order to optimize sensitivity.] The column temperature is maintained at 40° for 20 minutes, then raised at a rate of 10° per minute to 240°, and maintained at 240° for 20 minutes. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Chromatograph the *Class 1 Standard Solution, Class 1 System Suitability Solution,* and *Class 2 Mixture A Standard Solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio of 1,1,1-trichloroethane in the *Class 1 Standard Solution* is not less than 5; the signal-to-noise ratio of each peak in the *Class 1 System Suitability Solution* is not less than 3; and the resolution, *R,* between acetonitrile and methylene chloride in the *Class 2 Mixture A Standard Solution* is not less than 1.0.

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S *(USP32)*
Separately inject (use headspace operating parameters 3 in *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard Solution,* and *Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the *Test Solution* is greater than or equal to a corresponding peak in either the *Class 1 Standard Solution* or either of the two *Class 2 Mixture Standard Solutions,* or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 Standard Solution,* proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

Procedure B—

Class 1 Standard Stock Solution, Class 1 Standard Solution, Class 1 System Suitability Solution, Class 2 Standard Stock Solutions, Class 2 Mixture A Standard Solution, and *Class 2 Mixture B Standard Solution, Test Stock Solution,* and *Test Solution*—Proceed as directed for *Procedure A.*

Chromatographic System—Proceed as directed for *Procedure B* under *Water-Soluble Articles* with a split ratio of 1 : 3. [NOTE—The split ratio can be modified in order to optimize sensitivity.]

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S *(USP32)*
Separately inject (use headspace operating parameters 3 in *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Class 1 Standard Solution, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard Solution,* and *Test Solution,* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in *Test Solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to a corresponding peak(s) in either the *Class 1 Standard Solution* or any of the two *Class 2 Mixture Standard Solutions,* proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

Procedure C—

Class 1 Standard Stock Solution, Class 1 Standard Solution, Class 1 System Suitability Solution, Class 2 Standard Stock Solution A, and *Class 2 Mixture A Standard Solution*—Proceed as directed for *Procedure A.*

Standard Stock Solution—[NOTE—Prepare a separate ~~*Standard Solution*~~

■*Standard Stock Solution*■1S *(USP33)*
for each peak identified and verified by *Procedures A* and *B.*

In-Process Revision

■For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 Standard Stock Solution* in *Procedure A.*]■1S (*USP33*)
Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak identified and verified by *Procedures A* and *B* to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in *Table 1* or *Table 2* (under *Concentration Limit*).

Standard Solution—Transfer 1.0 mL of the *Standard Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Test Stock Solution—Proceed as directed for *Procedure A.*

Test Solution—Transfer 1.0 mL of the *Test Stock Solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

Spiked Test Solution—[NOTE—Prepare a separate *Spiked Test Solution* for each peak identified and verified by *Procedures A* and *B.*] Transfer 1.0 mL of *Test Stock Solution* to an appropriate headspace vial, add 1 mL of *Standard Stock Solution* and 4.0 mL of water, apply the stopper, cap, and mix.

Chromatographic System—Proceed as directed for *Procedure C* under *Water-Soluble Articles.*

Procedure—

■[NOTE—It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.]■1S (*USP32*)
Separately inject (use headspace operating parameters 3 in *Table 5* with a vial pressure of 10 psi) equal volumes of headspace (about 1.0 mL) of the *Standard Solution, Test Solution,* and *Spiked Test Solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in ppm, of each residual solvent found in the article under test by the formula:

$$10(C/W)[r_U/(r_{ST} - r_U)]$$

in which C is the concentration, in μg per mL, of the appropriate USP Reference Standard in the ~~*Standard Solution;*~~

■*Standard Stock Solution;*■1S (*USP32*)
W is the weight, in g, of the article under test taken to prepare the *Test Stock Solution;* and r_U and r_{ST} are the peak responses of each residual solvent obtained from *Test Solution* and *Spiked Test Solution,* respectively.

Class 3 Residual Solvents

If Class 3 solvents are present, the level of residual solvents may be determined as directed under *Loss on Drying* ⟨731⟩ when the monograph for the article under test contains a loss on drying procedure

■specifying an upper limit of no more than 0.5% (per *Option 1*),■1S (*USP33*)
or a specific determination of the solvent may be made. If there is no loss on drying procedure in the monograph for the article under test or if a Class 3 solvent limit in an individual monograph is greater than 50 mg per day (corresponding to 5000 ppm or 0.5% under *Option 1*), the individual Class 3 residual solvent or solvents present in the article under test should be identified and quantified, and the procedures as described above, with appropriate modifications to the standard solutions, are to be applied wherever possible. Otherwise an appropriate validated procedure is to be employed. USP Reference Standards, where available, should be used in these procedures. ~~A flow diagram for the application of residual solvent limit tests is shown in *Figure 1.*~~

■■1S (*USP32*)

In-Process Revision

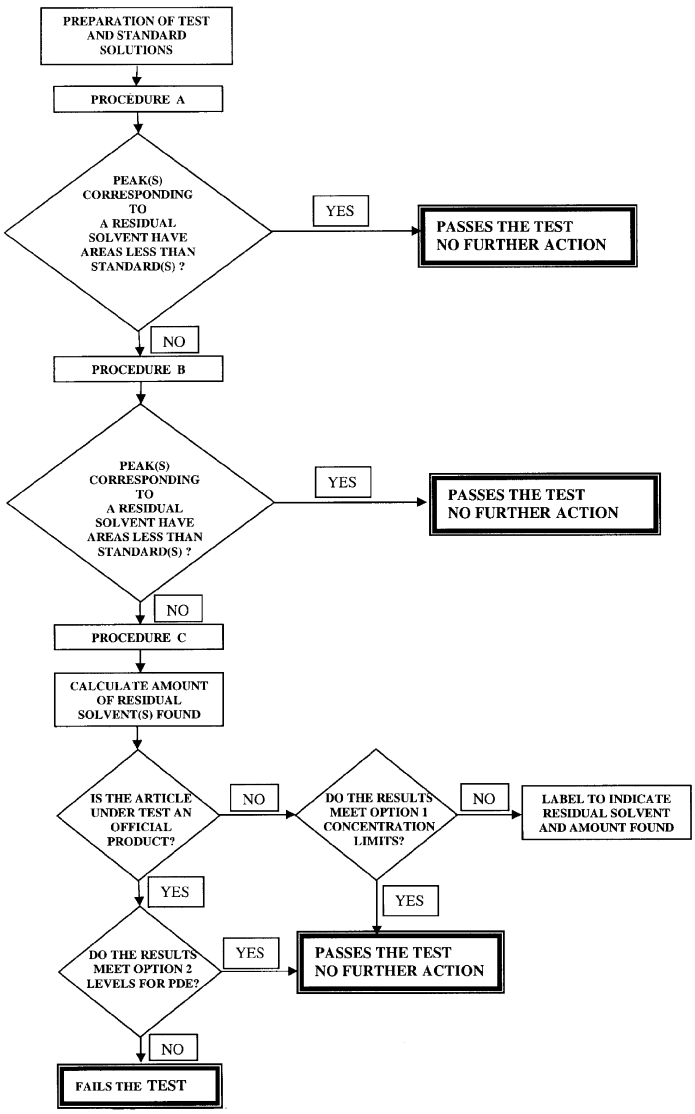


Figure 1. Diagram relating to the identification of residual solvents and the application of limit tests.

Delete the following:

▪~~OTHER ANALYTICAL PROCEDURES~~

~~The following procedures, with any necessary variations, are used where specified in the individual monographs.~~

~~Method I~~

~~A gas chromatograph capable of temperature programming and equipped with a wide-bore, wall-coated open tubular column and a flame-ionization detector is used in the following procedure.~~

~~**Standard Solution**— Prepare a solution, in organic-free water, or the solvent specified in the monograph, containing in each mL, 12.0 µg of methylene chloride, 7.6 µg of 1,4-dioxane, 1.6 µg of trichloroethylene, and 1.2 µg of chloroform. [NOTE—Prepare fresh daily.]~~

~~**Test Solution**— Dissolve in organic-free water, or the solvent specified in the monograph, an accurately weighed portion of the material to be tested to obtain a final solution having a known concentration of about 20 mg of the test material per mL.~~

~~**Chromatographic System** (see *Chromatography* ⟨621⟩)— The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused-silica analytical column coated with a 5-µm chemically cross-linked G27 stationary phase and a 0.53-mm × 5-m silica guard column deactivated with phenylmethyl siloxane. The carrier gas is helium with a linear velocity of about 35 cm per second. [NOTE—When a makeup gas is used, nitrogen is recommended.] The injection port temperature and the detector temperature are maintained at 70° and 260°, respectively. The column temperature is programmed as follows. Initially, the column temperature is maintained at 35° for 5 minutes, then increased at a rate of 8° per minute to 175°, followed by an increase at a rate of 35° per minute to 260°, and maintained at 260° for at least 16 minutes.~~

~~Inject the *Standard Solution*, and record the peak responses as directed for *Procedure*: a suitable system is one that yields chromatograms in which all of the components in the *Standard Solution* are resolved; the resolution, *R*, between any two components is not less than 1.0; and the relative standard deviation of the individual peak responses from replicate injections is not more than 15%.~~

Procedure— Separately inject equal volumes (about 1 µL) of the *Standard Solution* and the *Test Solution* into the chromatograph, record the chromatograms, and measure the peak responses.

Identify, on the basis of retention time, any peaks present in the chromatogram of the *Test Solution*. The identity and peak response in the chromatogram may be established as being from any of the organic volatile impurities listed in the table shown below or from some other volatile impurity eluting with a comparable retention time as determined by mass spectrometric relative abundance procedures or by the use of a second validated column containing a different stationary phase.

Unless otherwise specified in the individual monograph, the amount of each organic volatile impurity present in the material does not exceed the limit given in the table shown below.

Organic Volatile Impurity	Limit (µg per g)
Chloroform	60
1,4-Dioxane	380
Methylene Chloride	600
Trichloroethylene	80

Method IV

Standard Solution— Prepare as directed for *Standard Solution* in *Method I*. Pipet 5 mL of the solution into a vial fitted with a septum and crimp cap, containing 1 g of anhydrous sodium sulfate, and seal. Heat the sealed vial at 80° for 60 minutes.

Test Solution— Transfer 100 mg, accurately weighed, of the material under test to a vial, add 5.0 mL of water, or the solvent specified in the monograph, and 1 g of anhydrous sodium sulfate, and seal with a septum and crimp cap. Heat the sealed vial at 80° for 60 minutes, or as specified in the individual monograph.

Chromatographic System and Procedure— [NOTE—The use of headspace apparatuses that automatically transfer a measured amount of headspace is allowed. Also, the use of a guard column in this headspace procedure is not necessary.] Proceed as directed for *Method V*, except to inject, using a heated gas-tight syringe, 1 mL of the headspace.

Method V

Standard Solution and Test Solution— Prepare as directed for *Method I*.

Chromatographic System ⟨see *Chromatography* ⟨621⟩⟩— The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused silica analytical column coated with a 3.0-µm G43 stationary phase, and a 0.53-mm × 5-m silica guard column deactivated with phenylmethyl siloxane. The carrier gas is helium with a linear velocity of about 35 cm per second. The injection port and detector temperatures are maintained at 140° and 260°, respectively. The column temperature is programmed according to the following steps: It is maintained at 40° for 20 minutes, then increased rapidly to 240°, and maintained at 240° for 20 minutes.

Inject the *Standard Solution*, and record the peak responses as directed for *Procedure*: a suitable system is one that yields chromatograms in which all of the components in the *Standard Solution* are resolved; the resolution, *R*, between any two components is not less than 3; and the relative standard deviation of the individual peak responses from replicate injections is not more than 15%.

Procedure— Proceed as directed for *Method I*, the injection volume being about 1 µL.

Method VI

Standard Solution and Test Solution— Prepare as directed for *Method I*.

Chromatographic System ⟨see *Chromatography* ⟨621⟩⟩— The gas chromatograph is equipped with a flame-ionization detector. The column and column temperature conditions, as chosen from the list below (see *Table 6*), are specified in the individual monograph. The carrier gas, linear velocity or flow rate, and detector and injection port temperatures are appropriate to the column dimensions and column temperatures chosen from the list below.

Inject the *Standard Solution*, and record the peak responses as directed for *Procedure*: a suitable system is one that yields the chromatograms in which all of the components in the *Standard Solution* are resolved; the resolution, *R*, between any two components is not less than 1.0; and the relative standard deviation of the individual peak responses from replicate injections is not more than 15%.

Procedure— Proceed as directed for *Method I*, the injection volume being about 1 µL.

Table 6. Chromatographic Conditions for Method VI

Chromatographic Conditions	USP Column Designation	Column Size	Column Temperature
A	S3	3 mm × 2 m	190°
B	S2	3 mm × 2.1 m	160°
C	G16	0.53 mm × 30 m	40°
D	G39	3 mm × 2 m	65°
E	G16	3 mm × 2 m	70°
F	S4	2 mm × 2.5 m	Hold 120° (35 min.) Gradient 120°–200° (2°/min.) Hold 20 min.
H	G14	2 mm × 2.5 m	Hold 45° (3 min.) Gradient 45°–120° (8°/min.) Hold 15 min.
I	G27	0.53 mm × 30 m	Hold 35° (5 min.) 35°–175° (8° /min.) 175°–260° (35°/min.) Hold 16 min.
J	G16	0.33 mm × 30 m	Hold 50° (20 min.) 50°–165° (6°/min.) Hold 20 min.

■2S (*USP32*)

In-Process Revision

BRIEFING

⟨**525**⟩ **Sulfur Dioxide,** page 3697 of the *Second Supplement.* On the basis of comments and data received, it is proposed to make the following revisions:

1. Add *Method IV,* which is being used extensively in many starch monographs as the current method. *Method I* does not work well for modified starches.
2. Add *Method V,* which is being widely used in excipient monographs that also have applications in the food industry. Sulfur dioxide, a potential allergenic agent as stated in federal regulations, is limited by the food industry. In general, the labeling is required to indicate the presence of sulfur dioxide if the residual sulfur dioxide concentration is greater than 10 ppm.

(EGC: H. Wang.) RTS—C70002

Add the following:

▪METHOD IV

In this test, sulfur dioxide is released from the test specimen in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution, in which the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali, using a pH meter to control the pH value and titration. This test is performed under conditions such that the requirements specified in the system suitability test are met.

Special Reagents

Carbon Dioxide—Use carbon dioxide with a flow regulator that will maintain a flow of 100 ± 10 mL per minute.

Hydrogen Peroxide Solution—Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Neutralize the 3% hydrogen peroxide solution with 0.01 N sodium hydroxide to a pH of 4.1 determined potentiometrically.

Potassium Metabisulfite Solution—Transfer 0.87 g of potassium metabisulfite ($K_2S_2O_5$) and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Dilute with water to volume before mixing. [NOTE—Edetate disodium is used to protect sulfite ion from oxidation.]

Apparatus

A suitable apparatus for sulfur dioxide determination is shown in the accompanying diagram (*Figure 1*). The apparatus consists of a 500-mL three-neck, round-bottom boiling flask, *A;* a separatory funnel, *B,* having a capacity of 100 mL or greater; a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask; a reflux condenser, *C,* having a jacket length of 200 mm; and a delivery tube, *E,* connecting the upper end of the reflux condenser to the bottom of a receiving test tube, *D.* Apply a thin film of stopcock grease to the sealing surfaces of all joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

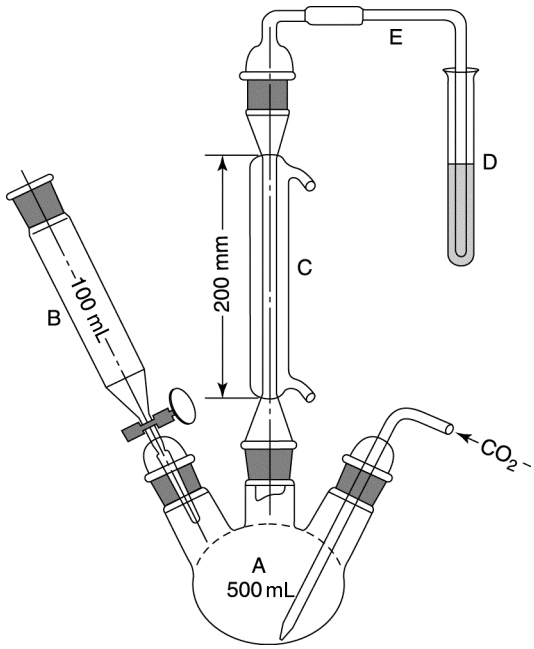


Figure 1. Apparatus for Method IV

System Suitability Test

Test A—Using the *Potassium Metabisulfite Solution* as the standard, proceed as directed for *Procedure*, except replace the 25.0 g of test substance with 20 mL of *Potassium Metabisulfite Solution*. Calculate the content, in μg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* taken by the formula:

$$1000(32.03)VN / V_P$$

in which the factor 1000 converts mg to μg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and V_P is the volume, in mL, of the *Potassium Metabisulfite Solution* taken for the test.

Test B—In a 100-mL conical flask, add 20 mL of 0.02 N iodine solution and 5 mL of 2 N hydrochloric acid. Add 1 mL of starch TS, and titrate with the *Potassium Metabisulfite Solution* until the first discoloration is observed. Calculate the content, in μg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* by the formula:

$$1000(32.03)V_IN_I / V_P$$

in which 1000 and 32.03 are defined above; V_I is the volume, in mL, of the iodine solution used in the test; N_I is the normality of the iodine solution; and V_P is the volume, in mL, of the *Potassium Metabisulfite Solution* consumed.

The difference between the sulfur dioxide contents obtained from *Test A* and *Test B* is not more than 5% of their mean value. *Test B* shall be performed within 15 minutes after completion of *Test A*. [NOTE—This avoids a potential variation of the sulfur dioxide content in the *Potassium Metabisulfite Solution* when stored at room temperature.]

Procedure

Add 150 mL of water to the boiling flask (*A*). Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL per minute through the apparatus. Start the condenser coolant flow. Place 10 mL of *Hydrogen Peroxide Solution* in the receiving test tube (*D*). After 15 minutes, without interrupting the flow of carbon dioxide, remove the separatory funnel (*B*) from the boiling flask, and transfer 25.0 g of the test specimen to the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 hour. Open the stopcock of the funnel, stop the flow of carbon dioxide, discontinue heating the flask, and turn off the cooling water in the condenser. Remove the receiving test tube, and transfer

its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 minutes, and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue, with the color change lasting for at least 20 seconds. Perform a blank determination and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the content, in μg per g, of sulfur dioxide in the test specimen taken by the formula:

$$1000(32.03)VN/W$$

in which the factor 1000 converts mg to μg; 32.03 is the milliequivalent weight of sulfur dioxide; *V* is the volume, in mL, of titrant consumed; *N* is the normality of the titrant; and *W* is the weight, in g, of the test specimen taken. ■1S (*USP33*)

Add the following:

▪METHOD V

In this method, similar to *Method IV,* sulfur dioxide is released from the test specimen in a boiling acid medium and is removed by a stream of nitrogen. The separated gas is collected in a dilute hydrogen peroxide solution, in which the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali, using methyl red as an indicator. This test is performed under conditions such that the requirements specified in the system suitability test are met.

Special Reagents

Hydrogen Peroxide Solution—Dilute a portion of 30 percent hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of methyl red TS, and neutralize to a yellow endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.

Nitrogen—Use high-purity nitrogen with a flow regulator that will maintain a flow of 200 ± 10 mL per minute. Guard against the presence of oxygen by passing the nitrogen through a scrubber, such as alkaline pyrogallol, prepared as follows: add 4.5 g of pyrogallol to a gas-washing bottle, purge the bottle with nitrogen for 3 minutes, and add a solution containing 85 mL of water and 65 g of potassium hydroxide while maintaining an atmosphere of nitrogen in the bottle.

Potassium Metabisulfite Solution—Transfer 0.87 g of potassium metabisulfite ($K_2S_2O_5$) and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Dilute with water to volume before mixing. [NOTE—Edetate disodium is used to protect sulfite ion from oxidation.]

Apparatus

The apparatus (see *Figure 2*) is designed to effect the selective transfer of sulfur dioxide from the specimen in boiling aqueous hydrochloric acid to the *Hydrogen Peroxide Solution* in vessel *G.* The backpressure is limited to the unavoidable pressure due to the height of the *Hydrogen Peroxide Solution* above the tip of the bubbler, *F.* Keeping the backpressure as low as possible reduces the likelihood that sulfur dioxide will be lost through leaks. Preboil vinyl and silicone tubing. Apply a thin film of stopcock grease to the sealing surfaces of all joints, except the joint between the separatory funnel and the flask, and clamp the joints to ensure tightness. The separatory funnel, *B,* has a capacity of 100 mL or greater. The inlet adapter,

A, with a hose connector, provides a means of applying headpressure over the solution. [NOTE—A pressure-equalizing dropping funnel is not recommended because condensate, which may contain sulfur dioxide, is deposited in the funnel and the side arm.]

The round-bottom flask, *C,* is a 1000-mL flask with three 24/40 tapered joints. The gas inlet tube, *D,* is long enough to permit introduction of the nitrogen to within 2.5 cm of the bottom of the flask. The Allihn condenser, *E,* has a jacket length of 300 mm. The bubbler, *F* (see *Figure 3*), is fabricated from glass according to the dimensions given in *Figure 3.* The *Hydrogen Peroxide Solution* is contained in a vessel, *G,* having an inside diameter of about 2.5 cm and a depth of about 18 cm. Circulate coolant, such as a mixture of water and methanol (4 : 1) maintained at 5°, to chill the condenser.

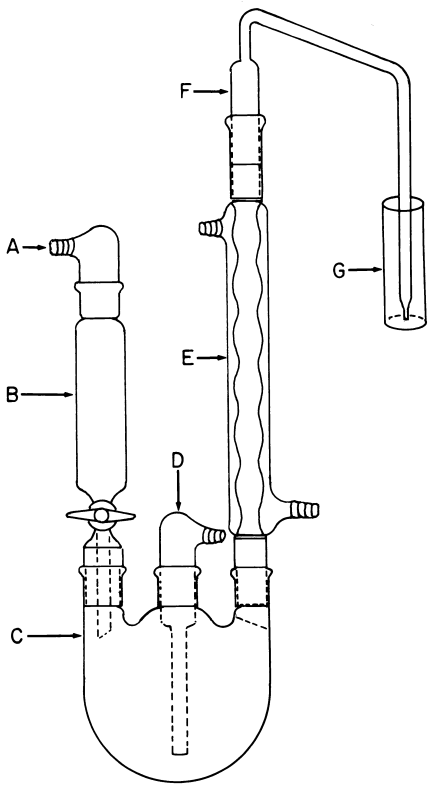


Figure 2. Apparatus for Method V

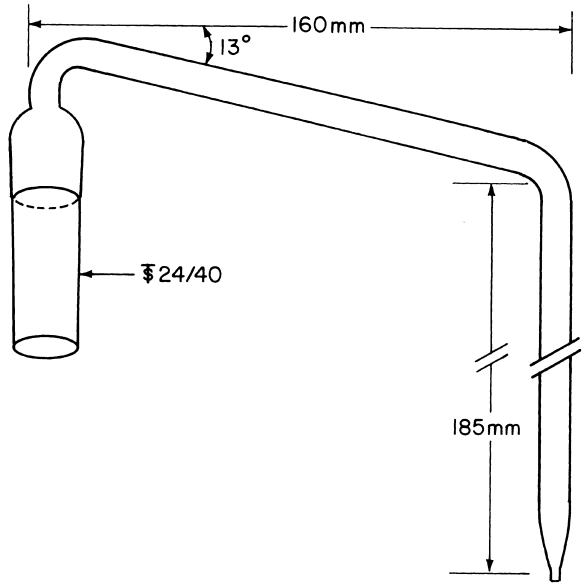


Figure 3. Bubbler (F) for apparatus in Method V

System Suitability Test

Test A—Using the *Potassium Metabisulfite Solution* as the standard, proceed as directed for *Procedure*, except replace the 50.0 g of test substance with 20 mL of *Potassium Metabisulfite Solution*. Calculate the content, in μg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* taken by the formula:

$$1000(32.03)VN / V_P$$

in which the factor 1000 converts mg to μg; 32.03 is the milliequivalent weight of sulfur dioxide; *V* is the volume, in mL, of titrant consumed; *N* is the normality of the titrant; and V_P is the volume, in mL, of *Potassium Metabisulfite Solution* taken for the test.

Test B—In a 100-mL conical flask, add 20 mL of 0.02 N iodine solution and 5 mL of 2 N hydrochloric acid. Add 1 mL of starch TS, and titrate with the *Potassium Metabisul-*

fite Solution until the first discoloration is observed. Calculate the content, in μg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* by the formula:

$$1000(32.03)V_I N_I / V_P$$

in which 1000 and 32.03 are defined above; V_I is the volume, in mL, of iodine solution used in the test; N_I is the normality of the iodine solution; and V_P is the volume, in mL, of *Potassium Metabisulfite Solution* consumed.

The difference between the sulfur dioxide contents obtained from *Test A* and *Test B* is not more than 5% of their mean value. *Test B* shall be performed within 15 minutes after completion of *Test A*. [NOTE—This avoids a potential variation of the sulfur dioxide content in the *Potassium Metabisulfite Solution* when stored at room temperature.]

Procedure

Position the apparatus in a heating mantle controlled by a power-regulating device. Add 400 mL of water to the flask. Close the stopcock of the separatory funnel, and add 90 mL of 4 N hydrochloric acid to the separatory funnel. Begin the flow of nitrogen at a rate of 200 ± 10 mL per minute. Start the condenser coolant flow. Add 30 mL of *Hydrogen Peroxide Solution* to the vessel *(G).* After 15 minutes, remove the separatory funnel, and transfer a mixture of 50.0 g of the test specimen, accurately weighed, and 100 mL of alcohol solution (5 in 100) to the flask. Apply stopcock grease to the outer joint of the separatory funnel, return the separatory funnel to the tapered joint flask, and concomitantly resume the nitrogen flow. Apply headpressure above the hydrochloric acid solution in the separatory funnel with a rubber bulb equipped with a valve. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the flask. Continue to maintain sufficient pressure above the hydrochloric acid solution to force it into the flask. [NOTE—The stopcock may be temporarily closed, if necessary, to increase the pressure.] To guard against escape of sulfur dioxide into the separatory funnel, close the stopcock before the last few mL of hydrochloric acid drain out. Apply power to the heating mantle sufficient to cause about 85 drops of reflux per minute. After refluxing for 1.75 hours, remove the vessel *(G),* add 3 drops of methyl red TS, and titrate the contents with 0.01 N sodium hydroxide VS, using a 10-mL buret with an overflow tube and a hose connection to a carbon dioxide-absorbing tube, to a yellow endpoint that persists for at least 20 seconds. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the quantity, in μg, of SO_2 in each g of the test specimen taken by the formula:

$$1000(32.03)VN / W$$

in which the factor 1000 converts mg to μg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and W is the weight, in g, of the test specimen taken.■1S (*USP33*)

Physical Tests and Determinations

BRIEFING

⟨**921**⟩ **Water Determination,** *USP 31* page 370, and page 761 of *PF* 34(3) [May–June 2008]. On the basis of comments received, in the *Procedure* sections under *Method 1a (Direct Titration)* and *Method 1b (Residual Titration),* it is proposed to change the text to reflect the transfer of 30 to 40 mL of methanol or suitable solvent to the titration vessel, ensuring that the volume is sufficient to cover the electrodes. The following revisions are proposed for *Method Ic (Coulometric Titration)*: under *Principle*, it is proposed to revise the text to indicate that introduction of solids into the cells may require precautions; under *Test Preparation*, it is proposed to revise the text to accommodate various sample handling techniques; and under *Procedure*, it is proposed to revise the text to accommodate sample introduction and blank determination, as needed, and make any necessary corrections.

(GC: A. Hernandez-Cardoso.) RTS—C66277

Change to read:

METHOD I (TITRIMETRIC)

Determine the water by *Method Ia,* unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle—The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the *Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the *Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Apparatus—Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds to 30 minutes, depending upon the solution being titrated. The time is shortest for substances that dissolve in the reagent. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. Commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant, and the titration vessel may be purged by means of a stream of dry nitrogen or current of dry air.

Reagent—Prepare the Karl Fischer Reagent as follows. Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and, keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 hour before use, or daily if in continuous use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light, and under refrigeration.

A commercially available, stabilized solution of Karl Fischer type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine or alcohols other than methanol may be used also. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol or other suitable solvent, such as ethylene glycol monomethyl ether, may be used as the diluent.

Test Preparation—Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 2 to 250 mg of water. The amount of water depends on the water equivalency factor of the *Reagent* and on the method of endpoint determination. In most cases, the minimum amount of specimen, in mg, can be estimated using the formula:

$$FCV / KF$$

in which *F* is the water equivalency factor of the *Reagent,* in mg per mL; *C* is the used volume, in percent, of the capacity of the buret; *V* is the buret volume, in mL; and *KF* is the limit or reasonable expected water content in the sample, in percent. *C* is

■generally■2S (*USP32*) between 30% and 100% for manual titration, and between 10% and 100% for the instrumental method endpoint determination.

■Note ■it is recommended■1S (*USP33*) that the product of *FCV* ~~must~~ ■■1S (*USP33*) be greater than or equal to 200 for the calculation to ensure that the minimum amount of water titrated is greater than or equal to 2 mg.■2S (*USP32*)

Where the specimen under test is an aerosol with propellant, store it in a freezer for not less than 2 hours, open the container, and test 10.0 mL of the well-mixed specimen. In titrating the specimen, determine the endpoint at a temperature of 10° or higher.

Where the specimen under test is capsules, use a portion of the mixed contents of not fewer than 4 capsules.

In-Process Revision

Where the specimen under test is tablets, use powder from not fewer than 4 tablets ground to a fine powder in an atmosphere of temperature and relative humidity known not to influence the results.

Where the monograph specifies that the specimen under test is hygroscopic, use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into a tared container, and shake to dissolve the specimen. Using the same syringe, remove the solution from the container and transfer it to a titration vessel prepared as directed for *Procedure.* Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured, add this washing to the titration vessel, and immediately titrate. Determine the water content, in mg, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed for *Standardization of Water Solution for Residual Titrations,* and subtract this value from the water content, in mg, obtained in the titration of the specimen under test. Dry the container and its closure at 100° for 3 hours, allow to cool in a desiccator, and weigh. Determine the weight of specimen tested from the difference in weight from the initial weight of the container.

Standardization of the Reagent—Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Reagent* to give the characteristic endpoint color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential.

For determination of trace amounts of water (less than 1%), it is preferable to use *Reagent* with a water equivalency factor of not more than 2.0.

■*Purified Water,* sodium tartrate dihydrate, ■a USP Reference Standard,■1S (*USP33*) or commercial standards with a certificate of analysis traceable to a national standard may be used to standardize the *Reagent.* The reagent equivalency factor, the recommended titration volume, ~~and the amount of standard to measure are factors to consider when deciding which standard and how much~~■buret size, and amount of standard to measure are factors to consider when deciding which standard and how much■1S (*USP33*) to use.[1] For *Purified Water* or water standards, quickly add the equivalent of between 2 and 250 mg of water. Calculate the water equivalency factor, *F,* in mg of water per mL of reagent, by the formula:

$$W / V$$

in which *W* is the weight, in mg, of the water contained in the aliquot of standard used; and *V* is the volume, in mL, of the *Reagent* used in the titration.■2S (*USP32*)
~~Sodium tartrate may be used as a convenient water reference substance. Quickly add 75~~

■For sodium tartrate, quickly add 20■2S (*USP32*) to 125 mg of sodium tartrate ($C_4H_4Na_2O_6 \cdot 2H_2O$), accurately weighed by difference, and titrate to the endpoint. The water equivalence factor *F,* in mg of water per mL of *Reagent,* is given by the formula:

$$2(18.02/230.08)(W/V)$$

in which 18.02 and 230.08 are the molecular weights of water and sodium tartrate dihydrate, respectively; *W* is the weight, in mg, of sodium tartrate dihydrate; and *V* is the volume, in mL, of the *Reagent* consumed in the second titration.

■Note that the solubility of sodium tartrate dihydrate in methanol is such that fresh methanol may be needed for additional titrations of the sodium tartrate dihydrate standard.■2S (*USP32*)

~~For the precise determination of significant amounts of water (1% or more), use *Purified Water* as the reference substance. Quickly add between 25 and 250 mg of water, accurately weighed by difference, from a weighing pipet or from a precalibrated syringe or micropipet, the amount taken being governed by the reagent strength and the buret size, as referred to under *Volumetric Apparatus* ⟨31⟩. Titrate to the endpoint. Calculate the water equivalence factor, *F,* in mg of water per mL of reagent, by the formula:~~

~~W/V~~

~~in which *W* is the weight, in mg, of the water; and *V* is the volume, in mL, of the reagent required.~~

■■2S (*USP32*)

Procedure—Unless otherwise specified, transfer ~~35~~

■30■1S (*USP33*) to 40 mL of methanol or other suitable solvent to the titration vessel,

■ensuring that the volume is sufficient to cover the electrodes,■1S (*USP33*) and titrate with the *Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed, since it does not enter into the calculations.) Quickly add the *Test Preparation,* mix, and again titrate with the *Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$SF$$

in which *S* is the volume, in mL, of the *Reagent* consumed in the second titration; and *F* is the water equivalence factor of the *Reagent.*

[1] ~~Consider a setup in which the reagent equivalency factor is 5 mg per mL, and the buret volume is 5 mL. Also, what must be considered is an instrumental endpoint.~~ ■Consider a setup in which the reagent equivalency factor is 5 mg/mL, and the buret volume is 5 mL and an instrumental endpoint.■1S (USP33) Standard amounts equivalent to between 2.5 mg and 22.5 mg of water (10 to 90% of buret capacity) could be used based on the buret and the reagent equivalency factor. The upper end of this range would involve an excessive amount of sodium tartrate dihydrate. If Purified Water or a standard is weighed, an analytical balance appropriate to the amount weighed is required.

Method Ib (Residual Titration)

Principle—See the information given in the section *Principle* under *Method Ia.* In the residual titration, excess *Reagent* is added to the test specimen, sufficient time is allowed for the reaction to reach completion, and the unconsumed *Reagent* is titrated with a standard solution of water in a solvent such as methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, Reagent, and Test Preparation—Use *Method Ia.*

Standardization of Water Solution for Residual Titration—Prepare a *Water Solution* by diluting 2 mL of water with methanol or other suitable solvent to 1000 mL. Standardize this solution by titrating 25.0 mL with the *Reagent,* previously standardized as directed under *Standardization of the Reagent.* Calculate the water content, in mg per mL, of the *Water Solution* taken by the formula:

$$V'F/25$$

in which V' is the volume of the *Reagent* consumed, and F is the water equivalence factor of the *Reagent.* Determine the water content of the *Water Solution* weekly, and standardize the *Reagent* against it periodically as needed.

Procedure—Where the individual monograph specifies that the water content is to be determined by *Method Ib,* transfer ~~35~~ ■30■1S (*USP33*) to 40 mL of methanol or other suitable solvent to the titration vessel, ■ensuring that the volume is sufficient to cover the electrodes,■1S (*USP33*) and titrate with the *Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation,* mix, and add an accurately measured excess of the *Reagent.* Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$F(X' - XR)$$

in which F is the water equivalence factor of the *Reagent; X′* is the volume, in mL, of the *Reagent* added after introduction of the specimen; X is the volume, in mL, of standardized *Water Solution* required to neutralize the unconsumed *Reagent;* and R is the ratio, $V'/25$ (mL *Reagent*/mL *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration.*

Method Ic (Coulometric Titration)

Principle—The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the endpoint. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer solution after each determination is not necessary since individual determinations can be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen is compatible with the other components, and no side reactions take place. Samples are usually transferred into the vessel as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system; thus, the introduction of solids into the cell ~~is not recommended, unless elaborate precautions are taken,~~ ■may require precautions,■1S (*USP33*) such as working in a glove-box in an atmosphere of dry inert gas. Control of the system may be monitored by measuring the amount of baseline drift. This method is particularly suited to chemically inert substances like hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micro-method.

Apparatus—Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary, as the current consumed can be measured absolutely.

Reagent—See ~~*Reagent* under *Method Ia.*~~ ■manufacturer's recommendations.■2S (*USP32*)

~~**Test Preparation**—Where the specimen is a soluble solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or other suitable solvents. Liquids may be used as such or as accurately prepared solutions in appropriate anhydrous solvents.~~

~~Where the specimen is an insoluble solid, the water may be extracted using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas, this gas being then passed into the cell.~~

~~**Procedure**—Using a dry syringe, quickly inject the *Test Preparation,* accurately measured and estimated to contain 0.5 to 5 mg of water, or as recommended by the instrument manufacturer into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, and make any necessary corrections.~~

■**Test Preparation**—Where the specimen is a soluble solid, an appropriate quantity, accurately weighed, may be dissolved in anhydrous methanol or other suitable solvents.

In-Process Revision

Where the specimen is an insoluble solid, an appropriate quantity, accurately weighed, may be extracted using a suitable anhydrous solvent, and may be injected into the anolyte solution. Alternatively, an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas. The gas is then passed into the cell.

Where the specimen is to be used directly without dissolving in a suitable anhydrous solvent, an appropriate quantity, accurately weighed, may be introduced into the chamber directly.

Where the specimen is a liquid, and is miscible with anhydrous methanol or other suitable solvents, an appropriate quantity, accurately weighed, may be added to anhydrous methanol or other suitable solvents.

Procedure—Using a dry device, inject or add directly an accurately measured amount of the sample or sample preparation estimated to contain between 0.5 to 5 mg of water, or an amount recommended by the instrument manufacturer into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the liquid *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, as needed, and make any necessary corrections.■1S (*USP33*)

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1033**⟩ **Biological Assay Validation.** Because there is currently no information in *USP* regarding this topic, it is proposed to add this new general information chapter to provide perspective on bioassay validation.

(STAT: T. Morris.) RTS—C65692

Add the following:

■⟨1033⟩ BIOLOGICAL ASSAY VALIDATION

INTRODUCTION

[NOTE—See the *Definitions* section for terms used throughout this chapter.]

Biological assays (bioassays) are an integral part of the quality assessment required for the development and manufacturing of biological and biotechnological drugs. Rather than relying on classical or instrumental chemical methods, bioassay procedures rely on methods that measure biochemical or physiological activities in whole animals, tissues, and cells, including enzymatic reaction rates or biological responses induced by immunological interactions, and ligand- and receptor-binding assays (ICH Guideline Q6B, section 2.1.2). While bioassays may be used for a variety of purposes in the development and manufacture of biological and biotechnological products, the bioassay tests addressed in this chapter are those used for estimation of relative potency. Bioassays display variability that stems from the influences of

multiple operational and biological factors. This variability usually exceeds that of chemical-based measurements. As new biological products and new technologies emerge, these definitions are likely to expand; therefore, this chapter will emphasize validation approaches in a study protocol that provide flexibility for adoption of new bioassay procedures and methods, new biological product types, or both together, for assessment of drug potency.

U.S. Food and Drug Administration current Good Manufacturing Practice (cGMP) regulations [21 CFR 211.194(a)] require that test methods used for assessing compliance of pharmaceutical products meet appropriate standards for accuracy and reliability. Assay validation is the process of demonstrating and documenting that the performance characteristics of the method meet the requirements for the intended application and that the assay is thereby suitable for its intended use. *USP* general information chapter *Validation of Compendial Procedures* ⟨1225⟩ describes the assay performance characteristics that should be evaluated during chemical assay validation. Although these validation parameters are straightforward for many types of analytical methods, for some types of bioassays the interpretation and applicability of these validation parameters have not been clearly delineated. Because of the nature of bioassays arising from their use in practice, the validation described in ⟨1225⟩ may be difficult to execute. Although there is disagreement about establishing accuracy in bioassays, some laboratories may use dilutional linearity to verify the relative accuracy of the method. Dilutions of the reference provide a series of levels to establish relative accuracy and range. Alternatively, similarity of dilution response in the presence of process intermediates, stressed samples, and the reference demonstrates selectivity of the method.

This general information chapter *Biological Assay Validation* ⟨1033⟩ describes validation goals pertaining to relative potency assays in which a test sample is compared to a designated reference sample to yield a sample relative potency. Specifically, relative accuracy or selectivity, intermediate precision, and range will be discussed. This chapter also describes proper approaches for validation design (sample selection and replication strategy), validation acceptance criteria, data analysis and interpretation, and finally assay performance monitoring.

FUNDAMENTALS OF A VALIDATION STUDY FOR RELATIVE POTENCY ASSAYS

The goals of a validation study for relative potency bioassays are to confirm the operating characteristics of the procedure in order to make informed decisions about a test sample. For bioassays that measure relative potency, multiple doses (concentrations) of both the test sample and the reference are included in a single assay run. The amount of activity (potency) of the reference typically is assigned 1.0 or 100%, and the potency of the test sample is calculated by relating the dose-response curves for the test sample and reference pairs. This results in a unitless measure, the potency of the test sample relative to that of the reference. In some cases the reference is assigned a value according to another property such as protein concentration. In that case, the potency of the test sample is reported as the relative potency times the assigned value of the reference. The parallel line bioassay assumes that the dose-response curves that are generated using a reference and a test sample have similar (parallel) curve shapes that are distinguished only by a horizontal shift in the log dose. For slope ratio bioassay, the curves generated for reference and test sample should be linear and should pass through a common intercept. The curves are thus distinguished only by their slopes.

Sample materials that represent some relative potency significantly different from the reference may be created by concentration or dilution of the initial reference material, prior to adjustment to assay doses. Dilutional linearity is observed when measured relative potencies are proportional to the initial manipulation of the reference material. Dilutional linearity may be used to establish the validity of the procedure by demonstration of reliable potency measurement across a range. Alternatively, the analyst may choose to demonstrate similarity of dose response to the reference using samples that have different matrix components or experimentally varied potencies following deliberate degradation.

In addition, the validation study should be directed toward obtaining a representative estimate of the variability of the relative potency determination. Because the assay potentially can be influenced by factors such as multiple analysts, instruments, or reagent sources, the design of the bioassay validation study should include these factors in order to effectively evaluate their impact on overall biological assay variability. The overall variability from these combined elements defines the intermediate precision of the biological assay. Although the effects of intra-assay (robustness) factors usually are determined during assay development, key factors such as incubation time and temperature may be included in the validation in order to establish the interactions between these and other inter-assay (ruggedness) factors. An appropriate study of the variability of the biological assay, including the impact of intra-assay and inter-assay factors, can help the laboratory establish an adequate testing strategy. This enables forecast of the inherent variability of the reportable value (which may be the average of multiple potency determinations), as well as the sizes of differences that can be distinguished between samples tested in the biological assay for various numbers of replicate assays.

BIOLOGICAL ASSAY VALIDATION STUDY PROTOCOL

A biological assay validation protocol should state the number and types of samples that will be studied in the validation, the study design (including ruggedness and robustness factors), the replication strategy, the intended validation parameters, justified target acceptance criteria for each parameter, and a proposed data analysis plan. Failure to find a statistically significant effect is not an appropriate basis for defining acceptable performance in a bioassay. Conformance to acceptance criteria should be evaluated using an equivalence approach.

In addition, assay and sample validity criteria such as system suitability and parallelism should be specified before the formal validation exercise. Depending on how extensively the bioassay has been developed, these criteria may be proposed as tentative values that will be updated with data from the validation. Assay or sample failures may be reassessed in an appropriate fashion and, with sound justification, included in the overall validation assessment. Additional validation trials may be required to support changes to the procedure.

The assay validation study protocol should include target acceptance criteria for the proposed validation parameters. Failure to meet a target acceptance criterion may result in a limit on the range of potencies that can be measured in the assay or a modification to the replication strategy in the assay procedure.

DESIGN OF A BIOLOGICAL ASSAY STUDY VALIDATION PROTOCOL

The bioassay validation should include samples that are representative of materials that will be tested in the assay. In addition, the validation protocol should establish the performance characteristics of the procedure. If relative accuracy is to be explored, sample concentrations should bracket the range of potencies expected of

materials that will be tested in the assay. Thus samples covering a wide range of potencies might be studied for a drug or biological that is inherently unstable, but a narrower range can be studied for a more stable product. A minimum of three potency levels is required, and five are recommended for a reliable assessment of the behavior of a bioassay. The potency levels chosen will constitute the range of the assay if the criteria for relative accuracy and intermediate precision are met during the validation. A limited range will result when concentrations at the lower or higher end of the range fail to meet their target acceptance criteria. Alternatively, a range of potencies may be generated by stressing a high-potency sample to a range of levels that may be observed in routine practice. Where there is no clear expectation regarding how a sample might behave in the bioassay (compared to a reference), similarity to the reference (selectivity) and intermediate precision may be used to validate the assay. In addition, the influences of the sample matrix (excipients, process constituents, or combination components) can be studied by intentionally varying these together with the target analyte using a multifactorial approach.

The replication strategy used in the validation study should reflect knowledge of the factors that may influence the measurement of potency. Intra-run variability may be affected by a variety of biological assay operating factors (temperature, pH, incubation times, etc.) as well as by the biological assay design (number of animals, number of dilutions, replicates per dilution, dilution spacing, etc.). Operating restrictions and assay design (intra- and inter-run formulae that result in a reportable value for a test material) are usually determined during development and become a part of the biological assay operating procedure. Intermediate precision is studied during the validation by independent runs of the procedure, which may be varied on the basis of an experimental design to evaluate factors that may have long-term impact on the performance of the procedure. Multifactor design of experiments (DOE) and nested designs can be utilized to help identify important sources of variability in the procedure, as well as to ensure a representative estimate of the long-term variability of the procedure. Replication in the inter-run configuration required to achieve the reportable value for a test sample is not needed. A well-designed validation study can be used to estimate the independent components of the biological assay variability, which can then be used to verify or forecast the variability of the test sample format.

A thorough analysis of the validation data includes graphical and statistical summaries that address the validation parameters and their conformance to target acceptance criteria. The analysis should follow the specifics of the data analysis plan outlined in the validation study protocol. In most cases, log potency is analyzed in order to satisfy the assumptions of the statistical methods used in the analysis (see *Statistical Considerations, Scale of Analysis*). Those assumptions include normality of the distribution from which the data were sampled and homogeneity of variability across the range of results observed in the validation. These assumptions can be explored using graphical and inferential techniques such as box plots, probability plots, and statistical tests of normality. Alternative methods of analysis should be sought when the assumptions of normality and/or homogeneity are not met. Confidence intervals are calculated for the validation parameters using methods described here and in the general information chapter *Analytical Data—Interpretation and Treatment* ⟨1010⟩.

VALIDATION STRATEGIES FOR ASSAY PERFORMANCE CHARACTERISTICS

Parameters that should be verified in a bioassay are relative accuracy or selectivity, intermediate precision, and range. Following are strategies for addressing these parameters.

Relative Accuracy

The concept of relative accuracy in a relative potency assay applies to the relationship between measured log relative potency and known log relative potency. Relative accuracy in bioassay refers to a linear relationship with unit slope between log measured relative potency and log known relative potency. The most common approach to demonstrating relative accuracy for relative potency biological assays is by construction of ''known'' levels by dilution of the reference material or a test sample with known potency. This type of study is often referred to as a dilutional linearity study. The results from a dilutional linearity study can be assessed from the estimated relative bias at individual levels or by trends in relative bias across levels. The relative bias at individual levels is calculated as:

$$\textit{Relative Bias} = 100 \cdot \left(\frac{\textit{Measured Potency}}{\textit{Target Potency}} - 1 \right) \%$$

If there is no trend in relative bias across levels, the estimated relative bias at each level may be held to a target acceptance criterion that has been defined in the study validation protocol (see *A Bioassay Study Validation Example*).

Selectivity

When validation samples come from intermediates or as a result of chemical, physical, or temperature stress, selectivity may be explored by similarity (parallelism) of the dose-response profiles of the test samples and the reference. One or more measures of similarity, such as the ratio of slopes, should be held to a target acceptance criterion that has been defined under selectivity in the validation protocol.

Intermediate Precision

Because factors such as analysts, instruments, or reagent lots can potentially influence results from biological assays, the bioassay validation study protocol should include evaluation of these factors to capture their impact on bioassay variability. The variability from measurements taken under a variety of normal test conditions within a laboratory defines the intermediate precision of the biological assay. Intermediate precision is commonly referred to as ''inter-assay'' variability and is called ruggedness in *USP* text.

Intermediate precision measures the influence of factors that vary over time. Intermediate precision is distinguished from robustness, which measures the influence of intra-assay factors (e.g., ranges of operating factors) that can and should be controlled if they affect the assay. Although robustness studies normally are conducted during assay development, key robustness factors such as incubation time and temperature may be included in the validation using multifactor design of experiment (DOE) approaches in order to capture the interactions between these and other inter-assay (ruggedness) factors. An appropriate study of the variability of the bioassay, including the impact of intra-assay and inter-assay factors, can help the laboratory confirm an adequate test strategy for purposes of control. This allows forecast of

the inherent variability of the reportable value as well as critical differences that can be distinguished between samples tested in the biological assay.

When the validation has been planned using multifactor DOE, the contribution of each factor first may be explored graphically to establish important contributions to the variability of the strategic qualification procedures for inter-assay factors such as analysts and instruments.

Variability of the reportable value also can be controlled by a strategic test plan. Contributions of validation study factors to the overall intermediate precision of the biological assay can be determined by performing a variance component analysis (5) on the validation results. Variance component analysis is best carried out using a statistical software package that is capable of performing a mixed model analysis with restricted maximum likelihood (REML) estimation.

A variance component analysis yields variance component estimates such as:

$$\hat{\sigma}^2_{\text{Intra}} \text{ and } \hat{\sigma}^2_{\text{Inter}}$$

corresponding to intra- and inter-run variation. These can be used to estimate the intermediate precision of the bioassay, as well as forecast the variability of the reportable value (format variability) for different assay designs. Intermediate precision expressed as percent geometric relative standard deviation (%GRSD) is given by (using the natural log of the relative potency in the analysis):

$$\textit{Intermediate Precision} = 100 \cdot \left(e^{\sqrt{\hat{\sigma}^2_{Inter} + \hat{\sigma}^2_{Intra}}} - 1 \right) \%$$

The variability of the reportable value from testing performed with *n* replicates in each of *k* runs (format variability) is equal to:

$$\textit{Format Variability} = 100 \cdot \left(e^{\sqrt{\hat{\sigma}^2_{\text{Inter}}/k + \hat{\sigma}^2_{\text{Intra}}/n \cdot k}} - 1 \right) \%$$

Range

The range of the bioassay is defined as the true or known potencies for which it has been demonstrated that the analytical procedure has a suitable level of relative accuracy and intermediate precision. The range normally is derived from the dilutional linearity study and must minimally cover the product specification range for potency. However, for purposes of stability testing and to avoid diluting hyper- or hypo-potent test samples into the functional assay range and retesting after an unsupported value is obtained, there is value in validating the assay over a broad range.

VALIDATION TARGET ACCEPTANCE CRITERIA

The validation target acceptance criteria should be determined by the uses made of the bioassay measurements or by ''the capability of the art'' of the procedure. When a product specification exists, acceptance criteria may be justified on the basis of the risk that measurements may fall outside of the acceptance criteria. Considerations from a process capability index (Cpk) can be used to establish bounds on the relative bias (RB) and the intermediate precision of the biological assay (4):

$$Cpk = \min\left(\frac{Mean - LSL}{3\cdot\sqrt{\hat{\sigma}^2_{Product} + RB^2 + \hat{\sigma}^2_{Assay}}}, \frac{USL - Mean}{3\cdot\sqrt{\hat{\sigma}^2_{Product} + RB^2 + \hat{\sigma}^2_{Assay}}}\right)$$

where *USL* and *LSL* are the upper and lower limits of the acceptance criteria, *Mean* is the process mean, *RB* is a bound on the degree of RB in the assay, and

$$\hat{\sigma}^2_{Product} \text{ and } \hat{\sigma}^2_{Assay}$$

are product and assay variances, respectively. This formula requires prior knowledge regarding product variability or the inclusion of a random selection of lots to estimate this characteristic as part of the validation. The choice of a bound on Cpk is a business decision and is related to the risk of obtaining an out-of-specification (OOS) result. Some laboratories require process capability corresponding to Cpk $\geq$1.3. This corresponds to a 1 in 10,000 chance that a lot with potency at the center of the specification range will be OOS. The proportion of lots that are predicted to be OOS is a function of Cpk.

When acceptance criteria have not yet been established, criteria based on relative bias or intermediate precision can be formulated on the basis of the ''capability of the art'' of the bioassay technology. For example, although chemical assays and immunoassays often are capable of achieving near-single-digit %GRSD, more liberal acceptance criteria might be placed on biological assays, such as animal potency assays, that operate with much larger %GRSD. Alternatively, the goal of the bioassay validation might be to characterize the method using the validation results to establish an inter-run assay format that is predicted to yield reliable product measurements. In either case a sound scientific justification for target acceptance criteria or use of characterization should be included in the validation protocol.

BIOASSAY MAINTENANCE

A validated bioassay procedure is used to assess the potency of a biological or biotechnological product. The performance of a bioassay should be reassessed periodically. This is most easily accomplished by maintaining statistical process control (SPC) charts on assay suitability parameters, including control sample potency. These charts help the analyst to identify any shift or drift in the assay at an early stage. If trending occurs in an SPC chart, the reason for the trending should be identified. If the resolution requires a modification to the assay or if a serious modification of the assay has occurred for other reasons (e.g., a major technology change), the modified assay should be revalidated or linked to the original assay by an adequately designed bridging study.

STATISTICAL CONSIDERATIONS

Several statistical considerations are associated with the design of a bioassay validation study protocol and analysis of the data. These relate to the properties of bioassay measurements, as well as the statistical tools that can be used to summarize and interpret bioassay validation results.

Scale of Analysis

The scale of analysis studied during bioassay validation must be maintained in order to obtain meaningful conclusions from the study. This general information chapter ⟨1033⟩ assumes that appropriate methods are already in place to reduce the raw bioassay response data to relative potency (see general chapter *Design and Analysis of Biological Assays* ⟨111⟩). Relative potency measurements are

generally log-normally distributed. Log-normally distributed measurements are skewed and are characterized by heterogeneity of variability such that the standard deviation is proportional to the level of response. The statistical methods outlined in this chapter require that the data be symmetric, approximating a normal distribution, and some of the procedures require homogeneity of variability in measurements across the potency range. Log transformation provides data that more closely fulfill both of these requirements. The base of the log transformation does not matter as long as the same base is maintained throughout the analysis. For example, if the natural log (log to the base *e*) is used to transform relative potency measurements, summary results are converted back to the assay scale utilizing base *e*.

As a consequence of the log transformation of relative potency measurements, there are advantages (balance and representativeness) if the levels selected for the validation study are geometrically scaled. An example of geometric scaling with five levels would be 0.50, 0.71, 1.00, 1.41, and 2.00. The range should approximate the expected range of potencies for product tested in the bioassay. Intermediate levels are obtained as the geometric mean (GM) of two adjacent levels. For example, the *GM* between 0.50 and 1.00 is derived as follows:

$$GM = \sqrt{0.50 \cdot 1.00} = 0.71$$

Similarly to relative potency measurements, validation summary measures conform to the log-normal distribution. Average response should be reported as the GM of individual relative potency measurements, and variability expressed as %GRSD. An example of the calculation of GM and associated RB, with %GRSD, is presented in *Table 1* for a series of relative potency (RP) measurements performed on samples tested at the 1.00 level (taken from an example that follows). The log base *e* is used in the illustration. Here the *GM* of the relative potency measurements is calculated as the antilog of the average of the log relative potency measurements then expressed as *RB*, the percent deviation from the target potency:

$$GM = e^{\text{Average}} = e^{0.0485} = 1.050$$

$$RB = 100 \cdot \left(\frac{GM}{\text{Target}} - 1 \right) = 100 \cdot \left(\frac{1.050}{1.00} - 1 \right)\% = 5.0\%$$

and the *%GRSD* is calculated as:

$$\%GRSD = 100 \cdot (e^{SD} - 1)\% = 100\ (e^{0.0715} - 1)\% = 7.2\%$$

Table 1. Illustration of Calculations of GM and %GRSD

RP	log RP	
1.1299	0.1221	
0.9261	-0.0768	
1.1299	0.1221	
1.0143	0.0142	
1.0027	0.0027	
1.0316	0.0311	
1.1321	0.1241	
1.0499	0.0487	
Average	0.0485	GM = 1.050
		RB = 5.0%
SD	0.0715	%GRSD = 7.2%

Reporting Validation Results Using Confidence Intervals

An estimate of a bioassay validation parameter should be presented as a point estimate together with its confidence interval. A point estimate is the numerical value obtained from statistical calculations, such as the geometric mean or %GRSD. A confidence interval, the inter-

val estimate, represents the likely range of the true value of the validation parameter. The previous example yields a 90% confidence interval on average log relative potency as follows:

$$CI_{\ln=} \bar{x} \pm t_{df} \cdot s / \sqrt{n}$$

$$= 0.0485 \pm 1.89 \cdot 0.0358 / \sqrt{8}$$

$$= (0.0128, 0.0843)$$

$$CI_{RB} = 100 \cdot \left(\frac{e^{0.0128}}{1.00} - 1 \right), 100 \cdot \left(\frac{e^{0.0843}}{1.00} - 1 \right) = (1.3\%, 8.8\%)$$

The statistical constant (1.89) is from a table of *t* values, with degrees of freedom (df) equal to the number of measurements minus one (df = 8 – 1 = 7). Thus the true relative bias falls between 1.3% and 8.8% with 90% confidence. A confidence interval for intermediate precision or format variability can be formulated using methods described in Burdick (2).

Assessing Conformance to Acceptance Criteria

Bioassay validation study results are compared to target acceptance criteria to demonstrate that the bioassay is fit for use. The process of establishing conformance of validation parameters to validation acceptance criteria should not be confused with establishing conformance of relative potency measurements to product specifications. Product specifications should inform the process of setting validation acceptance criteria (see *Validation Target Acceptance Criteria*).

A common practice is to apply acceptance criteria to the estimated validation parameter. This does not account, however, for the uncertainty in the estimated validation parameter. A solution is to hold the confidence interval on the validation parameter to the acceptance criterion. This is a standard statistical approach used to demonstrate conformance to expectation and is called an equivalence test (1). An equivalence test should not be confused with the practice of performing a significance test, such as a *t* test, that seeks to establish a difference from some target value (e.g., 0% relative bias). A significance test that yields a P value > 0.05 (equivalent to a confidence interval that includes the target value for the parameter) indicates that there is insufficient evidence to conclude that the parameter is different from the target value. This is not the same as concluding that the parameter conforms to its target value. The study design may have too few replicates, or the validation data may be too variable to discover a meaningful difference from the target. Additionally, a significance test may detect a small deviation from the target that is practically insignificant. These scenarios are illustrated in *Figure 1*.

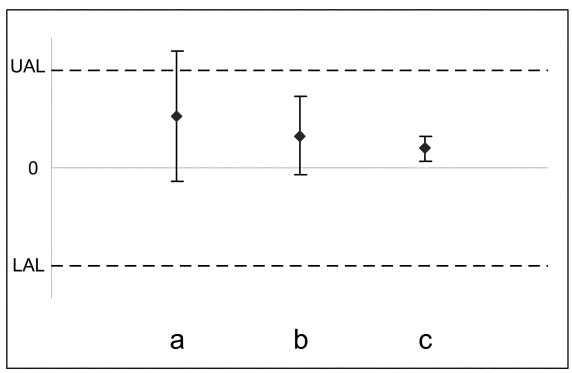


Figure 1. Illustration of use of confidence intervals to establish conformance of validation results to an acceptance criterion.

The solid horizontal line represents the target value (perhaps 0% relative bias), and the dashed lines depict the lower (LAL) and upper (UAL) acceptance limits. In scenario *a*, the confidence bound includes the target; one might conclude there is insufficient evidence to conclude a difference from the target (the significance test approach). However, although the point estimate (the solid diamond) falls within the acceptance range, the interval estimate falls outside the range demarcated by the

LAL and UAL, signifying that the true relative bias may be an unacceptable value. In scenario *b*, the interval estimate falls within the acceptance range, signifying conformance to the acceptance criterion. In scenario *c*, although the interval estimate excludes the target, it falls within the acceptance range, thus yielding an acceptable result.

Using the 90% confidence interval calculated previously, we can establish whether the assay has acceptable relative bias at the 1.00 level against a target acceptance criterion of no greater than 12% relative bias. Because the 90% confidence interval (1.3%, 8.8%) falls within

$$100 \cdot [(1/1.12) - 1]\% = -11\% \text{ and } 12\%$$

we conclude that there is acceptable relative bias at the 1.00 level. Note that the interval excludes 1.00. As discussed previously, this does not indicate a failure to meet the acceptance criterion. Note also that a 90% confidence interval is used in an equivalence test rather than a conventional 95% confidence interval. This is common practice and is the same as the two 1-sided t-test (with 5% test on both sides) approach seen in pharmaceutical bioequivalence testing (2).

Risks in Decision Making and Number of Validation Runs

All statistical tests, including the assessment of conformance of a validation parameter to its acceptance criteria, involve risk. There is the risk that the parameter will not meet its acceptance criterion when the property associated with that parameter is satisfactory (type II error), as well as a risk that the parameter conforms when the parameter is unsatisfactory (type I error). A consideration related to these risks is sample size. The two types of risk can be simultaneously controlled by strategic design, including determining the number of runs that will be conducted during the validation. Specifically, the number of runs needed to establish conformance to an acceptance criterion on relative bias is given by:

$$n \geq \frac{\left(t_{\alpha,\mathrm{df}} + t_{\beta,\mathrm{df}}\right)^2 \sigma_{\mathrm{IP}}^2}{\Delta^2}$$

where $t_{\alpha,\,\mathrm{df}}$ and $t_{\beta,\,\mathrm{df}}$ are distributional points from a Student's t distribution, α and β are the one-sided type I and type II errors, respectively, df are the degrees of freedom associated with the study design (usually $n - 1$),

$$\hat{\sigma}_{\mathrm{IP}}$$

is a preliminary estimate of intermediate precision, and Δ is the acceptable deviation (target acceptance criterion).

For example, if the acceptance criterion on relative bias is ± 0.10 log (i.e., $\Delta = 0.10$) and the bioassay variability is

$$\hat{\sigma}_{\mathrm{P}} = 0.071 \log$$

using $t_{\alpha,\,\mathrm{df}} = t_{\beta,\,\mathrm{df}} = 2$ ($\alpha = \beta \approx 0.05$),

$$n \geq \frac{(2+2)^2\, 0.071^2}{0.10^2} = 8 - runs$$

These eight runs can be designed in a factorial manner to incorporate key robustness and ruggedness factors.

Modeling Validation Study Results Using Mixed Effects Models

Many of the analyses associated with bioassay validation studies must account for multiple design factors. The factors in a typical bioassay validation are composed

of fixed effects such as potency level, as well as random effects such as operator, run, and replicate. Models composed of both fixed and random effects are called mixed effects models and usually require sophisticated statistical software for analysis. The results of the analysis may be summarized in an analysis of variance (ANOVA) table or a table of variance component estimates. The primary goal of the analysis is to estimate critical parameters rather than establish the significance of an effect.

The mixed effects model output provides parameter estimates with their standard errors, which can be used to establish conformance of a validation parameter to its acceptance criterion. An example is given by the analysis of RB. The mixed effects model provides an average and associated variability for RB at each level of the effect ''dose''. These confidence intervals are compared to RB acceptance criteria, and whether or not confidence intervals across doses can be pooled, can be determined. If it is established that variances across levels can be pooled, statistical modeling also can determine the overall relative bias and intermediate precision by combining information across levels performed in the validation. Similarly, mixed effects models can be utilized to obtain variance components for validation study factors and to combine results across validation study samples and levels.

Statistical Design

Statistical design, such as multifactor DOE or nesting can be used to organize runs in a bioassay validation. Such designs are useful for evaluating factors that are believed to influence the assay and that vary throughout long-term use of the procedure. These also are used to better characterize the sources of variability and to develop a strategic test plan that can be used to describe the variability of the bioassay.

An example of a multifactor DOE that incorporates multiple analysts, multiple cell culture preparations, and multiple reagent lots into the validation plan is illustrated in *Table 2*. In this design each analyst performs the bioassay with both cell preparations and both reagent lots. This is an example of a full factorial design because all combinations of the factors are performed in the validation study. In order to reduce the number of runs in the study, fractional factorial designs (3) can be employed when more than three factors have been identified. To mitigate the potential influences of run order or time, validation runs should be randomized whenever possible.

Table 2. Example of a Multifactor DOE Involving Three Factors

Run	Analyst	Cell Prep	Reagent Lot
1	1	1	1
2	1	1	2
3	1	2	1
4	1	2	2
5	2	1	1
6	2	1	2
7	2	2	1
8	2	2	2

An example of a validation using nesting is illustrated in *Figure 2*.

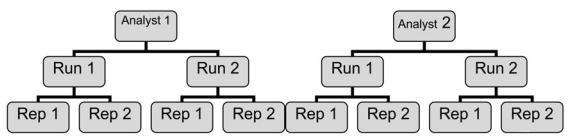


Figure 2. Example of a nested design using two analysts.

Full and fractional factorial designs as well as combinations of the two can be used to estimate components of variability. These estimates can identify significant sources of variability and contribute to the development of an assay format that meets the procedure's requirements for precision.

A BIOASSAY STUDY VALIDATION EXAMPLE

An example illustrates the principles described in this chapter. The bioassay will be utilized to support a specification range of 0.7 to 1.4 for the product. Use the process capability index described in the section on *Validation Target Acceptance Criteria* is used to derive a table showing the projected rate of OOS results for various restrictions on RB and intermediate precision. In the calculations product variability is assumed to be equal to 0. This shows that acceptable performance ($<$1% chance of obtaining an OOS result due to bias and variability of the bioassay) may be expected if the intermediate precision is $\leq$8% and the relative bias is $\leq$12% (*Table 3*). The sample size formula given in the section on *Risks in Decision Making and Number of Validation Runs* can be used to derive the number of runs required to establish conformance to a relative bias equal to 12% (using σ_{IP} = 8%):

$$n \geq \frac{(2.0+2.0)^2 \cdot \log(1.08)^2}{\log(1.12)^2} = 7.4$$

Thus eight runs would be needed to have a 95% chance of passing the target acceptance criterion for relative bias if the true relative bias is 0.

Table 3. CP and Probability of OOS for Various Restrictions on RB and Intermediate Precision

LSL–USL	Intermediate Precision (%)	RB (%)	CP	Prob (OOS)
0.7–1.43	20.0	20.0	0.56	(9.30%)
0.7–1.43	8.0	12.0	0.98	(0.33%)
0.7–1.43	10.0	5.0	1.62	(0.0001%)

Five levels of the target analyte are studied in the validation: 0.5, 0.71, 1.00, 1.41, and 2.00. Runs are generated by two trained analysts using two cell preparations. Each analyst performs two independent runs using each cell preparation. A run consists of a single titration of the reference and two titrations of the test sample. This yields duplicate measurements of relative potency at each level in each validation run. The example dataset is presented in *Table 4*.

Table 4. A Bioassay Validation Example Using Two Analysts and Two Cell Preparations

	Analyst/Cell Preparation							
Level	1/1	1/1	1/2	1/2	1/1	1/1	1/2	1/2
0.50	0.52	0.45	0.57	0.51	0.52	0.52	0.53	0.51
0.50	0.50	0.45	0.56	0.54	0.50	0.51	0.54	0.55
0.71	0.76	0.67	0.68	0.70	0.70	0.75	0.69	0.74
0.71	0.71	0.62	0.82	0.71	0.64	0.69	0.77	0.74
1.00	1.11	0.98	1.15	0.99	1.09	1.03	1.15	1.03
1.00	1.16	0.88	1.11	1.04	0.92	1.03	1.12	1.07
1.41	1.52	1.28	1.53	1.45	1.42	1.35	1.47	1.50
1.41	1.52	1.33	1.56	1.42	1.40	1.43	1.55	1.54
2.00	2.35	1.89	2.35	2.29	2.24	2.14	2.37	2.04
2.00	2.23	1.98	2.40	2.17	2.10	2.15	2.17	2.31

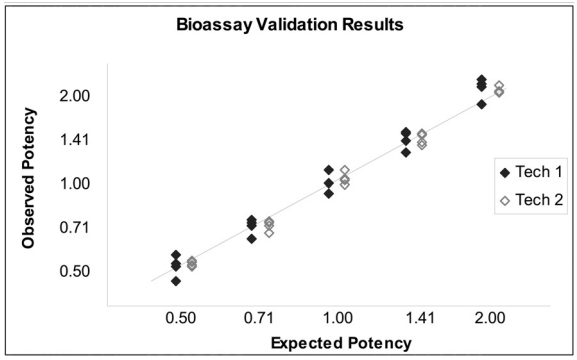


Figure 3. Plot of the validation results vs. the sample levels.

The plot (*Figure 3*) reveals potential irregularities in the experimental results. In particular, a properly prepared plot can reveal a failure in agreement of validation results with validation levels, as well as heterogeneity of variability across levels (see discussion of the log transformation in *Statistical Considerations*). The example plot includes the unit line (line with slope equal to 1, passing through the origin).

A formal analysis of the validation data could be undertaken in a stepwise manner: first, variability (intermediate precision) is assessed in order to establish conformance to the assumption of poolability of variances across sample levels; second, relative accuracy is evaluated either at separate levels or using a combined analysis, depending on whether or not variability can be pooled across levels. These steps will be demonstrated using the example validation data and for illustrative purposes will include some details of the calculations. [NOTE—The calculations illustrated in the following sections are appropriate only with a balanced dataset. Unbalanced designs or datasets with missing relative potency measurements should be analyzed using a mixed model analysis with restricted maximum likelihood estimation (REML).]

Intermediate Precision

Data at each level can be analyzed using variance component analysis (5). *Table 5* presents an example of the calculation performed at a single level (0.50) based on the output of a standard statistical package.[1] The top of the table represents a standard analysis of variance (ANOVA). The expected mean square is the linear combination of variance components that generates the measured mean square for each source. The variance component estimates are derived by solving the equation ''Expected Mean Square = Mean Square'' for each component. To start, the mean square for Error estimates VAR(Error), the within-run component of variability:

$$\text{Var(Error)} = \text{MS(Error)} = 0.00076$$

The between-run component of variability, Var(Run), is subsequently calculated by setting the mean square for *Run* to the mathematical expression for the expected mean square, then solving the equation for *Var(Run)* as follows:

$$MS(Run) = Var(Error) + 2 \cdot Var(Run) = 00076 + 2 \cdot Var(Run)$$

$$Var(Run) = \frac{MS(Run) - Var(Error)}{2}$$

$$= \frac{0.0079 - 0.00076}{2} = 0.00357$$

These variance component estimates are combined to establish the overall intermediate precision of the assay at 0.50:

[1] For example: SAS, Cary, NC.

$$\text{Overall Variability} = 100 \cdot \left(e^{\sqrt{\text{Var(Run)} + \text{Var(Error)}}} - 1 \right)$$

$$= 100 \cdot \left(e^{\sqrt{0.00357 + 0.00076}} - 1 \right) = 6.8\%$$

The same analysis is performed at each level of the validation, and resulting data are presented in *Table 6*.

A combined analysis can be performed if it can be established that the variance components are similar across levels. Similarity is arbitrarily defined. For example, one might restrict the estimate of the ratio of the maximum variance to the minimum variance to no greater than 10-fold; i.e., the ratio of the maximum variance to the minimum variation can be no more than 10 (10-fold is used because of the limited number of runs performed in the validation). In the current example, the ratio associated with the between-run variance component is 0.00364/0.00065 = 5.6, and the ratio associated with the within-run component is 0.0043/0.00058 = 7.4. These ratios (5.6 and 7.4) do not differ by a factor of 10 or more and thus meet the less than 10-fold criterion. If the ratio exceeded 10 and was due to excess variability in one or the other of the extremes in the levels tested, that extreme would be eliminated from further analysis and the range accordingly would be limited to exclude that level.

The analysis could be performed utilizing statistical software that is capable of applying a mixed effects model to the validation results. That analysis should account for any imbalance in the design, as well as other random effects such as analyst and cell preparation (see *Modeling Validation Study Results Using Mixed Effects Models* in *Statistical Considerations*). Variance components can be determined separately for analyst and cell preparation in order to characterize their contributions to the overall variability of the bioassay.

Table 5. Variance Component Analysis Performed on Log Relative Potency Measurements at the 0.5 Level.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
Run	7	0.05533	0.0079	Var(Error) + 2 Var(Run)
Error	8	0.00612	0.00076	Var(Error)
Corrected Total	15	0.06145	—	—

	Variance Component Estimate
Var(Run)	0.00357
Var(Error)	0.00076

Table 6. Variance Component Estimates and Overall Variability for Each Validation Level and the Average.

	Level					
Component	0.50	0.71	1.00	1.41	2.00	Average
Var(Run)	0.00357	0.00065	0.00364	0.00314	0.00262	0.00272
Var(Error)	0.00076	0.0043	0.00295	0.00058	0.00226	0.00217
Overall	6.8%	7.3%	8.5%	6.3%	7.2%	7.2%

In the example, variance components can be averaged across levels to show the intermediate precision of the bioassay. This method of combining estimates is exact only if a balanced design has been employed in the validation (i.e., an equal number of replicates has been performed at each level) and is approximate if an unbalanced design has been employed. A balanced design was employed for the example validation; thus the intermediate precision can be reported as 7.2% GRSD.

Relative Accuracy

The analysis may proceed with an assessment of relative accuracy at each level. *Table 7* shows the average and 90% confidence interval of validation results in the log scale, as well as corresponding potency and relative bias. The analysis has been performed on the average of the duplicates from each run (n = 8 runs). Because duplicate measurements are correlated within a run they should not be considered separately. A plot of relative bias vs. level can be used to look for patterns in the experimental results and to establish conformance to the target acceptance criterion on relative bias (12%; see *Figure 4*).

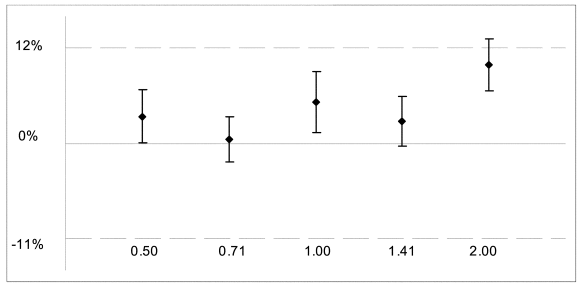


Figure 4. Plot of 90% confidence intervals on relative bias vs. the acceptance criterion.

[NOTE—The lower acceptance criterion is equal to $100 \cdot [(1/1.12) - 1]\% = -11\%$.]

Figure 4 shows an overall average positive bias across sample levels (i.e., the average relative bias is positive at all levels). This is due in part to the lack of independence of assay results across levels. In addition, there does not appear to be a trend in relative bias across levels. A trend in relative bias would indicate that a comparison of samples with different measured relative potency such as stability samples is biased, resulting perhaps in an erroneous conclusion. The lowest estimated relative bias occurs at an intermediate level (0.5% at 0.71), and the highest estimated relative bias occurs at an extreme (9.7% at 2.00). The estimated trend across these levels is thus $(100.0 - 9.7/(100.0 - 0.5) = 0.096$ (9.6% per 2.00/0.71 = 2.8-fold range). This might be held to an acceptance criterion such as no greater than 15% difference in relative bias across the range. A more formal analysis could be performed using a regression of log relative potency vs. log level (4).

Table 7. Average Potency and Relative Bias at Individual Levels.

Level	n[a]	log Potency		Potency		Relative Bias	
		Average	(90% CI)	Average	(90% CI)	Average	(90% CI)
0.50	8	–0.6613	(–0.6928, –0.6299)	52%	(50, 53)	3.2%	(0, 6.5)
0.71	8	–0.3419	(–0.3684, –0.3154)	71%	(69, 73)	0.5%	(-2.2, 3.2)
1.00[b]	8	0.0485	(0.0128, 0.0843)	105%	(101, 109)	5.0%	(1.3, 8.8)

Table 7. Average Potency and Relative Bias at Individual Levels. *(Continued)*

Level	n[a]	log Potency		Potency		Relative Bias	
		Average	(90% CI)	Average	(90% CI)	Average	(90% CI)
1.41	8	0.3723	(0.343, 0.4016)	145%	(141, 149)	2.6%	(-0.4, 5.7)
2.00	8	0.7859	(0.7553, 0.8166)	219%	(213, 226)	9.7%	(6.4, 13.1)

[a] Analysis performed on averages of duplicates from each run.
[b] Calculation illustrated in section on *Statistical Considerations, Scale of Analysis.*

After the lack of a trend across levels has been established, relative accuracy at each level may be assessed. The assay has acceptable relative bias at levels from 0.50 to 1.41, yielding 90% confidence bounds (equivalent to a two one-sided t test) that fall within the acceptance region of –11% to 12% relative bias. The 90% confidence interval at 2.00 falls outside the acceptance region, indicating that the relative bias may exceed 12%.

A combined analysis can be performed utilizing statistical software that is capable of applying a mixed effects model to the validation results. That analysis accurately accounts for the validation study design. The analysis also accommodates random effects such as operator, cell preparation, and run (see *Modeling Validation Study Results Using Mixed Effects Models* in *Statistical Considerations*).

Range

The conclusions derived from the assessment of intermediate precision together with those for relative accuracy can be used to establish the assay range that demonstrates satisfactory performance. Using the acceptance criterion for intermediate precision of 8% GRSD (see *Table 6*) and for relative bias of 12% (see *Table 7*), the range of the assay is determined to be 0.50 to 1.41. This range is slightly higher than the acceptable intermediate precision ($\leq$ 8.0%) observed at 1.0, which may be due to the variability of the estimate that results from the small dataset.

Use of Validation Results for Bioassay Characterization

When the validation protocol states that the study has been performed to estimate the characteristics of the assay (characterization), the variance component estimates also can be used to predict the variability for different assay formats and thereby determine a format that has a desired level of precision. The predicted variability for *k* independent runs, with *n* individual dilution series of the test preparation within a run, is given by the following formula for format variability:

$$Format\ \ Variability = 100 \cdot \left(e^{\sqrt{Var(Run)/k + Var(Error)/n \times k}} - 1 \right)$$

For example, if the assay is performed in three independent runs, with a single dilution series of the test within each run, the predicted variability of the reportable value (geometric mean of the replicate relative potency results) is equal to:

$$Format\ \ Variability = 100 \cdot \left(e^{\sqrt{0.00272/3 + 0.00217/3}} - 1 \right) = 4.1\%$$

In-Process Revision

This calculation can be expanded to include various combinations of runs and replicates within runs as in *Table 8*:

Table 8. Format Variability for Different Combinations of Number of Runs (*k*) and Number of Replicates Within Run (*n*)

	Number of Runs (*k*)			
Replicates (*n*)	1	2	3	6
1	7.2%	5.1%	4.1%	2.9%
2	6.4%	4.5%	3.6%	2.6%
3	6.0%	4.2%	3.4%	2.4%
6	5.7%	4.0%	3.3%	2.3%

The most effective means of reducing the variability of the reportable value (the geometric mean potency across runs and replicates) is through independent runs of the assay procedure. In addition, a confidence interval can be calculated for the overall variability, using methods for confidence intervals on variance components (2).

APPENDIX I

DEFINITIONS

Analysis of Variance (ANOVA)—A statistical tool used to assess contributions of variability from experimental factors.

Bioassay Format—Intra-run and inter-run elements including number of independent runs that form the basis for a reportable value.

Biological Assay/Bioassay—Analysis (as of a drug) to quantify the biological activity(ies) of one or more components by determining its capacity for producing an expected biological activity, expressed in terms of units.

Characterization—Validation strategy where the study is intended to characterize the properties of the bioassay.

Confidence Interval—A statistical interval expressing the likely value of a parameter.

Design of Experiments (DOE)—A systematic approach for studying multiple factors in a validation study.

Dilutional Linearity—Conformance to relative bias using a dilution series of a test sample or reference.

Equivalence Test—A test of conformance to interval-based target acceptance criteria.

Expected Mean Square—A mathematical expression of variances estimated by an ANOVA mean square.

Fixed Effect—An experimental factor that can be reproduced exactly, such as dose.

Format Variability—Predicted variability for a particular assay format.

General Linear Model—A statistical model that relates study factors to experimental responses.

Geometric Relative Standard Deviation (%GRSD)—Similar to relative standard deviation; the variability in log normal response expressed as a percent.

Heterogeneity of Variability—The property of changes in variability with change in level of a factor(s) or response.

Intermediate Precision—The precision of an assay, including ruggedness factors that create variability over long term performance.

Log Normal Distribution—A skewed distribution, characterized by increased variability with increased level of response; a normal distribution is generated by taking the log of the response.

Mean Square—A calculation in ANOVA representing the variability associated with an experimental factor.

Mixed Effects Model—A statistical model including both fixed and random effects.

Multiplicity—The property of compound risk with multiple independent events, all with fixed risk.

Nested Design/Nesting—A statistical design in which some factors are embedded in others, such as analyst within laboratory.

Out of Specification—The property of a measurement in which it falls outside its acceptable range.

P **Value (Significance Probability)**—A statistical calculation representing the probability associated with observing an experimental outcome that is different from expectation.

Point Estimate—A single-value estimate obtained from statistical calculations, such as the average and standard deviation.

Random Effect—An experimental factor that varies randomly, such as analyst and instrument.

Range—The range of potencies that have been demonstrated in the validation to have acceptable relative bias and precision.

Relative Accuracy—Relative accuracy in bioassay refers to linear relationship with unit slope between log measured relative potency versus known log level.

Relative Bias—Degree of difference from the true value expressed as a percent.

Repeatability—A measure of intra-run variability.

Replicate—A within-run repeat of a dilution series of a test sample and/or reference.

Reportable Value—The measured value for a test sample; it represents complete testing of the sample and is compared to the specification.

Run—The enactment of the assay, including independent preparations of a test sample and reference.

Sample Size—The number of independent runs of the validation experiment that are performed.

Selectivity—The ability of an assay to measure the analyte of interest in the presence of other constituents in the sample.

Similarity—In parallel line bioassay, similarity of shape of dilution kinetics between the test and reference samples.

Standard Error of Estimate—The variability associated with an estimate of a reportable value or other parameter.

Statistical Process Control (SPC)—A set of statistical tools used to monitor for shifts and trends in a process.

Two One-Sided T Test—A test procedure associated with equivalence testing.

Type I Error (α)—The error made in judging data analysis, wherein the alternative hypothesis is accepted when it is false.

Type II Error (β)—The error made in judging data analysis, wherein the alternative hypothesis is rejected when it is true.

Variance Component Analysis—A statistical analysis that divides total variability into its component parts.

APPENDIX II

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BRIEFING

⟨**1097**⟩ **Bulk Powder Sampling Procedures.** Because there is no information in *USP–NF* on this subject, it is proposed to add this general information chapter that discusses the theory and practice of sampling. The chapter is the result of a combined effort between the Statistics and the Excipient General Chapters Expert Committees.

(STAT: H. Pappa) RTS—C66302

Add the following:

■⟨1097⟩ BULK POWDER SAMPLING PROCEDURES

INTRODUCTION

The purpose of a sampling plan is to obtain a representative sample of a population so that reliable inferences about the population sampled can be drawn to a certain level or degree of confidence. Acquiring a representative sample from a lot is critical because without a representative sample all further analyses and data interpretations about the lot are in doubt. A perfect sampling process is a process in which every particle or at least every equal-size portion of the population has an equal probability of being chosen in the sample. In addition, sampling procedures should be reproducible, i.e., if the sampling protocol were repeated, a high probability should exist of obtaining similar results. Also, the integrity of the sample should be preserved during and after sampling. The details of how to sample depend on a variety of factors. For example, criteria for sampling to evaluate particle segregation may differ from criteria for evaluating moisture content or identification.

Because of the propensity of a powder to segregate, heterogeneous powder systems can make it difficult to obtain a perfect sample. Thus, to extract representative samples requires careful development of a sampling plan that accounts for and mitigates the segregation tendencies of a particular powder system. Developing a general guidance for bulk powder sampling is challenging because every situation is different, and therefore different approaches must be used to deal with each situation. Thus, the goal of this general information chapter is to outline recommended steps for developing a sampling scheme or plan for a particular system that is consistent with good sampling practices.

The primary difficulty in acquiring a representative sample is that the size of the sample for measurement, typically a few milligrams to grams, must be withdrawn from a large population on the order of hundreds to thousands of kilograms. The few milligrams analyzed in a laboratory must be taken from a large population of particles in a warehouse in such a manner that the measurement sample is representative of all the particles in the lot. Any bias or error in the sampling process will cause all future inferences to be in error. Over the years methods have been developed and refined to attempt to ensure that the measurement sample is representative of the whole population. A typical strategy is shown in *Figure 1*. The strategy is to sample in stages, starting with the initial gross or primary sample withdrawn directly from the received containers. In the laboratory, the gross sample must be reduced in size until it is the appropriate size for measurement. This should be done in a manner that minimizes the introduction of sampling errors. Randomness is the key to reducing the sampling error because it ensures that every particle of the population has an equal probability of being included in the sample. However, because of segregation or the nonrandom nature of powders, many obstacles can cause bias and contribute to sampling errors. Following the flow chart in *Figure 1* and the steps outlined in subsequent discussions will help to minimize sampling errors.

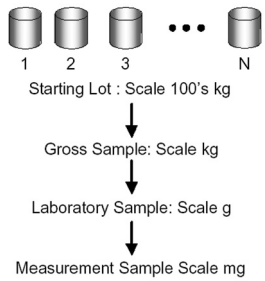

Figure 1. Overall sampling strategy for reducing the sample size from the hundreds of kg scale to the mg scale.

To acquire a representative sample, a suitable sampling plan must be developed and implemented. A good sampling plan includes (1) population determination and sample size selection, (2) a sample collection procedure and a method for sample size reduction, and (3) summary calculations that demonstrate that the sampling plan will yield samples that accurately characterize the population. In addition, an infrastructure is needed to maintain the integrity of the samples and sampled materials.

This chapter begins with a brief introduction to sampling theory and terminology. The technical content of the chapter requires a basic scientific understanding of physical particle characteristics (e.g., mass, density, shape, and size) and statistics (e.g., acceptance sampling and binomial distribution).

SAMPLING THEORY AND TERMINOLOGY

Fundamental Sample Size (Sample Mass)

Sample size is considered from two perspectives: (1) the mass of the sample intended to represent the entire population, sometimes termed the composite sample, and (2) the number of samples taken with a mass sufficient to independently evaluate, compare, or provide confidence to ensure the reproducibility of the composite or the uniformity of the population. The key to obtaining a good sample is to understand and account for the degree of heterogeneity of the characteristic being evaluated in the system under study. For example, heterogeneity of a particle system arises from two sources: the intrinsic, constitutive, or compositional heterogeneity and the spatial distribution heterogeneity. The intrinsic heterogeneity of the powder system reflects the fundamental differences in the individual particles. Statistical heterogeneity (differences between individuals), or variance, is expected to maintain assumed properties. For a normal population the general expression for a statistical sample size suggests that the number of independent samples is proportional to the square of the confidence level (Z) and the bell-shaped population variation (σ) and is inversely proportional to the square of the difference required (δ), as shown in equation 1:

$$n \propto \frac{Z^2\sigma^2}{\delta^2} \qquad (1)$$

For a heterogeneous bulk material, such as a bulk powder, the sample mass required to ensure adequate representation of the intrinsic or fundamental population heterogeneity or variation is determined by the size, shape, and density of the particles. The total sampling error (*TSE*), or fundamental sampling error for a perfect sampling method, measures the difference between the analyte sample concentration (a_{sample}) and the analyte lot concentration (a_{lot}) relative to the average analyte lot concentration (a_{lot}), as shown in equation 2:

$$TSE = \frac{a_{sample} - a_{lot}}{a_{lot}} \qquad (2)$$

The fundamental sampling error (S_{fse}^2) has been experientially estimated in particle size applications by characterizing the critical particle mass, heterogeneity, size (diameter), shape, density, and weights of the material. Experiential estimates require a thorough and complete knowledge of the material and process. Established material characterization and methods are critical aspects of avoiding unacceptable estimates. As shown in equation 3 below:

$$S_{fse}^{\ 2} \propto f_{shape}\, g_{CF}\, c_{max}\, l\, d_{max}^{\ 3} \left(\frac{1}{m_{sample}} - \frac{1}{m_{lot}} \right) \qquad (3)$$

where f_{shape} is a measure of cubicity or shape factor of the analyte particles; g_{Cf}, the granulometric factor, is an experiential correction factor of differences in diameter of the particles; c_{max} is the compositional maximum heterogeneity and is expressed when the analyte or particle exists as two fractions; l, the liberation factor, is an experiential factor representing the proportion of critical content particles within the particles of the lot; d_{max} is the maximum particle diameter; m_{sample} is the mass of the sample; and m_{lot} is the mass of the lot being sampled. [NOTE—A liberation factor is needed when the analyte is not completely liberated. A high liberation value (1.0) suggests heterogeneity of particles. A low liberation value (0.05) suggests very homogeneous particles. See *Appendix I* for examples of potential applications of equation 3 in the estimation of the fundamental sample mass needed to account for constitutional heterogeneity of the powder mixture.]

Segregation Error

Distribution heterogeneity is the difference between samples or groups of particles spatially or temporally. For example, small particles are located preferentially in the lower portion of a powder bed. This type of situation can arise as a result of powder bed segregation and is common in some particle systems with a broad particle size distribution. In other words, smaller particles may not be randomly distributed throughout the lot. This spatial heterogeneity introduces variation in the sample and is a source of variation that contributes to the total variation. Together, fundamental and segregation error give rise to sampling error, which dictates how variable the samples will be, how large the sample size and numbers of samples should be (e.g., 10 containers, sampled at top and bottom, with sample sizes of 50 g each), and how hard it will be to obtain a representative sample.

Minimizing the effects of segregation error during lot material characterization while still ensuring a representative sample mass requires collecting many small samples that average out the variation of the segregation error. Segregation error is difficult to control because segregation may be the result of changes in particle size, shape, and density, as well as inputs into the determination of sample mass. Minimizing the effects of segregation error when reducing the primary sample size requires adequate physical mixing or randomization of the primary samples before analysis, thus providing equal selection probability.

Total Sampling Method Error

Intrinsic or compositional heterogeneity is a function of the powder system and represents the true characteristics of the material (e.g., equation 3). Thus, intrinsic heterogeneity is often the minimal variance a system can have. The difference between the true state of the system and what is actually measured is called the fundamental error (equation 2). The total sampling error (s_{Total}^2) is represented in equation 4 as the sum of all error components:

$$s_{Total}^2 = s_{fundamental}^2 + s_{segregation}^2 + s_{extraction}^2 + s_{delimitation}^2 + s_{preparation}^2 + s_{trends,\,shifts}^2 + s_{cycles}^2 + s_{analytical\ method}^2 \qquad (4)$$

Total error can be reduced by correct sampling. Correct sampling will limit or adjust for the effects of error contributed by particle segregation, extraction error created by the sampling device, delimitation error created by not considering the three-dimensional nature of the bulk material, and sample preparation and handling errors such as product degradation. The total variation is the sum of these sources of error, illustrated in equation 4 as independent, additive components. To the end of reducing these errors, an important goal of material characterization by sampling is the determination of the relevant errors within the bulk sample. Knowing the source of the error helps determine how to best minimize these errors.

Fundamental error is the intrinsic distributional variation of particles within a sample of the material population. Reducing fundamental error requires changing the intrinsic characteristics of the material, such as subsampling or reducing the particle size by milling or grinding. Segregation or grouping error is the spatial distributional error of particles across the population and this type of error can be minimized by mixing or randomization. Segregation error is affected by the characteristics of fundamental error. Additionally, for the determination of both fundamental and segregation error, it is assumed that mechanical sampling is carried out correctly and is not invasive, i.e., that mechanical sampling does not alter the characteristics being measured and provides a true representation. In instances where sampling of the bulk material does not provide a true representation or is so invasive that it alters material characteristics, then, in order to obtain noninvasive, true samples, operators may need to change sampling from a bulk form to a stream form of processing, either upstream or downstream from the sample point (see *Appendix 2*). The mechanical sampler may need to mix the sample sufficiently to facilitate random sampling with equal probability of selection in order to obtain an accurate representation of the entire bulk lot. The process may also require mixing or sampling from a location in the process that will provide a random sample from material that is susceptible to segregation.

Extraction, delimitation, and preparation or handling errors occur as a result of the mechanical sampler and sample handling prior to analysis, which also are affected by fundamental error. Trends, shifts, and cycles are temporal sources of error that affect fundamental error. The analytical error of the method of analysis contributes to the total error of the reported result. In addition to obtaining representative subsamples from the bulk material, the method must also obtain a representative subsample from the particulate laboratory sample before analysis.

Sampling Strategy

A typical sampling strategy consists of two basic steps: (1) the primary or gross sample, followed by (2) the secondary sample, which reduces the primary sample to a size that is suitable for laboratory measurement. In short, the goal is to select from the lot a quantity of material suitable for measurement without significantly changing the attribute for which one is sampling. In parallel with the sample size reduction, sample size calculations must be done in such a way that the sampling strategy has sufficient statistical power to determine whether the attributes of interest lie within the specification ranges with a reasonable degree of certainty. Each step must be done correctly, or the sampling strategy as a whole will not provide a sample that is representative of the original population.

To successfully withdraw a sample from a bulk container that is representative of the population, one needs to have an idea of the population's heterogeneity, i.e., how segregated or stratified the system is. Knowing what factors can accentuate segregation and knowing the patterns of segregation that are likely will help one to account for segregation in a powder bed and to take better samples. Many factors can affect the degree of powder bed segregation. For segregation to occur, sufficient energy needs to be put into the powder bed to induce motion between particles. When a sufficient amount of energy is supplied, segregation can occur via three modes: percolation (in the powder bed), rolling (on the free surfaces of a powder bed), and free flight (when the powder bed is fluidized). These modes are illustrated in *Figure 2*.

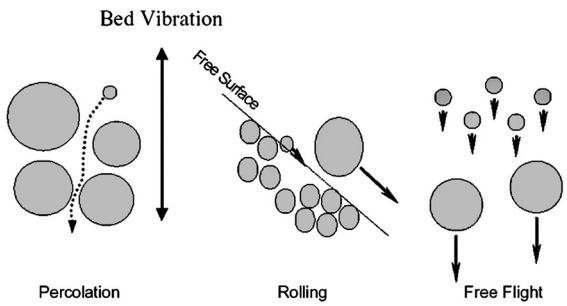

Figure 2. Illustration of the three modes of particle segregation: percolation, rolling, and free flight.

Within the powder bed, segregation can occur by means of percolation, also called sifting segregation, as well as through the movement of coarse particles to the top via vibration. During sifting segregation, smaller particles acting under the influence of gravity can more easily migrate downward into the void spaces between larger particles when the particle bed is perturbed. The net effect of these movements is that the smaller particles percolate down into the powder bed, resulting in the top of the powder bed having a higher proportion of larger particles. A common example of sifting segregation is unpopped corn kernels that are found at the bottom of a bag of popped popcorn.

For free surfaces, rolling segregation can occur any time that particles can roll down a free surface. In other words, segregation can occur on any nonlevel surface that allows the relative movement of particles. When particles roll down these free surfaces, larger particles tend to tumble farther down the surface than the smaller particles (see *Figure 3*). For example, if a conical heap or pile is formed in the middle of a hopper during loading, larger particles are more likely to roll farther down the heap, toward the outer edge of the hopper. This creates a situation in which the smaller particles tend to be in the center of the hopper, and the larger particles accumulate toward the outer wall of the hopper. The formation of these free surfaces can be a major factor in segregation.

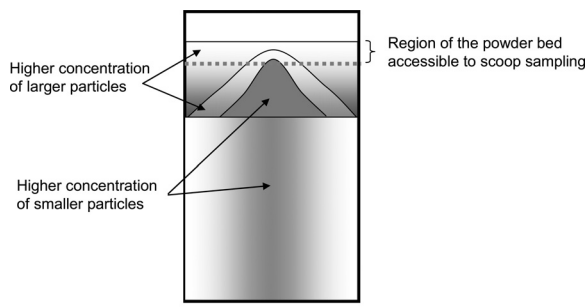

Figure 3. Example of extensive powder segregation within a drum.

When powder beds are fluidized, a large amount of air is incorporated into the powder bed and, when this air is moving, the air velocity may exceed the terminal velocity of the smaller particles. When this happens, the fine particles are suspended in the air stream while the coarse particles settle out. The fine particles eventually settle on top of the powder bed, forming a top layer that has a higher concentration of fine particles. This type of segregation, sometimes called elutriation segregation, can

occur when a powder is discharged from a hopper, or is poured into the top of a hopper, and a large volume of air is displaced.

In summary, for a highly segregating system, the powder bed could have a particle distribution similar to that shown in *Figure 3*, where, as a result of elutriation segregation, a layer of fine particles on the top overlies larger particles deposited by percolation segregation, and a radial distribution of larger particles appears toward the outer wall as a result of rolling segregation.

In general, the primary factors that affect segregation are particle size and size distribution, density, and shape and shape distribution. Of secondary importance are surface roughness, surface coefficient of friction, moisture content, and container shape and design. Particle size is the most important single factor, and subtle differences in particle size can cause measurable segregation. If the attribute of interest is associated with particle size, then this attribute will segregate along with the different particle sizes. For example, if a manufacturer makes a granulation in which the larger particles contain more drug than the smaller particles, then drug content can be very prone to segregation—i.e., drug content will show segregation patterns similar to those associated with particle size segregation.

Segregation can notably increase sampling error because it decreases the probability that certain particle types will be in the sample. In addition, the powder bed may already be segregated when material is received, and poor sample handling can also cause segregation. To avoid further segregation during sample handling, the operator should avoid situations that promote segregation, such as the following: pouring where the powder forms a sloping surface, pouring into the core of a hopper, vibrations, shaking, and stirring (unless done to promote mixing). In addition, the use of mass flow hoppers reduces segregation.

Two basic strategies help ensure that every element of the population of segregating systems has an equal probability of being in the sample: (1) use of a sampling thief and (2) sampling from a moving powder stream.

A sampling thief is a long spearlike probe that can be inserted into the powder bed and, once inserted, can collect powder samples from points adjacent to the spear. With a sampling thief, particles from almost any point in the powder bed can be included in the sample.

The second method relies on fundamental principles of sampling, namely that (1) a powder should always be sampled when in motion, and (2) the whole stream of powder should be sampled for many short periods rather than sampling a part of the stream for a longer period. For example, if the container to be sampled is emptied onto a conveyer belt, all the material will pass by a single point that can be sampled. Thus, no matter how segregated the system is, the collection of the powder at random time points ensures that every particle has an equal probability of being included in the sample. The second fundamental principle accounts for material segregation on the conveyer belt: by collecting the entire stream, one gets a cross section of all the particles, no matter how much segregation occurs on the conveyer belt.

Many methods are available for obtaining a sample from a powder system. Unfortunately, many of these methods involve setting the powder bed in motion or performing in-process sampling. Because of concerns about cross-contamination and containment of potentially toxic materials, most of these methods are impractical for the bulk sampling required for compliance with current Good Manufacturing Practices (cGMPs). Hence, most of the sampling done in the pharmaceutical industry is static sampling, done by either (1) scoop or grab sampling or (2) stratified sampling, typically employing a sampling thief. The choice of method is dictated by the distribution of the attribute being sampled in the container, as discussed below.

GENERAL SAMPLE COLLECTION: CONSIDERATIONS AND TOOLS

Types of Systems and General Considerations

Homogeneous Systems—For powder systems where the attribute of interest is uniformly distributed throughout the container—so that any sample is a true representation of the entire container, lot, or population of interest—scoop sampling is adequate. Scoop sampling is a straightforward procedure in which the operator, after selecting representative containers for sampling, opens a container, scoops out a sufficient amount of material from the top of the powder bed, and then seals the container. If a thin layer of material on top of the powder bed is suspected of being different from the bulk, samples should be taken from a point below this top layer. For example, in cases of elutriation segregation, a thin layer of fine particles may lie on top of the powder bed, and the operator should dig down into the powder bed to avoid sampling from this layer. The scoop should be large enough that no material is lost during handling, because lost material may result in sample bias. In other words, one should avoid the use of a heaping scoop from which material can roll off the sides. The advantages of scoop sampling are convenience and cost, and, for highly potent materials, low-cost disposable scoops that can be used to minimize cross-contamination.

Heterogeneous Systems—If the attribute of interest is spatially distributed in a heterogeneous manner throughout the sample, then scoop sampling is prone to potentially significant errors. Scoop sampling is a nonprobabilistic method because only the most accessible fraction of the container is sampled. Obviously, only the material in the top layer can be reached with a scoop. For example, a sample from the top outer edge of the drum shown in *Figure 3* could be biased because, in this example, the larger particles are preferentially distributed toward the top and outer edges of the drum. Hence the smaller particles have a lower probability of appearing in the sample. As a result, the smaller particles will be underrepresented in the sample, and any analysis of particle size will not reflect the true particle size distribution of the original population.

For heterogeneous systems, the initial primary sample is the most difficult to obtain. Use of a sampling thief, sometimes called a grain probe or sampling spear, is needed. The advantage of a sampling thief is that much more of the powder bed is accessible because the sampling thief can sample from different points in the powder bed, thus helping to reduce sampling bias. Many types of sampling thieves are available, including (1) the concentric sleeve with slotted compartments, (2) the concentric sleeve with grooves, sometimes called the open-handled probe, (3) the end sampler, and (4) the core sampler. Each type has its own unique operating procedures, as described below.

The concentric sleeve with slotted compartments is probably the most popular type of sampling thief used in the pharmaceutical industry. This type consists of two concentric tubes or cylinders in which the inner tube is divided into compartments. This design makes it possible to detect differences in the attribute of interest across the depth of the container. To collect a sample, the operator closes the compartments and inserts the sampling thief into the powder bed with the collection zone openings facing upward. The handle is turned to open the sample zones, then the handle is moved up and down with two quick short strokes to help fill the compartments. The sampling thief is then closed and removed from the powder bed. The operator should visually inspect the powder bed through its depth before emptying the sampling thief. The powder from the individual compartments can be combined on a clean sur-

face or in a collection container. In certain situations the material from each compartment may be analyzed separately, that is, without mixing.

In the concentric sleeve with grooves (open-handled probe), the inner tube is not divided into compartments. The probe is first inserted into the powder bed with the groove open, the outer sleeve is rotated to close, and the sampling thief is then withdrawn from the powder bed. The probe's contents are emptied from the handle end by holding the probe upright and letting the sample slide out from the handle, a method more convenient than the one using the thief with slotted compartments. However, this type of thief makes it more difficult to perform visual inspection to examine for material inconsistencies according to depth.

An end sampler probe, often used to sample slurries, has a single entry zone at the bottom of the sampling thief. Frequently the end sampling zone is larger than the rest of the sampling thief. This feature is a disadvantage because the larger the probe, the more it perturbs the powder bed, possibly resulting in the introduction of sampling bias.

Core samplers have a hollow outer cylinder with a tapered outer wall on the open end. This probe is inserted into the powder bed, and the intrinsic cohesion of the particles keeps them from flowing out when the probe is withdrawn. The contents of the cylinder are then emptied into a clear container.

General Considerations—The most reliable and reproducible results in powder size measurements are obtained when the particle size ranges from 2 to 10 μm; otherwise, the powder is too cohesive and does not flow properly into the sampling thief. In addition, particles larger than about one-third the width of the slot give poor results. Samples should be taken from several sites throughout the container. The probe should be long enough to penetrate at least three-quarters of the depth of the powder bed, ensuring that material from all depths can be captured in the sample. The choice of sites should be dictated by an understanding (often subjective) of the powder bed's degree of heterogeneity, which may have been caused by handling or movement during transport. Sampling plans can call for the insertion of the probe either at random locations and random angles or at predetermined locations and angles. For example, the plan may call for the probe to be inserted at the center and at two locations near the edges. Also, many operators recommend that probes always be inserted at a 10° angle from vertical, which increases the range of locations sampled.

Some of the disadvantages of sampling thieves include the labor-intensive nature of the procedure. The probe must be physically inserted into the powder bed, often multiple times; the contents of the probe must be emptied; and then the probe must be thoroughly cleaned. For settled powder beds, the sampling probe can be difficult to insert. In addition, the sampling probe can introduce errors as a result of the following: fine particles can lodge between the inner and outer tubes; particles can fracture; fines can compact and not flow well into the sampling compartments; segregation can occur during flow into the sampling zone; and the act of inserting the probe can disrupt the powder bed by dragging powder from the top layers of the bed down through the bed.

Representative Lot Sampling

The number of samples should be of sufficient mass to give the desired accuracy. Statistically-based sampling plans are based on statistical principles and depend on the population's spatial heterogeneity and intrinsic variability. Statistically-based plans are efficient and allow the collection of a sufficient number of samples to yield the desired degree of certainty without collecting too many or too few samples for the test method, scale, product variation, risk requirements, and tolerance for a stated product's quality level or specification. For example, the $\sqrt{N} + 1$ sampling plan given in *Table 1* may result in collection of too few samples for small populations and too many samples for large populations. However, the primary advantage of convenience sampling is that sample size can be chosen with little forethought or a priori knowledge of the population to be sampled.

The sample size selection scheme shown in *Figure 4* can be used to determine the sample size. The first choice is whether to use a statistical or nonstatistical sampling plan. Statistical plans are preferred when a variable attribute like particle size or drug content is being determined. General sampling approaches are outlined in USP general information chapter *Analytical Data—Interpretation and Treatment* ⟨1010⟩. Statistically based lot acceptance sampling plans require a valid rationale with known quality levels for the determination of product lot characteristics. As noted, the application of statistical sampling plans, including lot acceptance sampling plans, requires specific and thorough knowledge of the material being sampled. Reference statistical sampling plans state the rationale for sampling as part of the sampling scheme. Manufacturers who use a statistically-based lot acceptance sampling method should refer to an appropriate standard such as ANSI/ASQ Z1.9-2003 for bulk materials or ANSI/ASQ Z1.4-2003 for multiunit or discrete populations. These standards are readily available via sources such as the American Society for Quality (http://www.asq.org/) or the American National Standards Institute (http://www.ansi.org/).

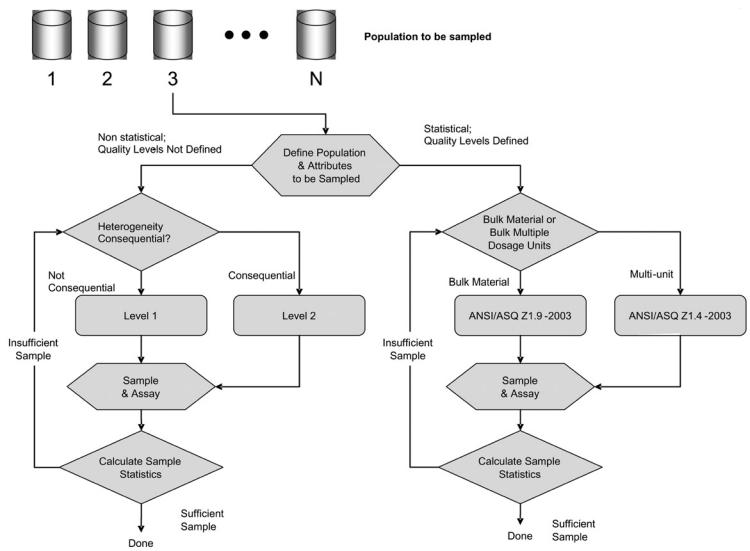

Figure 4. Sample size selection scheme.

In-Process Revision

If one is developing a nonstatistical sampling plan for which the quality level is not known, *Table 1* gives suggested sample sizes for the number of containers in the lot that should be sampled.

The *Level 1* sampling plan is relevant to materials when heterogeneity does not affect the analysis and the customer seeks to sample more than one container, when the sampling plan can be proportional to the square root of the number of containers received, and when the material comes from a known and trusted source. In such cases, the sample can be withdrawn from any point in the container. For homogenous systems, scoop sampling from the top of the container is suitable.

The *Level 2* sampling plan involves a 50% increase in sample size when compared with *Level 1* and is used when a larger proportion of the number of containers is needed, for example, when a material's heterogeneity is suspected of being consequential and acceptance sampling quality levels are not defined, or when the material comes from a less trusted source. Depending on the material's degree of heterogeneity, a sampling thief may be used. However, if the degree of heterogeneity will not significantly affect the results for the attribute being sampled, then scoop sampling from the top of the drum may still be suitable.

Table 1 shows the number of containers, *n*, to be sampled for a lot size *N.* Note that the value of *n* from the formula is rounded at 0.5 up to the next higher integer. For example, if $N = 6$: for *Level 1,* $n = \sqrt{6} + 1 = 3.45$ rounds to $n = 3$; for *Level 2,* $n = 1.5 \times \sqrt{6} = 3.67$, which rounds to $n = 4$.

Table 1

N (Population Size)	Sample Size *n*	
	Level 1	Level 2
$N \leq 3$	All	All
$N \geq 4$	$\sqrt{N} + 1$	$1.5 \times \sqrt{N}$

These initial decisions, as illustrated in *Figure 4*, are often difficult and sometimes must be made without sufficient information. If there is uncertainty about which method or level is appropriate, sometimes a quick, small-scale informal test of the system may help determine the best way to proceed. In addition, for some systems and attributes, the *Level 1* and *Level 2* sampling plans may result in oversampling. For example, when one is sampling for identification from the same lot, the suggested levels may result in collecting more samples than are statistically needed; in such cases, the statistically-based sampling plans referenced in *Figure 4* can be used.

Sample Collection

Acquiring a representative sample from a lot of bulk powder is a difficult procedure that requires special consideration, and the basic procedures for acquiring a representative sample are discussed below. Note that every situation requires techniques that are appropriate for the given population to be sampled. The methods presented here are applicable to the sampling of static powders stored in midsize bulk containers such as 1-ton super sacks, 50-kg drums, or 50-pound bags. These methods are not necessarily applicable to the sampling of liquids, large storage containers such as train cars or silos, or in-process systems such as blenders or moving conveyer belts. In addition, the procedures described here are most applicable to particles in the size range

from approximately ~1 μm to approximately ~1000 μm. Significantly smaller or larger particles require special procedures that are not covered here.

PRIMARY SAMPLE COLLECTION

Lot acceptance samples are generally transferred or delivered in containers. To collect a representative primary or gross sample (see *Figure 1*), the appropriate container or containers must first be selected from the population of *N* containers; second, a representative sample must be withdrawn from each of the selected containers.

Container Selection

To avoid bias and other sampling errors, the containers to be sampled must be randomly selected. To make a random selection, first number all containers in the lot, then use a random number table (or computer-generated random numbers) to choose from which container or containers to withdraw the samples. (See *Appendix 3* for an example showing the use of random number tables.)

For systems in which containers are grouped together in such a manner that many of the individual containers are not practically accessible (e.g., 50-pound bags stacked and bound in shrink wrap on a pallet), the sampling plan may need to take into accout the larger container, in addition to the smaller container, as a sampling unit, in order to ensure a representative sample.

Withdrawing Sample from a Container

Container Types—The three most popular container types are the bag, drum, and super sack. Because bags are generally closed and not resealable, special sampling thieves, sometimes called bag triers, have been designed to puncture the bag. If the system to be sampled is heterogeneous, the samples should be obtained from the bottom, center, and top of the bag; and, depending on how the bags are stacked on the pallet, they should also be sampled from the front and the back. When sampling from bags, particular attention should be paid to the corners, because they can disproportionately trap fine particles. If no bag trier is available, use a knife to cut open the bag for sampling. When sampling from a bag, be sure to clean the external surface sufficiently that the sample is not contaminated and foreign material is not introduced into the bulk material. Once the sample has been taken, place a compatible material over the hole in the bag, then fix this patch with an appropriate adhesive tape. Depending on the heterogeneity of the drum, a scoop or a sampling thief is used. Super sacks are large sack containers that usually have a fill spout on the top and a discharge spout on the bottom. For homogeneous material, scoop sampling is appropriate; but if there is any concern about the heterogeneity of the material, a thief should be used. The large size of super sacks makes the use of a thief more important for representative sampling than in the case of a drum or bag, in order to limit potential delimitation error.

Sample Handling

The samples collected can be either assayed individually or combined; then a subset of the gross sample can be assayed, as depicted in *Figure 1* and described below. Sample increments should be combined on a clean, dry surface or in a suitable container or bag. All containers with which the sample comes into contact should be inert and should not chemically or physically react with the sample. In addition, samples should be accurately labeled and good records kept. A portion should be kept for possible future analysis.

PRIMARY SAMPLE SIZE REDUCTION

As mentioned above, the primary sample typically consists of multiple samples taken from containers and mixed together. To obtain an analysis or measurement sample (*Figure 1*), the gross or primary sample must be reduced to a size appropriate for the analytical method. Gross or primary sample size reduction is an often overlooked aspect of a sampling plan, but it is an important step. The factors that cause segregation in a container can also cause segregation in the primary sample, and any bias in the size reduction method for the primary sample will lead to erroneous results. The advantage of secondary samples is that the mass has been reduced to a point at which it is much easer to obtain a representative unbiased sample because every element in the powder bed is readily accessible. Such accessibility makes it easier to adhere to sampling best practices. Generally speaking, sample measurement takes place under either wet or dry conditions; the choice is dictated by the requirements of the analytical method. For example, the Coulter counter requires that samples be uniformly suspended in an electrolyte, but other methods, like sieving, are typically performed with dry powders.

Before dividing an agglomerated sample, the agglomerates should be broken apart by a suitable technique such as sieving.

Dry Analysis Methods

Many laboratory devices are available for the reduction of the primary sample to an analytical sample. The three most important methods used in the pharmaceutical industry are (1) scoop sampling, (2) cone and quartering, and (3) the spinning riffler or rotary sample divider (manual method of fractional shoveling); see *Figure 5*.

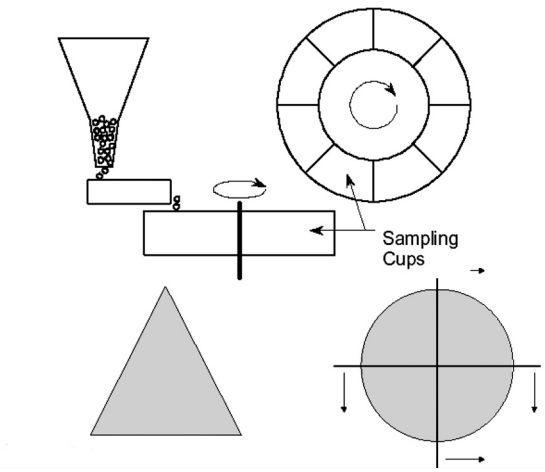

Figure 5. Two procedures for dividing samples. *Top:* spinning riffler, in which a circular holder rotates at a constant speed, and the sample is loaded at a constant rate into the containers via a vibratory chute, which is fed by a mass-flow hopper. *Bottom:* cone and quartering. (Cone, left, is flattened and quartered; quarters can be formed into cones and further subdivided.)

Scoop Sampling—Scoop sampling is done as previously described, but generally with a smaller scoop or spatula. Great care must be taken when removing material from the primary sample, because this material could be highly segregated as a result of handling. Scoop sampling is appropriate for homogeneous or cohesive powders. However, if the powder is prone to segregation, scoop sampling can introduce significant errors. Moreover, scoop sampling has several serious disadvantages. First, the method depends on the operator's deciding from which part of the primary sample to scoop the material and what quantity of the sample to extract, features that can introduce operator bias. Second, in scoop sampling, operators have a natural tendency to withdraw the sample from the free surface, which is highly prone to segregation and is not representative of the bulk. Third, operators need to avoid creating a heap where rolling segregation can occur, because material could fall off the edges of the spatula or scoop and bias the sample. Ideally, the operator should make some attempt to mix

the primary sample before using the scoop, but this too can exacerbate segregation problems and should be done only with great caution.

Cone and Quartering—Cone and quartering is done by pouring the primary sample into a symmetric cone on a flat surface. The cone is then flattened by a flat surface such as a spatula and is divided into four identical quarters (*Figure 5*). One quarter is taken as the sample. This procedure can be repeated (e.g., quarter-samples can be subdivided into quarters) until the desired sample size is obtained. The theory of this method is that when a symmetric cone is created, all the segregation processes also occur symmetrically around the cone, and hence symmetry is used to mitigate the effect of segregation. In practice, it is very difficult to actually make a symmetric powder cone, and the method becomes very operator-dependent and often unreliable. Differences in how operators form the heap and subdivide it can lead to a lack of precision and significant errors. In addition, if the method is done more than once, errors can propagate each time the cone and quartering is performed. Some experts do not recommend this method.

Spinning Riffler—A spinning riffler (*Figure 5*) includes a series of containers mounted on a circular holder. The circular holder rotates at a constant speed, and the sample is loaded at a constant rate into the containers via a vibratory chute, which is fed by a mass-flow hopper. Once the material has been divided among the different holders, an individual holder can be removed for testing or further sample division. The angular velocity of the circular holders and the amplitude of the vibratory feeder can be controlled to accommodate powders with different flow properties. The holder velocity and feed rate should be adjusted so that the containers fill uniformly and so that a heap does not form on the vibratory feeder. Spinning rifflers are available in different sizes, making possible subdivisions of powders ranging from a few milligrams to hundreds of grams. The only drawbacks of the spinning riffler are the time required to process the sample and the capital expense. Despite these minor disadvantages, the spinning riffler is by far the best method for subdivision of free-flowing powders.

Fractional shoveling is the manual version of the spinning riffler. In this method, scoop samples are taken from the original sample and placed into a sufficient number of aliquots, and then subsequent scoops are taken from the original sample and placed into one of the aliquots in sequential order. This process is repeated until the original samples are gone. Then one of the aliquots is randomly taken as the reduced sample. As is the case with all manual methods, operator error and variability can be significant factors.

Wet Analysis Methods

Wet analysis methods require dispersing the sample in a liquid suitable for analysis and then withdrawing an aliquot using a syringe or pipet. Effective secondary sampling requires making a stable homogenous suspension (i.e., the sample must be stable from the time of formation of a suspension to the time when the analysis is complete). Some important factors in wet analysis are sample solubility in the dispersion vehicle, aggregation of sample, the use of suspending agents, and deaggregation of primary particles in the dispersion vehicle. Even though a uniform suspension is created, the sample should be homogenized, typically by shaking, immediately before withdrawing the sample with a syringe or pipet. The diameter of the syringe or pipet should be large enough so that particles are not excluded and clogging does not occur. The diameters of the largest particles should not exceed 40% of the syringe or pipet tip diameter. If for practical reasons the amount of material from the primary sample is too large, the sample size should be reduced before a suspension is made. To reduce the sample size, use the methods described above in the

Dry Analysis Methods section. As a precaution, collect and retain enough sample to repeat all tests a minimum of five times.

APPENDIX 1: SUBSAMPLING EXAMPLES

The examples provided below describe the importance of material particle characterization during the selection of an appropriate sample mass. Four examples are presented. In the first example, similarity in the fundamental or intrinsic material characteristics is assumed. In the second example, the density of the heavy metal analyte being measured is changed. In the third example, the effect of changing the particle size is evaluated. In the fourth example, the adequacy of the fundamental particle characteristics in a formulation needed for a given unit dose or mass is evaluated.

Example 1. Sample Mass Determination

Assuming the lot size is 1 kg, the maximum particle diameter is 1000 μm, and the concentration of the analyte is expected to be 1%, what sample mass of round, equal-sized and -shaped 1000-μm particles with a density of 1 g/cm^3 would be needed to estimate the average concentration of the analyte with a percent relative standard deviation (%RSD) of 5%?

Rearranging equation 3, one can estimate the sample mass as shown in equation 5:

$$m_{sample} \approx \frac{1}{\frac{s_{fse}^{\,2}}{f_{shape}\, g_{CF}\, c_{max} / d_{max}^{\,3}} + \frac{1}{m_{lot}}} \qquad (5)$$

The compositional maximum heterogeneity (c_{max}) can be estimated by considering the analyte and matrix density (λ_a and λ_m, respectively) and analyte concentration (a_L) (equation 6):

$$c_{max} \approx \frac{(1-a_L)^2 \lambda_a \lambda_m}{\bar{a}_L} \qquad (6)$$

For low analyte concentrations, the compositional maximum heterogeneity is simplified by equation 7:

$$c_{max} \approx \lambda_a / a_L \qquad (7)$$

For high analyte concentrations, the compositional maximum heterogeneity is simplified by equation 8:

$$c_{max} \approx \lambda_m (1-a_L) \qquad (8)$$

The shape factor is approximated by equation 9:

$$f_{shape} \approx Volume / d^3 \qquad (9)$$

For a sphere, the shape factor is [(4/3)π/8], or approximately 0.5.

The granulometric factor can be approximated by the typical minimum diameter noted as the 5th percentile size, divided by the typical maximum diameter noted as the 95th percentile size, as shown in equation 10:

$$g_{CF} \approx {}^{d_{5\%}} / _{d_{95\%}} \qquad (10)$$

Because all particles are the same size, the granulometric factor, (g_{CF}), is 1.0. Because the analyte exists in a state liberated from the matrix particles, the liberation factor is also 1.0. The sample mass for a 5% RSD (using equation 5) is then:

$$m_{sample} = \frac{1}{\frac{0.05^2}{1x1x100x1x0.1^3} + \frac{1}{1000}} = 19.6\,g$$

A sample mass of 19.6 g will provide a sampling error of approximately 5% RSD. Note that in this example the particle characteristics are simplified to demonstrate that a lot mass of 1000 g contains 2×10^6 particles of 0.5 mg mass. The sample mass of 19.6 g contains approximately 39,216 particles, yielding a 5% RSD, using the binomial distribution where p is the concentration of the analyte (a_L) and n is the number of particles sampled, as shown in equation 11.

$$Binomial\ RSD = \sqrt{(1-p)/np} = \sqrt{(1-0.01)/39,216 \times 0.01} = 0.0498 \approx 0.05 \qquad (11)$$

(See *Table 2* for a summary of calculations.)

In determining the required sample mass, it is assumed that the sample is representative of the population. Moreover, when using a single representative sample, it is assumed that the uniformity of the sample mass is consistent with the remaining population. Note that the granulometric and liberation factors allow proportional adjustment of the sample size, depending on the nature of the particles. The inclusion of a liberation factor in the equation allows for particles to exist with a proportion of the analyte residing within every particle or a proportion thereof. The granulometric factor permits adjustment of the sample mass by accounting for the relationship in size between the smallest and largest particles represented in the lot.

Table 2. Summary of Calculations for *Example 1*, Equal-Sized and -Shaped Particles

m_{Lot} (g)	d (cm)	f_{shape}	g_{CF}	c_{max}	a_L	λ_a (g/cm³)	l	m_s (g)
1000	0.1	0.5	1	100.0	0.01	1	1	19.6

Mass per Particle P_s	Particles in 19.6 g	Binomial RSD
$d^3 f_{shape} \lambda_a$	$m_s/(P_s/g_{CF})$	$p = a(L) = 0.01$
		n = 32,916 (Eq. 11)
0.005	39,216	0.05

In-Process Revision

This approximation also can be applied to liquid suspensions in which each particle is considered discrete and the sample can be characterized with respect to size, density, mass, and volume.

Example 2. Heavy Metal

In this example, it is assumed that the analyte is the heavy metal lead, with a density of 11.34 g/cm^3, with a limit of not more than 50 ppm, where the shapes of the particles are cubes (f_{shape} = 1.0), the particles are approximately 50 μm, and a 5-g sample is taken from screened material (g_{CF} = 0.55). On the basis of equation 3, the %RSD is 17.7%. Using equation 5, one finds that a sample mass of approximately 60 g is needed to achieve a 5% RSD, assuming that a_L is equal to the limit allowed and that the analyte cannot be assumed to be liberated from the material (l = 1.0). (See *Table 3* for a summary of calculations.)

If the sample were assumed to be homogeneous (l = 0.1) with respect to presence of the analyte with all particles, then a sample mass of 6.2 g would be required. Moreover, if the shape of the particles were between round and cubic (f_{shape} = 0.8), then a sample mass of 5 g would be required to complete the analysis.

Example 3. Subsampling

Correct sampling, as noted earlier, is fundamental to understanding the important role of subsampling. In many instances it is desirable to reduce the sample size in a manner that results in a representative sample and lessens the need to test a large sample mass. In some cases the particle size and compositional heterogeneity can result in an unwieldy sample mass. This may occur with larger-sized particles or when a composite sample of many containers is required. Samples with larger-sized particles may need to be physically reduced.

For example, using *Example 2* above, if the maximum particle size were 1000 μm or 1 mm, then a 997-g sample would be suggested by equation 3. Reducing the particle size by grinding and subsampling to achieve a predetermined sampling %RSD may require subsampling more than once to achieve the desired particle size. For example, the entire sample may be reduced to 100 μm to reduce the %RSD to approximately 3%; then, with correct sampling, a subsample could be selected and entirely reduced to 50 μm to achieve a 5% RSD. Finally, a 5-g subsample could be correctly taken and tested. If certain particles have a large size with high concentration of the analyte, then samples should be selected to ensure that at least 1, but preferably at least 5–6, particles would be selected with 95% probability or chance of selection.

Example 4. Minimum Unit Dosage Mass

A formulator would like to know the minimum mass required for a dosage form to ensure with 95% confidence a unit dosage of 1% active drug powder. The drug and the excipient have a similar round shape (f_{shape} = 0.5) and a density of 0.33 g/cm^3. The active drug is milled to 1 μm, but the size of excipients can be as large as 200 μm. The value for g_{CF} is taken from equation 10 using the expected range of the excipient that accounts for 99% of the formulation, as 10 μm/200 μm, or g_{CF} = 0.05. The quantity c_{max} from equation 7 is taken as 0.33/0.01. The drug particles are completely liberated from the excipient. The batch size is 100 kg.

Table 3. Summary of Calculations for *Example 2,* Heavy Metal

m_{Lot} (g)	*d (cm)*	f_{shape}	g_{CF}	c_{max}	a_L	λ_a (g/cm³)	*l*	m_S (g)
1000	0.005	1.0	0.55	2.3×10^6	5×10^{-6}	11.34	1.0	58.71
Mass per Particle P_S			Particles in 58.7 g			Binomial RSD		
$d^3 f_{shape} \lambda_a$			$m_s/(P_s/g_{CF})$			$p = a_L = 5 \times 10^{-6}$		
						n = 75,303,331		
0.0000014175			75,303,331			0.0515		

Table 4. Summary of Calculations for *Example 4*, Minimum Unit Dose Mass

m_{Lot} (g)	*d (cm)*	f_{shape}	g_{CF}	c_{max}	a_L	λ_a (g/cm³)	*l*	m_S (g)
10^5	0.02	0.5	0.05	33	0.01	0.33	1.0	0.00264
Mass per Particle P_s				Number of Particles in 2.64 mg			Binomial RSD	
$d^3 f_{shape} \lambda_a$				$m_s / (P_s/g_{CF})$			n=40,000; p=a_L=0.01	
0.000001326				40,000			0.0497	

A minimum sample mass of approximately 3 mg is needed to ensure with 95% confidence (2 RSDs) that the average drug content is 0.9% to1.1%. The proposed dosage form has an active concentration of 100 μm per 10 mg total unit mass. The unit dosage form mass is adequate, but the formulation requires that the mixing process, unit dosage production, bulk sampling device, and lab sample preparation or subsampling from bulk samples result in equal probability of selection of drug particles. Only if these conditions for mixing, production, sampling, and testing are met can it be reliably demonstrated that the unit dosage and test determination acceptance criteria of 1% (0.01 μg/mg) are met. Acceptable outcomes of such testing also indicate that the particle size, shape, and density must be controlled. For example, an increase in the sizes of particles to 500 μm results in a need for a 42-mg sample mass and dose. Assuming a cubic, as opposed to a rounded, particle increases the sample mass to 5 mg, which for a fixed dosage form mass may result in less room for the variation contributed by other characteristics, or in lesser confidence. If the acceptance criteria were changed to 0.95% to 1.05%, requiring a 1% RSD, then the minimum sample mass would increase to approximately 70 mg. (See *Table 4* for a summary of calculations.)

APPENDIX 2: MATERIAL CHARACTERIZATION AND SAMPLING

Specific and thorough knowledge of the material's synthesis, composition, and usage is critical to developing a bulk material sampling plan. Material characterization is important because bulk material can exist in many forms throughout the material process flow. As illustrated in *Figure 6*, the type of sampling can vary by process step and ultimately affects the use of the material in the drug pro-

duct. Appropriate material characterization considers the material process step, the type of sampling, the objective of the process step, and ultimately the drug product.

For example, material can be synthesized or mixed in a large container where sampling may be limited or ideal for the characteristic needed. If the characteristic is important but the sampling conditions are not ideal, perhaps because of the heterogeneity of a powder mixture, then sampling for that characteristic may be more appropriately performed at a different stage upstream or downstream to assess the heterogeneity of the contents and ensure correct sampling. Correct sampling is the technique that results in equal probability of selection, using truly random representative samples to accurately measure the population. This is sometimes referred to as reducing the sampling dimension to measure material characteristics. Reducing the sampling dimension allows for the application of simple random sampling of subgroups or sampling from a stream or conveyer belt that represents the three-dimensional bulk container.

Acceptance attributes (see *Table 5*) depend on material characterization and process. Acceptance attributes may be applicable throughout the life of the bulk material. Both the number and size of samples require an understanding of the material's variation.

Table 5. Examples of Acceptance Attributes

Acceptance Attributes			
Physical	Chemical	Microbiological	Packaging
Particle size	Purity	Sterility	Label accuracy
	pH		
Viscosity	Identity	Pyrogens	
Density	Strength	Microbial load	Integrity

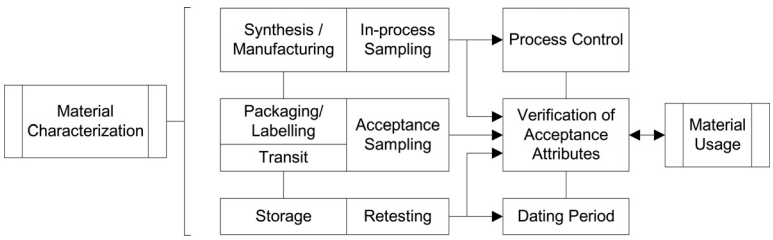

Figure 6. Material process flow.

APPENDIX 3: RANDOM NUMBER TABLES

1. Number all containers in the lot to be sampled.
2. Choose the random number table page, or generate the random numbers.
3. Choose a direction to read—up, down, right, or left.
4. To get a starting point, close your eyes and choose a point on the table.
5. Read numbers from the table in that predetermined direction. If the digits that you selected are between 01 and *N,* then this is the first sample element. If not, keep going in the preset direction until you find a suitable number.
6. Continue to move in the preset direction until you find the next number between 01 and *N*. That is the second element. Continue in this manner until you have enough samples. See *Table 6* for an example.

Table 6. Random Number Table

N^a = 500	*N* = 100
n^b= 50	*n* = 10
6977	69**77**
8**377**	8377 ←Sampling without replacement
3**034**	30**34**
9903	99**03**
6955	69**55**
5**483**	54**83**
5733	57**33**
0**126**	01**26**
4**329**	43**29**
3776	37**76**
etc.	etc.

Table 6. Random Number Table *(Continued)*

N^a = 500	*N* = 100
n^b= 50	*n* = 10
Containers to be sampled:	Containers to be sampled:
377, 34, 483, 126, 329, ...	77, 34, 3, 55, 83, 33, 26, 29, 76, ...

[a] *N* = population size.
[b] *n* = sample size.

APPENDIX 4. ADDITIONAL SOURCES OF INFORMATION

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BRIEFING

⟨**1180**⟩ **Human Plasma.** Because there is no information in *USP* regarding human plasma, this new general information chapter provides consolidated information on plasma classification, nomenclature, collection and processing procedures required for ensuring product safety, details of specific plasmas, and quality systems relating to plasma collection. This chapter emphasizes U.S. practices, but provides information regarding the requirements of the European Union, the United Kingdom, and Australia.

(BB BBP: A. Szajek) RTS—C55355

Add the following:

■⟨1180⟩ HUMAN PLASMA

SCOPE

This chapter provides a consolidated source of information regarding human plasma, with emphasis on plasma for fractionation. Specifically, the chapter addresses plasma classification and nomenclature; collection and processing procedures required for ensuring product safety; details of specific plasmas; and quality systems relating to plasma collection. The chapter also includes, at the end of the text sections, a glossary; a list of abbreviations used in the chapter; and appendices that provide plasma definitions, donor selection criteria, and testing requirements.

Plasma originating from US-licensed collection facilities provides the major supply for the global plasma derivative market. The U.S. Food and Drug Administration (FDA) regulates the collection and processing of plasma used for further manufacture. Title 21 of the Code of Federal Regulations (CFR) details Good Manufacturing Practice (GMP) requirements and product standards to protect the health of the blood donor and to ensure the safety and efficacy of blood products. CFR regulations are updated periodically, but between revisions, existing regulations may not address the most current issues and scientific developments. Therefore, the FDA periodically publishes guidance documents.[1] (For further information, see *Quality Systems*, below).

This chapter emphasizes U.S. practices, but because plasma and its derivatives are shipped globally, it is important to recognize that there are regional differences in recommendations, requirements, and regulations. Therefore this chapter provides information regarding the European Union (EU), the United Kingdom (UK), and Australia.

OVERVIEW

Composition of Plasma

Plasma constitutes approximately 55% of the total blood volume. It is a clear, straw-colored, complex liquid that is 7% protein, 91% water, and 0.9% mineral salts. The majority (approximately 70%) of total plasma protein is albumin. Additional plasma proteins relevant to fractionation include immunoglobulins, coagulation factors, fibrinolytic proteins, proteases, and protease inhibitors. These constituent plasma proteins can be isolated on the basis of the different solubility characteristics of each protein when subjected to specific conditions of pH, temperature, ionic strength, and ethanol concentration. The major products derived from fractionation are listed in *Table 1*.

Table 1. The Major Fractions and Products from the Cohn Process

Fraction	Product
Cryoprecipitate	Antihemophilic factor (FVIII)
Cryosupernatant	Antithrombin III, factor IX complex
Fraction I	Fibrinogen, factor XIII
Fraction II	Immune globulin G (IgG)
Fraction III	IgA, IgM, prothrombin, plasminogen

[1] FDA. Blood Guidances. www.fda.gov/cber/blood/bldguid.htm. FDA Memoranda to Blood Establishments available at www.fda.gov/cber/memo.htm.

Table 1. The Major Fractions and Products from the Cohn Process *(Continued)*

Fraction	Product
Fraction IV-1	Factor IX complex, activated factor IX complex
Fraction IV-4	Plasma protein fraction, alpha-1 proteinase inhibitor
Fraction V	Albumin

Plasma for Manufacture of Derivative Products

The two methods for collection of human plasma are automated apheresis (for definitions, see the *Glossary* preceding the appendices) and centrifugation of whole blood donations. Source plasma collected by apheresis constitutes the majority of plasma used in the manufacture of plasma derivatives in the United States. Plasma collected for transfusion but not so used (i.e., recovered plasma) also may be used for manufacture. Flow charts delineating how apheresis and whole blood–derived plasma can be used in the manufacture of plasma derivatives in the United States and Europe are presented in *Figures 1* and *2*, respectively.

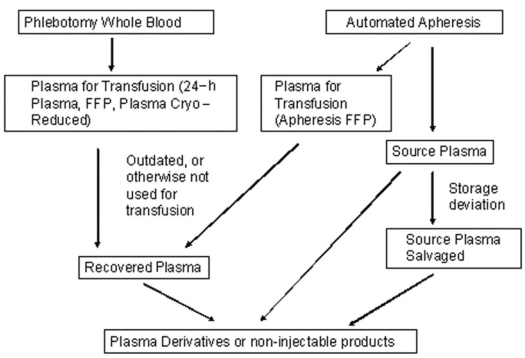

Figure 1. U.S. plasma derivative manufacture: FDA standards.

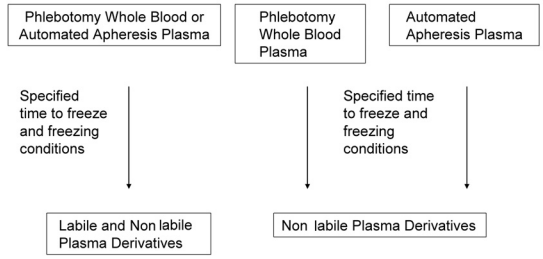

Figure 2. EU plasma derivative manufacture: EU standards.

Regardless of the collection method, plasma for fractionation should be a clear to slightly turbid liquid without visible sign of hemolysis; it may vary in color from light yellow to green; it should be $\pm$10% of the stated volume; and it should show no sign of clots. Residual cells should comply with the following limits: red cells $< 6.0 \times 10^9$/L; leukocytes $< 0.1 \times 10^9$/L; platelets $< 50 \times 10^9$/L.

Source Plasma

Licensed Source Plasma may be manufactured only in collection centers that are approved by the FDA for the collection and distribution of Source Plasma in interstate commerce. Federal regulations governing the manufacture of Source Plasma, including minimal requirements for donors, are found in 21 CFR 640, Subpart G. By definition, Source Plasma is plasma intended for further manufacture. Source Plasma donors can donate as often as twice a week and may be compensated. In addition to FDA requirements, most plasma collectors and fractionators also comply with voluntary standards established by the Plasma Protein Therapeutics Association (PPTA), a trade and standards-setting organization.[2] PPTA voluntary standards address several areas of donor, plasma unit, plasma pool, and center management, and are designed to supplement existing regulatory requirements.

[2] PPTA. www.pptaglobal.org.

Plasma for Transfusion

Plasma for transfusion is not intended for further manufacture but for direct transfusion to patients. It may be collected by either whole blood or apheresis donation. In the United States, plasma for transfusion comes from unpaid volunteer donors. Blood collecting facilities that collect plasma for transfusion comply with requirements of both the CFR and a voluntary trade organization, the American Association of Blood Banks (AABB). Sections 640.3 and 640.31 of 21 CFR outline requirements for donors of whole blood and therefore govern most donors of plasma for transfusion, regardless of the collection method. AABB voluntary standards include information contained in FDA regulations and guidance pertaining to blood and plasma, as well as additional standards.[3] Plasma for transfusion may be converted to recovered plasma, an unlicensed product that may be used for further manufacture.

Plasma for Ancillary Use in Biologics Manufacturing

Human plasma and its derivatives are used in the manufacture of other biologic products. In this role, the plasma or plasma derivative falls into the category of ancillary use. This is defined as use of a reagent or material as a processing or purification aid or a reagent that exerts an effect on the therapeutic substance but is not intended to be part of the final product formulation (see the *USP* general information chapter *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products* ⟨1043⟩).

Human plasma is commonly used in manufacturing processes that involve primary human cells or cell lines intended for therapeutic applications. In these applications, plasma provides a source of protein and possibly other factors that enhance expansion and differentiation of cell populations. A variety of methods have been used to prepare human plasma and derivatives for ancillary use, but the practices are not standardized. Allogeneic plasma typically is obtained from either apheresis or whole blood, using citrate anticoagulation. Allogeneic plasma typically is collected from paid donors who, like Source Plasma donors, have been screened for the absence of transfusion-transmissible diseases. Preferred donors may be blood type group AB, because they lack anti-A and anti-B isohemagglutinins. Other preferred donors include untransfused males, because this group is unlikely to have human leukocyte antigen (HLA) antibodies that could react with cells in a given culture system. Serum is prepared either from nonanticoagulated whole blood that has been allowed to clot or by the addition of calcium to plasma obtained from citrated whole blood or apheresis. Heating plasma or serum to 56° inactivates heat-labile complement and other proteins.

Although there are no standardized specifications for plasma products used as ancillary materials, assays often include safety testing associated with general biologics (e.g., bacterial/fungal cultures, endotoxin, and mycoplasma). In addition, characterization of the products may include tests for irregular erythrocyte and HLA antibodies, osmolality, pH, total protein and immunoglobulin concentrations, hemoglobin concentration, and chemistries such as Na, K, Cl, Ca, and glucose.

In some instances, plasma from bovine sources has been used instead of human plasma. The differences between bovine and human plasma products include factors relevant to their efficacy as ancillary materials, as well as safety of the final manufactured product when administered to humans (see the forthcoming *USP* general information chapter *Bovine Serum* ⟨1024⟩). Fetal bovine serum is preferred to human serum for some applications because it may be superior in promoting human cell growth *in vitro*. Safety considerations,

[3] AABB. *Standards for Blood Banks and Transfusion Services*. 25th ed. Bethesda, MD: AABB; 2008.

especially the risk of transmissible spongiform encephalopathy and allergic reactions related to antibovine antibodies, have led to recommendations that human plasma sources be used whenever possible instead of bovine sources. However, substitution of human for bovine plasma does not completely eliminate the risk of infectious and immunologic sequelae, even when human plasma is used as an ancillary material.

PLASMA COLLECTION AND PROCESSING

This section discusses the standard principles involved in plasma collection and the methods used to ensure the safety of the plasma and subsequently manufactured plasma derivatives. Principles for screening and testing of donors are presented in other parts of this chapter.

Collection

Source Plasma is collected by apheresis. Recovered plasma can be obtained either by apheresis or as a by-product of whole blood collection. Collection should take place via an FDA-approved, closed, sterile, pyrogen-free collection system that contains an anticoagulant. No antibacterial or antifungal agent should be added to the plasma. Donations must be collected aseptically. The skin of the donor must be prepared by a method known to ensure a sterile product. Source plasma is collected using 4% sodium citrate as the anticoagulant. The composition of sodium citrate is given in *Table 2*.

Three anticoagulant solutions are licensed in the United States for collection of whole blood: citrate phosphate dextrose (CPD), citrate phosphate double dextrose (CP2D), and citrate phosphate dextrose adenine (CPDA-1). The composition of blood collection bags containing these anticoagulants is shown in *Table 3*. Plasma for transfusion or further manufacture can be made from a unit of whole blood collected in any of the three anticoagulant solutions. Regulations relating to plasma make no distinction among the three anticoagulant solutions. Consequently, collection, storage, and transport requirements are identical regardless of the anticoagulant solution used in the primary collection.

Labeling

The labeling for Source Plasma should comply with 21 CFR 640.70. The labeling for whole blood should comply with 21 CFR 606.121 and 606.122.

A unique identification number is assigned so that the donation can be related to the individual donor records and test results. The origin of each donation in a plasma pool and the results of the corresponding donation and laboratory tests must be traceable while the required degree of confidentiality concerning the donor's identity is maintained. Whole blood must be labeled ''This Product may transmit infectious agents'' [21 CFR 121(c)(9)]. Source Plasma or recovered plasma must be labeled ''Caution: For Manufacturing Use Only'' if the product is intended for use in fractionation. For plasma to be used as a reagent or for in vitro use, the required labeling statement is ''Caution: For Use in Manufacturing Noninjectable Products Only'' [21 CFR 121(e)(5)(ii)].

Table 2. Anticoagulant Solution for Collection of Source Plasma by Apheresis (4% Sodium Citrate)

Volume	Sodium citrate dihydrate	Citric acid anhydrous	pH (25°)	Ratio of solution to whole blood
250 mL or 500 mL	40 g/L	As required for pH adjustment	6.4–7.5	1 : 16

Table 3. Anticoagulant Solutions Used during Whole Blood Collection for Recovery of Plasma (500-mL collection bags)

Anticoagulant	CPD[a]	CP2D[b]	CPDA-1[c]
Volume (mL)	70	63	63
Dextrose (mg)	1780	3220	2010
Sodium citrate dihydrate (mg)	1840	1660	1660
Citric acid anhydrous (mg)	209	206	206
Monobasic sodium phosphate (mg)	155	140	140
Adenine (mg)	—	—	17.3
pH (25°)	5.3–5.9	5.3–5.9	5.3–5.9
Ratio of solution to whole blood	1.4 : 10	1.4 : 10	1.4 : 10

Note: Collection of source plasma typically involves the use of sodium citrate as the anticoagulant. The specification for sodium citrate is given in *Table 2.* Plasma for transfusion is stored at 2° to 8° after collection. Plasma collected by apheresis should be frozen immediately at –18° or colder.
[a] Citrate phosphate dextrose.
[b] Citrate phosphate double dextrose.
[c] Citrate phosphate dextrose adenine.

Storage

Plasma for fractionation should be stored at or below –20°. The plasma can still be used for fractionation if its temperature exceeds –20° on (at most) one occasion for not more than 72 hours and if the plasma has been maintained at a temperature of –5° or lower at all times. Storage temperatures must be maintained during transport.

PLASMA SAFETY CONSIDERATIONS

Plasma is protected by five overlapping safeguards that the FDA has termed the ''Five-Layer Safety Net'': donor screening, blood testing, donor deferral, quarantine, and investigation.

Voluntary measures that provide an additional margin of safety include recruitment and retention of suitable donors, donor site locations in epidemiologically favorable environments, and inventory hold procedures. In the manufacture of plasma-derived products, steps taken for viral clearance are very important for ensuring safety.

None of these measures is sufficient by itself; the safety net is the overlapping combination of the activities.

Donor Screening

The selection of a suitable site for blood and plasma donation activities is a first and very important step to ensure safe donations. Areas with low disease prevalence are preferred as locations for donation centers, thereby reducing the likelihood of collecting plasma from an infected donor.

One of the PPTA voluntary standards is the viral marker standard, which requires plasma centers to report the prevalence of viral markers for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) in the donor populations. The prevalence at an individual center is compared to the industry average. Alert limits are set to take into account the

number of annual donations in the center. If a center exceeds the limit for any of these viruses or the aggregate of all three, the center must implement corrective actions that will bring the center into compliance with the standard.

Appropriate donor selection helps provide a safe plasma supply. A detailed donor history questionnaire in conjunction with a careful medical examination allows center personnel to recognize unsuitable donors whose behavior puts them at risk for transfusion-transmitted disease or who have underlying medical conditions that preclude donation.

An additional measure put in place by centers that collect source plasma is the PPTA National Donor Deferral Registry (NDDR). It lists donors throughout the United States who have been previously deferred from donation (although it provides no information about the reason for deferral). Other countries have different systems depending on their national regulations concerning personal data gathering. Any individual who tests positive for HIV, HBV, or HCV is entered into the national database (the National Donor Deferral Registry) used by all U.S. plasma centers that are certified under the International Quality Plasma Program (IQPP). All individuals who present at a U.S. plasma center for the first time are checked against the NDDR. In this manner, patients who have previously been deferred for positive test results at any participating facility can be identified and rejected quickly. This standard ensures that donors deferred for positive test results do not donate in other facilities.

A voluntary safety initiative, the Qualified Donor Standard, implemented by the plasma fractionation industry, builds on the fact that many plasmapheresis donors contribute plasma frequently. A donor who enters a plasmapheresis center for the first time is called an Applicant Donor, and the first donation is used for further manufacturing only if the donor returns a second time. Potential donors must pass two separate medical screenings and testing for HIV, HBV, and HCV on two different occasions. Only after satisfactory screenings and negative test results does that person become a Qualified Donor. If a donor does not return within 6 months, that person loses his/her Qualified Donor status and must qualify again. This standard means that plasma from a one-time-only donor (even when all test results are negative) cannot be used for further manufacture. This standard results in committed donors and eliminates the risk that plasma centers will accept so-called test seekers. The interval between permitted donations of whole blood is too long to allow a similar screening program, although quite a number of donors in whole-blood donor centers are regular and repetitive donors.

Another PPTA voluntary standard addresses donor management criteria. The Community-Based Donor Standard allows only donors who permanently reside within its defined donor recruitment area to donate at a given center. In addition, a Donor Education Standard requires new donors to engage in an educational program and follow-up assessment regarding HIV/acquired immune deficiency syndrome (AIDS) and activities that place them at risk for HIV/AIDS.

In addition to donor management strategies and standards, PPTA has issued a plasma unit management standard called Inventory Hold. This standard states that collected plasma will be held in inventory for at least 60 days from the time of collection. This allows the retrieval of units as a result of post-donation information (information that was not known at the time of donation) that would have disqualified the donor. This information could include admitting high-risk behavior; becoming reactive for HIV, HBV, or HCV; or providing incorrect information about international travel.

Blood Testing

Testing of donations is an important safety measure both for plasma intended for transfusion and plasma intended for further manufacturing. Both enzyme-linked immunosorbent assays (ELISA) and nucleic acid amplification technologies (NAT) are used to screen donations for the presence of infectious disease. Automation provides the necessary throughput to screen every donation for a variety of potential pathogens.

Testing strategies differ depending on whether the plasma donation is intended for transfusion or further manufacture. Plasma for transfusion requires more extensive infectious disease testing, because there are no pathogen inactivation/removal technologies licensed for this product in the United States. On the other hand, plasma for further manufacture is subjected to several pathogen inactivation/removal steps during manufacture, thereby obviating the need for some disease testing. *Table 4* outlines current infectious disease tests required by the FDA for plasma donations collected in the United States. *Appendix 3* compares EU and U.S. disease testing and donor deferral requirements.

NAT, of which polymerase chain reaction (PCR) is the most widely used form, does not rely on the detection of antibodies produced by the infected host after exposure, but targets the nucleic acid of the infecting agent. By means of the selection of suitable priming molecules (the so-called primers), the assay is highly specific for the infecting virus (see the *USP* general information chapter *Nucleic Acid-Based Techniques—Amplification* ⟨1127⟩). Through several cycles of amplification, the polymerase enzyme can repetitively generate copies of the targeted fragment of the viral nucleic acid, providing an exponential amplification of a very short stretch of the viral deoxyribonucleic acid (DNA) [or ribonucleic acid (RNA)]. The exponential amplification leads to the generation of many copies of the target molecule and allows the subsequent detection of this virus-specific fragment, even if the original viral load was exceedingly low. This methodology has brought a new degree of safety.

NAT testing, because of its complexity and expense, is difficult to conduct on individual donations. Generally, aliquots from several donations are combined into a single pool, often called a minipool. Testing in pooled format remains more sensitive than serological ELISA screening of individual donations. In addition, the NAT principle circumvents several of the limitations in detecting pathogens by means of serological methods. Pooling can influence overall sensitivity, depending on the pool size and the analytical sensitivity of the NAT assay employed.

In many countries, the maximal load of a pathogen acceptable for a single donation defines the overall NAT sensitivity required. Assays of higher analytical sensitivity can use larger pools, but those of lower analytical sensitivity must test smaller pools in order to comply with regulations. The availability of commercial NAT test kits with defined analytical sensitivity has made minipools of 12 or 24 very common, because these pool sizes, in combination with the analytical sensitivity of the assays used, comply with common regulations on overall sensitivity.

Effective NAT screening requires that the viral load of the plasma pool at the beginning of production be less than the inactivation capacity of the process. Differences between the plasma transfusion and fractionation industries have led to different applications of NAT. For individual donations intended for transfusion, where there is no inactivation process and where testing is the only method to interdict a contaminated donation, the safety of each individual donation must be ensured by testing with the most sensitive assays possible. Plasma intended for further manufacturing, in contrast, is pooled and serves as the starting material for a multistep process that has built-in pathogen inactivation method-

Table 4. FDA Disease Test Requirements for Plasma for Transfusion and Plasma for Further Manufacturing

Disease	Plasma for Transfusion	Plasma for Further Manufacturing[a]
Hepatitis B	Hepatitis B surface Antigen (HBsAg) Hepatitis B core antibody	HBsAg
Hepatitis C	Anti-HCV HCV RNA	Anti-HCV HCV RNA
HIV	Anti-HIV I/II HIV RNA	Anti-HIV I/II HIV RNA
Human T-lymphotropic virus (HTLV) I/II	Anti-HTLV I/II	Not required
Syphilis	Serologic test for syphilis, every donation	Serologic test for syphilis, every 4 months
West Nile virus (WNV)[b]	WNV RNA	Not required

[a] The FDA also encourages in-process NAT testing for parvovirus B19 and hepatitis A. HBV NAT testing also is performed on most Source Plasma.
[b] Testing for WNV is recommended in an FDA draft guidance. The FDA is considering recommendations regarding testing for *Trypanosoma cruzi* (Chagas disease).

ologies. Therefore, NAT screening for plasma for further manufacture is focused on ensuring safe donations and limiting the viral load of the plasma pool to levels less than the known viral inactivation/removal capacity of the inactivation process.

To avoid the loss of large amounts of plasma from a reactive pool, the fractionation industry has implemented a prescreening strategy, the minipool screening concept mentioned earlier. Aliquots of plasma donations are combined to form minipools, and the minipools are tested by NAT. If a minipool is reactive for a virus tested, the individual donation that gave rise to this positive result can be identified and interdicted. The other donations demonstrated to be free of infection can be used for further manufacturing. The donations are then combined into a production pool, a sample of which is subjected to NAT testing as required by regulations.

As indicated, the NAT test portfolio is not uniform and depends on the intended use of the donation and the regulatory environment. Although screening for HCV RNA is done in most countries, screening for HIV is not universally required. NAT detection of HBV is used mainly for plasma for manufacturing. Screening for B19 virus or hepatitis A virus (HAV) is performed only on plasma for manufacturing. HBV screening using NAT detection is more widespread in European and Asian countries than in the United States.

Donor Deferral

A donor may be deferred from further donation as a result of answers provided on the donor history questionnaire, a medical examination performed at the time of donation, or positive tests for infectious diseases. These processes ensure both the eligibility of the donor and the suitability of the donation. In the event that either is not acceptable, standard processes permanently remove the donor and interdict unused units previously donated. The donor registries mentioned earlier are one means of ensuring that a donor deferred at one center cannot donate elsewhere.

Quarantine

Each individual unit of plasma, whether for transfusion or further manufacture, is held in quarantine until all the required tests have been completed. If all required tests have been performed and found acceptable, the unit can be released; if not, the unit must be destroyed. The plasma industry has voluntarily implemented the Inventory Hold protocol (also discussed in the previous section *Donor Screening*) for plasma for further manufacture. According to inventory hold requirements, during a 60-day hold period an individual plasma donation cannot be used for further manufacture. The rationale for the hold is that donors who have been recently infected with a pathogen may not have developed levels of antibody at the time of donation, thereby donating an infectious unit despite negative disease tests. The hold provides sufficient time for an infectious donor to develop levels of antibody that will be detected during a subsequent donation if the plasma was intended for transfusion. The 60-day hold also reduces the chance of releasing an infectious unit into the manufacturing process.

The introduction of NAT may have decreased the need for inventory hold, because NAT targets the infecting virus directly and thus does not rely on the time-delayed production of antibodies. Because NAT cannot detect all viruses and because even NAT has a certain (although very low) limit of detection, inventory hold is still of value and thus remains in place in the plasma fractionation industry.

Investigation

Each plasma donation must be traceable from donation to ultimate disposition in order to minimize the potential transmission of an infectious agent. Traceability encompasses all data concerning donation site, donor identifying information, test results, and data regarding transport, storage, and consignee(s).

Look-back is a process to identify and interdict (quarantine) previous donations from a donor who, at a subsequent donation event, has been found to be (1) infected with a transmissible agent or (2) unsuitable for donating plasma because of history, physical examination, or post-donation information. Although look-back strategies are similar in most countries, specific procedures may vary.

QUALITY SYSTEMS

The intent of this section is to outline the general principles and regulations that are the basis of quality systems relating to plasma collection. Collection centers must follow cGMPs that originate in CFR and are elaborated in FDA regulations, guidance documents, and industry standards.

The GMP guidelines specifically governing plasma are found in 21 CFR 600, Biological Products: General; and 606, Current Good Manufacturing Practice (cGMP) for Blood and Blood Components. More general cGMP regulations are found in 21 CFR 210, Current Good

Manufacturing Practice in Manufacturing, Processing, Packing, or General Holding of Drugs, General; and 21 CFR 211, Current Good Manufacturing Practice for Finished Pharmaceuticals. Although quality systems regulations are part of 21 CFR for medical devices, they have been extended to other manufacturing as part of the ''c'' in cGMP and FDA Guidance documents.[4]

The quality system is divided into four major parts: management responsibility, resources, manufacturing operations, and evaluation activities. These are the foundation of the five manufacturing systems: production, facilities and equipment, laboratory control, materials, and packaging and labeling. The procedures of each system are designed to allow operations that facilitate implementation of cGMP requirements. In many instances, these requirements relate to providing facilities and expertise to achieve the requirements for the Five-Layer Safety Net (donor screening, blood testing, donor deferral, quarantine, and investigation), discussed above.

Management responsibilities include providing leadership; building a quality system for the organization that meets requirements; establishing policies, objectives, and plans; and reviewing the quality systems with defined frequency. Resources include having sufficient resources for operational activities, personnel development plans, adequate facilities, and suitable equipment; and controlling outsourced operations. Manufacturing includes designing, developing, and documenting product and processes, performing and monitoring operations, and addressing nonconformities. Evaluation activities include analyzing data for trends, conducting internal audits, and initiating corrective and preventive actions.

A number of required routine activities related to collection and release of plasma or blood units are linked to both cGMP guidelines and quality systems. These include the requirement for having SOPs to cover all aspects of collection, testing, and release. It is also necessary to validate equipment and systems used by the collection center, including temperature-controlled areas, laboratory equipment, water systems, and computer systems. The design and operation of the facility must be adequate to perform the tasks at hand and prevent cross-contamination. Plasma collection facilities must have an adequate number of knowledgeable and trained staff as well as procedures for acceptance and release of raw materials. The requirements for a collection facility do not differ from those for a pharmaceutical manufacturing facility.

GLOSSARY

Apheresis—A method of obtaining one or more blood components by machine processing of whole blood; the residual components of the blood are returned to the donor during or at the end of the process.

Blood component—A therapeutic constituent of human blood: red cells, white cells, platelets, or plasma.

Blood establishment—Any structure or organization responsible for any aspect of the collection and testing of human blood or blood components, whatever their intended purpose, and their processing, storage, and distribution. This does not include hospital transfusion services.

Blood product—Any therapeutic product derived from human blood or plasma.

[4] FDA. Guidance for Industry: Quality Systems Approach to Pharmaceutical cGMP Regulations. 2006. Available at www.fda.gov/cber/gdlns/qualsystem.htm.

Center—Collection site or location where blood or plasma is collected (and also may be processed and stored). *Center* is also applicable to a testing *Site* (see entry in this glossary).

Cryoprecipitate—A plasma component prepared from fresh-frozen plasma by freeze-thaw precipitation of proteins and subsequent concentration and resuspension of the precipitated proteins in a small volume of the plasma.

Deferral—Temporary or permanent suspension of the eligibility of an individual to donate blood or blood components.

Distribution—The act of delivery of blood and blood components to other blood establishments, hospital blood banks, and manufacturers of blood products.

Manufacturing pool—A combination of a specified number of plasma donations used as the first step in the manufacture of plasma derivatives.

Donation minipool—A combination of a small number of units or samples representative of donations used for pretesting prior to full-scale manufacture.

Quarantine—The physical isolation of blood components or incoming materials/reagents over a variable period of time while awaiting acceptance, issuance, or rejection of the blood components or incoming material/reagents.

Site—Any location at which a blood establishment carries out blood collection, not including any location not owned or managed by the blood establishment at which blood is collected or any mobile blood collection unit.

Validation—The establishment of documented and objective evidence that the particular requirements for a specific intended use can be consistently fulfilled.

ABBREVIATIONS

AABB	American Association of Blood Banks
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
CFR	Code of Federal Regulations
CJD	Creutzfeldt-Jakob disease
CMV	Cytomegalovirus
CNS	Central nervous system
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EIA	Enzyme immunoassay
EU	European Union
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FFP	Fresh-frozen plasma
FP24	Plasma frozen within 24 hours after phlebotomy
cGMP	Current Good Manufacturing Practice
HAV	Hepatitis A virus
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus
HCT	Hematocrit
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HTLV	Human T-lymphotropic virus
IgA, IgG, IgM	Immunoglobulins A, G, and M, respectively
IOPP	International Quality Plasma Program
IU	International Unit
NAT	Nucleic acid amplification technology
NDDR	National Donor Deferral Registry
PCR	Polymerase chain reaction
PPTA	Plasma Protein Therapeutics Association
PRP	Platelet-rich plasma
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
TEP	Therapeutic exchange plasma
WBDP	Whole blood-derived plasma
WNV	West Nile virus

APPENDICES

Appendix 1

NOTE—The collection, processing, and uses of plasma have generated a large number of terms and definitions that reflect the diversity of operations. In addition to these FDA standards and terms, industrywide voluntary standards are discussed in the *Plasma Safety Considerations* section.

Advisory Note: These terms are not meant as regulatory definitions, because plasma term definitions can vary from region to region and among industry sectors. The reader is advised to consult with regulatory authorities responsible for the region and industry sector. Often specific process variables must be considered.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions

Plasma Type and Agency or Agency Type	Specification
Recovered plasma	
CFR	Plasma derived from single units of whole blood as a by-product in the preparation of blood components from whole blood collection and intended for further manufacturing. Compliance Policy Guides Manual (CPG 7134.12), Sec. 230.100.
AABB	Plasma for use in manufacturing and prepared from allogenic donations. Plasma selected for manufacture that has been collected from whole blood or apheresis plasma collected for transfusion that has expired.
Inter-region, Inter-sector	Plasma separated from whole blood most often by manual centrifugation or by apheresis. The priority for the blood collected is usually for the production of red blood cells. However, the plasma can be suitable for further manufacture of biotherapeutics and transfusion. The time from collection to freezing can vary depending on the distance of collection and processing sites. Volunteer donors typically are used.
Source plasma	
CFR	Fluid portion of human blood collected by plasmapheresis and intended as source material for further manufacturing use (21 CFR 640.60).
Inter-region, Inter-sector	Plasma separated from whole blood by plasmapheresis where the cellular components can be returned to the donor. The priority for the plasma usually is for further manufacture of biotherapeutic products. However, the plasma can be suitable for transfusion. It is rapidly frozen after collection.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
Fresh-frozen plasma (FFP)	
CFR	Fresh-frozen plasma shall be prepared from blood collected by a single uninterrupted venipuncture with minimal damage to and minimal manipulation of the donor's tissue. The plasma shall be separated from the red blood cells and placed in a freezer within 8 hours or within the timeframe specified in the directions for use for the blood collecting, processing, and storage system and stored at –18° or colder [21 CFR 640.34(b)].
AABB	Plasma separated from the blood of an individual donor and placed at –18° or colder within 6 to 8 hours of collection from the donor or within the timeframe specified by the manufacturer's instructions.
Inter-region, Inter-sector	Plasma that is collected and frozen quickly after preparation. Transfusion is the primary intended use. However, FFP can be suitable for further manufacture of biotherapeutic products.
Council of Europe	A component for transfusion or for fractionation prepared either from whole blood or from plasma collected by apheresis, frozen within a period of time and to a temperature that will adequately maintain labile coagulation factors in a functional state (Chapter 21).
UK	Supernatant plasma separated from a whole blood donation or plasma collected by apheresis, frozen, and stored.
Australia	Plasma separated from whole blood and frozen within 18 hours after collection of whole blood. FFP also is prepared from anticoagulated blood that is separated into components by a suitable apheresis machine with retention of the plasma and return of the remaining elements to the donor. Freezing commences within 6 hours of collection. Storage takes place at –25° or below.
Concurrent plasma	
Inter-region, Inter-sector	Plasma collected concurrently with cellular components. Concurrent plasma may be suitable for transfusion or for further manufacture of biotherapeutics.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
Applicant plasma	
Inter-region, Inter-sector	Source Plasma obtained during the first collection from a new donor. The plasma is reserved for testing, and any remainder or products derived from the remainder are not allowed for use in humans or are quarantined until the donor passes appropriate tests and returns for a second donation.
Platelet-rich plasma (PRP)	
CFR	PRP shall be prepared from blood collected by a single uninterrupted venipuncture with minimal damage to and manipulation of the donor's tissue. The plasma shall be separated from the red blood cells by centrifugation within 4 hours after completion of the phlebotomy or within the timeframe specified in the directions for use for the blood collecting, processing, and storage system. The time and speed of the centrifugation shall have been shown to produce a product with at least 250,000 platelets per µL. The plasma shall be stored at a temperature between 20° and 24° immediately after filling the final container. A gentle and continuous agitation of the product shall be maintained throughout the storage period if stored at a temperature of 20° to 24° [21CFR 640.34(d)].
Inter-region, Inter-sector	Plasma that is a product of the first centrifugation of blood where it is separated from red cells. Platelets are fractionated into the plasma layer.
Platelet-poor plasma	
Inter-region, Inter-sector	Plasma that is further purified from platelets by a second centrifugation of PRP.
Cryo-poor plasma	
CFR	Plasma that remains after both platelets and cryoprecipitated AHF have been removed may be labeled "Plasma, Cryoprecipitate Reduced" [21CFR 640.34(e)(2)].

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
AABB	Plasma Cryoprecipitate Reduced; Fresh-frozen Plasma from which cryoprecipitate has been removed.
Inter-region, Inter-sector	Plasma that has been thawed by maintaining the temperature just above freezing (usually 4°). A large portion of certain plasma proteins (e.g., FVIII, cryoprecipitate, fibrinogen, fibronectin, or FXIII) has been precipitated from the plasma.
Council of Europe	Plasma, Fresh-Frozen, Cryoprecipitate-Depleted (Chapter 23). A component prepared from plasma by the removal of cryoprecipitate.
UK	Plasma cryoprecipitate-depleted for transfusion means a plasma component prepared from a unit of plasma, fresh-frozen. It comprises the residual portion after the cryoprecipitate has been removed.
Australia	Cryo-depleted Plasma is the supernatant remaining after cryoprecipitate has been removed from fresh-frozen plasma. It contains most clotting factors in amounts similar to those in FFP but is deficient in Factor VIII, fibrinogen, von Willebrand factor, Factor XIII, and fibronectin. Storage: 12 months at –25° or below.
Cryo-rich plasma	
Inter-region, Inter-sector	Plasma that has been thawed by gentle heat input (e.g., in a 37° water bath) where the cryoprecipitate remains dissolved.
Plasma for labile products	
Inter-region, Inter-sector	Plasma that has been collected and best maintains the activity and integrity of labile plasma proteins as exemplified by clotting Factor VIII. Generally the time from collection through processing to freezing is rapid.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
Plasma for stable products	
Inter-region, Inter-sector	Plasma that has been collected where conditions for preservation of labile products was not achieved, however conditions were sufficiently moderate so relatively stable products like IgG and albumin would not be impacted.
Less than 6-hour plasma	
Inter-region, Inter-sector	This is generally recovered plasma that has been collected, processed, and frozen prior to 6 hours after collection. This plasma generally is considered to be acceptable for the production of labile products.
6- to 12-hour plasma	
Inter-region, Inter-sector	Generally this is recovered plasma that has been collected, processed, and frozen more than 6 hours and less than 12 hours after collection. This plasma is generally considered to be acceptable for the production of labile products but is inferior to less than 6-hour plasma for this purpose.
12- to 24-hour plasma	
Inter-region, Inter-sector	Generally this is recovered plasma that has been collected, processed, and frozen more than 12 hours and less than 24 hours after collection. This plasma may be acceptable for the production of labile products but is inferior to less than 6-hour plasma and 6- to 12-hour plasma for this purpose.
Less than 12-hour plasma	
Inter-region, Inter-sector	Generally this is recovered plasma that has been collected, processed, and frozen less than 12 hours after collection. This plasma may be acceptable for the production of labile products but is inferior to less than 6-hour plasma and 6- to 12-hour plasma for this purpose.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
More than 24-hour plasma	
Inter-region, Inter-sector	Generally this is recovered plasma that has been collected, processed, and frozen more than 24 hours after collection and usually less than 72 hours after collection. This plasma generally is not acceptable for the production of labile products.
Pooled plasma	
Inter-region, Inter-sector	Plasma that has been pooled from several donors. Some plasma pools for further manufacture of biotherapeutic products can be derived from several hundred to a few thousand donors.
Single-donor plasma	
Inter-region, Inter-sector	Plasma derived from a single donor. It can be a single unit or a pool of several units derived from multiple collections from the same donor.
Hyperimmune plasma	
Inter-region, Inter-sector	Plasma derived from donors with high titers to specific disease agents. Titers are elevated in these donors mostly as a result of immunization with a vaccine (e.g., hepatitis B, tetanus, or rabies) or exposure to disease agents (e.g., HCV or SARS). Hyperimmune plasma usually is intended for the preparation of IgG to provide passive immunity against target disease agents.
S/D plasma	
Inter-region, Inter-sector	Plasma that has been treated with solvent/detergent, an inactivation method effective against envelope virus disease agents (e.g., HIV, HBV, or HCV). Some plasma protein components are inactivated or damaged by the process (e.g., alpha-1 proteinase inhibitor, Protein S, anti-plasmin, or FVIII).

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
EU (PharmEuropa)	Human Plasma Pooled and Treated for Virus Inactivation is a frozen or freeze-dried, sterile, nonpyrogenic preparation obtained from human plasma derived from donors belonging to the same ABO blood group. The preparation is thawed or reconstituted before use to give a solution for infusion. The human plasma used complies with the monograph on Human Plasma for Fractionation.
Therapeutic exchange plasma (TEP)	
Inter-region, Inter-sector	Similar to Source Plasma in its collection. However, the donors are patients who are having their plasma replaced with electrolytes, protein solutions, or plasma from another donor. The objective usually is to remove disease elements from the patient's plasma. Generally, TEP is not advisable for further manufacture of biotherapeutic products. However, there may be cases where a specialty product may propose a specific TEP as a source material.
Quarantine plasma	
Inter-region, Inter-sector	Plasma that has been collected and stored as part of a controlled donor program. The donor is retested for disease agents (e.g., 6 months after collection). If the donor again is negative for the tested disease agents, then the plasma is released for use for further manufacture and/or use in humans.
Quarantine residual plasma	
Inter-region, Inter-sector	Plasma that has been collected and stored as part of a controlled donor program. The donor was not retested for disease agents (e.g., 6 months after collection). An example would be that the donor did not return to the collection facility to permit the later test. Quarantine Residual Plasma is not recommended for further manufacture and/or use in humans.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
Salvaged plasma	
Inter-region, Inter-sector	Plasma that has experienced a storage or transport temperature deviation but may still be useful for the preparation of nonlabile products such as albumin or IgG.
Plasma for fractionation	
Australia	The liquid part of human blood remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure. It is intended for the manufacture of plasma-derived products. When the plasma is intended for the recovery of proteins that are labile in plasma, it is cooled rapidly at –30° or below as soon as possible and at the latest within 24 hours of collection. When the plasma is intended for nonlabile proteins, the plasma should be frozen at –20° or below as soon as possible and at the latest within 72 hours of collection.

Appendix 1: Plasma Types and Specifications as Assigned by Regulatory Agencies in Selected Jurisdictions *(continued)*

Plasma Type and Agency or Agency Type	Specification
EU (PharmEuropa)	Human Plasma for Fractionation is the liquid part of human blood remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure. It is intended for the manufacture of plasma-derived products. When obtained by plasmapheresis or from whole blood (after separation from cellular elements), plasma intended for the recovery of proteins that are labile in plasma is frozen within 24 hours of collection by cooling rapidly in conditions validated to ensure that a temperature of –25° or below is attained at the core of each plasma unit within 12 hours after it is placed in the freezing apparatus. When obtained by plasmapheresis, plasma intended solely for the recovery of proteins that are not labile in plasma is frozen by cooling rapidly in a chamber at –20° or below as soon as possible and at the latest within 24 hours of collection. When obtained from whole blood, plasma intended solely for the recovery of proteins that are not labile in plasma is separated from cellular elements and is frozen in a chamber at –20° or below as soon as possible and at the latest within 72 hours of collection. Frozen plasma is stored and transported in conditions designed to maintain the temperature at or below –20°. For accidental reasons, the storage temperature may rise to above –20° on one or more occasions during storage and transport, but the plasma is nevertheless considered suitable for fractionation if all the following conditions are fulfilled: the total period of time during which the temperature exceeds –20° does not exceed 72 hours; the temperature does not exceed –15° on more than one occasion; the temperature at no time exceeds –5°.
Plasma frozen within 24 hours after phlebotomy	
CFR	Plasma manufactured from whole blood should be frozen within 24 hours after phlebotomy. Blood component must be labeled “Plasma Frozen Within 24 Hours after Phlebotomy.”

Appendix 2: Donor Criteria

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
General criteria for blood donation				
Appearance	Donor should appear to be in good health (AABB)	Donor should be in good health	Only donors in good health accepted	Nothing specific noted
Underlying medical conditions	Most serious medical conditions are grounds for deferral under United States, EU, and UK guidelines. FDA guidelines require deferral only if a person has used bovine insulin manufactured from UK cattle. However, AABB guidelines require deferral for cancer, heart, liver, or lung disease, and bleeding tendency unless approved by a medical director. Cancer and cardiac disease and diabetes treated with insulin require permanent deferral under EU and UK guidelines			Most serious medical conditions are grounds for deferral. Cancer: accepted 5 years of remission. Cardiac disease: varies depending on clinical condition. Diabetes: acceptable if controlled.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Age	≥16 or conform to applicable state law (AABB)	Between 18 and 65; donation at 17 permitted if in accord with national legislation; first-time donors >60 only if permitted by physician	Whole blood: between 17 and 65; no first-time donors. >60 Apheresis: first-time donors between 18 and 60; may donate up to age 65	Whole blood: can start at 16–17 with consent of parents and continue to 80, but medical review required at > 70. Apheresis: accept new donors 18–65, with medical evaluation required when >60. Existing donors require annual medical review if >65.
Weight	None stated: no more than 10.5 mL/kg may be withdrawn (AABB) FDA requires Source Plasma donors to weigh at least 110 lb	Whole blood: ≥50 kg Apheresis: no specific weight requirement	Whole blood: ≥50 kg Apheresis: ≥50 kg	<45 kg—defer. Medical opinion required for unexplained weight loss

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Blood pressure	Systolic ≤180 mm Hg Diastolic ≤100 mm Hg (AABB)	Systolic ≤180 mm Hg Diastolic ≤100 mm Hg	No specified blood pressure parameters; donors with high blood pressure may donate provided (1) they have not suffered any complications caused by high blood pressure, (2) they are taking only beta blockers and/or diuretics, and (3) their disease is stable.	Acceptable ranges: Systolic 90–180 mm Hg. Diastolic 60–180 mm Hg. Hypertension 180–100—defer. Hypotension 90–60—defer.
Pulse	Between 50 and 100 beats per minute and regular; lower pulses acceptable at discretion of physician (AABB)	Between 50 and 100 beats per minute and regular	No specific pulse rate parameters stated	Regular pulse between 50 and 100: accept Pulse between 40 and 49: accept if donor is physically fit and not on medication
Temperature	≤37.5° taken orally at time of donation (AABB)	Donors who have had a temperature of ≥38° may not donate for 2 weeks	Donors who have had a temperature of ≥38° or flu-like symptoms may not donate for 2 weeks	No requirement

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
HgB/HCT	≥125 g/L or Hct 38%	Males: ≥135 g/L Females: ≥125 g/L	Males: ≥135 g/L Females: ≥125 g/L	Whole blood females, 120–165 g/L; males, 130–185 g/L. Apheresis females, 115–165 g/L; males, 125–185 g/L. If high, defer. If low, defer for 6 months and test for ferritin.
Skin examination	Free of infectious skin disease at site of phlebotomy; no skin punctures or scars indicative of addiction to self-injected narcotics (CFR) Free of infectious diseases (AABB)	Skin at venipuncture site should be clear of lesions, including eczema	There should be no skin disease at venipuncture site	Avoid venesection where there is evidence of inflammation or infection

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Pregnancy	Defer for 6 weeks after delivery (AABB)	Defer 6 months after delivery	Defer for 1 week for every completed week of pregnancy	Current: defer 9 months from estimated date of confinement. After third-trimester delivery: defer 9 months. Miscarriage or termination: defer 3, 6, or 9 months, respectively for 1st, 2nd, and 3rd trimester.
Underlying medical conditions that require deferral and that do not pose a risk of transfusion-transmissible infection				
Cancer	Permanent deferral unless deemed suitable by medical director (AABB)	Permanent deferral although physician may make exceptions. Permitted after cervical cancer or basal cell carcinoma if successfully treated	Malignant neoplasms, including leukemias and myeloproliferative disorders, are cause for permanent deferral; exceptions may be made for certain conditions after successful therapy	Permanent deferral for haematological malignancies. Skin cancer–basal–basal cell carcinoma: accept. Other cancers: defer 5 years after completion of treatment

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Cardiac disease	Free of major organ disease (heart, liver, and lungs) unless deemed suitable by medical director (AABB)	Permanent deferral for persons with a history of heart disease, especially coronary disease, angina pectoris, severe cardiac arrhythmia, arterial thrombosis, or recurrent venous thrombosis. 2-year deferral for rheumatic heart disease with no evidence of chronic disease	Permanent deferral for persons with active or past serious cardiovascular disease, except congenital abnormalities with complete cure	Permanent deferral for arrhythmias, endocarditis, ischaemic heart disease, heart surgery, myocardial disease. Accept: congenital heart disease if surgically corrected. Heart murmurs: accept, subject to medical opinion. Accept after full recovery: pericardial disease, rheumatic heart disease.
Cerebrovascular diseases	No specific guideline other than that the donor must be free of major organ disease (AABB)	Permanent deferral for history of cerebrovascular diseases	Permanent deferral for donors with a history of serious CNS disease	Permanent deferral

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Epilepsy	No deferral	Must be free of epileptic attack for 3 years and have been taken off all medication	Permanent deferral unless at least 3 years have elapsed since the date that donor last took anticonvulsant medication and there has been no recurrence of symptoms	Defer for 2 years from last seizure
Gastrointestinal disease	No specific deferral; free of major organ disease unless deemed suitable by medical director (AABB)	No specific deferral	Deferral (not noted to be a permanent deferral) for disease that renders the individual liable to impaired iron absorption or blood loss	Ulcers: defer indefinitely

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Genitourinary and renal disease	No specific deferral. Free of major organ disease unless deemed suitable by medical director (AABB)	Five-year deferral after complete recovery from acute glomerulonephritis	Permanent deferral for donor with serious genitourinary or renal disease	Permanent deferral: chronic pyelonephritis, chronic kidney infection, chronic dialysis. Accept if resolved: haematuria, acute kidney infection. Urinary catheter present: plasma only for fractionation if underlying condition acceptable. Acute dialysis: defer for 12 months. Acute glomerulonephritis: defer 5 years after recovery.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Diabetes	No specific deferral except that receipt of bovine insulin manufactured in the UK requires permanent deferral (FDA). Free of major organ disease unless deemed suitable by medical director	Permanent deferral if insulin therapy required	Permanent deferral for donors on insulin treatment	Permanent deferral if diabetes-associated complications present Accept if disease is controlled, even if patient is taking insulin

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Respiratory disease	Free of acute respiratory disease (CPR). Free of major organ disease (lungs) unless deemed suitable by medical director (AABB)	Permanent deferral for chronic bronchitis; common cold acceptable	Permanent deferral for serious disease	Permanent deferral: chronic abscess, bronchiectasis, or emphysema with respiratory insufficiency. Accept if mild and controlled: asthma, bronchiectasis without respiratory insufficiency Plasma for fractionation only: chronic bronchitis. Acute bronchitis: defer 2 weeks after recovery and being off antibiotics for 5 days. Pleurisy, pneumonia: defer 4 weeks after recovery. Acute pulmonary embolism: defer 12 months after recovery

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Hematologic disorders	Free of abnormal bleeding tendency unless determined suitable by medical director (AABB) Free of major organ disease (cancer) unless deemed suitable by medical director (AABB)	No specific deferral. Donors who are heterozygous for beta thalassemia eligible if HGB values are within normal limits	Permanent deferral for donors with serious hematologic diseases	Permanent deferral: donors with serious hematologic diseases (sickle cell disease, thalassaemia major). Accept: thalassaemia minor. Defer: anaemia Plasma for fractionation only: elliptocytosis, glucose-6-phosphate dehydrogenase (G6PD) deficiency, spherocytosis. Apheresis only permitted for patients with G6PD deficiency

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Immunologic disorders	No specific deferral. Free of major organ disease unless deemed suitable by medical director (AABB)	Permanent deferral for donors with autoimmune disease that affects more than one organ. Defer: documented history of anaphylaxis	Permanent deferral for donors with serious immunologic diseases	Permanent deferral: autoimmune disorders. Accept: If asymptomatic, only one organ system involved, not on immunosuppressive therapy. Accept: Sjogren's syndrome
Metabolic disease	No specific deferral. Free of major organ disease unless deemed suitable by medical director (AABB)	No specific deferral	Permanent deferral for serious metabolic disease	No specific reference
Bone disease	No specific deferral. Free of major organ disease unless deemed suitable by medical director (AABB)	Two-year deferral after having been declared cured of osteomyelitis	No specific deferral	No specific reference

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Surgery	No specific deferral unless blood was transfused; in which case, a 12-month deferral applies (CFR)	Major surgery requires evaluation of risk for transfusion-transmissible disease	Permanent deferral for persons with history of resection of the stomach. Major surgery requires a 6-month deferral; minor surgery requires a 1-week deferral	Minor (e.g., skin lesions, arthroscopy): defer until recovered. Routine minor (e.g., appendectomy, laparoscopy): defer for 2 months. Major surgery (if donor received autologous blood only): defer 6 months. Neurosurgery; medical assessment required.
Medications that require deferral				

Appendix 2: Donor Criteria *(continued)*

	Region			
Criterion	**United States**	**United Kingdom**	**European Union**	**Australia**
Antibiotics	As defined by medical director (AABB)	Donors treated with any prescribed drug should be deferred for a period consistent with the pharmacokinetic properties of the drug	Defer for 2 weeks from full recovery or 1 week from cessation of antibiotic therapy, whichever is longer	Acute treatment: defer until recovered and off antibiotics for 5 days. Prophylactic: accept plasma only for fractionation. Topical: accept if skin is unbroken and not infected.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Drugs with teratogenic potential	Etretinate: permanent deferral (FDA). Acitretin: 3-year deferral from last dose (FDA). Dutasteride: 6-month deferral from last dose (FDA). Isotretinoin: 1-month deferral from last dose (FDA). Finasteride: 1-month deferral from last dose (FDA).	Donors treated with drugs with proven teratogenic effect should be deferred for a period consistent with the pharmacokinetic properties of the drug	Donors taking drugs that are proven or potential teratogens or who are taking drugs that accumulate in the tissues over long periods of time should not be used as blood donors	Raloxifene (Evista): defer for 6 months after completion of treatment. Acitretin (Neotigason): defer for 3 years after completion of treatment. Etretinate (Tigason): permanent deferral. Finasteride (Proscar): 7 days after completion of treatment. Isotretinoin: defer for 8 weeks after completion of treatment.
Growth hormone from human pituitary glands	Permanent deferral (FDA)			Permanent deferral

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Other drugs	No other deferrals by FDA or AABB. Any other deferral at discretion of medical director of blood center (AABB). Warfarin: 7-day deferral for plasma donation (AABB).	Recommended that a list of commonly used drugs with rules for acceptability of donors, approved by the medical staff of the transfusion center, be available.	Other drugs acceptable as long as the underlying condition for which the drug is taken is acceptable.	Most medications that are taken by donors are not harmful to recipients; therefore people taking medications can be acceptable as blood donors. Eligibility is based on the assessment of the underlying condition and specific medication guidelines.
Insulin	Permanent deferral: bovine insulin made in UK (FDA).	Permanent deferral if treated with insulin	Permanent deferral if treated with insulin	Defer if diabetes is poorly controlled
Immunizations				
Toxoids	No deferral	No deferral	No deferral	
Licensed killed bacterial vaccines	No deferral	No deferral		No deferral
Licensed inactivated viral vaccines	No deferral	No deferral	No deferral except 1-week deferral after hepatitis B vaccination	No deferral except 1-week deferral after hepatitis B vaccination

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Unlicensed killed vaccines	1-year deferral (AABB)			Defer 3 months after vaccination
Inactivated rickettsial vaccines	No deferral	No deferral		
Live attenuated bacterial and viral vaccines	4 weeks for varicella and rubella 2 weeks for rubeola, yellow fever, mumps, polio (oral), typhoid (oral) (AABB)	4 weeks	8 weeks	Plasma only for fractionation for 4 weeks after vaccination
Transfusion-transmissible infections that require deferral				

Appendix 2: Donor Criteria *(continued)*

	Region			
Criterion	**United States**	**United Kingdom**	**European Union**	**Australia**
HIV infection/AIDS and sexual partners	Permanent deferral if present or past clinical or laboratory evidence of HIV infection/AIDS: positive EIA with positive or indeterminate confirmatory test; positive NAT test; clinical signs include unexplained weight loss, night sweats, blue or purple spots in mouth or on skin, white spots or unusual sores in the mouth, swollen lymph nodes for more than 1 month, persistent cough or shortness of breath, persistent diarrhea, fever for more than 10 days; sexual partners deferred for 1 year from time of last contact (FDA)	Permanent deferral for donors found to have a confirmed positive marker for HIV. Donors found to have a repeat positive marker for HIV that cannot be confirmed should be informed according to the nationally agreed algorithm.	Donors with HIV I or II must be permanently deferred	Infection: permanent deferral. Relevant symptoms within the last 6 months: defer for 12 months. Sexual contact with HIV-positive partner: defer for 12 months after last sexual contact.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Hepatitis and sexual partners and household contacts	Permanent deferral for the following: History of viral hepatitis after the 11th birthday. Confirmed positive test for HBsAg. Positive test for anti-HBc on more than 1 occasion (testing is not required for Source Plasma donors). Present or past laboratory or clinical evidence of infection with HCV. Sexual partners of patients with hepatitis deferred for 1 year from last contact; household contacts of persons with hepatitis B deferred for 1 year from last contact	Permanent deferral for donors whose blood gives a positive reaction for the presence of HBsAg and/or anti-HCV. Donors with a history of jaundice or hepatitis may, at the discretion of the appropriate competent medical authority, be accepted as blood donors, provided that an approved test for HBsAg and anti-HCV is negative.	Permanent deferral for hepatitis B and C: donors with history of hepatitis B may donate after 12 months after recovery, provided that all markers are negative or core antibody positive, HBsAg is negative, and anti-HbS ≥100 IU/L; donors with documented current or past infection with hepatitis C are permanently deferred; donors with hepatitis A are deferred for 12 months.	Hepatitis B acute or past infection: defer for 12 months after recovery, then perform hepatitis testing. Hepatitis B chronic carrier: permanent deferral. Hepatitis B contact, sexual, mucosal, household: defer for 12 months from last exposure unless immune. Hepatitis B other contact: accept. Hepatitis C positive past infection: permanent deferral. Hepatitis C contact, sexual, mucosal, household: defer for 12 months from last exposure; other contact: accept.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
	(CFR).			
HTLV	Present or past clinical or laboratory evidence of infection with HTLV I/II (positive EIA on 2 occasions). [HTLV not tested in source plasma donors] (FDA)	Permanent deferral for carriers of HTLV I/II	Permanent deferral for donors with HTLV I/II	Infection: permanent deferral. Repeat reactive status: plasma only for fractionation. Sexual contact: defer for 12 months after last contact. Household contact: accept.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
West Nile virus	Donors with symptoms suspicious of or actual diagnosis of WNV deferred for 120 days. Donor testing positive on WNV NAT deferred for 120 days. Donor who develops symptoms of WNV within 2 weeks of donation should be deferred for 120 days. Donors implicated in possible transfusion-transmitted WNV infection should be deferred for 120 days (FDA).	Defer for 28 days after donor leaves an area with ongoing transmission to humans.	Defer for 6 months if donor was in area endemic for WNV and was diagnosed with or had symptoms consistent with WNV. Defer for 28 days after donor returns from endemic area, provided donor has no symptoms of WNV.	Infection: defer for 3 months after full recovery. Area exposure: plasma only for fractionation for 8 weeks after leaving risk area.

Appendix 2: Donor Criteria *(continued)*

	Region			
Criterion	**United States**	**United Kingdom**	**European Union**	**Australia**
Chagas disease and babesiosis	Permanent deferral for history of Chagas disease; current FDA regulations do not require testing for Chagas disease. However, although not required, most facilities collecting blood for transfusion perform EIA test for Chagas and permanently defer following positive test. (AABB) FDA probably will not require antibody screening for fractionated or recovered plasma used for further manufacture. FDA has granted exemptions and permitted the collection and distribution of source plasma for further	Permanent deferral for individuals with Chagas disease or history of Chagas disease; blood of persons who were born or have been transfused in areas where the disease is endemic should be used only for plasma fractionation products unless a validated test for infection is negative	Permanent deferral for individuals with Chagas disease. Individuals in the following categories may donate 6 months after leaving an endemic area, provided that a validated test for Chagas disease is negative (if a validated test is positive or not performed, the donor is permanently deferred): born in South or Central America, mother born in South or Central America, transfused in South or Central America; lived or worked in a rural subsistence community in South or Central America for 4 weeks or more	Infection: permanent deferral. Contact: accept. Chagas disease area resident: plasma only for fractionation, permanently. Chagas disease area visitor: plasma only for fractionation for 12 months after leaving endemic area

Appendix 2: Donor Criteria *(continued)*

	Region			
Criterion	**United States**	**United Kingdom**	**European Union**	**Australia**
	manufacture into noninjectable products from a donor known to have Chagas disease (CFR). Permanent deferral for history of babesiosis (AABB)			

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Creutzfeld-Jakob disease (CJD) and new variant CJD	Permanent deferral if donor: has diagnosis of CJD or nvCJD; has a blood relative diagnosed with CJD; received dura mater graft; received human-derived pituitary growth hormone; received bovine insulin made in UK; spent a cumulative 3 months in UK between 1980 and 1996; received a blood transfusion in the UK at any time since 1980; spent 6 months between 1980 and 1990 on a US military base in Northern Europe;	Permanent deferral if donor: treated with extracts derived from human pituitary glands; has been recipient of dura mater or corneal graft; has a family risk of CJD or any other TSE. For vCJD: Member states should determine on the basis of the prevalence of BSE within individual countries, of the endogenous exposure of the population to bovine products imported from countries with a high BSE prevalence, and of the incidence of cases of vCJD what precautionary measures they	Permanent deferral if donor: Diagnosed with CJD, vCJD, or any other prion-associated disease; has family risk of CJD; at increased risk from surgery, transfusion, or transplant of tissues or organs; received a dura mater graft; received a corneal, scleral, or ocular graft; received human-derived pituitary extract; received a blood transfusion in UK since 1980; received intravenous immunoglobulin (IVIg) of UK origin; donated unit of blood implicated in possible	Permanent deferral if diagnosed with any prion-related disease. Permanent deferral if donors have spent a cumulative time of 6 months in England, Wales, Scotland, Northern Ireland, the Channel Islands, or the Isle of Man between 1 January 1980 and 31 December 1996. Permanent deferral if donors received a blood transfusion or blood products in England, Wales, Scotland, Northern Ireland, the Channel Islands, or the Isle of Man from 1 January 1980 onwards unless the blood products were

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
	spent 6 months between 1980 and 1996 on a US military base elsewhere in Europe; spent a cumulative 5 years in Europe (Source Plasma donors are not deferred for the latter) (FDA)	may need to take to minimize the risk of transmission of vCJD via blood transfusion	case of transfusion-related vCJD. Additionally, all plasma from British donors cannot be used for fractionation	processed plasma products and were given after 31 December 2001. Permanent deferral if the donor had ear surgery performed between 1972 and 1989 and dura mater was used. Permanent deferral for donors who received human-derived pituitary growth and gonadotrophic fertility hormones prior to 1986.
Visceral leishmaniasis	Donors who have been to Iraq are deferred for 1 year. Permanent deferral for signs and symptoms of visceral leishmaniasis (FDA)	No specific deferral	Permanent deferral for visceral leishmaniasis	Cutaneous: plasma only for fractionation, permanently Visceral: permanent deferral Contact: accept.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Medical conditions and behaviors that place an individual at risk for a transfusion-transmissible infection and require deferral.				
Xenotransplant	No current deferral required for blood donation, but draft regulations for tissue and organs require permanent deferral.	Permanent deferral	Permanent deferral	Permanent deferral

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Blood transfusion organ and tissue transplant; treatment with plasma-derived clotting factor concentrates	One-year deferral (dura mater graft is permanent deferral); permanent deferral if patient received clotting factor concentrates and sexual partner deferred for 1 year after last contact	Six-month deferral: if NAT test for hepatitis C is negative, may donate after 4 months	Permanent deferral if donor ever received clotting factor concentrate or was transfused after 1 January 1980; 1 year for tissue or organ transplant	Blood transfusion homologous: defer 12 months Coagulation factor, blood derived, short term: defer 12 months from last treatment. Coagulation factor, blood derived, continuous: permanent deferral. Human tissue recipients: Organ/haematological: permanent deferral. Homologous, bone, tendon, skin: accept. Collagen: accept. Corneal: permanent deferral for iatrogenic cCJD risk.

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Surgery or use of endoscope with biopsy	No specific deferral criteria; general health and transfusion criteria apply	Six-month deferral for major surgery; stomach resection requires permanent deferral; 1-week deferral for minor surgery; 6-month deferral for endoscope with biopsy; if NAT test for hepatitis C is negative, may donate after 4 months	Six-month deferral for major surgery or procedure using an endoscope; 1-week deferral for minor surgery	Defer 6 months
Nonsterile skin penetration or mucous membrane exposure to blood or body fluids not the donor's own	Twelve-month deferral	Six-month deferral; if NAT test for hepatitis C is negative, may donate after 4 months	Twelve-month deferral	Defer 12 months
Acupuncture, tattoo, body piercing, etc.	Twelve-month deferral unless performed by a state-regulated entity, using sterile needles and disposable dyes	Six-month deferral; if NAT test for hepatitis C is negative, may donate after 4 months; exception can be made according to national risk assessment	Twelve-month deferral; 6-month deferral if validated test for hepatitis B core antibody is negative; for acupuncture, no deferral if performed by state-regulated entity	If using single-use items: plasma only for fractionation for 12 months If not single-use or unsure: defer for 12 months

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Injection of medications or steroids not prescribed by a physician	Permanent deferral; sexual partner deferred for 1 year	All blood donors should be provided with accurate and updated information about HIV transmission and AIDS so that persons who have unsafe sex practices or other risk behavior exposing them to potential infection will refrain from donating. The information provided may vary among countries according to local epidemiological data.	Permanent deferral if donor ever injected or has been injected with drugs; sexual partner deferred for 1 year	Permanent deferral
Males who have sexual contact with another male	Permanent deferral for sexual contact, even once, since 1977; female sexual partner deferred for 1 year from last contact		Permanent deferral for oral or anal sexual contact even if protection used; female sexual partner deferred for 1 year from last contact.	Defer for 12 months after last sexual contact
Accepted money or drugs or other payment in exchange for sex since 1977	Permanent deferral; sexual partner deferred for 1 year from last contact		Permanent deferral; sexual partner deferred for 1 year from last contact.	Defer for 12 months after last sexual contact

Appendix 2: Donor Criteria *(continued)*

Criterion	Region			
	United States	**United Kingdom**	**European Union**	**Australia**
Incarceration for more than 72 hours in the past year	One-year deferral		No deferral	Defer for 12 months after release
Born in or lived in Africa	Permanent deferral if born or lived in countries where HIV I subtype O is endemic (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger, Nigeria); sexual partner deferred for 1 year from last contact guidance unless tested with a test validated to detect Group O		No specific deferral for Africa; malaria rules apply; however, sexual partners of persons who were sexually active in areas where HIV is endemic deferred for 1 year from last contact	No specific deferral: donors who have visited a malaria-endemic area are subject to a plasma-only restriction period of at least 12 months. The restriction period is extended to 3 years if residence has been for 6 continuous months or more within the past 3 years. With a negative malaria test, the restriction period can be reduced to 4 months. Donors who have traveled to an HIV risk area must be asked if they had sexual contact with a resident of that area.

Appendix 3: Disease Testing

Disease Test	United States	European Union
Hepatitis		
HBsAg	Permanent deferral for confirmed positive test results (FDA)	*European Pharmacopoeia:* **Human Plasma for Fractionation**
Hepatitis B core antibody	Permanent deferral if reactive on 2 or more separate occasions; permanent deferral if core antibody positive results are coupled with prior or concurrent repeatedly reactive HBsAg test (FDA). Testing is not required for Source Plasma donors	Laboratory tests are carried out for each donation to detect the following viral markers: (1) antibodies against HIV-1 (anti-HIV-1). (2) antibodies against HIV-2 (anti-HIV-2). (3) hepatitis B surface antigen (HBsAg). (4) antibodies against hepatitis C virus (anti-HCV). If a repeat-reactive result is found in any of these tests, the donation is not accepted.
Hepatitis C antibody EIA	Permanent deferral if repeatedly reactive for hepatitis C antibody; may reenter donor after 6 months if confirmatory test is negative (FDA)	**Blood Directive 2002/98/EC (Annex IV): Basic Testing Requirements for Whole Blood and Plasma Donations**

Appendix 3: Disease Testing *(continued)*

Disease Test	United States	European Union
Hepatitis C NAT testing	Permanent deferral if positive on single testing (FDA)	The following tests must be performed for whole blood and apheresis donations, including autologous predeposit donations: ABO group (not required for plasma intended only for fractionation). Rh D group (not required for plasma intended only for fractionation). Testing for the following infections is required in donors: Hepatitis B (HBsAg), Hepatitis C (Anti-HCV), HIV 1 and 2 (Anti-HIV 1 and 2). Additional tests may be required for specific components or donors or epidemiological situations. **Blood Directive 2004/33/EC (Annex III): Permanent Deferral Criteria (excerpt):** Hepatitis B, except for HBsAg-negative persons who are demonstrated to be immune Hepatitis C HIV 1 or 2
HIV		

Appendix 3: Disease Testing *(continued)*

Disease Test	United States	European Union
HIV NAT	Permanent deferral if positive on single testing (FDA)	
HIV I or II Antibody (EIA)	Permanent deferral for repeatedly reactive HIV EIA test; may reenter after 6 months if confirmatory test negative (FDA). Testing is not required for Source Plasma donors	*European Pharmacopoeia:* **Human Plasma for Fractionation** Laboratory tests are carried out for each donation to detect the following viral markers: (1) antibodies against HIV-1 (anti-HIV-1) 2) antibodies against HIV-2 (anti-HIV-2) 3) hepatitis B surface antigen (HBsAg) 4) antibodies against hepatitis C virus (anti-HCV) If a repeat-reactive result is found in any of these tests, the donation is not accepted **Blood Directive 2002/98/EC (Annex IV): Basic Testing Requirements for Whole Blood and Plasma Donations** The following tests must be performed for whole blood and apheresis donations, including autologous predeposit donations: ABO group (not required for plasma intended only for fractionation) Rh D group (not required for plasma intended only for fractionation) Testing for the following infections are required in donors:

Appendix 3: Disease Testing *(continued)*

Disease Test	United States	European Union
		Hepatitis B (HBsAg) Hepatitis C (Anti-HCV) HIV 1 or 2 (Anti-HIV 1 and 2) Additional tests may be required for specific components or donors or epidemiological situations. **Blood Directive 2004/33/EC (Annex III), Permanent Deferral Criteria (excerpt)** Hepatitis B, except for HBsAg-negative persons who are demonstrated to be immune Hepatitis C HIV 1 or 2 ■1S (*USP33*)

BRIEFING

〈**1225**〉 **Validation of Compendial Procedures,** *USP 31* page 683 and page 794 of *PF* 34(3) [May–June 2008]. The General Chapters Expert Comittee proposes to include in this informational chapter, references to the validation of physical tests procedures. Other minor changes are also introduced.

(GC: H. Pappa) RTS—C70462; C68775

Change to read:

VALIDATION

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. Typical analytical performance characteristics that should be considered in the validation of the types of procedures described in this document are listed in *Table 1*. Because opinions may differ with respect to terminology and use, each of the performance characteristics is defined in the next section of this chapter, along with a delineation of a typical method or methods by which it may be measured.

■The definitions refer to ''test results.'' The description of the analytical procedure should define what the test results for the procedure are. As noted in ISO 5725-1 and 3534-1, a test result is ''the value of a characteristic obtained by carrying out a specified test method. The test method should specify that one or a number of individual measurements be made, and their average, or another appropriate function (such as the median or the standard deviation), be reported as the test result. It may also require standard corrections to be applied, such as correction of gas volumes to standard temperature and pressure. Thus, a test result can be a result calculated from several observed values. In the simple case, the test result is the observed value itself.'' A test result also can be, but need not be, the final, reportable value that would be compared to the acceptance criteria of a specification.■1S (*USP32*)

■Validation of physical property methods may involve the assessment of chemometric models. However, the typical analytical characteristics used in method validation can be applied to the methods derived from the use of the chemometric models.

The effects of processing conditions and potential for segregation of materials should be considered when obtaining a representative sample to be used for validation of procedures.■1S (*USP33*)

Table 1. Typical Analytical Characteristics Used in Method Validation

Accuracy
Precision
Specificity
Detection Limit
Quantitation Limit
Linearity
Range
Robustness

In the case of compendial procedures, revalidation may be necessary in the following cases: a submission to the USP of a revised analytical procedure; or the use of an established general procedure with a new product or raw material (see below in *Data Elements Required for Validation*).

The ICH documents give guidance on the necessity for revalidation in the following circumstances: changes in the synthesis of the drug substance; changes in the composition of the drug product; and changes in the analytical procedure.

■Chapter 〈1225〉 is intended to provide information that is appropriate to validate a wide range of compendial analytical procedures. The validation of compendial procedures may use some or all of the suggested typical analytical characteristics used in method validation as outlined in *Table 1* and categorized by type of analytical method in *Table 2*. For some compendial procedures the fundamental principles of validation may extend beyond characteristics suggested in chapter 〈1225〉. For these procedures the user is referred to the individual compendial chapter for those specific analytical validation characteristics and any specific validation requirements.■1S (*USP33*)

In-Process Revision

Analytical Performance Characteristics

ACCURACY

Definition—The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range.

■[NOTE—The definition of accuracy in ⟨1225⟩ and ICH Q2 corresponds to unbiasedness only. In the International Vocabulary of Metrology (VIM) and documents of the International Organization for Standardization (ISO), ''accuracy'' has a different meaning. In ISO, accuracy combines the concepts of unbiasedness (termed ''trueness'') and precision.]■1S (*USP32*)

Determination—In the case of the assay of a drug substance, accuracy may be determined by application of the analytical procedure to an analyte of known purity (e.g., a Reference Standard) or by comparison of the results of the procedure with those of a second, well-characterized procedure, the accuracy of which has been stated or defined.

In the case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure. If it is not possible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product (i.e., ''to spike'') or to compare results with those of a second, well-characterized procedure, the accuracy of which has been stated or defined.

In the case of quantitative analysis of impurities, accuracy should be assessed on samples (of drug substance or drug product) spiked with known amounts of impurities. Where it is not possible to obtain samples of certain impurities or degradation products, results should be compared with those obtained by an independent procedure. In the absence of other information, it may be necessary to calculate the amount of an impurity based on comparison of its response to that of the drug substance; the ratio of the responses of equal amounts of the impurity and the drug substance (relative response factor) should be used if known.

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals.

The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration).

Assessment of accuracy can be accomplished in a variety of ways, including evaluating the recovery of the analyte (percent recovery) across the range of the assay, or evaluating the linearity of the relationship between estimated and actual concentrations. The statistically preferred criterion is that the confidence interval for the slope be contained in an interval around 1.0, or alternatively, that the slope be close to 1.0. In either case, the interval or the definition of closeness should be specified in the validation protocol. The acceptance criterion will depend on the assay and its variability and on the product. Setting an acceptance criterion based on the lack of statistical significance of the test of the null hypothesis that the slope is 1.0 is not an acceptable approach.

■Accuracy of physical property methods may be assessed through the analysis of standard reference materials, or alternatively, the suitability of the above approaches may be considered on a case-by-case basis.■1S (*USP33*)

PRECISION

Definition—The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or of repeatability of the analytical procedure under normal operating conditions. In this context, reproducibility refers to the use of the analytical procedure in different laboratories, as in a collaborative study. Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment.

Determination—The precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (coefficient of variation). Assays in this context are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result.

The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration

■) ■1S (*USP33*)
or using a minimum of six determinations at 100% of the test concentration.~~)~~

■■1S (*USP33*)

SPECIFICITY

Definition—The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. [NOTE—Other reputable international authorities (IUPAC, AOAC-I) have preferred the term ''selectivity,'' reserving ''specificity'' for those procedures that are completely selective.] For the tests discussed below, the above definition has the following implications:

Identification Tests: ensure the identity of the analyte.

Purity Tests: ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte (e.g., related substances test, heavy metals limit, organic volatile impurities).

Assays: provide an exact result, which allows an accurate statement on the content or potency of the analyte in a sample.

Determination—In the case of qualitative analyses (identification tests), the ability to select between compounds of closely related structure that are likely to be present should be

demonstrated. This should be confirmed by obtaining positive results (perhaps by comparison to a known reference material) from samples containing the analyte, coupled with negative results from samples that do not contain the analyte and by confirming that a positive response is not obtained from materials structurally similar to or closely related to the analyte.

In the case of analytical procedures for impurities, specificity may be established by spiking the drug substance or product with appropriate levels of impurities and demonstrating that these impurities are determined with appropriate accuracy and precision.

In the case of the assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials.

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure (e.g., a Pharmacopeial or other validated procedure). These comparisons should include samples stored under relevant stress conditions (e.g., light, heat, humidity, acid/base hydrolysis,

■and■1S (*USP33*)
oxidation). In the case of the assay, the results should be compared; in the case of chromatographic impurity tests, the impurity profiles should be compared.

The ICH documents state that when chromatographic procedures are used, representative chromatograms should be presented to demonstrate the degree of selectivity, and peaks should be appropriately labeled. Peak purity tests (e.g., using diode array or mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.

■For validation of specificity for qualitative and quantitative determinations by spectroscopic methods, chapters related to topics such as near-infrared spectrophotometry, raman spectroscopy, and X-ray powder diffraction should be consulted.■1S (*USP33*)

DETECTION LIMIT

Definition—The detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit tests merely substantiate that the amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample.

Determination—For noninstrumental procedures, the detection limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

For instrumental procedures, the same approach may be used as for noninstrumental procedures. In the case of procedures submitted for consideration as official compendial procedures, it is almost never necessary to determine the actual detection limit. Rather, the detection limit is shown to be sufficiently low by the analysis of samples with known concentrations of analyte above and below the required detection level. For example, if it is required to detect an impurity at the level of 0.1%, it should be demonstrated that the procedure will reliably detect the impurity at that level.

In the case of instrumental analytical procedures that exhibit background noise, the ICH documents describe a common approach, which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can reliably be detected is established. Typically acceptable signal-to-noise ratios are 2 : 1 or 3 : 1. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses. Whatever method is used, the detection limit should be subsequently validated by the analysis of a suitable number of samples known to be near, or prepared at, the detection limit.

QUANTITATION LIMIT

Definition—The quantitation limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample.

Determination—For noninstrumental procedures, the quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision.

For instrumental procedures, the same approach may be used as for noninstrumental procedures. In the case of procedures submitted for consideration as official compendial procedures, it is almost never necessary to determine the actual quantitation limit. Rather, the quantitation limit is shown to be sufficiently low by the analysis of samples with known concentrations of analyte above and below the quantitation level. For example, if it is required that an analyte be assayed at the level of 0.1 mg per tablet, it should be demonstrated that the procedure will reliably quantitate the analyte at that level.

In the case of instrumental analytical procedures that exhibit background noise, the ICH documents describe a common approach, which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can reliably be quantified is established. A typically acceptable signal-to-noise ratio is 10 : 1. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses. Whatever approach is used, the quantitation limit should be subsequently validated by the analysis of a suitable number of samples known to be near, or prepared at, the quantitation limit.

LINEARITY AND RANGE

Definition of Linearity—The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Thus, in this section, ''linearity'' refers to the linearity of the relationship of concentration and assay measurement. In some cases, to attain linearity, the concentration and/or the measurement may be transformed. (Note that the weighting factors used in the regression analysis may change when a transformation is applied.) Possible transformations may include log, square root, or reciprocal, although other transformations are acceptable. If linearity is not attainable, a nonlinear model may be used. The goal is to have a model, whether linear or nonlinear, that describes closely the concentration-response relationship.

Definition of Range—The range of an analytical procedure is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity

using the procedure as written. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure.

Determination of Linearity and Range—Linearity should be established across the range of the analytical procedure. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares). Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, *y*-intercept, slope of the regression line, and residual sum of squares should be submitted.

The range of the procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.

ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used. It is also recommended that the following minimum specified ranges should be considered:

Assay of a Drug Substance (or a finished product): from 80% to 120% of the test concentration.

Determination of an Impurity: from 50% to 120% of the acceptance criterion.

For Content Uniformity: a minimum of 70% to 130% of the test concentration, unless a wider or more appropriate range based on the nature of the dosage form (e.g., metered-dose inhalers) is justified.

For Dissolution Testing: ±20% over the specified range (e.g., if the acceptance criteria for a controlled-release product cover a region from ~~20%~~

■30%,■1S (*USP33*)
after 1 hour, and up to 90%, after 24 hours, the validated range would be ~~0%~~

■10%■1S (*USP33*)
to 110% of the label claim).

■The traditional definition of linearity, i.e. the establishment of a linear or mathematical relationship between sample concentration and response is not applicable to particle size analysis. For particle size analysis, a concentration range is defined (instrument- and particle size-dependent) such that the measured particle-size distribution is not affected by changes in concentration within the defined concentration range. Concentrations below the defined concentration range may introduce an error due to poor signal-to-noise ratio, and concentrations exceeding the defined concentration range may introduce an error due to multiple scattering.■1S (*USP33*)

ROBUSTNESS

Definition—The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage. Robustness may be determined during development of the analytical procedure.

SYSTEM SUITABILITY

If measurements are susceptible to variations in analytical conditions, these should be suitably controlled, or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness and ruggedness should be that a series of system suitability parameters is established to ensure that the validity of the analytical procedure is maintained whenever used. Typical variations are the stability of analytical solutions, different equipment, and different analysts. In the case of liquid chromatography, typical variations are the pH of the mobile phase, the mobile phase composition, different lots or suppliers of columns, the temperature, and the flow rate. In the case of gas chromatography, typical variations are different lots or suppliers of columns, the temperature, and the flow rate.

System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being evaluated. They are especially important in the case of chromatographic procedures. Submissions to the USP should make note of the requirements under the *System Suitability* section in the general test chapter *Chromatography* ⟨621⟩.

Data Elements Required for Validation

Compendial test requirements vary from highly exacting analytical determinations to subjective evaluation of attributes. Considering this broad variety, it is only logical that different test procedures require different validation schemes. This chapter covers only the most common categories of tests for which validation data should be required. These categories are as follows:

Category I—Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

Category II—Analytical procedures for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These procedures include quantitative assays and limit tests.

Category III—Analytical procedures for determination of performance characteristics (e.g., dissolution, drug release,

■etc.■1S (*USP33*)
).

Category IV—Identification tests.

For each category, different analytical information is needed. Listed in *Table 2* are data elements that are normally required for each of these categories.

Table 2. Data Elements Required for Validation

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes

Table 2. Data Elements Required for Validation *(Continued)*

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

* May be required, depending on the nature of the specific test.

Already established general procedures (e.g., titrimetric determination of water, bacterial endotoxins) should be ▲verified to establish their suitability for use, such as▲*USP31* their accuracy (and absence of possible interference) when used for a new product or raw material.

■When validating physical property methods, consider the same performance characteristics required for any analytical procedure. Evaluate use of the performance characteristics on a case-by-case basis, with the goal of determining that the procedure is suitable for its intended use. The specific acceptance criteria for each validation parameter should be consistent with the intended use of the method.

Physical methods may also be classified into the four validation categories. For example, validation of a quantitative spectroscopic method may involve evaluation of *Category I* or *Category II Analytical Performance Characteristics*, depending on the method requirements. Qualitative physical property measurements, such as particle size, surface area, bulk and tapped density, which could impact performance characteristics, often best fit in *Category III*. *Category IV Analytical Performance Characteristics* usually applies to validation of qualitative identification spectroscopic methods. However, the various techniques may be used for different purposes, and the specific use of the method and characteristics of the material being analyzed should be considered when definitively applying a category to a particular type of method.■1S *(USP33)*

The validity of an analytical procedure can be verified only by laboratory studies. Therefore, documentation of the successful completion of such studies is a basic requirement for determining whether a procedure is suitable for its intended application(s). Current compendial procedures are also subject to regulations that require demonstration of suitability under actual conditions of use ▲(see *Verification of Compendial Procedures* ⟨1226⟩ for principles relative to the verification of compendial procedures).▲*USP31* Appropriate documentation should accompany any proposal for new or revised compendial analytical procedures.

BRIEFING

⟨**1251**⟩ **Weighing on an Analytical Balance,** *USP 31* page 712 and page 798 of *PF* 34(3) [May–June 2008]. On the basis of comments received, it is proposed to make additional changes to this general information chapter.

(GC: H. Pappa) RTS—C68766; C68773; C68911; C68912; C69216

Change to read:

~~Weighing is a frequent step in analytical procedures, and the balance is an essential piece of laboratory equipment in most analyses. In spite of this, weighing is a common source of error that can be difficult to detect in the final analytical results. The procedure described here applies directly to electronic balances; therefore, certain portions of the procedure are not applicable to other types of balance. The weighing procedure can be separated into three basic steps: planning, checking the balance, and weighing the material.~~

~~PLANNING~~

~~The initial step is to assemble the proper equipment, such as containers for weighing, receiving vessels, forceps, pipets, spatulas of proper size, and so forth. Use containers of size such that the loading capacity of the balance is not exceeded. Make sure that the containers selected to receive the weighed material are clean and dry. Assemble the necessary chemicals if solutions or reagents are required.~~

~~Preparation of the material to be weighed is often necessary. The material may require grinding or drying. Some materials may have been heated or stored in a refrigerator. Materials must be brought to the temperature of the balance before they are weighed. To avoid condensation of moisture, refrigerated materials must be allowed to come to room temperature before the container is opened.~~

~~CHECKING THE BALANCE~~

~~In the next step it is important to remember that, unless the balance is checked before each weighing operation is performed, errors can easily occur, resulting in faulty analytical da-~~

In-Process Revision

ta. The balance user should check the *Balance Environment*, *Calibration*, and *Balance Uncertainties*. Do not assume that the balance has been left in the proper operating condition by the previous user.

Balance Environment

The balance is placed in a suitable location with sufficiently low levels of vibration and air current. It must have a constant electrical supply. The balance and the surrounding work area have to be kept neat and tidy. It is good practice to use a camel's hair brush or its equivalent to dust the balance pan before any weighing so as to remove any materials that may have been left by the previous operator. [NOTE—Individuals must clean up debris, dispose of any spilled materials or paper, and remove the vessels and apparatus used in making the measurements.] When a balance is moved, it must be allowed to adjust to the temperature of its new environment and be recalibrated.

Calibration

If necessary, turn on the power, and allow the balance to equilibrate for at least 1 hour before proceeding with the calibration. (Microbalances may require up to 24 hours to reach equilibrium.) If the balance power has gone off and then has come back on, as in a power outage, certain types of balance may display a message indicating that the balance must be calibrated before a weighing is made. If the operator touches the balance bar, the message may be cleared and the balance may display zeros; however, the balance will not give the correct weighing until it has been calibrated. Electronic analytical balances have an internal calibration system based on an applied load. The calibration applies for the current ambient temperature.

Balance Uncertainties

DRIFT REDUCTION

Drift is one of the most common errors, and it is also one of the easiest to reduce or eliminate. Balance drift can be present without the operators being aware of the problem. Check the sample, the balance, and the laboratory environment for the following causes of errors, and eliminate them:

1. A balance door is open.
2. Temperatures of the balance and the material to be weighed are not the same.
3. The sample is losing or gaining weight.
4. The balance has been recently moved but has not been allowed to equilibrate to its surroundings or has not been recalibrated.
5. Air currents are present in the laboratory.
6. Temperatures in the laboratory vary.
7. The balance is not properly leveled.
8. Laboratory operations are causing vibration.
9. Hysteresis of the mechanical parts occurs during weighing.

MECHANICAL HYSTERESIS

Hysteresis in the balance is caused by excessive stretching of the springs, and it is primarily due to overloading or to the accidental dropping of an object onto the pan. Microbalances are very sensitive to overload and shock. When using a microbalance, set the lever to the rest position when adding or removing material; turn the lever to the weigh position to register the weight. In some cases, drift due to hysteresis can be eliminated by allowing the balance to stand without weighing long enough for it to recover. If stretching of the springs is excessive, an expensive balance overhaul may be needed. In the case of electronic force restoration balances, springs are replaced by flextures, and the term *creep* is more appropriate than *hysteresis*.

QUALITY ASSURANCE PROCEDURE FOR MEASUREMENT OF BALANCE DRIFT

Over an extended period of time, balance drift and other day-to-day variations are monitored by weighing a fixed check weight on a regular basis; this check should be performed after the balance has been calibrated at the ambient laboratory temperature. The check should be made before the first weighing of the day or after any event that might disturb the balance's calibration (power failure, moving the balance to a new location, etc.). The check weight may be any object whose mass remains constant and does not exceed the load limit of the balance. A balance weight makes a reliable check weight. Each balance should be provided with a check weight, which should be stored in a protective container near the balance.

Perform the following procedures to reduce balance errors and the possibility of an incorrect reading because of drift:

1. Make certain that the electrical power to the balance is on and that the level bubble is in the center of the indicator.
1. Calibrate the analytical balance or the microbalance. [NOTE—Some balances have a calibration lever, which must be returned fully to its original weighing position. Do not depend upon any prior calibration.]
3. The first person to use the balance each day should weigh the check weight and record the weight in the log book for comparison with previous readings. If a deviation greater than those indicated below for *Analytical Balances* and *Microbalances* is observed, the balance should be reported for service. [NOTE—Check weights tend to gain weight upon standing because of mishandling and exposure to contaminants in the atmosphere. These weights can be cleaned by wiping with a lint-free cloth moistened with a small amount of an appropriate solvent such as diethyl ether.]

Analytical Balances—Select a check weight of an appropriate mass to examine an analytical balance. If possible, set the balance to read to 5 decimal places. Follow the manufacturer's operating instructions. Pick up the check weight with a forceps, place it carefully on the balance pan, and weigh it. [NOTE—Do not drop the weight on the balance pan, because damage to the balance could result.] Place the weight in the center of the pan to eliminate corner weighing differences. The accuracy of the weight is not important; the only factor of interest is whether any drift has occurred. If no drift has taken place, the value should remain constant. Periodic weighing of a fixed weight will determine whether the boards (or knife edges in mechanical balances) in the instrument are defective. The check for drift at the most sensitive position will show whether a problem exists; the variation in the observed weight does not exceed ±0.2 mg. For example, with a 20-g weight, if the mean value of the readings were 19.9984, the tolerance would be from 19.9982 to 19.9986 g. Thus, several readings must be taken before one can establish a tolerance. [NOTE—The check weight need not be of high accuracy, but it is essential that its mass remain constant. In addition, the tolerance does not correspond to the value of 0.1%, specified under *Weights and Balances* ⟨41⟩, for weighing material accurately. Rather, the tolerance is purposefully tight to reveal possible drift or calibration errors; this tolerance is readily achievable with modern electronic balances.]

Microbalances—Proceed as directed for *Analytical Balances*, but use a check weight appropriate for the particular balance. For example, a 100-mg check weight might be selected for a balance that has a load limit of 150 mg; or a 10-mg check weight might be used for an ultramicrobalance with a load limit of 15 mg. (The operator must know the maximum capacity of the balance to select the correct check weight.) The balance in-

dicates the weight in milligrams. Record the weight as soon as the reading is stable for a few seconds. The variation in weighings ought to be within a range commensurate with the specifications given by the balance manufacturer, but not greater than 0.1% of the amount of material typically weighed on the particular balance. For example, if 10-mg samples are routinely weighed, the variation in the weighings of the check-weight cannot exceed 0.01 mg.

WEIGHING THE MATERIAL

In this final step, select the number of decimal places required for the analytical procedure. In most pharmaceutical analyses small quantities of material are used, requiring the balance reading to be set to the fifth decimal place to achieve the necessary accuracy. Weighing read with four decimal places is preferred for weighing near-gram quantities. Do not allow the material to remain on the balance for an extended period of time because changes, caused by interaction with atmospheric water or carbon dioxide, may take place.

Load Limit

Select the appropriate balance for the quantity and accuracy needed. Each balance has a load limit, which should not be exceeded. Each balance manufacturer supplies the maximum loading condition, and this limit varies with the type of balance. The operator should know this limit so that the balance will not be damaged. [NOTE—Electronic balances operate on a ''load cell'' principle that produces an electrical output proportional to the movement of the strain gauge and is linear over the range.]

Receivers

The proper receiver for the material must be selected. The receiver's weight plus the weight to be measured must not exceed the maximum load for the balance; the size and shape of the receiver should permit it to fit into the space and on the balance pan without interfering with any operation. It is important that the receiver be clean and dry. Common receivers are weighing bottles, weighing funnels, flasks, and weighing paper. The correct receiver depends upon the quantity and type of material (liquid, solid, or powder) to be weighed. All other things being equal, a vessel of low mass should be chosen when small amounts of material are to be weighed. It is recommended that gloves, forceps, or another type of gripping device be used when handling receivers, because oils from the hands will add weight.

The weighing funnel is often the most satisfactory receiver, because it can function as both a weighing dish and a transfer funnel, allowing easy transfer to volumetric flasks. Weighing funnels come in various sizes; the size suitable for the operation should be selected.

Weighing paper may be used for solids. Paper receivers must be handled by hand, and great care must be used to prevent spills.

Weighing by Difference

Weighing is usually done by difference. The following methods are acceptable for good analytical results.

METHOD 1

Tare the empty receiver as follows. Place the receiver on the balance in the center of the pan, and press the appropriate tare key on the balance. This operation electrically sets the signal from the strain gauge to zero so that the weight of the receiver is no longer indicated. Add the material to the receiver, and record the weight. Transfer the weighed material to the final flask or receiver; then reweigh the original weighing receiver by placing it in the same position on the pan. [NOTE—Do not change the set tare of the balance between these two weighings.] The second weight represents the untransferred material and is subtracted from the total material weight to determine the weight of the transferred material.

METHOD 2

If the empty receiver is not going to be tared, add the material to the receiver, and place the receiver on the balance in the center of the pan. Record the weight, and transfer the weighed material to the final flask or receiver; then reweigh the original weighing receiver by returning it to the same position on the pan. The second weight represents the sum of the weights of the receiver and the untransferred material; subtract this sum from the sum of the total material weight and the receiver weight to determine the weight of the transferred material.

METHOD 3

This method may be described as quantitative transfer. The material is added to the tared receiver, the amount is determined by difference, and then the whole amount is transferred quantitatively (e.g., by using a solvent) to the final receiver.

Materials-Handling Safety Procedures

The operator must be familiar with precautions described in the Material Safety Data Sheet for the substance before weighing it. Hazardous materials must be handled in an enclosure having appropriate air filtration. Many substances are extremely toxic, are possibly allergenic, and may be liquids or finely divided particles. A mask that covers the nose and mouth should be used to prevent any inhalation of chemical dust. Gloves should be used to prevent any contact with the skin. [NOTE—The use of gloves is good practice for handling any chemical. If it is necessary to handle the container being weighed, the operator should put on gloves, not only for self-protection but also to prevent moisture and oils from being deposited on the weighed container.] During a weighing, the operator may be exposed to high concentrations of the pure substance; therefore, the operator must carefully consider these possibilities at all times.

Weighings are made on many different types of materials, such as large solids, finely divided powders, and liquids (viscous or nonviscous, volatile or nonvolatile). Each type of material requires its own special handling.

Weighing Solids

Solids come in two forms: large chunks, with or without powdery surface, and finely divided powders or small crystals. If large chunks with a powdery surface are to be weighed, at least a piece of weighing paper must be placed on the balance pan to protect it from damage. Large nonreactive chunks that have no powdery surface may be placed directly on the pan (for example, a coated tablet). [NOTE—Solid pieces must be handled with forceps, never by hand.]

STATIC CHARGE

Fine powders have a tendency to pick up static charge, which will cause the particles to fly around. This static charge must be eliminated before a suitable weighing can be made. An antistatic device may be used to minimize this problem. [NOTE—Such devices may use piezoelectric components or a very small amount of a radioactive element (typically polonium) to generate a stream of ions that dissipate the static charge when passed

~~over the powder to be weighed.] The static charge depends upon the relative humidity of the laboratory, which in turn depends upon the atmospheric conditions. In certain conditions, static charge is caused by the type of clothing worn by the operator; this charge causes large errors in the weighing when discharged.~~

~~WEIGHING PROCEDURE~~

~~Place the receiver on the balance pan, close the balance door, and weigh as indicated for *Weighing by Difference*, with the following additions. Carefully add the powdered material from a spatula until the desired amount is added. Use care to avoid spilling. Close the balance door, and record the weight as soon as the balance shows a stable reading.~~

~~SPILLS~~

~~If solids are spilled, remove the receiver, and sweep out all of the spilled material from the balance. The spilled material must be properly disposed of and must not be swept out onto the balance table where other operators may come in contact with the chemical. Then either start the process over or reweigh the remaining material. [NOTE—Never return any excess material to the original container. Any excess material must be disposed of in a proper manner.]~~

~~Weighing Liquids~~

~~Liquids may be volatile or nonvolatile and viscous or nonviscous. Each type requires special attention.~~

~~WEIGHING PROCEDURE~~

~~Weigh as directed for *Weighing by Difference*, with the following additions. Liquids should always be weighed into a container that can be closed so that none of the material is lost. It is best if the liquid can be added to its receiving container outside the balance because of the possibility of a spill. [NOTE—Liquids spilled within the balance housing can cause serious damage to the balance, and they may be difficult to remove.]~~

~~Nonviscous liquids can be handled with a Pasteur capillary pipet equipped with a small rubber bulb such as a medicine dropper bulb. The liquid is discharged into its receiver, the top is closed or stoppered, and the receiver and contents are weighed. Small quantities of viscous liquids can be handled by touching a glass stirring rod to the surface of the liquid and then carefully touching the rod to the side of the receiving vessel, which allows some of the material to be transferred.~~

~~Weighing Corrosive Materials~~

~~Many chemicals, such as salts, are corrosive, and materials of this nature should not be spilled on the balance pan or inside the balance housing. Extreme care is essential when materials of this nature are being weighed.~~

~~CONCLUSION~~

~~By carefully following the procedures outlined above, laboratory personnel will eliminate many errors that might be introduced into weighing procedures. However, it is important for each balance to be serviced and calibrated regularly by a specially trained internal or external service person. The balance should be tested using weights traceable to standardization by the National Institute of Standards and Technology. No repairs should be made to any balance by anyone other than a qualified maintenance person.~~

▪INTRODUCTION

Weighing is a frequent step in analytical procedures, and the balance is an essential piece of laboratory equipment in most analyses. In spite of this, weighing is a common source of error that can be difficult to detect in the final analytical results. The general information described here applies directly to electronic balances used in analytical procedures, and certain portions of the chapter are not applicable to other types of balances. This chapter should not be considered all-inclusive, and there are other sources of information that may be useful and applicable (e.g., NIST, FDA, and balance manufacturers)[1] when performing a weighing operation or implementing a weighing procedure. The recommendations in this chapter are not intended to meet all the standards stated in *Weights and Balances* ⟨41⟩ but instead to provide for recommendations for balances used in all analytical procedures.

QUALIFICATION

Users are advised to consult *Analytical Instrument Qualification* ⟨1058⟩, standard operating procedures, and recommendations from manufacturers in devising qualification plans.

Installation

A balance's performance is dependent on the conditions of the facility where it is installed. Information provided by the manufacturer should be consulted prior to installing a balance.

[1] Also see: Ted Scorer, Michael Perkin, and Mike Buckley, ''Good Practice Guide No. 70, Weighing in the Pharmaceutical Industry,'' National Physical Laboratory, Teddington, Middlesex, United Kingdom, for additional details. Nomenclature in this chapter tends to follow this document, except where in conflict with USP terms.

SUPPORT SURFACE

The balance should be ~~mounted~~ installed on a solid, level, nonmagnetic surface that minimizes the transmission of vibration (e.g., a floor-mounted, ~~polished~~ granite weigh bench). If a metallic support surface is used, the surface should be grounded in order to prevent the buildup of static electricity.

LOCATION

The balance should be located in a room that is temperature and humidity controlled. The location should have a clean, consistent electrical power supply. The location should be free of drafts and should not be near ovens, furnaces, air conditioner ducts, or cooling fans from equipment or computers. The balance should be positioned away from outside windows so that direct sunlight does not strike the balance. The balance should not be installed near sources of electromagnetic radiation such as radio frequency generators, electric motors, hand-held communication devices (including cordless telephones, cellular telephones, and walkie-talkies). The balance should not be located near magnetic fields induced by laboratory instrumentation or other equipment.

In some situations, it may not be possible to position the balance in an optimum environment. Examples of potential facility issues include the following:

1. Air currents are sometimes present in the laboratory.
2. Temperatures in the laboratory vary excessively (check manufacturer's literature on temperature sensitivity).
3. Humidity is either very low or very high. Either condition may increase the rate at which the sample weight varies due to pickup or loss of water. Low humidity increases buildup of static electricity.
4. Adjacent facility operations are causing vibration.
5. Corrosive materials are used nearby or are routinely weighed.
6. The balance is located within a fume hood because it is used to weigh corrosive or hazardous materials.
7. The balance is adjacent to equipment producing a magnetic field (e.g., a magnetic stirrer).

In these cases, the performance of the balance should be assessed following installation and prior to use in order to demonstrate adequate performance. In situations where the balance is located near equipment or systems that induce vibration, drafts, electromagnetic radiation, magnetic fields, or changes in temperature or humidity, the assessment should be conducted with those systems operating in order to duplicate a worst-case scenario.

Qualification and Routine Checks

Calibration is defined as a set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards.

~~If necessary, turn~~ Turn on the power and allow the balance to equilibrate according to the manufacturer's instructions (1 to 24 hours, depending on type of balance) prior to ~~performing calibrations~~ use.

Several types of electronic analytical balances use internal weights for automatic calibration adjustment. This adjustment can also be applied for changes in the ambient temperature and is intended to reduce the effect of the drift of the balance over time. If the balance system does not provide any clear indication regarding the state of calibration, the calibrated state can only be verified by use of external weights with known masses.

Calibration is normally performed prior to any other qualification measurements and periodically prior to use of the balance. In *Weights and Balances* ⟨41⟩, a check of the state of calibration is typically performed each day the balance is used or at appropriate intervals based on applicable standard operating procedures. ~~and the measured weight is within 0.1% of the certified value.~~

Operational Qualification

Operational qualification is often performed by the manufacturer of the analytical balance but may also include specific requirements not tested by the manufacturer. Depending on the balance model, these tests may include testing of the following:

- tare facility
- maximum capacity of the balance
- autocalibration feature
- operation of ancillary equipment, e.g., printer

Performance Qualification

Table 1 provides examples for accomplishing performance qualification requirements. The examples in the table are not all-inclusive. Any procedures used should be consistent with in-house standard operating procedures, applicable for the specific balance, and adequately justified. If there is evidence that linearity, hysteresis, and eccentricity deviations are negligible in the operating range of the balance, these tests may be skipped.Performance qualification would be performed before placing the balance into routine use and then periodically as described in standard operating procedures.

Table 1. Suggested Performance Qualification Tests

Test	Example	Considerations for Criteria
Linearity	From 3 to 10 points over the range of the balance.	No more than 0.1% deviation where *Weight and Balances* ⟨41⟩ is applicable; for other uses consider how the balance is used, but errors should not exceed 0.1% of load.
Repeatability	10 replicates (test weight a few percent of the maximum capacity of the balance).	Manufacturer's specification; *Weights and Balances* ⟨41⟩ requirements, as applicable.
Hysteresis	Three measurements covering the range of the balance, e.g., 1) 0% of load 2) 50% of load 3) 100% of load	Consider how the balance is used, but errors should not exceed 0.1% of load.
Eccentricity	Eccentricity test should be performed at four ~~corners~~ quadrants of the balance pan at approximately 50% load.	Consider how the balance is used, but errors should not exceed 0.1% of load.

Quality Control Checks (Weight Check)

Analytical balances vary greatly in the features they offer to ensure that the balance is maintained in a calibrated state. A weight check using an internal or external check weight ensures that the balance is suitable to use. The weight check is typically performed each day the balance is used or at appropriate intervals based on applicable standard operating procedures. ~~and the measured weight~~ The test is passed if the weighing value indicated by the balance is within 0.1% of ~~the certified value~~its nominal value. Before weighing, the weight should be placed ~~by the balance~~ in the vicinity of the balance for at least thirty minutes to reach thermal equilibrium prior to testing. *Table 2* provides suggested information on balance checks using check-weights.

Routine weight checks using external weights should be recorded in a manner where the data can be used to easily track balance performance in order to allow for setting of meaningful action and failure limits (such as control charts) and to assist in laboratory investigations as needed.

The check weights that are used should be stored and handled in such a manner as to minimize contamination. Procedures should be in place to address check-weight results that are observed to be outside acceptable ranges and to provide assurance that the balance cleanliness and environment have not affected the result. Also, a procedure for removing a balance from service due to observed results outside acceptable ranges should be in place.

Table 2. Suggested Balance Checks

Topic	Example	Criteria
Type of material	Stable but not necessarily a NIST-traceable external weight.	Possible need for calibration certificate or weight history.
Mass of weight	Large enough to minimize variation from handling, e.g., 200 mg for a 4- or 5-place balance, near the upper limit for microbalances (6–7 place).	Below the maximum weight for the balance and above the minimum weight for the balance (if established).
Frequency of use	Time-of-use, daily, or other periodic check.	A justified fixed interval needs to be used.
Measurement result	Statistical process control, ~~and~~ control charts with limits based on measured performance, and log books	Justified action limit (e.g., no more than 0.1% deviation from check weight) based on control charts with the failure limit based on balance application.

In-Process Revision

Minimum Weight

In addition, *Weights and Balances* ⟨41⟩ gives specific requirements for balances used in pharmacopeial procedures with a reportable value expressed in at least three significant figures[2] (see §7.1 *Interpretation of Requirements* under §7 *Test Results* in the *General Notices*), when substances are to be ''accurately weighed''. The information used for repeatability requirements in ⟨41⟩ may also be used to calculate a minimum weight from the formula:

$$(k / U_{rel})s$$

in which k is the coverage factor ($\geq$2); U_{rel} represents the required maximum relative uncertainty ~~factor of~~ 0.001; and s is the standard deviation~~, in mg,~~ (in a mass unit e.g., in mg), of not less than 10 replicate measurements of a mass ~~near the low end of the operating range~~ of a few percent of the capacity of the balance or less. The relative uncertainty ~~factor~~ of 0.001 is for a reportable value expressed in at least three significant figures. ~~and corresponding uncertainty factors~~ Corresponding relative uncertainties of 0.01 and 0.1 could be used for reportable values with two and one significant figures; respectively. An example of minimum weights are shown in the *Table 3*.

An amount of sample (i.e., net weight), equal to or larger than the minimum weight, satisfies the required weighing uncertainty. The minimum weight applies to the sample weight, not to the tare or gross weight.

~~**Table 3.**~~

~~Reportable limit (number of significant figures)~~	~~s = 0.002~~	~~s = 0.02~~	~~s = 0.2~~
~~3~~	~~4~~	~~40~~	~~400~~
~~2~~	~~0.4~~	~~4~~	~~40~~
~~1~~	~~0.04~~	~~0.4~~	~~4~~

Table 3.

Reportable Limit (number of significant figures	Relative Uncertainty	Relative Uncertainty (%)	Standard Deviation (mg) s = 0.002	s = 0.02	s = 0.2
			Minimum Sample Weight (mg)		
3	0.001	0.1%	4	40	400
2	0.01	1%	0.4	4	40
1	0.1	10%	0.04	0.4	4

[2] Zeros within a number are always significant. Zeros that do nothing but set the decimal point are not significant. Trailing zeros that aren't needed to hold the decimal point are significant.
Examples of where this requirement would apply are (1) procedures with specification limits: 90.0% to 110.0%, 98.0% to 102.0%; (2) procedures where the result is reported in mg: mg limits for 5-mg tablet where the specification is 90.0% to 110.0% are 4.50 mg to 5.50 mg; (3) limits expressed with three significant figures: not more than 1.50%, not more than 0.00100%.
Examples of where this requirement would not necessarily apply are procedures with specification limits: 90% to 110%; and limits expressed with less than three significant figures: not more than 1.5%, not more than 0.0010%, not more than 150 ppm.

OPERATION OF THE ANALYTICAL BALANCE

Select the appropriate balance for the quantity and accuracy needed. *Weights and Balances* ⟨41⟩ provides requirements when the reportable value from a Pharmacopeial analysis is expressed on at least three significant figures.

The balance user should check the balance environment (vibration, air currents, cleanliness) and status of calibration.

Receivers

To ensure suitable accuracy in measuring the weight of a specimen, consideration should be given to selecting a proper receiver for the material.

GENERAL CHARACTERISTIC

All receivers must be clean, dry, and inert. The total weight of the receiver plus the specimen must not exceed the weight capacity of the balance. With a properly maintained and adjusted laboratory balance, weighing uncertainty is essentially determined by the repeatability for ''small samples'', i.e., net weights with a mass not exceeding typically a few percent of the capacity of the balance. However, repeatability is dependent on the gross load, and even more on the size and surface area of the weighed object; for that reason large or heavy receivers introduce a deviation from the conditions where the repeatability was determined (see *Weights and Balances* ⟨41⟩). Therefore, receivers of a low mass and small surface are the most desirable, especially in cases where specimens of low weight are being measured. Receivers should be constructed from nonmagnetic materials in order to prevent magnetic interference of electronic balance components. Receivers should be at ambient temperature in order to prevent the formation of air currents within the balance cell.

SOLID SAMPLES

Receivers for weighing solid materials include weighing paper, weighing dishes, weighing funnels, or enclosed vessels, including bottles, vials, and flasks. Papers that are hygroscopic in nature are not recommended for weighing because they may have a detrimental effect on the observed results.

Weighing dishes are typically constructed from a polymer or from aluminum. Specialty ''anti-static'' weighing dishes are available for measuring the weights of materials that retain static electricity.

Weighing funnels are typically constructed from glass or from a polymer. The design of this type of receiver combines attributes of a weighing dish and a transfer funnel, which can simplify the analytical transfer of a weighed powder to a narrow-necked vessel, such as a volumetric flask.

For solid samples that are volatile or deliquescent, it is necessary to weigh the material into an enclosed vessel. Where practical, an enclosed vessel with a small opening should be used in order to reduce sample weight loss from volatilization or weight gain from the adsorption and absorption of water from the atmosphere.

LIQUID SAMPLES

Receivers for liquid samples will typically be inert, enclosed vessels. For liquid samples that are volatile or deliquescent, an enclosed vessel with a small opening should be used, and the enclosure should be replaced rapidly following the transfer of material. Special precautions should be taken to be certain that the receiver and the enclosure are constructed from a material that is compatible with the liquid sample. The receiver and enclosure must have a seal sufficient to prevent leaks from a liquid that is of low viscosity or has low surface tension or a low boiling point.

Types of Weighing

QUANTITATIVE ANALYSIS

The initial step for many quantitative analyses is to accurately weigh a specified amount of a sample. Errors introduced during the weighing of a sample will affect the accuracy of all subsequent analytical measurements.

ADDITION WEIGHING

Addition weighings are typically used for the weighing of solid samples. The receiver is placed on the balance. After the balance display stabilizes, tare the balance. Add the desired amount of material to the receiver, and allow the balance display to stabilize. Record the weight, and quantitatively transfer the material to an appropriate vessel.

DISPENSE WEIGHING

Dispense weights are typically used for the weighing of emulsions or viscous liquids such as a topical ointment. In these situations, it is not practical to weigh the material into a typical receiver. Tare the balance. Fill a small transfer pipet or a syringe with an amount of sample greater than the desired specimen weight. Wipe the outside of the pipet or syringe clean and place on the balance. After the balance display stabilizes, record the weight. Transfer the desired amount of sample to an appropriate receiving vessel, such as a volumetric flask. Place the pipet or syringe back onto the balance. The difference in the two weighings is equal to the weight of the transferred specimen.

Alternatively, the sample can be weighed directly into a tared vessel such as a volumetric flask, provided that the weight of the vessel is sufficiently small to allow an accurate tare and that the total weight of the vessel and the sample specimen do not exceed the accurate range of the balance.

Problem Samples

ELECTRICALLY CHARGED SAMPLESAND RECEIVERS

Dry, finely divided powders may be charged with static electricity. The static charge will make the powder either attracted to or repelled by the receiver or the balance. This can cause inaccurate weight measurements and specimen loss during transfer. A ~~rapid change~~ drift in the balance readings should alert the operator to the possibility that the material has a static charge. Commercially available balances with a built-in anti-static device can be used to remedy the problem. Such devices may use piezoelectric components or a very small amount of a radioactive element (typically polonium) to generate a stream of ions that dissipate the static charge when passed over the powder to be weighed. Anti-static weigh boats, anti-static guns, and anti-static screens are also commercially available. The static charge depends also on the relative humidity of the laboratory, which in turn depends on atmospheric conditions. In certain conditions, static charge is caused by the type of clothing worn by the operator; this charge causes large errors in the weighing. ~~Plastic containers will have a greater tendency~~ Borosilicate glassware and plastic receivers are notorious for picking up a static charge, especially at low relative humidity. The gloves used to protect the operator may also increase the potential for a static charge problem. Placing the ~~plastic~~ container in a metal holder may help to ~~dissipate~~shield the static charge; special anti-static gloves also may help to alleviate the problem.

VOLATILE SAMPLES

When weighing a liquid that has a low boiling point, the specimen must be received in a vessel with a gas-tight enclosure of small diameter. Tare the vessel and enclosure. Add the desired amount of sample, and replace the enclosure. After the balance display stabilizes, record the specimen weight.

WARM OR COOL SAMPLES

Samples that are warm or cool should be equilibrated in the laboratory, or the weight readings may be erroneous. With regard to warm samples, the apparent weight will be ~~higher~~ smaller than the true weight because of heat convection. For example, a flask warmer than its ambient air warms up this air, which then flows upwards along the flask and reduces its apparent weight by viscous friction.

HYGROSCOPIC SAMPLES

Hygroscopic materials readily absorb moisture from the atmosphere and will steadily gain weight if left exposed. Therefore, hygroscopic samples must be either weighed promptly or placed in a vessel with a gas-tight enclosure. For a gas-tight vessel, tare the vessel and enclosure. Add the desired amount of sample and replace the enclosure. After the balance display stabilizes, record the specimen weight.

ASEPTIC OR BIOHAZARDOUS SAMPLES

The weighing of sterile or biohazardous samples will take place within the confines of a ~~laminar flow hood~~ clean bench, biosafety cabinet, isolator or similar containment ~~cabinet.~~ device. The flow of air within the hood will potentially cause balance instability. It is recommended that, upon installation of the balance in the hood, a rigorous qualification study be performed with suitable weight artifacts (see *Weights and Balances* ⟨41⟩) in order to determine the acceptability of the balance performance in this environment.

WEIGHING CORROSIVE MATERIALS

Many chemicals, such as salts, are corrosive, and materials of this nature should not be spilled on the balance pan or inside the balance housing. Extreme care is essential when materials of this nature are being weighed. The use of sealed containers such as weighing bottles or syringes should be considered. In the event of a spill, requalification of balance may be necessary, depending on the nature of the spill.

SAFETY CONSIDERATIONS WHEN WEIGHING

During a weighing, the operator may be exposed to high concentrations of the pure substance. The operator must carefully consider this possibility at all times and should be familiar with the precautions described in the Material Safety Data Sheet for a substance before weighing it. Hazardous materials should be handled in an enclosure having appropriate air filtration. Many extremely toxic—and possibly allergenic—substances present as liquids or finely divided particles. When weighing these substances, a mask that covers the nose and mouth should be used to prevent any inhalation of the substance, and gloves should be used to prevent any contact with the skin. [NOTE—The use of gloves is good practice for handling any chemical. If it is necessary to handle the container being weighed, the operator should put on gloves, not only for self-protection but also to prevent moisture and oils from being deposited on the weighed container.]

BUOYANCY CORRECTION

Buoyancy corrections are not generally needed for typical pharmaceutical applications; however, this information is provided as a guide to users that may have a need to apply abuoyancy correction to their weighing operation. In general, buoyancy corrections are applied to mass measurements by calculating the difference in volume between the material being weighed and the weight standard, multiplying the volume difference by the density of air (which is dependent on temperature, relative humidity, and atmospheric pressure) at the balance or scale, and adding the product to the mass of the standard. The correction also depends on the type of balance and method of calibration.[3]

For powders, the buoyancy correction strictly applies only to the true density (gas pycnometer) and not the bulk density. The true density of most organic solids is greater than 1 g/cm^3. Many balances have a program to correct for buoyancy that uses a value for sample density provided by the user. The manufacturer's instructions should be consulted for the proper way to correct for buoyancy. For single-pan electronic balances, the error is generally $\leq$0.1% for densities $\geq$1 g/cm^3. Buoyancy corrections are crucial only when the weighing error must be minimized or when the densities of the weighed materials are low. Examples of buoyancy errors for a single-pan electronic balance are shown in the accompanying table.

Table 4

Density (g/cm^3)	Error (%)
2	0.05
0.8	0.1
0.5	0.2
0.4	0.3
0.2	0.6
0.1	1

■1S (*USP33*)

[3] NIST SOP 2, ''Recommended Standard Operations Procedure for Applying Air Buoyancy Corrections.''

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

BRIEFING

Heptyl *p*-Hydroxybenzoate. It is proposed to add this new reagent used as an internal standard in the monographs for *Tacrolimus* and *Tacrolimus Capsules*, which appear elsewhere in this issue.

(HDQ: M. Marques) RTS—C70856

Add the following:

▪**Heptyl *p*-Hydroxybenzoate** *(Heptyl 4-Hydroxybenzoate; n-Heptyl 4-Hydroxybenzoate; Benzoic Acid, 4-Hydroxy-, Heptyl Ester),* $C_{14}H_{20}O_3$—**236.31** [*1085-12-7*]—Use a suitable grade with a content of NLT 98%.▪1S *(USP33)*

BRIEFING

Pectate Lyase. It is proposed to add this new reagent used in the *Identification* test in the monograph for *Pectin*.

(HDQ: M. Marques) RTS—C72151

Add the following:

▪**Pectate Lyase** [*9015-75-2*]—An enzyme obtained from *Aspergillus sp*. Light brown, viscous liquid. Specific gravity is about 1.5. It is readily soluble in water. It is supplied at approximately 14 units per mL (at pH 8.0 in Tris-HCl buffer [50 mM of Tris(hydroxymethyl)aminomethane containing 1 mM of $CaCl_2$, pH 8.0] in a solution of 50% glycerol and 0.02% sodium azide. One unit is defined as the enzyme activity that produces 1 μmol of unsaturated product per minute.

Activity

Pectin solution—Transfer a quantity of Pectin [NOTE—Pectin has a molecular weight of 103,000 Da; its degree of esterification (percentage of galacturonic acid groups substituted with methyl) is 12.], equivalent to 0.05 g on the dried basis, to a 100-mL volumetric flask. Moisten with 0.1 mL of 2-propanol. Add 50 mL of water to the flask, and mix the solution with a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the solution to a pH of 12. Stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 minutes. Adjust the solution with 0.5 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Tris buffer solution—Transfer 6.055 g of Tris(hydroxymethyl)aminomethane and 0.147 g of calcium chloride ($CaCl_2 \cdot H_2O$) to a 1000-mL volumetric flask containing 950 mL of water, and mix. Adjust the solution with 1 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Diluted pectate lyase—Transfer 0.5 mL of Pectate Lyase to a 50-mL volumetric flask, dilute with *Tris buffer solution* to volume, and mix.

Procedure—Add the solutions set forth in the table below to quartz cuvettes. Perform the test on the solutions so obtained, using a suitable ultraviolet/visible spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩) and using water as the blank. Mix the solutions well at time 0, and immediately measure the absorbances at 235 nm. Record the value for the *Enzyme blank*, A_{0-EB}; for the *Test blank*, A_{0-TB}; and for the *Test solution*, A_{0-TS}. After incubation at room temperature for 30 minutes, determine the absorbance again at 235 nm for the *Enzyme blank*, A_{30-EB}; for the *Test blank*, A_{30-TB}; and for the *Test solution*, A_{30-TS}. One unit is defined as the enzymatic activity that produces 1 μmol of unsaturated

Label	*Tris buffer solution* (mL)	*Pectin solution* (mL)	*Diluted pectate lyase* (mL)	Water (mL)
Enzyme blank	0.5	1.0	0	1.0
Test blank	0.5	0	0.5	1.5
Test solution	0.5	1.0	0.5	0.5

product from pectin per minute. Calculate the Pectate Lyase activity, in units per mL, using the following formula:

$$50(10^3)[(A_{30-TS} - A_{30-EB} - A_{30-TB}) - (A_{0-TS} - A_{0-EB} - A_{0-TB})]/30\epsilon_{235}L$$

in which 50 is the volume, in mL, of *Diluted pectate lyase*; 10^3 is the unit conversion factor; 30 is the time, in minutes, of the reaction; ϵ_{235} is the molar extinction coefficient, in M^{-1} cm^{-1}, of the reaction product (4600 M^{-1} cm^{-1}); and L is the path length, in cm, of the reaction cuvette (1 cm). Alternatively, these solutions, after being mixed in the cuvettes, can be immediately measured at 235 nm continuously in a recording UV-Vis spectrophotometer set up for kinetic assays. The result is obtained by correcting the blank determination, using the *Enzyme blank* and the *Test blank*.■1S (*USP33*)

BRIEFING

Sodium Acetate, *USP 31*, page 796. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques) RTS—C70892

Change to read:

Sodium Acetate, $NaC_2H_3O_2 \cdot 3H_2O$—**136.08** ~~[127-09-3]~~ ■[*6131-90-4*]■1S (*USP33*)
—Use ACS reagent grade Sodium Acetate Trihydrate.

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 31* page 831, page 3789 of the *Second Supplement,* and page 186 of *PF* 35(1) [Jan.–Feb. 2009].

(HDQ) RTS—C46270; C53605; C61576; C62529; C66222

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	$T_{▲USP33}$
Add the following:	
■Amlodipine Besylate Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
▲Arginine Capsules	T, $LR_{▲USP32}$
Add the following:	
▲Arginine Tablets	T, $LR_{▲USP32}$
Add the following:	
■Azithromycin Tablets	$T_{■2S\ (USP32)}$
Add the following:	
▲Bicalutamide Tablets	$T_{▲USP32}$
Add the following:	
■Cabergoline Tablets	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Calcium Citrate Tablets	$W_{■1S\ (USP32)}$
Add the following:	
▲Cefdinir Capsules	T, $LR_{▲USP32}$

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
■Clonazepam Orally Disintegrating Tablets	W, $LR_{■1S\ (USP32)}$
Add the following:	
▲Curcuminoids Capsules	W, $LR_{■USP32}$
Add the following:	
▲Curcuminoids Tablets	W, $LR_{▲USP32}$
Change to read:	
Dantrolene Sodium Capsules	T $_{■2S\ (USP32)}$
Add the following:	
■Doxycycline Hyclate Tablets, Delayed-Release	T, $LR_{■1S\ (USP32)}$
Add the following:	
▲Fenofibrate Capsules	$W_{▲USP32}$
Add the following:	
▲Flavoxate Hydrochloride Tablets	W, $LR_{▲USP32}$
Add the following:	
■Fluconazole Tablets	$W_{■2S\ (USP32)}$
Add the following:	
■Granisetron Hydrochloride Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Guggul Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Ivermectin and Pyrantel Pamoate Tablets	$T_{■1S\ (USP32)}$
Add the following:	
▲Ketoprofen Capsules, Extended-Release	$T_{▲2S\ (USP32)}$
Add the following:	
■Lamivudine and Zidovudine Tablets	W, $LR_{■1S\ (USP33)}$

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
▲Lisinopril and Hydrochlorothiazide Tablets	W▲*USP32*
Add the following:	
▲Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	LR▲2S (*USP32*)
Add the following:	
■Loratadine Orally Disintegrating Tablets	T■2S (*USP32*)
Add the following:	
■Losartan Potassium Tablets	T■2S (*USP32*)
Add the following:	
■Metronidazole Capsules	W, LR■1S (*USP32*)
Add the following:	
▲Mirtazapine Orally Disintegrating Tablets	LR▲*USP32*
Add the following:	
■Nateglinide Tablets	T■1S (*USP33*)
Add the following:	
■Olanzapine Tablets	T, LR■1S (*USP33*)
Add the following:	
■Ondansetron Tablets	T, LR■2S (*USP32*)
Add the following:	
■Orbifloxacin Tablets	T■1S (*USP32*)
Add the following:	
■Orphenadrine Citrate Tablets, Extended-Release	T, LR■1S (*USP32*)

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
▲Oseltamivir Phosphate Capsules	W▲*USP33*
Add the following:	
▲Oxcarbazepine Tablets	W▲*USP33*
Add the following:	
▲Pantoprazole Sodium Delayed-Release Tablets	W▲*USP32*
Add the following:	
■Pilocarpine Hydrochloride Tablets	T■1S (*USP32*)
Add the following:	
■Potassium Citrate Tablets	W■1S (*USP32*)
Add the following:	
■Risedronate Sodium Tablets	W■2S (*USP32*)
Add the following:	
▲Soy Isoflavones Capsules	T, LR▲*USP32*
Add the following:	
■Tacrolimus Capsules	T■1S (*USP33*)
Add the following:	
■Tamsulosin Hydrochloride Capsules	T■2S (*USP32*)
Add the following:	
■Tranylcypromine Sulfate Tablets	W■1S (*USP33*)
Add the following:	
▲Valganciclovir Tablets	T▲2S (*USP32*)
Add the following:	
■Zinc Citrate Tablets	W■1S (*USP32*)
Add the following:	
▲Zolpidem Tartrate	W▲*USP33*

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 31* page 840, page 3790 of the *Second Supplement,* page 266 of *PF* 29(1) [Jan.–Feb. 2003], page 591 of *PF* 31(2) [Mar.–Apr. 2005], page 1193 of *PF* 31(4) [July–Aug. 2005], page 188 of *PF* 32(1) [Jan.–Feb. 2006], page 285 of *PF* 33(2) [Mar.–Apr. 2007], page 1053 of *PF* 33(5) [Sept.–Oct. 2007], page 1270 of *PF* 33(6) [Nov.–Dec. 2007], page 166 of *PF* 34(1) [Jan.–Feb. 2008], page 450 of *PF* 34(2) [Mar.–Apr. 2008], page 817 of *PF* 34(3) [May–June 2008], page 1046 of *PF* 34(4) [July–Aug. 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], and page 188 of *PF* 35(1) [Jan.–Feb. 2009].

(HDQ) RTS—C49304; C68850; C64168; C66403; C66404; C53605; C62528; C63300

Add the following:

▪**Epirubicin Hydrochloride:** Orange-red powder. Soluble in water and in methanol; slightly soluble in anhydrous ethanol; practically insoluble in acetone.▪1S (*USP33*)

Add the following:

▪**Ethylene Glycol and Vinyl Alcohol Graft Copolymer:** White or slightly yellowish powder. Very soluble in water; practically insoluble in anhydrous alcohol, and in acetone. It dissolves in dilute acids and dilute solutions of alkali hydroxides. *NF category:* Coating agent; tablet binder.▪1S (*NF28*)

Add the following:

▪**Salmeterol Xinafoate:** White to off-white powder. Soluble in methanol; slightly soluble in alcohol, in isopropanol, and in chloroform; practically insoluble in water (pH 8), and in saline solution (0.9% w/w).▪1S (*USP33*)

Add the following:

▪**Sucrose Palmitate:** White or almost white, unctuous powder. Sparingly soluble in ethanol (96%); very slightly soluble in water. *NF category:* Suspending and/or viscosity-increasing agent.▪1S (*NF28*)

Add the following:

▪**Sucrose Stearate:** White or almost white, unctuous powder. Sparingly soluble in ethanol (96%); very slightly soluble in water. *NF category:* Tablet and/or capsule lubricant; emulsifying and/or solubilizing agent.▪1S (*NF28*)

Add the following:

▪**Tacrolimus:** White crystals or white crystalline powder. Very soluble in methanol; freely soluble in *N*,*N*-dimethylformamide, and in alcohol; practically insoluble in water.▪1S (*USP33*)

Add the following:

▪**Tranylcypromine Sulfate:** White or almost white crystalline powder. Freely soluble in water; very slightly soluble in alcohol and in ether; practically insoluble in chloroform.▪1S (*USP33*)

Change to read:

Tromethamine: White, crystalline powder, having a slight, characteristic odor. Freely soluble in water. ~~and in low molecular weight aliphatic alcohols; practically insoluble in chloroform, in benzene, and in carbon tetrachloride.~~

▪▪1S (*USP33*)

In-Process Revision

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* or an *IRA.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents; Indicators; and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

	***PF* Volume, Issue, and Page Numbers of Pending Proposals**		
Title and Proposal	***Vol.***	***No.***	***Page(s)***
General Notices (entire *General Notices and Requirements* revised)	34	1	40
<u>*USP Monographs*</u>			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetaminophen and Tramadol Hydrochloride Tablets (new)	35	1	56
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Albendazole—*Assay*	34	1	69
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Sulfate—*USP Reference standards, Assay*	34	2	242
Albuterol Tablets—*Assay*	31	3	726
Alendronate Sodium Tablets—*Dissolution*	35	1	59
Alfuzosin Hydrochloride (new)	34	1	69
Allopurinol—*Related compounds, Limit of hydrazine* (add)	34	1	70
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Aluminum Acetate Topical Solution—*Identification*	34	2	242
Aluminum Subacetate Topical Solution—*Identification*	34	2	242
Amantadine Hydrochloride Capsules—*Labeling* (add), *Dissolution*	35	1	61
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Aminophylline—*CAS number*	34	1	72
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	35	1	62
Amodiaquine Hydrochloride—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	2	243

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Amodiaquine Hydrochloride Tablets—*USP Reference standards, Identification, Assay*	34	3	558
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140
Ampicillin Sodium—*Dimethylaniline*	35	1	65
Anastrozole (new)	34	2	244
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Arginine Capsules (new)	33	6	1160
Arginine Tablets (new)	33	6	1161
Aspirin—*Readily carbonizable substances*	34	5	1143
Atenolol Tablets—*Dissolution*	35	1	66
Atorvastatin Calcium (new)	35	1	66
Atovaquone—*Assay*	34	2	247
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam for Injection—*Assay*	34	4	906
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Benzoin—*Botanic characteristics, Identification*	35	1	70
Betamethasone Oral Solution—*Packaging and storage, Thin-layer chromatographic identification test* (delete), *Identification A, B* (add), *Microbial limits* (add), *pH* (add), *Deliverable volume* (add), *Related compounds* (add), *Assay*	34	3	567
Bicalutamide Tablets (new)	34	5	1147
Bisoctrizole (new)	32	2	309
Bisoprolol Fumarate Tablets—*Dissolution*	34	3	570
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Related compounds*	34	4	907
Bupivacaine Hydrochloride—*CAS number*	34	1	75
Bupropion Hydrochloride Extended-Release Tablets—*Dissolution, Related compounds*	35	1	70
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Cabergoline (new)	34	1	75
Cabergoline Tablets (new)	34	3	572
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Capecitabine Tablets—*Dissolution*	35	1	72
Carbidopa—*Specific rotation*	35	1	73
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Cefaclor Capsules—*Identification, Related compounds, Assay*	34	2	248
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefdinir (new)	33	6	1162
Cefdinir for Oral Suspension (new)	34	1	81
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Chlorhexidine Acetate (new)	34	3	582
Chlorhexidine Gluconate Oral Rinse—*Labeling, USP Reference standards*	34	2	250
Chlorhexidine Gluconate Solution—*USP Reference standards, Limit of p-chloroaniline, Assay*	34	2	250
Chlorhexidine Hydrochloride (new)	34	3	585
Chloroquine—*Assay*	34	1	86

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Chloroquine Phosphate—*USP Reference standards, Identification, Assay*	34	2	251
Chloroquine Phosphate Tablets—*USP Reference standards, Identification, Assay*	34	3	587
Cilostazol—*Loss on drying*	34	3	589
Cisapride (new)	34	2	253
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Anhydrous Citric Acid *(Harmonization)*—*Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)*—*Sulfate*	31	3	750
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49
Clarithromycin Tablets—*Dissolution*	35	1	73
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Climbazole (new)	33	5	891
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clonazepam Orally Disintegrating Tablets (new)	34	2	254
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Clozapine Tablets—*Uniformity of dosage units* (add)	34	3	589
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclazuril (new)	35	1	73
Diclofenac Potassium—*Identification*	34	1	87
Diclofenac Sodium Delayed-Release Tablets—*Identification*	31	3	751
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine—*USP Reference standards, Related compounds*	34	1	87
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Diethylstilbestrol Diphosphate (delete entire monograph)	33	6	1165
Diethylstilbestrol Diphosphate Injection (delete entire monograph)	33	6	1167
Dimethyl Sulfoxide—*Definition*, *Congealing temperature* (delete), *Substances darkened by potassium hydroxide* (delete), *Limit of dimethyl sulfone* (delete), *Limit of nonvolatile residue, Related compounds* (add), *Assay* (add)	34	1	88
Dipivefrin Hydrochloride—*Assay*	34	1	89
Disopyramide Phosphate—*Assay*	34	1	90
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Doxycycline Hyclate Delayed-Release Tablets (new)	34	3	589
Dronabinol—*Packaging and storage, Related compounds, Assay*	34	1	90
Dyclonine Hydrochloride—*Chemical information*	33	6	1167
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Enalaprilat Injection (new)	34	3	593
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Epinephrine—*Assay*	34	1	91
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Estradiol—*Chemical information, Labeling* (add)	33	6	1167
Estradiol Tablets—*USP Reference standards, Chromatographic purity* (add)	34	3	596
Estradiol Vaginal Inserts (new)	34	3	597
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Ethinyl Estradiol Tablets—*Dissolution* (add)	31	4	1067
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Eucatropine Hydrochloride (delete entire monograph)	33	6	1168
Eucatropine Hydrochloride Ophthalmic Solution (delete entire monograph)	33	6	1168
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate (new)	31	3	763
Fenofibrate Capsules (new)	34	2	258
Fenoprofen Calcium—*Chromatographic purity*	34	3	601
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931
Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets—*Labeling, USP Reference standards, Identification, Dissolution, Related compounds, Assay*	34	6	1445
Flavoxate Hydrochloride (new)	33	6	1172
Flavoxate Hydrochloride Tablets (new)	34	3	607
Fluconazole Injection (new)	34	2	266
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766
Fluticasone Propionate Cream (new)	34	3	609
Fluticasone Propionate Ointment (new)	34	3	611
Fluvestrant (new)	33	5	99
Formoterol Fumarate (new)	33	3	402
Foscarnet Sodium (new)	34	1	97
Fosinopril Sodium—*Related compounds*	34	3	613
Fosphenytoin Sodium—*Related compounds, Assay*	34	2	270
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Labeling* (add), *Dissolution, Related compounds*	34	6	1452
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide Tablets—*Dissolution*	33	4	651
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride (new)	33	6	1176
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163
Haloperidol Decanoate (new)	34	3	614
Heparin Sodium—*Definition, Anti-factor X_a activity, Assay*	33	2	238
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxyzine Pamoate—*Identification, Residue on ignition, Heavy metals, Pamoic acid content* (delete), *Assay*	34	2	271
Hydroxyzine Pamoate Capsules—*Identification, Assay*	34	2	272
Hydroxyzine Pamoate Oral Suspension—*Identification, Assay*	34	2	273
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Iopamidol—*Chemical structure, Reference standards, Identification, Related compounds*	33	6	1179
Iopamidol Injection—*Assay*	33	6	1182
Irbesartan—*Limit of azide*	34	5	1164
Isopropyl Alcohol—*Reference standards*	33	6	1182
Isotretinoin Capsules—*Labeling* (add), *Chromatographic purity, Assay*	34	4	942
Itraconazole (new)	34	4	947
Ivermectin and Pyrantel Pamoate Tablets (new)	34	2	277
Ketoprofen—*USP Reference standards, Chromatographic purity*	34	3	617
Ketoprofen Extended-Release Capsules (new)	34	4	951

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamotrigine (new)	34	3	617
Levonorgestrel—*USP Reference standards, Chromatographic purity, Assay*	34	3	620
Levorphanol Tartrate—*Assay*	34	2	280
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lipid Injectable Emulsion—*Definition, Limit of free fatty acids*	34	3	621
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium—*Limit of cyclohexane and isopropyl alcohol* (delete)	34	3	626
Losartan Potassium Tablets (new)	34	5	1164
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Mafenide Acetate Cream—*Identification*	34	2	280
Mafenide Acetate for Topical Solution—*Content of acetic acid*	34	3	627
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263
Meclizine Hydrochloride Tablets—*Related compounds, Assay*	33	6	1186
Meclocycline Sulfosalicylate—*Assay*	34	3	627
Meclocycline Sulfosalicylate Cream—*Assay*	34	3	628
Mefenamic Acid—*Heavy metals*	34	2	281
Megestrol Acetate Oral Suspension—*Dissolution*	35	1	75
Meradimate—*Assay*	34	1	100
Mesna (new)	34	5	1168
Metformin Hydrochloride Extended-Release Tablets—*Dissolution*	35	1	76
Methacholine Chloride—*Identification, Melting range* (delete)	34	3	629
Methotrexate—*USP Reference standards, Chromatographic purity*	34	3	630
Methoxsalen Capsules—*Assay*	34	1	101
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Methylprednisolone—*Chromatographic purity, Assay*	33	6	1189
Metronidazole—*Packaging and storage, USP Reference standards, Identification, Melting range* (delete), *Non-basic substances* (delete), *Chromatographic purity* (delete), *Related compounds* (add), *Assay*	34	3	631
Metronidazole Capsules (new)	34	3	633
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Mirtazapine Orally Disintegrating Tablets (new) (*Water, Method 1a,* canceled)	33	6	1189
Mometasone Furoate Cream—*Packaging and storage, Related compounds* (add), *Assay*	35	1	82
Mometasone Furoate Ointment—*Packaging and storage, Related compounds* (add), *Assay*	35	1	84

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Mometasone Furoate Topical Solution—*Packaging and storage, Related compounds* (add), *Assay*	35	1	87
Morantel Tartrate—*pH*	32	6	1735
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Calcium—*Identification, Related compounds, Assay*	34	1	101
Mupirocin Cream—*Related compounds, Assay*	34	2	281
Mupirocin Nasal Ointment (new)	34	4	966
Mycophenolate Mofetil—*Identification, Melting range* (delete), *Related compounds, Assay*	35	1	89
Naltrexone Hydrochloride—*Related compounds*	34	2	283
Naproxen Delayed-Release Tablets—*Drug release*	33	6	1192
Naratriptan Hydrochloride Oral Suspension (new)	35	1	90
Nateglinide (new)	34	6	1463
Niacinamide—*Readily carbonizable substances*	34	5	1176
Nitrofurantoin—*Packaging and storage*	35	1	92
Nitrofurantoin Capsules—*Packaging and storage*	35	1	92
Nitrofurantoin Oral Suspension—*Packaging and storage*	35	1	92
Nitrofurantoin Tablets—*Packaging and storage*	35	1	92
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethindrone Acetate and Ethinyl Estradiol Tablets—*Dissolution*	33	3	432
Norethindrone Acetate and Ethinyl Estradiol Tablets—*Identification*	33	6	1194
Norethynodrel (delete entire monograph)	35	1	92
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine (new)	34	3	641
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Orbifloxacin (new)	34	2	283
Orbifloxacin Tablets (new)	34	2	286
Orlistat Capsules (new)	32	6	1739
Orphenadrine Citrate Extended-Release Tablets (new)	34	3	643
Oseltamivir Phosphate (new)	34	6	1468
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin for Injection (new)	34	6	1473
Oxcarbazepine (new)	34	5	1177
Oxcarbazepine Tablets (new)	34	6	1478
Oxybutynin Chloride Tablets—*Dissolution*	35	1	93
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxytocin—*Definition, USP Reference standards, Identification, Vasopressor activity* (delete), *Acetic acid content* (add)	34	3	647
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Paricalcitol—*Identification, Assay*	33	2	252
Pectin—*Identification*	31	3	783
Penicillamine Capsules—*Dissolution*	31	2	436
Pentazocine and Acetaminophen Tablets—*Title, Assay for pentazocine, Assay for acetaminophen*	33	6	1200
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Pergolide Oral Suspension, Veterinary (new)	34	2	289
Permethrin (new)	32	4	1100
Permethrin Cream (new)	34	1	103
Petrolatum (new)—*Harmonization*	28	2	569
White Petrolatum (new)—*Harmonization*	28	2	570
Liquefied Phenol—*Identification* (add), *Other requirements*	35	1	93
Phenylephrine Hydrochloride—*Assay*	34	2	291
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride Tablets (new)	34	2	291
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980
Piperazine—*USP Reference standards* (add), *Identification, Primary amines and ammonia* (delete), *Chromatographic purity* (add)	34	1	105
Piperazine Adipate (new)	33	6	1201
Piperazine Citrate—*USP Reference standards* (add) *Identification*, *Primary amines and ammonia* (delete) *Chromatographic purity* (add), *Assay*	34	1	106
Piperazine Dihydrochloride (new)	33	6	1202
Piperazine Phosphate (new)	33	6	1204
Polyethlene Glycol 3350 and Electrolytes for Oral Solution—*Reference standards, Assay for potassium and sodium*	33	6	1205
Polyvinyl Alcohol—*Definition, Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification* (add), *Viscosity, Residue on ignition, Heavy metals* (add), *Acid value* (add), *Water-insoluble substances, Limit of methanol (methyl alcohol) and methyl acetate* (add)	33	6	1206
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Bromide Oral Solution, Veterinary (new)	33	5	936
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180
Pravastatin Sodium—*Chromatographic purity, Assay*	34	2	294
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Prednisolone Sodium Phosphate—*Definition*, *Free prednisolone* (delete) *Related compounds* (add), *Assay*	34	1	108
Proguanil Hydrochloride (new)	34	2	296
Propafenone Hydrochloride—*USP Reference standards, Chromatographic purity* (delete), *Related compounds* (add)	35	1	94
Propofol Injectable Emulsion (new)	33	6	1208
Propoxycaine and Procaine Hydrochloride and Norepinephrine Bitartrate Injection—*Assay for norepinephrine*	34	1	110
Pseudoephedrine Hydrochloride—*Definition, USP Reference standards, Ordinary impurities* (delete), *Chromatographic purity* (add), *Assay*	34	2	298
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ramipril—*Definition, Assay*	31	3	787
Ranitidine Hydrochloride—*Chromatograpic purity, Assay*	34	2	299
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Ritonavir—*Identification*	35	1	95
Rocuronium Bromide (new)	34	3	648
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Simethicone Emulsion—*Assay*	34	3	652

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

	***PF* Volume, Issue, and Page Numbers of Pending Proposals**		
Title and Proposal	***Vol.***	***No.***	***Page(s)***
Simethicone Tablets—*Disintegration*	34	3	652
Sodium Bromide Injection, Veterinary (new)	33	5	949
Sodium Bromide Oral Solution, Veterinary (new)	33	5	950
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Fluoride—*Assay*	34	3	653
Sodium Sulfate—*Assay*	34	5	1192
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Stavudine—*Specific rotation*	34	3	653
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfadoxine and Pyrimethamine Tablets—*Assay*	34	2	301
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfasalazine—*Identification*	34	3	653
Sulfazalazine Tablets—*Identification*	34	3	653
Sulfinpyrazone—*Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	34	6	1483
Tamsulosin Hydrochloride (new)	33	6	1211
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Tazobactam—*Identification, Specific rotation, Related compounds, Organic volatile impurities* (delete), *Assay*	34	2	302
Terbinafine Hydrochloride—*Melting range*	34	5	1197
Terbinafine Oral Suspension (new)	35	1	96
Terbutaline Oral Suspension (new)	35	1	97
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride—*Chromatographic purity*	34	2	306
Tiagabine Hydrochloride Oral Suspension (new)	35	1	98
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798
Tobramycin Inhalation Solution—*Identification* (add), *Osmolarity, Chromatographic purity, Other requirements* (delete), *Assay*	34	2	307
Topiramate Tablets (new)	34	5	1197
Torsemide—*Water*	33	6	1213
Tramadol Hydrochloride (new)	34	5	1200
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Trandolapril (new)	34	2	310
Travoprost (new)	32	4	1115
Travoprost Ophthalmic Solution (new)	32	4	1118
Trenbolone Acetate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Limit of trenbolone acetate 17α-isomer* (delete), *Related compounds* (add), *Assay*	35	1	100
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800
Triamterine Capsules—*USP Reference standards, Related compounds* (add), *Assay*	34	3	654
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Valganciclovir Tablets (new)	33	1	89

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Valrubicin—*Definition, USP Reference standards, Identification, Loss on drying* (delete), *Water* (add), *Limit of residual solvents* (delete), *Related compounds, Assay*	35	1	103
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486
Vancomycin Hydrochloride—*Labeling* (add), *USP Reference standards, Chromatographic purity, Other requirements* (add)	34	1	111
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Labeling* (add), *Identification* (add), *Water* (add), *pH* (add), *Uniformity of dosage units* (add), *Chromatographic purity, Assay*	34	4	992
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Vincristine Sulfate Injection—*Identification*	35	1	106
Vincristine Sulfate for Injection—*Identification*	35	1	106
Sterile Water for Injection—*Oxidizable substances*	31	3	803
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Zidovudine—*Assay*	34	3	656
Zidovudine Capsules—*Related compounds, Assay*	34	3	657
Zidovudine Injection—*Related compounds, Assay*	34	3	658
Zolpidem Tartrate (new)	34	6	1487
Zonisamide (new)	34	6	1489
Dietary Supplements Monographs			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
N-Acetyltyrosine (new)	35	1	107
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Calcium Citrate Tablets (new)	34	2	312
Curcuminoids (new)	33	6	1215
Curcuminoids Capsules (new)	33	6	1217
Curcuminoids Tablets (new)	33	6	1218
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Glucosamine Hydrochloride—*Assay*	33	4	691
Glucosamine Sulfate Potassium Chloride—*Assay*	33	4	692
Glucosamine Sulfate Sodium Chloride—*Assay*	33	4	692
Glutamic Acid (new)	34	4	997
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004
Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Powdered Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Omega-3 Acid Triglycerides (new)	34	3	662
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Potassium Citrate Tablets (new)	34	2	313
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Powdered Soy Isoflavones Extract (new)	33	6	1224
Soy Isoflavones Capsules (new)	33	6	1227
Soy Isoflavones Tablets (new)	33	6	1228
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Citrate (new)	34	2	315
Zinc Citrate Tablets (new)	34	2	316
Zinc and Vitamin C Lozenges (new)	34	2	317
USP General Test Chapters			
⟨1⟩ Injections—*Ingredients*	34	4	1020

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
⟨11⟩ USP Reference Standards—	29	6	2022
	30	5	1674
	31	2	507
	31	6	1680
	32	1	407
	33	1	95
	33	3	497
	33	4	716
	33	5	981
	33	6	1256
	34	1	142
	34	2	332
	34	3	680
	34	4	1021
	34	5	1230
	34	6	1531
	35	1	144
⟨41⟩ Weights and Balances—*Introduction, Repeatability* (add), *Verification of Accuracy* (add), *Calibration Check* (add)	34	3	682
⟨63⟩ Mycoplasma Tests (new)	35	1	146
⟨85⟩ Bacterial Endotoxins Test—*Harmonization*	33	3	539
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨191⟩ Identification Tests—General—*Introduction*	34	2	333
⟨197⟩ Spectrophotometric Identification Tests (entire chapter revised)	35	1	153
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨223⟩ Dimethylaniline—*Chromatographic System, Procedure*	35	1	156
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨271⟩ Readily Carbonizable Substances Test—*Introduction*	33	6	1258
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨401⟩ Fats and Fixed Oils—*Ester Value, Hydroxyl Value, Iodine Value, Peroxide Value, Saponification Value, Polyunsaturated Fatty Acids Determination and Profile* (add), *Trace Metals* (add), *Sterol Composition* (add)	34	3	736
⟨429⟩ Light Diffraction Measure of Particle Size (new)—*Harmonization*	31	4	1234
⟨467⟩ Organic Volatile Impurities—*Identification, Control, and Quantification of Residual Solvents*	34	3	747
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	34	5	1232
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density—*Harmonization*	31	3	909
⟨621⟩ Chromatography—*System Suitability, Glossary of Symbols*	34	5	1238
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨661⟩ Containers—Plastics—*Introduction, Polyethylene Containers, Polypropylene Containers*	34	2	335
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533
⟨671⟩ Containers—Performance Testing—*Introduction, Moisture Permeation, Light Transmission Test*	34	2	337
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	34	2	341
⟨731⟩ Loss on Drying—*Introduction*	34	3	760
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨851⟩ Spectrophotometry and Light-Scattering—(entire chapter revised)	35	1	157
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

	***PF* Volume, Issue, and Page Numbers of Pending Proposals**		
Title and Proposal	***Vol.***	***No.***	***Page(s)***
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	34	3	761
⟨941⟩ X-Ray Diffraction (new)—*Harmonization*	31	4	1241
General Information Chapters			
⟨1010⟩ Analytical Data—Interpretation and Treatment—*Prerequisite Laboratory Practices and Principles, Measurement Principles and Variation, Comparison of Analytical Methods, Appendixes B, C, D, E, F*	34	3	764
⟨1024⟩ Bovine Serum (new)	34	3	776
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients (entire chapter revised)	34	2	343
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1113⟩ Microbial Identification (new)	35	1	167
⟨1121⟩ Nomenclature—*General Nomenclature Forms*	34	1	159
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients (new)	34	2	375
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation*	30	5	1729
⟨1225⟩ Validation of Compendial Procedures—*Validation*	34	3	794
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1235⟩ Vaccines for Human Use—General Considerations (new)	34	5	1297
⟨1237⟩ Virology Test Methods (new)	34	2	391
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	34	3	798
⟨1788⟩ Particulate Matter Determination in Parenteral and Ophthalmic Products (new)	34	2	421
Reagents, Indicators, and Solutions			
Reagents, Indicators, and Solutions—*Introduction*	35	1	176
Acetic Acid	33	6	1259
Acetylactone	34	3	808
Alcohol	35	1	177
Alcohol, Denatured (new)	34	3	808
8-Amino-6-methoxyquinoline (new)	34	1	162
p-Aminophenol	34	2	442
Ammonium Molybdate	35	1	177
α-Amylase	34	1	162
Barium Chloride	34	2	442
Beclomethasone (new)	34	3	808
Bismuth Subnitrate (new)	34	1	162
1-Butanesulfonic Acid Sodium Salt (new)	33	4	766
Calcium Chloride	34	3	808
Activated Charcoal	33	6	1259
Chloramine T	34	2	442
Chromotropic Acid	35	1	177
Chromotropic Acid Disodium Salt	35	1	177
Diatomaceous Earth (new)	34	3	809
Diethylene Glycol	33	6	1259
2,7-Dihydroxynaphthalene (new)	34	3	809
N,N-Dimethyldecylamine (new)	34	4	1041
Dimethyltin Dibromide (new)	34	2	442
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Ethylenediamine (new)	34	2	442
Ferric Chloride	34	2	443

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

	PF Volume, Issue, and Page Numbers of Pending Proposals		
Title and Proposal	***Vol.***	***No.***	***Page(s)***
Ferrous Sulfate	33	6	1260
Hexylamine (new)	33	6	1260
Hydrogen Peroxide, 30 Percent	34	2	443
Hydrogen Peroxide, 30 Percent, Unstabilized (new)	34	3	809
Hydrogen Peroxide, 50 Percent in Water (new)	34	3	809
Lead Acetate	34	2	443
Maltotriose (new)	34	3	809
7-Methoxycoumarin (new)	34	2	443
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Methyl Red	35	1	177
Morin (new)	34	2	443
Naphthalene	33	6	1260
4-(*p*-Nitrobenzyl)pyridine	33	6	1260
1-Octanol (new)	32	6	1804
Phloxine B (new)	33	6	1260
Phosphatase Enzyme, Alkaline	34	3	809
Phosphorous Acid (new)	35	1	178
Potassium Metabisulfite (new)	35	1	178
Potassium Sodium Tartrate	35	1	178
Salicylaldehyde	33	6	1260
Silver Nitrate	34	3	810
Sodium Biphenyl	35	1	178
Sodium Cholate Hydrate (new)	34	3	810
Sodium 1-Decanesulfonate	34	5	1319
Sodium Phosphite Pentahydate (new)	34	1	162
Sorbitol (new)	34	3	810
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Tetrabutylammonium Hydroxide 30-Hydrate (new)	34	3	810
Tetrabutylammonium Hydroxide, 40 Percent in Water (delete)	34	3	810
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled	34	3	810
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled	34	3	811
Tetrahexylammonium Hydrogen Sulfate (new)	34	1	162
Tetrahydro-2-furancarboxylic Acid	33	6	1261
Triethylamine Phosphate (new)	33	6	1261
Triethylenediamine (new)	34	2	443
Trimethyltin Bromide (new)	34	2	444
Test Solutions			
Acetic Acid, Glacial, TS	35	1	179
Alcoholic TS (new)	34	3	811
Denatured Alcoholic TS (new)	35	1	179
Ammonia TS 2 (new)	34	2	444
Cupric Citrate TS 2, Alkaline	35	1	179
Iodine and Potassium Iodide TS 3 (new)	34	2	444
Lanthanum Nitrate TS (new)	34	2	444
Methyl Red TS 2 (new)	34	2	445
Potasssium Pyroantimonate TS	34	3	812
Volumetric Solutions			
Bismuth Nitrate, 0.01 mol/L	34	4	1041
Hydrochloric Acid, Normal (1 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N) in Methanol	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid	35	1	181
Potassium Iodate, Twentieth-Molar (0.05 M)	34	3	813
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Sodium Tetraphenylboron, Fiftieth-Molar (0.02 M)	34	2	447
Chromatographc Reagents			
Chromatographic Reagents—*Title, Packings*	35	1	182
Reference Tables			
Container Specifications for Capsules and Tablets	35	1	186
Description and Solubility	29	1	266
	31	2	591
	32	1	188
	33	5	1053
	33	6	1270
	34	1	166

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
	34	2	450
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
	35	1	188
Atomic Weights—*Standard Atomic Weights of the Elements*	35	1	189
<u>*Excipients*</u>			
USP and *NF* Excipients, Listed by Category	35	1	109
<u>*NF General Notices and Requirements*</u>—*Title* (delete), *''Official'' and ''Official Articles''* (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
<u>*NF Monographs*</u>			
Acetone—*USP Reference standards* (add), *Water, Assay*	34	1	120
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
rAlbumin Human (new)	34	1	121
Alfadex—*USP Reference standards, Identification, Heavy metals Reducing sugars, Related compounds, Assay*	34	1	126
Alpha-Lactalbumin (new)	34	3	670
Amino Methacrylate Copolymer—*Definition, Packaging and storage, Viscosity, Limit of monomers*	34	2	326
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Betadex—*Structure* (add), *Packaging and storage, USP Reference standards, Identification, Microbial limits, pH, Heavy metals, Reducing substances, Light-absorbing impurities* (add), *Related compounds* (add), *Assay*	34	1	127
Butylated Hydroxytoluene—*USP Reference standards* (add), *Identification, Related compounds* (add)	34	1	130
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Carbomer 934—*Title, Definition, Packaging and storage, Viscosity*	34	1	131
Carbomer 934P—*Title, Definition, Packaging and storage, Viscosity*	34	1	132
Carbomer 940—*Title, Definition, Packaging and storage, Viscosity*	34	1	133
Carbomer 941—*Title, Definition, Packaging and storage, Viscosity*	34	1	133
Carbomer Copolymer—*Definition, Labeling, Viscosity, Limit of benzene, Limit of acrylic acid*	34	1	134
Carbomer Homopolymer—*Title, Definition, Labeling, Viscosity, Residue on ignition, Limit of benzene, Limit of arylic acid*	34	1	136
Carbomer Interpolymer—*Definition, Labeling, Viscosity, Limit of benzene, Limit of acrylic acid*	34	1	138
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Harmonization*	33	3	537
Silicified Microcrystalline Cellulose (new)	34	5	1218
Chitosan (new)	35	1	115
Hydrogenated Coconut Oil (new)	34	2	327
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
High Fructose Corn Syrup—*Total solids, Assay*	34	2	329

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals		
	Vol.	No.	Page(s)
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone—*Harmonization*	28	4	1257
Cystine (new)	35	1	122
Desoxycholic Acid (new)	34	6	1523
Egg Phospholipids (new)	33	4	703
Erythorbic Acid (new)	33	6	1246
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Identification, Viscosity, Coagulum content*	35	1	123
Ethyl Maltol (new)	34	5	1224
Ethylparaben—*Harmonization*	34	6	1594
Liquid Glucose—*CAS number* (add), *Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification, Assay for reducing sugars (dextrose equivalent)* (add)	33	6	1248
Glyceryl Monooleate—*Chemical name, Reference standards, Identification, Saponification value*	33	6	1248
Hydrogenated Palm Oil (new)	34	2	330
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Hydroxypropyl Cellulose—*Identification*	35	1	124
Low-Substituted Hydroxypropyl Cellulose—*Harmonization*	30	1	338
Anhydrous Lactose—*Harmonization*	32	6	1847
Lecithin—*CAS number* (add), *Packaging and storage, Labeling* (add), *Reference standards* (add), *Identification* (add), *Acid value, Peroxide value* (add), *Hexane-insoluble matter, Lead, Heavy metals, Content of acetone-insoluble matter*	33	6	1249
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methylacrylic Acid Copolymer—*Definition, Packaging and storage, Labeling, Viscosity, Heavy metals, Limit of monomers*	33	6	1251
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Viscosity, Limit of monomers, Coagulum content*	35	1	124
Methyl Alcohol—*USP Reference standards* (add), *Identification, Readily carbonizable substances, Assay*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Definition, Packaging and storage, Assay*	31	4	1145
Nitrogen 97 Percent—*Definition, Packaging and storage, Assay*	31	4	1146
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Olive Oil—*CAS number* (add), *Definition, Packaging and storage, Identification* (add), *Fatty acid composition* (add), *Specific gravity* (delete), *Cottonseed oil* (delete), *Peanut oil* (delete), *Sesame oil* (delete), *Teaseed oil* (delete), *Absence of sesame oil* (add), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Specific absorbance* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add)	35	1	126
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete) *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227
Polyethylene Glycol—*Harmonization*	31	3	897
Polyoxyl 15 Hydroxystearate (new)	35	1	128
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polyvinyl Acetate (new)	34	6	1526
Polyvinyl Acetate Dispersion (new)	35	1	134
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Hydrogenated Starch Hydrolysate (new)	35	1	136
Pea Starch (new)	35	1	140
Rice Starch (new)—*Harmonization*	30	2	721
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Tagatose (new)	30	5	1672
Tetrafluoroethane (new)	31	6	1672
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	***PF Volume, Issue, and Page Numbers of Canceled Proposals***		
	Vol.	***No.***	***Page(s)***
USP Monographs			
†Carvedilol Tablets—*Title* (add), *Definition* (add), *Packaging and storage* (add), *USP Reference standards* (add), *Identification* (add), *Uniformity of dosage units* (add), *Related compounds* (add), *Assay* (add)	33	5	888
Conjugated Estrogens—*Definition*	30	3	840
†Desogestrel and Ethinyl Estradiol Tablets—*Related compounds*	30	5	1604
†Estradiol Vaginal Inserts—*Dissolution*	31	6	1617
†Flavoxate Hydrochloride Tablets—*Dissolution* (add)	33	6	1174
†Hydrocodone Bitartrate and Homatropine Methylbromide Tablets—*Dissolution*	30	3	853
†Ketoprofen Extended-Release Capsules—*Drug release*	31	5	1378
†Leflunomide Tablets—*Dissolution*	31	5	1383
†Mirtazapine Orally Disintegrating Tablets—*Water, Method Ia* (add)	33	6	1189
†Norethindrone Tablets—*Dissolution* (add)	32	6	1736
Norethindrone Tablets—*Dissolution* (add)	33	6	1193
†Promethazine Hydrochloride—*USP Reference standards, Related substances*	32	2	365
†Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
†Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add), *Assay*	32	2	367
†Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add)	32	4	1107
Dietary Supplements			
†Asian Ginseng Capsules (entire submission)	30	2	571
USP General Test Chapters			
<191> Identification Tests—General—*Acetate, Ammonium*	33	4	719
USP General Information Chapters			
†<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire submission)	31	2	524
NF Monographs			
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
Sucralose—*Related compounds*	33	6	1255

† New cancellations in *PF* 35(2).

HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization is composed of several steps **(*Stages*)**.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors.*

INSTRUCTIONS TO AUTHORS

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication.

All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current copy of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current copy of *Pharmacopeial Forum* will offer examples of reference formats.

Copyright—Copyright transfer documents will be sent to authors after manuscripts have been accepted for publication.

Contact Person—USP will designate a Scientific Liaison in the Documentary Standards Division as the corresponding author. This ensures that USP receives all comments generated by the *Stimuli* article. Authors should contact the Scientific Liaison if they would like to receive copies of comments generated by their *Stimuli* articles.

Submission Instructions—Manuscripts must be submitted both as an electronic file and as a printed copy of the electronic file. Submit the text in Microsoft® Word or another current word-processing application. The preferred format for graphics submitted electronically is tagged image file format (TIFF). Photocopies are not acceptable. Manuscripts submitted for publication should be addressed to:

Pharmacopeial Forum
Executive Secretariat, USP
12601 Twinbrook Pkwy.
Rockville, MD 20852

Pharmacopeial Forum
Vol. 35(2) [Mar.–Apr. 2009]

A Discussion of Net Water Gain for Water Vapor Transmission Rate Determinations

S. Yoon, *Eli Lilly and Company*, D. Sparks, *Eli Lilly and Company*, S. Selke, *Michigan State University*[a]

ABSTRACT This *Stimuli* article discusses the USP General Chapter *Containers—Permeation* ⟨671⟩ method to determine net water gain. This article introduces a proposal to change the procedure based on an analysis of the steady-state conditions of the plastic walls of containers. The analysis discussed in this paper shows that the plastic walls of *controls* have a higher water concentration than do the plastic walls of *test containers* and that the current Water Vapor Transmission Rate calculation in *USP* ⟨671⟩ is not theoretically correct. Although this proposal does not have an important impact on test results, this method change advances the scientific basis by providing more accurate test results.

INTRODUCTION

USP General Chapter *Containers—Permeation* ⟨671⟩ (*1*) provides a method to determine the net water gain of anhydrous calcium chloride or other desiccant in a plastic bottle container–closure system. The net water gain is used to calculate water vapor transmission rate (WVTR) of the sealed container–closure system. The current method employs test container–closure systems filled with desiccant (*test containers*) and container–closure systems filled with glass beads (*controls*). The net water gain is determined by subtracting the weight gain of *controls* from the weight gain of *test containers*. This is then used as the weight gain of desiccant only. This method is based on two assumptions: 1) the glass beads do not absorb water, and 2) the *test container* walls and *control* walls absorb the same amount of water.

However, the net water gain calculated in this manner is not theoretically correct because the water content of the plastic walls of the *controls* is not the same as the water content of the plastic walls of *test containers*. Theoretically, the plastic walls of *controls* achieve a higher water concentration than do the plastic walls of *test containers* at the steady-state conditions of the test. Therefore, the subtraction of a higher water concentration in *controls* from the lower concentration in *test containers* results in a net water gain that is lower than the actual net water gain. Further, this would result in a WVTR that is lower than the actual WVTR.

Plastic (e.g., polyethylene and polypropylene) container walls reach a steady state of water concentration. The steady-state condition is a gradient that depends on the storage conditions and the conditions of the headspace in the container. This gradient is maintained as constant during testing if the conditions of storage and the container headspace are constant. This *Stimuli* article proposes an alternative approach for determining net water gain and calculating WVTR of plastic container–closure systems (bottles and blisters).

DISCUSSION

1. Water Vapor Transmission into Plastic Container Walls

Theory—Water vapor from a high vapor pressure environment permeates plastic container walls of a sealed container that has a low water vapor pressure internal environment. This is described by Equation (1), Fick's Second Law of Diffusion:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (1)$$

where C is the concentration of water in the plastic container wall, t is time, D is the diffusion coefficient, and x is the spatial coordinate in the direction of transfer (*2*,*3*). >

Figure 1 describes Fick's Second Law of Diffusion graphically and shows a graphical representation of water concentration across the plastic container wall as a function of time (t). The total change in water concentration across the plastic container wall with time is directly proportional to the change in concentration gradient with the permeant penetration depth. At a steady-state rate of water transmission, the concentration gradient remains constant across the plastic wall.

[a] Correspondence should be addressed to: Desmond G. Hunt, PhD, Scientist, Documentary Standards Division, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8341; e-mail dgh@usp.org.

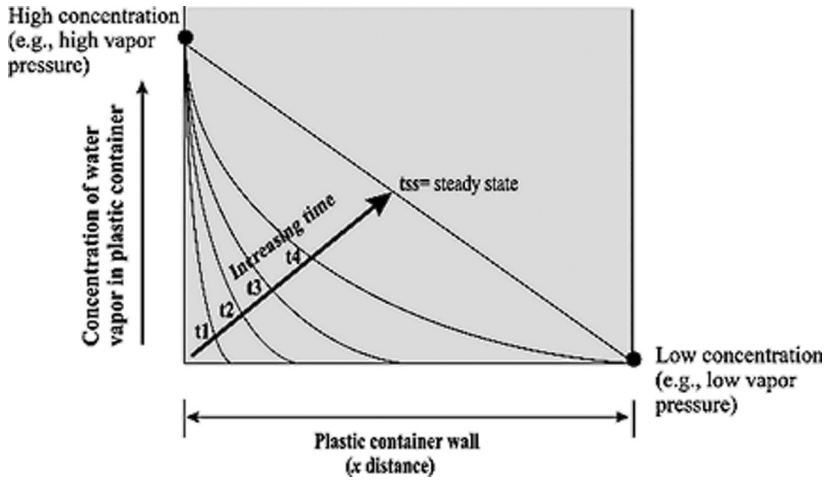

Figure 1. Graphical representation of water concentration across the plastic container wall as a function of time (*t*).

Example—In the standard test, *test containers* with desiccant and *controls* with glass beads are prepared at ambient conditions [e.g., 21 °C, 30% relative humidity (RH)] with the following assumptions:

- The container itself reaches equilibrium at 21 °C/30% RH.
- The desiccant is completely dried.

A sufficient amount of desiccant is inserted in the *test containers* to achieve and maintain an atmosphere inside the container close to 0% RH during the test. RH is used to express the water concentration in the headspace and in the walls of the container. *Figure 2* shows the initial water concentration across the plastic wall of a *test container* and a control. Because the plastic wall does not provide a good temperature barrier, the temperature across the plastic wall, inside and outside, is 21 °C.

Desiccant is assumed to absorb the water immediately in the headspace of *test containers*. Therefore, the RH of *test container* headspace with desiccant is close to 0% and the RH of the plastic wall of *test containers* is close to 30%. It is assumed that glass beads do not absorb water in *controls*. Therefore, the RH of the *control* headspace with glass beads is close to 30% and the RH of the plastic wall of the *controls* is close to 30%.

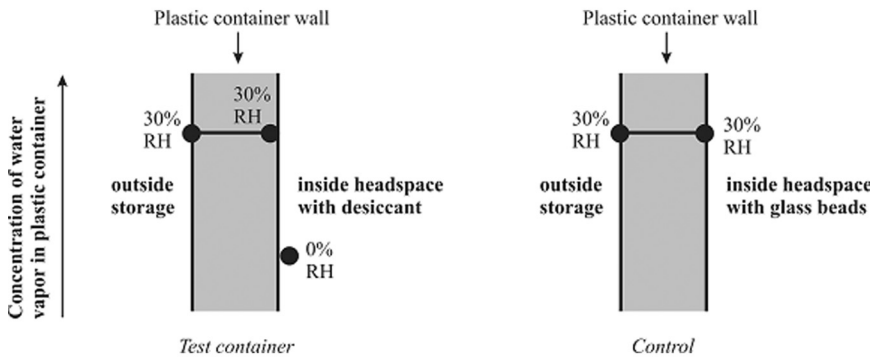

Figure 2. Diagram showing initial RH (i.e., water concentration) outside the container, across the plastic container wall, and inside the plastic container: initial state at sample preparation.

In the standard test, *test containers* and *controls* are placed in a storage chamber (e.g., 23 °C/75% RH) to allow study of the weight gain of desiccant due to water transmission into the containers and absorption of water by the desiccant. *Figure 3* shows the water concentration across the plastic wall of the *test containers* and *controls* after the containers are placed in the 23 °C/75% RH chamber. The water in the storage chamber first adsorbs on the surface of the plastic container wall. Therefore, the RH of the surface will change immediately to that of the storage RH condition (75% RH). Because the plastic walls do not provide a good temperature barrier, the temperature across the plastic wall, inside and outside, reaches 23 °C essentially immediately.

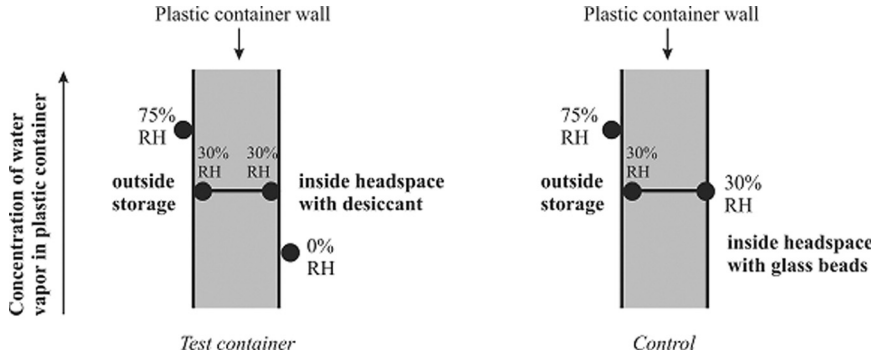

Figure 3. Diagram showing RH (i.e., water concentration) outside the container, across the plastic container wall, and inside the plastic container immediately after containers are placed in the 23° C/75% RH chamber: initial state upon storage.

Water will transmit through the plastic wall because the vapor pressure outside the container is greater than the vapor pressure of the wall itself and of that inside the container. *Figure 4* shows the water transmission and the resulting water concentration across the plastic wall. The arrows show a progression with increasing time. For *test containers*, the plastic material has a lower water concentration than the storage condition and has a higher water concentration than the container headspace. Therefore, the plastic wall absorbs water from the 75% RH chamber environment and desorbs water to the 0% RH headspace. The absorbed water diffuses through the plastic wall. The diffused water desorbs from the plastic wall to the 0% RH container headspace, where it is immediately absorbed by the desiccant, maintaining the container headspace at 0% RH. This results in the water concentration gradient shown in *Figure 4*. This water concentration gradient across the plastic walls may reach a steady-state condition (equilibrium) within a few days for typical pharmaceutical packages such as high-density polyethylene (HDPE) bottles and polychlorotrifluoroethylene (PCTFE) blisters. See *Appendix* for more information about the equilibrium time.

For *controls*, the plastic material has a lower water concentration than the storage condition and has the same water concentration as the container headspace. Therefore, the plastic wall absorbs water from the 75% RH chamber environment, and initially there is no transfer between the plastic wall and package headspace. The absorbed water diffuses through the plastic wall. The diffused water desorbs from the plastic wall to the container headspace, where it is not absorbed by the glass beads, resulting in the water concentration gradient shown in *Figure 4*. The water concentration gradient across the plastic wall reaches a steady-state condition (equilibrium). The time to reach equilibrium for *controls* is longer than for *test containers* because the water concentration gradient changes dynamically as the inside vapor pressure increases. It is assumed that the equilibrium time for *controls* is not more than 2 weeks. At the equilibrium condition, the water concentration across the plastic wall is constant at 75% RH.

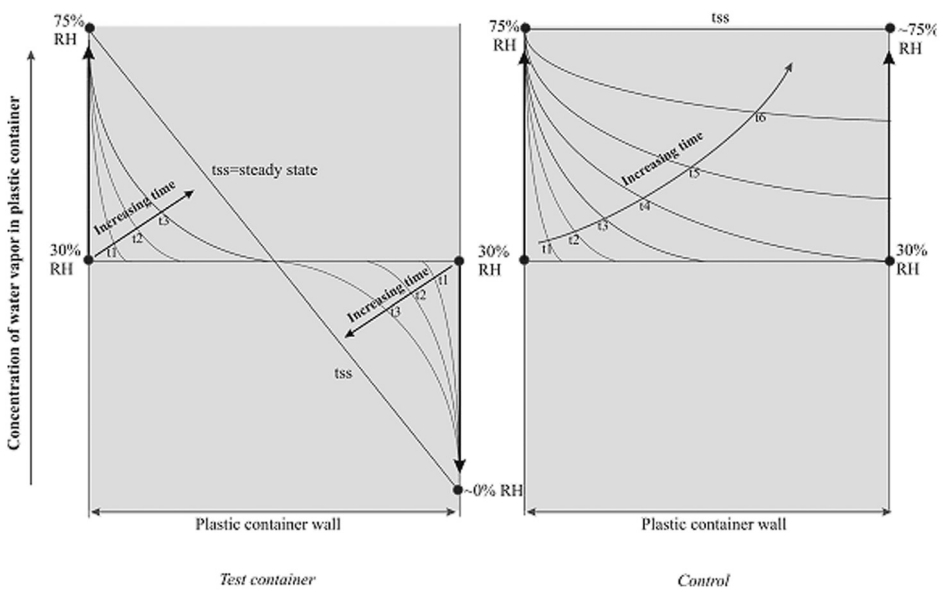

Figure 4. Graphical representation of water concentration across the plastic container wall as a function of time (*t*) after containers are placed in the 23 °C/75% RH chamber.

Conclusion, Part 1—*USP* ⟨671⟩ uses 14 days or 28 days to determine the net weight gain. After 14 or 28 days of storage, the water concentration gradient in the plastic material itself reaches steady-state condition. The water concentration (hence, the weight gain) of the plastic wall of *controls* is greater than the concentration (hence, the weight gain) of the plastic wall of *test containers*. Practically, for small or thin-walled containers, the moisture gain of container walls may not be significant and may not affect the accuracy of experimental results provided by *USP* ⟨671⟩.

2. Correction of Current Net Water Weight Gain Equation

The analysis of the water weight gain of plastic bottle container–closure systems has been important to the pharmaceutical industry for many years. *USP* ⟨671⟩ prescribes this analysis, currently, through the use of Equation (2) to calculate the net water gain of containers. The weight gain of *controls* (*C*) filled with glass beads is subtracted from the weight gain of *test containers* (*T*) filled with desiccant:

$$W_{net} = (T_F - T_I) - (C_F - C_I) \qquad (2)$$

where W_{net} is the net weight gain determined by subtracting the difference between the final (C_F) and initial (C_I) weights of the *controls* from the difference between the final (T_F) and initial (T_I) weights of *test containers*.

Equation (2) assumes that the water concentrations in the plastic walls of *test containers* and *controls* are equal at the same time of storage. However, the water concentration is not equal, as explained in the previous section. The plastic wall of *controls* has a higher water concentration than the plastic wall of *test containers*. Therefore, it is not correct to subtract the water weight gain of *controls* from the water weight gain of *test containers* to determine the net water gain of the *test containers*. That is, the water gain of containers calculated by Equation (2) results in a value that is less than actual. Hence, Equation (2) is not theoretically correct.

Instead, the steady-state (equilibrium) condition of *test containers* described in the previous section can be utilized in this determination without using the water weight gain of *controls* to determine the net water gain of containers. *Test containers* are placed at the desired condition for preconditioning to achieve a steady-state concentration gradient. (See *Test container* in *Figure 4*.) Typical plastic containers [e.g., HDPE, PP, polyethylene terephthalate (PET), and PCTFE] used in the pharmaceutical industry can achieve the steady-state concentration gradient within 1 or 2 days. When a sufficient amount of completely dried desiccant is used in *test containers*, the preconditioning does not affect the water vapor pressure of the container headspace. The constant water vapor pressure difference can be achieved during the test. Therefore, the net water gain can be measured by Equation (3) after preconditioning.

$$W_{net} = (T_F - T_{IC}) \qquad (3)$$

where W_{net} is the net weight gain determined from the difference between the final (T_F) and initial (T_{IC}) weights of *test containers*. The initial weight (T_{IC}) is determined after preconditioning.

CONCLUSION

This article has reviewed the use of *controls* to determine the net water gain for plastic container–closure systems provided in *USP* ⟨671⟩ and has shown that the procedure is not theoretically correct. Theoretically, the net water weight gain would be underestimated by subtracting the weight gain of *controls* from the weight gain of *test containers*. Therefore, it may result in a WVTR that is lower than the actual WVTR. The newly proposed simple equation with preconditioning of plastic containers determines net water gain more accurately, theoretically, and offers an improvement to the precision of the WVTR method.

ACKNOWLEDGMENTS

The authors would like to acknowledge Drs. Maria Rubino and Rafael Auras at Michigan State University for the WVTR experiments using the moisture analyzer equipped with a microbalance as referenced in this paper. The authors also would like to thank Randy Thackrey (Eli Lilly and Company) for constructive technical comments that have been incorporated in this paper.

REFERENCES

1. USP. *USP 30–NF 25, Containers—Permeation* ⟨671⟩. Rockville, MD: USP; 2007:267–269.
2. Crank J. *The Mathematics of Diffusion*. 2nd ed. Bristol, UK: Clarendon Press; 1979.
3. Selke S. *Plastic Packaging*. 2nd ed. Munich, Germany: Hanser Publishing; 2004.

APPENDIX: MOISTURE PERMEATION OF 2-MIL PCTFE BLISTERS

Moisture permeation of 2-mil PCTFE blisters has been determined with a VTI moisture analyzer. The analyzer can maintain constant environmental conditions and weigh the sample during the storage time. If the sample package contains adequate desiccant to maintain the inside vapor pressure consistently at 0, then a constant vapor pressure difference between inside and outside of the package can be achieved. This is identical with the approach in *USP* ⟨671⟩ and ASTM E96 gravimetric WVTR methods. The benefit of this method is that the sample can be weighed without taking the sample out of the chamber. Therefore, the analyzer can show the weight change immediately after the sample is mounted on the balance. It shows the weight change from a dynamic (unsteady) state to the steady state. The time to reach the steady-state condition can be estimated from this experiment.

Materials and Equipment

The 2-mil PCTFE film (supplied by TekniPlex,) is structured as 2-mil PCTFE/2-mil PE/7.5-mil PVC. The lidding foil (supplied by Alcan) is structured as paper/adhesive/polyester/adhesive/1-mil aluminum foil/heat-seal coating. The size of the individual blister cavity is 11 mm diameter × 5 mm height. A 4-Å molecular sieve desiccant (supplied by SudChemie) was used.

A symmetrical gravimetric analyzer (SGA 100) was newly equipped with a CAHN 200 microbalance at Michigan State University. The moisture analyzer and balance can measure the weight change of the sample with ±5 μg accuracy, control the temperature with ±0.5 °C accuracy, and control the RH with ±1% RH accuracy.

Methods

Blister samples were prepared with a Klockner EAS blistering machine. The 2-mil PCTFE film was thermoformed, and completely dried molecular sieve desiccant tablets were inserted manually into the blister cavities. The desiccant-filled blisters were heat-sealed immediately with aluminum foil lidding.

Moisture permeation of 2-mil PCTFE blisters was determined gravimetrically by using the VTI moisture analyzer equipped with a microbalance. The blister sample (6 cavities, approximately 4 g weight) was mounted on the weighing dish in the chamber, which is connected to the microbalance. The system was set up to maintain the relative humidity at 60% and the temperature at 30 °C for 2000 minutes (1.4 days), and data were collected every 2 minutes. The test was replicated with 2 different samples.

Results

The system maintained the desired RH and temperature consistently during the entire testing period. The temperature was maintained between 29.83 °C and 29.91 °C, and the RH was maintained between 59.6% and 60.3% for 2000 minutes. *Figure 5* shows the weight change of samples stored at 30 °C/60% RH. Initially, the weight change rate was in an unsteady state, but the weight change rate reached a steady state before 0.5 day for both samples.

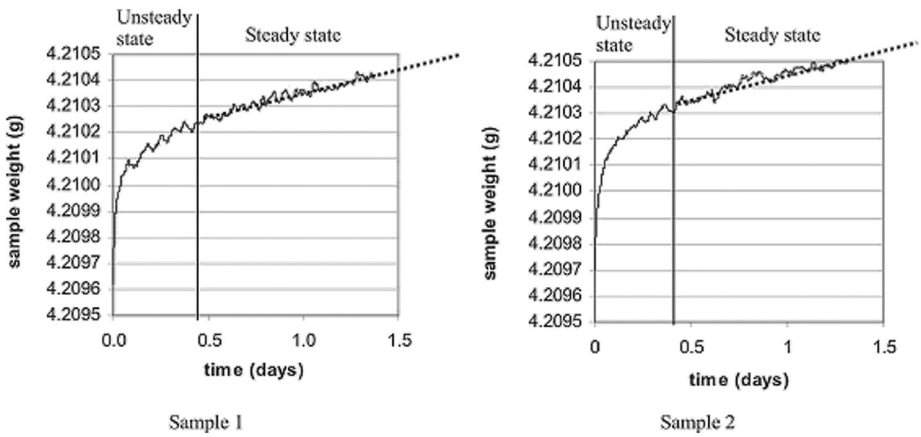

Figure 5. Recorded sample weight change from the VTI system.

Conclusions

A steady-state rate of weight change for 2-mil PCTFE blisters was obtained before 0.5 day at 30 °C/60% RH. If the storage condition is changed, the equilibrium time may be increased or decreased. In addition, if the material thickness and barrier property increase, the equilibrium time will be increased. The equilibrium time should be determined experimentally but probably will not require more than a few days with typical plastic package materials (e.g., HDPE, PP, PET, and PCTFE) at typical stability conditions such as 25 °C/60% RH, 30 °C/65% RH, and 40 °C/75% RH.

NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–218, Vol. 35 No. 2, pp. 219—498.*]

MONOGRAPHS

EXCIPIENTS

GENERAL CHAPTERS

REAGENTS, INDICATORS, AND SOLUTIONS

Index

Index

CHROMATOGRAPHIC REAGENTS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Columns Used in *USP-NF* and *Pharmacopeial Forum*
Mar.–Apr. 2009

DICLAZURIL (DSD Mgh #24944)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(1)	L1	Hypersil BDS C-18	Chromatographic purity	4.6 mm × 10 cm, 3 μm. Manufacturer: Thermo Scientific

ETHYLENE GLYCOL AND VINYL ALCOHOL GRAFT COPOLYMER (DSD Mgh #3827)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	G1	DB-1	Limit of	Limit of ethylene oxide and dioxane. 0.25 mm × 30 m, 1.0 μm. Manufacturer: J & W Scientific
35(2)	L1	AQUASIL C18	Limit of	Limit of vinyl acetate and Limit of acetic acid/acetate. Analytical column. 4 mm x 25 cm, 5 μm. Manufacturer: Thermo Scientific
35(2)	L1	Nucleosil 120 C18	Limit of	Limit of vinyl acetate. Pre-column. 4.0 mm × 3 cm, 5 μm. Manufacturer: Macherey-Nagel

MISOPROSTOL TABLETS (DSD Mgh #54310)

PF	LGS#	Reagent Brand	Type of Test	Comments
26(1)	L7	ZORBAX C8	Assay, Dissolution, Related compounds	4.6 mm × 15 cm. Manufacturer: Agilent Technologies

OLANZAPINE TABLETS (DSD Mgh #2645)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L10	ZORBAX SB-CN	Dissolution	4.6 mm × 15 cm. Manufacturer: Agilent Technologies

ORBIFLOXACIN (DSD Mgh #58770)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(2)	L1	Pecosphere-3CR C18	Assay, Identification, and Related compounds	4.6 mm × 3 cm, 3 μm. Manufacturer: PerkinElmer, Inc.

ORBIFLOXACIN TABLETS (DSD Mgh #58780)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(2)	L1	Pecosphere-3CR C18	Assay, Identification, and Chrom. purity	4.6 mm × 3.3 cm, 4 μm. Manufacturer: PerkinElmer, Inc.

OXALIPLATIN INJECTION (DSD Mgh #1903)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L1	Hypersil BDS C-18	Assay and Limit of	Limit of oxalic acid. 4.6 mm × 25 cm, 5 μm. Manufacturer: Thermo Scientific
35(2)	L1	Hypersil BDS C-18	Limit of	Limit of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N,N′*]platinum and unspecified impurities. 4.6 mm × 7.5 cm, 3 μm. Manufacturer: Thermo Scientific

PECTIN (DSD Mgh #61250)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	G43	Zebron ZB-624	Methanol, Ethanol, and Isopropanol	0.32 mm × 30 m, 1.8 μm. Manufacturer: Phenomenex
35(2)	S3	Porabond Q	Methanol, Ethanol, and Isopropanol	0.32 mm × 25 m, 5 μm. Alternative column. Manufacturer: Varian

PRAZIQUANTEL TABLETS (DSD Mgh #68458)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L1	Ultracarb C18	Dissolution	Dissolution test for veterinary products. 4.6 mm × 25 cm, 10 μm. Manufacturer: Phenomenex

TACROLIMUS CAPSULES (DSD Mgh #80283)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L1	TSK-GEL ODS-80Tm	Assay	4.6 mm × 15 cm, 5 μm. Manufacturer: Tosoh Bioscience
35(2)	L20	Supelcosil LC Diol	Organic impurities	4.6 mm × 25 cm, 5 μm. Manufacturer: Supelco
35(2)	L7	Nucleosil C8	Dissolution	4.6 mm × 15 cm, 5 μm. Manufacturer: Macherey-Nagel

Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 3 **MAY–JUNE 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *Pharmacopeial Forum* content to
USP Executive Secretariat
12601 Twinbrook Parkway,
Rockville, MD 20852
execsec@usp.org

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

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

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Rockville, MD 20852.
Fax: (301) 816-8148.

STANDARDS DEVELOPMENT

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

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

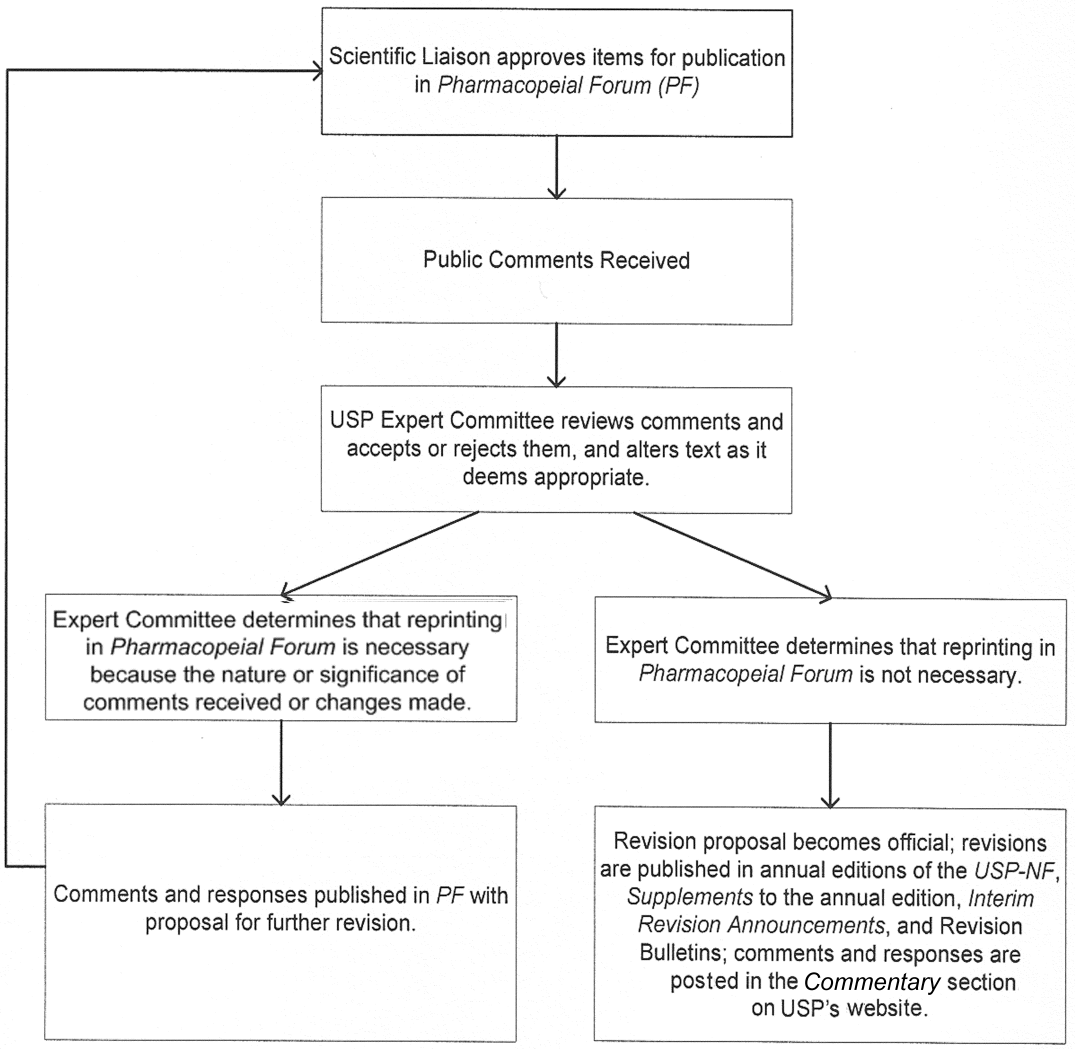
PF includes the following:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's typical Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that reprinting in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Accelerated Revisions**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP website) (e.g., *Interim Revision Announcements*, *Revision Bulletins*, and *Errata*). Accelerated Revisions allow for a revision to become official prior to the next *USP–NF* or *Supplement* and do not always require notice and comment. *Interim Revision Announcements* are first presented for a 60-day public comment period in the *Proposed Interim Revision Announcement* section before becoming official in a later *PF* in the *Interim Revision Announcements* section of the *PF*. Note that *Revision Bulletins* appear only on the USP website.

USP welcomes comments and data on proposed revisions. Comments, along with USP's responses, will be published in the *Commentary* section of the USP website (www.usp.org).

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

''How to Use PF'' describes the various parts of *Pharmacopeial Forum*, lists the *Committee Designations*, and includes the *Staff Directory*.

The content of the different sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
In-Process Revision Revisions targeted for adoption	•BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst such as the brand name of the column used in developing the proposed procedure and the USP scientific staff liaison who handled the issue. •New and revised standards that have been approved for publication by the appropriate USP Committee when it is considering whether to advance standards to official status (see *Standards Development*).	Review material and send comments promptly to USP staff liaison (see the *Staff Directory*) identified at the end of the briefing accompanying each item. For general inquiries or in cases where a particular liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-816-8373. Comment deadlines are found at the end of the *Policies and Announcements* section.
Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to harmonize internationally	•BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. The designated stage of harmonization. Two stages are printed in the *Pharmacopeial Forum Harmonization* section: Stage 4 is available for comment, whereas Stage 6 is the final official harmonized standard. Stage 4 and Stage 6 standards are separated into their own respective sections within *Pharmacopeial Forum* in order to clearly differentiate the stages. •New or revised text to Stage 6 documents is marked with symbols that indicate the publication in which the book or *Supplement* becomes official.	For Stage 4 harmonized documents, review material and provide comments to the appropriate staff liaison cited in the *Briefing* preceding each *In-Process Revision.* Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopeia, with a copy to USP, for a given article. The addresses for the European and Japanese Pharmacopoeias are as follows: EP Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp

Proposed and Adopted Revisions to the *USP–NF* (*Continued*)

Section	Content	How Readers Can Respond
Interim Revision Announcement Adopted standards	Standards that have been adopted and will become officially binding on the specified date. Effective date is specified in the section's introductory page or within parentheses following a particular item. New or revised text is marked by the symbols ‣•.	Review to see if affected by any of the changes. Note effective date when standards become official and ensure compliance.
Pending Proposals	In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *PF* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF*, its *Supplements*, or an *IRA*. Those items that have not yet been adopted are still pending.	Review items to track pending proposals.
Canceled Proposals	Canceled proposals are items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption into the *USP–NF*.	Review items to track canceled proposals.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including scientific liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Reagents Used in USP–NF and Pharmacopeial Forum
Update of chromatographic reagents based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* (*Continued*)

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Darrell Abernethy, M.D., Ph.D., Chief Science Officer	dra@usp.org	(301) 816-8184	
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, Senior Scientific Associate	kxb@usp.org	(301) 816-8462	Food Ingredients (FI)
Colleen Brennan, R.Ph. Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	USP–NF Spanish Edition
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D. Scientist	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards
Anthony DeStefano, Ph.D., Vice President, General Chapters	ajd@usp.org	(301) 230-6303	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	Document Disclosure
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE) Radiopharmaceuticals and Medical Imaging Agents Veterinary Drugs (VET)
James Griffiths, Ph.D., Vice President, Food and Dietary Supplement Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	USP Spanish Edition; General Chapters (GC), Pharmaceutical Waters
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products— Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexy Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B: Proteins and Polysaccharides (BB PP)
Randy Kiser, Program Manager	rwk@usp.org	(301) 816-8324	USP Reference Standards Acquisition
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Vice President, RSO	psl@usp.org	(301) 816-8337	Reference Standards Operations
Markus Lipp, Ph.D. Director, Food Ingredients	mxl@usp.org	(301) 816-6366	Food Ingredients
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Kate Meringolo, Manager, Publications Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Senior Scientific Associate	jm@usp.org	(301) 816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Senior Scientist	ktm@usp.org	(301) 816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Vice President, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements Non-Botanicals (DSN)
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Senior Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D. Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D. Manager, Pharmacy Compounding EC	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, Director, Excipients	cxs@usp.org	(301) 816-8262	Excipients
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB-VV), Proteins and Polysaccharides (BB PP) (small peptides)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Senior Scientist	dwv@usp.org	(301) 998-6828	Monograph Development—Pulmonary and Steriods (MD-PS)
Mary ''Jeanie'' Waddell Scientist	msw@usp.org	(301) 816-8124	Monograph Development—Pulmonary and Steriods (MD-PS)
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	rstech@usp.org	(301) 816-8129	USP Reference Standards Evaluation
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section provides general information resources for *USP–NF* standards and processes. Information resources include announcements on scientific and policy issues currently under consideration, schedules for USP publications, and schedules for comment periods for proposed standards.

USP ANNOUNCES REVISION TO AMANTADINE HYDROCHLORIDE CAPSULES MONOGRAPH. The Biopharmaceutics Expert Committee (BPC) has approved the addition of *Dissolution Test 2* to the Amantadine Hydrochloride Capsules *Interim Revision Announcement* monograph. This monograph was previously printed as an *In-Process Revision* in *Pharmacopeial Forum* volume 35(1). The approved *Interim Revision Announcement* supersedes the monograph printed in *USP 32–NF 27* until the monograph is printed in the *USP 32–NF 27, Second Supplement*, which will be released June 1, 2009 and will become official December 1, 2009. The chromatographic procedure in this test was validated using the RTX-1 or DB-1 brand of G1 phase. The retention times for naphthalene and amantadine hydrochloride are approximately 6 and 7 minutes, respectively.

Should you have any questions on the Amantadine Hydrochloride Capsules Monograph, please contact Margareth Marques, Ph.D. at 301-816-8106 or mrm@usp.org.

USP ANNOUNCES REVISION TO BUPROPION HYDROCHLORIDE EXTENDED-RELEASE TABLETS MONOGRAPH. The Biopharmaceutics Expert Committee (BPC) has approved the addition of *Dissolution Test 8* to the Bupropion Hydrochloride Extended-Release Tablets *Interim Revision Announcement* monograph. This monograph was previously printed as an *In-Process Revision* in *Pharmacoeial Forum* volume 35(1). The approved *Interim Revision Announcement* supersedes the monograph printed in *USP 32–NF 27, First Supplement* (released February 1, 2009 and official August 1, 2009) until it is printed in the *USP 32–NF 27, Second Supplement*, which will be released June 1, 2009 and will become official December 1, 2009.

Should you have any questions on the Bupropion Hydrochloride Extended-Release Tablets Monograph, please contact Margareth Marques, Ph.D. at 301-816-8106 or mrm@usp.org.

STAGE 4 AND STAGE 6 HARMONIZED STANDARDS ARE NOW FEATURED IN TWO SEPARATE PF SECTIONS. USP publishes monographs and general chapters that are undergoing international harmonization by the Pharmacopeial Discussion Group (PDG) at Stage 4 and Stage 6. The Stage 4 documents are published for public comment. The Stage 6 documents have received PDG approval. These documents are being published to inform readers of their PDG sign-off status.

To make clear the distinction between Stages 4 and 6, USP has revised the Harmonization section of *Pharmacopeial Forum (PF)* by separating the two types of documents.

Stage 4 documents are published in *PF* in draft form and are available for public comment. The Japanese and the European Pharmacopoeias and the USP analyze comments and revise the general chapters and monographs accordingly. The documents are revised based on the comments received and then forwarded to the next stage.

The Stage 6 documents are provided in *PF* as an informational resource, and these documents will be published in the next available official publication. The Stage 6 documents published in *PF* 35(3) will be official in *USP 33–NF 28*.

***PHARMACOPEIAL FORUM* PUBLIC REVIEW AND COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section and incorporated in the upcoming *Supplement* or book. They may also be published as *Revision Bulletins* on www.usp.org in the ''New Official Text'' section. The official publication in which an *IRA* is incorporated will depend upon publication deadlines. See the table below. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and, therefore, contains all official text up to and including the contents of the latest *Supplement.* The table below outlines the publications and their release and official dates, and the book or supplement which supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
USP 32–NF 27	November 1, 2008	May 1, 2009	*1st Supplement to USP 32–NF 27*
IRA [*PF* 35(1)]	January 1, 2009	February 1, 2009	*2nd Supplement to USP 32–NF 27*
1st Supplement to USP 32–NF 27	February 1, 2009	August 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*

PRIORITY NEW MONOGRAPH ITEMS. USP is seeking monographs for the following drug substances and drug products that are or soon will be off patent and thus are of the highest priority. USP also is seeking monographs for the excipients listed below. Monographs are marked ''Received'' upon receipt of monograph proposal. Received monographs are removed from this list upon publication in *Pharmacopeial Forum* or when posted in the Pending Monographs section of the USP website (http://www.usp.org/standards/pending/). This list has been updated as of February 20, 2009; monographs received since the last update to the list are noted in bold.

Monograph sponsors should consult USP's Guideline for Submitting Requests for Revision to the *USP–NF* at http://www.usp.org/USPNF/submitMonograph/subGuide.html.

For additional information, contact Karen A. Russo, Ph.D., kar@usp.org.

Small Molecules (Drug Substances)—As of February 20, 2009

1. Allopurinol Sodium	2. Aminopropazine Fumarate	3. Aminopterin Sodium
4. Anagrelide Hydrochloride ***(Received)***	5. Arsenic Trioxide	6. Auranfoin
7. Azelaic Acid ***(Received)***	8. Balsalazide Disodium ***(Received)***	9. Bentoquatam
10. Benzphetamine Hydrochloride	11. Bivalirudin ***(Received)***	12. Calcipotriene
13. Calcium Trisodium Pentetate	14. Calfactant	15. Candesartan Cilexetil ***(Received)***
16. Ceftibuten	17. Cetrorelix	18. **Cevimeline Hydrochloride** ***(Received)***
19. Chloroxine	20. Choline Salicylate	21. Cysteamine Bitartrate
22. Dalfopristin	23. Dapirazole Hydrochloride	24. Desirudin
25. Desonide ***(Received)***	26. Dexrazoxane	27. Difenoxin Hydrochloride
28. Entacapone ***(Received)***	29. Epoprostenol Sodium ***(Received)***	30. Erythromycin Phosphate
31. Erythromycin Thiocyanate	32. Esmolol Hydrochloride ***(Received)***	33. Estazolam ***(Received)***
34. Estramustine Phosphate Sodium	35. Ethanolamine Oleate	36. Etomidate ***(Received)***
37. Etoposide Phosphate	38. Exemestane	39. Famciclovir ***(Received)***
40. Felbamate ***(Received)***	41. Fluoromethane F 18	42. Fosfomycin Tromethamine ***(Received)***
43. Gadobenate Dimeglumine	44. Gadopentetic Acid	45. Gallium Nitrate

Small Molecules (Drug Substances)—As of February 20, 2009 *(Continued)*

46. Ganirelix	47. Guanidine Hydrochloride	48. Halobetasol Propionate ***(Received)***
49. Haloperidol Decanoate ***(Received)***	50. Hydrocodone Polistirex	51. Ibandronate Sodium
52. Imipramine Pamoate	53. Imiquimod	54. Isosulfan Blue
55. Latanoprost ***(Received)***	56. Lomustine ***(Received)***	57. Metipranolol Hydrochloride
58. Miglitol	59. Milrinone Lactate	60. Moexipril Hydrochloride
61. Nalbuphine Hydrochloride	62. Nedocromil Sodium	63. Nicardipine Hydrochloride
64. Nilutamide	65. Nisoldipine	66. Olsalazine Sodium ***(Received)***
67. Orlistat ***(Received)***	68. Oxiconazole Nitrate	69. Pemirolast Potassium
70. Pioglitazone Hydrochloride ***(Received)***	71. Piperonyl Butoxide	72. Pirbuterol Acetate ***(Received)***
73. Poractant Alpha	74. Porfimer Sodium	75. Pramiprexole Dihydrochloride ***(Received)***
76. Quetiapine Fumarate ***(Received)***	77. Ranitidine	78. Rivastigmine Tartrate ***(Received)***
79. Ropinirole Hydrochloride ***(Received)***	80. Rose Bengal Disodium	81. Rosiglitazone Maleate
82. Sodium Phenylbutyrate	83. Sodium Phosphates	84. Spectinomycin Sulfate
85. Streptozocin	86. Tenofovir Disoproxil Fumarate ***(Received)***	87. Tiludronate Disodium
88. Tiopronin	89. Trimetrexate Glucuronate	90. Venlafaxine Hydrochloride ***(Received)***
91. Voriconazole ***(Received)***	92. **Zaleplon** ***(Received)***	93. Zinc Tridosium Pentetate
94. Zoledronic Acid		

Small Molecules (Drug Products)—As of February 20, 2009

1. Abacavir Sulfate, Lamivudine, and Zidovudine Tablets	2. Acarbose Tablets	3. Acetaminophen, Butalbital, Caffeine, and Codeine Phosphate Capsules
4. Acetaminophen, Clemastine Fumarate and Pseudoephedrine Hydrochloride Tablets	5. Acetazolamide Extended-Release Capsules	6. Albuterol and Ipratropium Bromide Inhalation Aerosol
7. Albuterol and Ipratropium Bromide Inhalation Solution	8. Albuterol Extended-Release Tablets	9. Albuterol Inhalation Aerosol
10. Albuterol Sulfate Inhalation Solution	11. Albuterol Sulfate Oral Solution	12. Alendronate Sodium Oral Solution
13. Alfuzosin Extended-Release Tablets	14. Allopurinol for Injection	15. Alprazolam Extended-Release Tablets
16. Alprostadil Urethral Suppository	17. Aminopropazine Fumarate and Neomycin Sulfate Tablets	18. Aminopropazine Fumarate Injection
19. Aminopropazine Fumarate Tablets	20. Aminopterin Sodium Tablets	21. Amiodarone Hydrochloride Injection
22. Amlodipine and Benazepril Hydrochloride Capsules ***(Received)***	23. Amphotericin B Injection	24. Anagrelide Hydrochloride Capsules ***(Received)***
25. Arsenic Trioxide Injection	26. Atovaquone and Proguanil Hydrochloride Tablets	27. Atovaquone Tablets
28. Auranofin Capsules	29. Azatadine Maleate and Pseudoephedrine Sulfate Extended-Release Tablets	30. Azelaic Acid Cream
31. Azithromycin for Injection ***(Received)***	32. Azithromycin Tablets ***(Received)***	33. Baclofen Injection
34. Balsalazide Disodium Capsules ***(Received)***	35. Beclomethasone Dipropionate Inhalation Aerosol	36. Beclomethasone Dipropionate Nasal Suspension
37. Benazepril Hydrochloride and Hydrochlorothiazide Tablets	38. Bentoquatam Topical Suspension	39. Benzocaine and Cetylpyridinium Chloride Lozenges
40. Benzocaine and Menthol Lotion	41. Benzphetamine Hydrochloride Tablets	42. **Bivalirudin for Injection** ***(Received)***
43. Brompheniramine Maleate, Dextromethorphan Hydrobromide and Pseudoephedrine Hydrochloride Oral Solution	44. Budesonide Inhalation Aerosol	45. Bupivacaine and Lidocaine Hydrochlorides Injection

Small Molecules (Drug Products)—As of February 20, 2009 *(Continued)*

46. Buprenorphine Hydrochloride Injection	47. Butalbital and Acetaminophen Capsules	48. Butalbital and Acetaminophen Tablets
49. Calcipotriene Cream	50. Calcipotriene Ointment	51. Calcipotriene Topical Solution
52. Calcitriol Capsules	53. Calcitriol Oral Solution	54. Calcium Acetate Capsules
55. Calcium Trisodium Pentetate Injection	56. Calfactant Intratracheal Suspension	57. Carbidopa and Levodopa Tablets for Oral Suspension ***(Received)***
58. Carbidopa, Levidopa, and Entacapone Tablets	59. Carmustine Implant	60. Cefdinir Tablets
61. Cefditoren Pivoxil Tablets	62. Ceftibuten Capsules	63. Ceftibuten For Oral Suspension
64. Ceftiofur Hydrochloride Oral Suspension	65. Cetirizine Hydrochloride Tablets ***(Received)***	66. Cetrorelix Injection
67. Cevimeline Hydrochloride Capsules	68. Chloroxine Cream	69. Chlorpromazine Hydrochloride Extended-Release Capsules
70. Choline and Magnesium Salicylates Oral Solution	71. Choline and Magnesium Salicylates Tablets	72. Choline Salicylate Oral Solution ***(Received)***
73. Ciclopirox Shampoo	74. Ciclopirox Topical Gel	75. Ciclopirox Topical Solution ***(Received)***
76. Cimetidine Oral Solution	77. Ciprofloxacin Extended-Release Tablets	78. Ciprofloxacin Hydrochloride and Hydrocortisone Otic Suspension
79. Ciprofloxacin Otic Solution	80. Cisplatin Injection	81. Citalopram Hydrobromide Oral Solution
82. Citric Acid, Gluconolactone, and Magnesium Carbonate Irrigation	83. Cladribine Injection	84. Clemastine Fumarate Syrup
85. Clobetasol Propionate Gel	86. Clorazepate Dipotassium Capsules	87. Clorazepate Dipotassium Extended-Release Tablets
88. Clotrimazole and Betamethasone Dipropionate Lotion	89. Compound Undecylenic Acid Cream	90. Compound Undecylenic Acid Topical Powder
91. Conjugated Estrogens and Medroxyprogesterone Acetate Tablets	92. Cyclosporine Modified Capsules	93. Cyclosporine Modified Oral Solution
94. Cyclosporine Ointment	95. Cyclosporine Topical Solution	96. Cysteamine Bitartrate Capsules
97. Cytarabine Liposome Injection	98. Dalfopristin and Quinupristin Injection	99. Dantrolene Sodium Oral Suspension
100. Dapiprazole for Ophthalmic Solution	101. Desirudin for Injection	102. Desonide Cream
103. Dexrazoxane for Injection	104. Dextroamphetamine Sulfate Extended-Release Capsules	105. Dextromethorphan Polistirex Extended-Release Oral Suspension
106. Diazepam Injectable Emulsion	107. Diclofenac Sodium Ophthalmic Solution	108. Diethylpropion Hydrochloride Extended-Release Tablets
109. Difenoxin Hydrochloride and Atropine Sulfate Tablets	110. Difloxacin Hydrochloride Tablets	111. Dihydroergotamine Mesylate Metered Spray
112. Diltiazem Hydrochloride Injection	113. Dinoprostone Vaginal Suppositories	114. Diphenhydramine Hydrochloride and Acetaminophen Tablets
115. Divalproex Sodium Delayed-Release Capsules	116. Dorzolamide and Timolol Ophthalmic Solution	117. Dorzolamide Ophthalmic Solution
118. Doxepin Hydrochloride Cream	119. Doxycycline Oral Gel	120. Econazole Nitrate Cream
121. Edrophonium Chloride and Atropine Sulfate Injection	122. Enalapril Maleate and Felodipine Extended-Release Tablets	123. Entacapone Tablets ***(Received)***
124. Ephedrine Sulfate and Guaifenesin Tablets	125. Epirubicin Hydrochloride for Injection	126. Epirubicin Hydrochloride Injection
127. Epoprostenol for Injection	128. Epoprostenol Injection	129. Esmolol Hydrochloride Injection
130. Esomeprazole Magnesium Capsules	131. Estazolam Tablets ***(Received)***	132. Estramustine Phosphate Sodium Capsules
133. Ethanolamine Oleate Injection	134. Etidronate Disodium Injection Concentrate	135. **Etomidate Injection** ***(Received)***
136. Exemestane Tablets	137. Famotidine Orally Disintegrating Tablets	138. Felbamate Oral Suspension
139. Felbamate Tablets	140. Fentanyl Lozenges	141. Famciclovir Tablets
142. Fentanyl Transdermal System ***(Received)***	143. Ferrous Fumarate and Docusate Sodium Extended-Release Capsules	144. Fluconazole Oral Suspension
145. Flunisolide Inhalation Aerosol	146. Flunisolide Nasal Spray	147. Fluocinolone Acetonide Shampoo
148. Fluorescein Sodium Ophthalmic Solution	149. Fluorometholone Ointment	150. Fluticasone Propionate Inhalation Powder ***(Received)***

Small Molecules (Drug Products)—As of February 20, 2009 *(Continued)*

151. Fluticasone Propionate Pressurized Inhaler	152. Foscarnet Sodium Injection	153. Fosfomycin for Oral Solution
154. Gabapentin Oral Solution	155. Gadobenate Dimeglumine Injection	156. Gallium Nitrate Injection
157. Ganciclovir Capsules	158. Ganirelix Acetate Injection	159. Gatifloxacin Injection
160. Gatifloxacin Tablets	161. Gentamicin Sulfate Oral Solution	162. Gentamicin Sulfate Soluble Powder
163. Glipizide Extended-Release Tablets	164. Guaifenesin and Pseudoephedrine Hydrochloride Extended-Release Tablets	165. Guaifenesin and Salts of Dextromethorphan and Pseudoephedrine Oral Solution
166. Guanidine Hydrochloride Tablets	167. Halobetasol Propionate Cream	168. Halobetasol Propionate Ointment
169. Haloperidol Decanoate Injection	170. Haloperidol Lactate Injection	171. Haloperidol Lactate Oral Concentrate
172. Hydralazine Hydrochloride and Hydrochlorothiazide Capsules	173. Hydrochlorothiazide Capsules	174. Hydrochlorothiazide Oral Solution
175. Hydrocodone Bitartrate and Acetaminophen Capsules	176. Hydrocodone Bitartrate and Acetaminophen Oral Solution	177. Hydrocodone Bitartrate and Aspirin Tablets
178. Hydrocodone Bitartrate and Guaifenesin Oral Solution	179. Hydrocodone Bitartrate and Homatropine Methylbromide Syrup	180. Hydrocortisone Acetate Dental Paste
181. Hydrocortisone Acetate Rectal Foam Aerosol	182. Hydrocortisone Butyrate Lotion	183. Hydroflumethiazide and Reserpine Tablets
184. Hydromorphone Hydrochloride Oral Solution ***(Received)***	185. Hydroquinone Lotion	186. Ibandronate Sodium Tablets
187. Ibuprofen Capsules	188. Idarubicin Hydrochloride Injection	189. Imipramine Pamoate Capsules
190. Imiquimod Topical Cream	191. Ipratropium Bromide Inhalation Aerosol	192. Ipratropium Bromide Inhalation Solution
193. Irinotecan Hydrochloride Injection	194. Isosulfan Blue Injection	195. Isradipine Extended-Release Tablets
196. Itraconazole Injection	197. Itraconazole Oral Solution	198. Ketoconazole Cream
199. Ketoconazole Shampoo	200. Ketoprofen Capsules ***(Received)***	201. Ketoprofen Extended-Release Capsules
202. Ketoprofen Tablets	203. Ketotifen Fumarate Ophthalmic Solution	204. Lactic Acid Lotion
205. Lamotrigine Tablets ***(Received)***	206. Latanoprost Ophthalmic Solution	207. Leucovorin Calcium for Injection
208. Levetiracetam Tablets ***(Received)***	209. Levocabastine Ophthalmic Suspension	210. Levofloxacin Solution
211. Lincomycin Hydrochloride and Spectinomycin Sulfate Soluble Powder	212. Liothyronine Injection	213. Lomustine Capsules ***(Received)***
214. Lopinavir and Ritonavir Solution	215. Lopinavir Capsules	216. Lopinavir Solution
217. Melphalan for Injection	218. Mesalamine Suppositories	219. Mesoridazine Besylate Concentrate
220. Metaraminol Bitartrate Injection	221. Methacholine Chloride for Inhalation Solution	222. Methadone Hydrochloride Oral Concentrate
223. Methocarbamol and Aspirin Tablets	224. Methoxsalen Softgels	225. Methyclothiazide and Deserpidine Tablets
226. Methylphenidate Hydochloride Chewable Tablets	227. Metipranolol Ophthalmic Solution	228. Metronidazole Cream
229. Metronidazole Extended-Release Tablets	230. Metronidazole Hydrochloride for Injection	231. Metronidazole Lotion
232. Miconazole Nitrate Topical Aerosol	233. Mifepristone Tablets	234. Miglitol Tablets
235. Milrinone Injection	236. Misoprostol Tablets ***(Received)***	237. Moexipril Hydrochloride and Hydrochlorothiazide Tablets
238. Moexipril Hydrochloride Tablets	239. Molindone Hydrochloride Oral Solution	240. Morphine Sulfate for Injection Concentrate
241. Morphine Sulfate Oral Solution	242. Morphine Sulfate Oral Solution Concentrate	243. Morphine Sulfate Tablets
244. Nalbuphine Hydrochloride Injection	245. Mycophenolate Mofetil Oral Solution	246. Nalmefene Hydrochloride Injection
247. Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution	248. Naproxen Sodium Extended-Release Tablets	249. Nedocromil Sodium Inhalation Aerosol
250. Neomycin Sulfate Oral Powder	251. Nicardipine Hydrochloride Capsules	252. Nilutamide Tablets
253. Nimodipine Capsules	254. Nisoldipine Extended-Release Tablets	255. Nitroglycerin Solution In Acrylic Adhesive

Small Molecules (Drug Products)—As of February 20, 2009 *(Continued)*

256. Nitroglycerin Transdermal System	257. Nizatidine Tablets	258. Ofloxacin In Dextrose Injection
259. Ofloxacin Injection	260. Olsalazine Sodium Capsules	261. Orphenadrine Citrate Extended Release Tablets ***(Received)***
262. Orphenadrine Citrate, Aspirin, and Caffeine Tablets	263. Oxcarbazepine Suspension	264. Oxiconazole Cream
265. Pamidronate Disodium Injection	266. Pantoprazole Sodium for Injection	267. Pantoprazole Sodium Tablets
268. Paroxetine Hydrochloride Extended-Release Tablets	269. Paroxetine Oral Suspension	270. Pemirolast Potassium Ophthalmic Solution
271. Penicillin G Potassium Tablets for Oral Solution	272. Pentamidine Isethionate for Inhalation	273. Pentamidine Isethionate Injection ***(Received)***
274. Pentazocine Hydrochloride and Acetaminophen Tablets	275. Phendimetrazine Tartrate Extended-Release Capsules	276. Phenobarbital Capsules
277. Phentermine Resin Complex Capsules	278. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Capsules	279. Phenylephrine Hydrochloride, Chlorpheniramine Maleate, and Acetaminophen Extended-Release Tablets
280. Pilocarpine Hydrochloride Ophthalmic Gel	281. Pilocarpine Hydrochloride Ophthalmic Ointment	282. Pioglitazone Hydrochloride Tablets ***(Received)***
283. Piperonyl Butoxide and Pyrethrins Aerosol Foam	284. Pirbuterol Acetate Inhalation Aerosol	285. Poractant Alpha Suspension
286. Porfimer Sodium for Injection	287. Povacrylate Solution	288. Povacrylate–Iodine Topical Solution
289. Povidone–Iodine Gauze	290. Povidone-Iodine Swabsticks	291. Povidone–Iodine Topical Aerosol Foam
292. Povidone–Iodine Vaginal Suppositories	293. Pramipexole Dihydrochloride Tablets	294. Prednisolone Sodium Phosphate Oral Solution
295. Prochlorperazine Maleate Extended-Release Capsules	296. Progesterone Capsules	297. Propafenone Hydrochloride Tablets
298. Pseudoephedrine Hydrochloride and Brompheniramine Maleate Extended-Release Tablets	299. Pseudoephedrine Hydrochloride and Naproxen Sodium Extended-Release Tablets	300. Pseudoephedrine Hydrochloride, Chlorpheniramine Maleate, and Codeine Phosphate Oral Solution
301. Pseudoephedrine Hydrochloride, Guaifenesin, and Codeine Phosphate Oral Solution	302. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Extended-Release Tablets	303. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Oral Solution
304. Pseudoephedrine Sulfate, Dexbrompheniramine Maleate, and Acetaminophen Extended-Release Tablets	305. Pyrilamine Maleate Injection	306. Quinapril Hydrochloride and Hydrochlorothiazide Tablets
307. Quinidine Sulfate Injection	308. Ramipril Capsules ***(Received)***	309. Ranitidine Capsules
310. Rauwolfia Serpentina and Endroflumethiazide Tablets	311. Reserpine and Polythiazide Tablets	312. Rimantadine Hydrochloride Oral Solution
313. Risperidone Oral Solution ***(Received)***	314. **Risperidone Orally Disintegrating Tablets** ***(Received)***	315. Rivastigmine Tartrate Capsules ***(Received)***
316. Rivastigmine Tartrate Oral Solution ***(Received)***	317. Rocuronium Bromide Injection	318. Ropinirole Hydrochloride Tablets
319. Rosiglitazone Maleate Tablets	320. Salicylic Acid and Sulfur Cleansing Lotion	321. Salicylic Acid and Sulfur Lotion
322. Salicylic Acid and Sulfur Shampoo	323. Salicylic Acid Cream	324. Salicylic Acid Ointment
325. Salmeterol Inhalation Aerosol	326. Salmeterol Xinafoate Inhalation Powder	327. Scopolamine Transdermal System
328. Selegiline Hydrochloride Capsules	329. Sertraline Hydrochloride Oral Solution	330. Sibutramine Hydrochloride Capsules
331. Sodium Bicarbonate and Sodium Citrate for Oral Solution	332. Sodium Bicarbonate, Sodium Citrate, and Sodium Tartrate for Oral Suspension	333. Sodium Iodide Injection
334. Sodium Phenylbutyrate Oral Powder	335. Sodium Phenylbutyrate Tablets	336. Sodium Phosphates for Oral Suspension
337. Sodium Phosphates Tablets	338. Sodium Salicylate and Sulfur Shampoo	339. Sterile Talc Aerosol
340. Streptozocin for Injection	341. Sucralfate Oral Suspension	342. Sulconazole Nitrate Cream
343. Sulfacetamide Sodium and Fluorometholone Ophthalmic Suspension	344. Sulfacetamide Sodium and Prednisolone Sodium Phosphate Ophthalmic Solution	345. Sulfasalazine Oral Suspension
346. Sulisobenzone Lotion	347. Sumatriptan Injection	348. Sumatriptan Tablets ***(Received)***

Small Molecules (Drug Products)—As of February 20, 2009 *(Continued)*

349. Tacrolimus Injection	350. Tacrolimus Ointment	351. Technetium Tc 99m Teboroxime Injection
352. Tenofovir Disoproxil Fumarate Tablets ***(Received)***	353. Terazosin Capsules ***(Received)***	354. Terazosin Tablets ***(Received)***
355. Terbinafine Hydrochloride Cream	356. Terbinafine Tablets ***(Received)***	357. Terbinafine Topical Solution
358. Terconazole Vaginal Cream	359. Terconazole Vaginal Suppositories	360. Testosterone Transdermal Gel
361. Testosterone Transdermal System	362. Tetracycline Hydrochloride Periodontal Fiber	363. Theophylline Extended-Release Tablets
364. Tioconazole Vaginal Ointment	365. Tiopronin Tablets	366. Tolnaftate Topical Aerosol Solution
367. Topiramate Capsules ***(Received)***	368. Torsemide Injection	369. Torsemide Tablets ***(Received)***
370. Trandolapril and Verapamil Hydrochloride Extended-Release Tablets	371. Trandolapril Tablets	372. Tranexamic Acid Injection
373. Tretinoin Capsules	374. Tretinoin Microsphere Gel	375. Triamcinolone Acetonide Nasal Suspension
376. Trifluridine Ophthalmic Solution	377. Trimetrexate for Injection	378. Trimipramine Maleate Capsules
379. Triprolidine and Pseudoephedrine Hydrochlorides and Codeine Phosphate Syrup	380. Trolamine Salicylate Cream	381. Trolamine Salicylate Gel
382. Trolamine Salicylate Topical Emulsion	383. Undecylenic Acid Topical Foam Aerosol	384. Urea Cream
385. Vecuronium Bromide for Injection	386. Venlafaxine Extended-Release Capsules ***(Received)***	387. Venlafaxine Tablets ***(Received)***
388. Verapamil Hydrochloride Capsules	389. Verapamil Hydrochloride Extended-Release Capsules	390. Voriconazole Injection
391. Voriconazole Oral Suspension	392. Voriconazole Tablets	393. Yttrium Y-90 Chloride Solution
394. Yttrium Y-90 Glass Microspheres	395. Yttrium Y-90 Microspheres Injection	396. Zaleplon Capsules
397. Zidovudine and Lamivudine Tablets ***(Received)***	398. Zinc Acetate Capsules	399. Zinc Tridosium Pentetate Injection
400. Ziprasidone Hydrochloride Capsules	401. Zoledronic Acid for Injection	402. Zonisamide Capsules ***(Received)***

Excipients—As of February 20, 2009

1. Acetone Sodium Bisulfite	2. Acetylated Monoglycerides	3. Aconitic Acid (Achilleic Acid)
4. Acrylic Acid–Octyl Acrylate Copolymer	5. Albumin Colloidal	6. Aliphatic Polyesters
7. Allantoin–Sodium Pyrrolidone Carboxylate	8. Aluminum Ammonium Sulfate	9. Aluminum Lactate
10. Aluminum Oxide	11. Aluminum Potassium Sulfate	12. Aluminum Silicate
13. Aluminum Sodium Sulfate	14. Aluminum Stearate	15. Ammonium Bicarbonate
16. Ammonium Calcium Alginate	17. Ammonium Phosphate	18. Batylalcohol Monostearate
19. Beeswax, Synthetic	20. Benzododecinium Bromide	21. Benzyl Chloride
22. Benzyl Nicotinate	23. Beta Naphthol	24. Brominated Vegetable Oil
25. Butadiene–Styrene Rubber	26. Butyl Stearate ***(Received)***	27. Butylated Hydromethylphenol
28. Butylene Glycol	29. Butylphthalyl Butylglycolate	30. Calcium Acid Pyrophosphate
31. Calcium Alginate	32. Calcium Alginate and Ammonium Alginate	33. Calcium Bromide
34. Calcium Chloride Solution	35. Calcium Phosphate Monobasic	36. Calcium Propionate
37. Calcium Pyrophosphate	38. Calcium Sorbate	39. Calcium Stearoyl Lactylate
40. Caldiamide Sodium	41. Calteridol Calcium	42. Capric Acid
43. Caprylic/Capric Diglyceryl Succinate	44. Carbon	45. Carboxymethyl Starch
46. Carboxymethylamylopectin Sodium	47. Carboxymethylcellulose Potassium	48. Cetostearyl Isononanoate
49. Chlorodifluoroethane	50. Cholic Acid	51. Cinnamaldehyde
52. Cocamide Diethanolamine	53. Cocamide Oxide	54. Cocoyl Caprylocaprate
55. Crystal Gum	56. Cutina	57. Cystine
58. Dammar Gum	59. Decanoic Acid	60. Decyl Oleate

Excipients—As of February 20, 2009 *(Continued)*

61. Dextrin Palmitate	62. Dextrins Modified	63. Diacetyl Tartaric Acid Esters of Mono- and Diglycerides
64. Dicetyl Phosphate	65. Dichlorofluoromethane	66. Diethyl Sebacate ***(Received)***
67. Difluoroethane	68. Diglycol Stearate	69. Diisobutyl Adipate
70. Diisopropyl Adipate	71. Diisopropylbenzothiazyl-2-Sulfenamide	72. Dilauryl Thiodipropionate
73. Dimethyl Dicarbonate	74. Dimyristoyl Lecithin	75. Dimyristoyl Phosphatidylglycerol
76. Dipropylene Glycol	77. Disodium Edisylate	78. Disodium Guanylate
79. Disodium Inosinate	80. Disodium Monooleamide Sulfasuccinate	81. D-Mannose
82. Docusate Sodium/Sodium Benzoate	83. Erythrosine	84. Ethoxylated Mono- and Diglycerides
85. Ethoxyquin	86. Ethyl Hexanediol	87. Ethyl Linoleate
88. Ethyl Maltol ***(Received)***	89. Ethylene Dichloride	90. Ethylurea
91. Ferric Ammonium Citrate	92. Ferric Citrate	93. Ferric Oxide, Brown
94. Ferric Phosphate	95. Ferric Pyrophosphate	96. Ferrous Citrate
97. Ferrous Glycinate	98. Ferrous Lactate	99. Fluorochlorohydrocarbons
100. Formic Acid	101. Furcelleran	102. Gentistic Acid
103. Geraniol	104. Glutamic Acid Hydrochloride	105. Gluten
106. Glycerol Ester of Gum Rosin (Ester Gum)	107. Glyceryl Laurate	108. Glyceryl Palmitate
109. Glyceryl Ricinoleate	110. Glyceryl Tristearate	111. Glycine Hydrochloride
112. Glycofurol	113. Glycol Stearate	114. Heptafluoropropane
115. Heptylparaben	116. Hexadecyl Isostearate	117. Hexane
118. Hexanetriol(-1,2,6-)	119. Hydrocarbon Gel	120. Hydroxyethylmethylcellulose
121. Hydroxylated Lecithin	122. Indigotine	123. Iron Carbonyl
124. Iron Subcarbonate	125. Isobutylated-Isoprene Copolymer	126. Isooctylacrylate
127. Isopropyl Isostearate	128. Isopropyl Stearate	129. Isostearic Acid
130. Isostearyl Alcohol	131. Lactobionic Acid ***(Received)***	132. Lactose Ferrin, Bovine
133. Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol	134. Lactylic Esters of Fatty Acids	135. Lanolin (Wool Fat), Hydrogenated
136. Lanolin Alcohols, Acetylated	137. Lanolin Hydrous	138. L-Ascorbyl Stearate
139. Lauramine Oxide	140. Lauric Myristic Diethanolamide	141. Lauric Acid
142. Lauric Diethanolamide	143. Lavender Oil	144. L-Cysteine Monohydrochloride
145. Lecithin, Hydroxylated	146. L-Glutamic Acid ***(Received)***	147. Linoleic Acid ***(Received)***
148. L-Leucine	149. Macrogol Sorbitan Tristearate	150. Macrogolglycerol Cocoates
151. Macrogolglycerol Triisostearate	152. Magnesium Aluminum Silicate Hydrate	153. Magnesium Aspartame Dihydrate
154. Magnesium Aspartate	155. Magnesium Phosphate Tribasic	156. Magnesium Phosphate, Diabasic, Trihydrate
157. Magnesium Tartrate	158. Malt Syrup	159. Maltitol Syrup
160. Maltol Isobutyrate	161. Manganese Chloride	162. Manganese Citrate
163. Manganese Glycerophosphate	164. Manganese Hypophosphite	165. Medical Antifoam Emulsion C
166. Medronate Disodium	167. Medronic Acid	168. Methyl Chloride
169. Methylchloroisothiazolinone	170. Methylisothiazolinone	171. Microcrystalline Cellulose, Silicified ***(Received)***
172. Mineral Spirits	173. Monoisostearyl Glyceryl Ester	174. Monopotassium Glutamate Monohydrate
175. Monosodium Citrate	176. Mullein Leaf	177. Myristyl Gamma-Picolinium Chloride
178. Myristyl Lactate	179. *N,N*-Bis(2-Hydroxyethyl)Stearamide	180. *N*-Acetyl-L-Methionine
181. Naphtha	182. *N*-Methylpyrrolidone ***(Received)***	183. Non-Pareil Seeds
184. Nutmeg Oil	185. Octanoic Acid	186. Oxystearin
187. Pentasodium Triphosphate	188. Pentetate Calcium Trisodium	189. Pentetate Pentasodium
190. Phenprobamate	191. Phenylmercuric Acetate	192. Phenylmercuric Nitrate
193. Pine Oil	194. Polacrilin	195. Polyglycerol Esters of Fatty Acids

Excipients—As of February 20, 2009 *(Continued)*

196. Polyglycerol Polyricinoleic Acid	197. Polyoxyethylene Castor Oil (USP has 35)	198. Polyoxyl Stearate (USP has 40)
199. Polypropylene Oleate	200. Polypropylene Stearyl Ether	201. Polysorbate 65
202. Polyvinylacetal Diethylanoacetate	203. Polyvinylpolypyrrolidone	204. Polyvinylpyrrolidone Ethylcellulose
205. Potassium Acid Tartrate	206. Potassium Bromate	207. Potassium Carbonate Solution
208. Potassium Dichloroisocyanurate	209. Potassium Gibberellate	210. Potassium Glycerophospate
211. Potassium Iodate	212. Potassium Nitrite	213. Potassium Phosphate
214. Potassium Phosphate Tribasic	215. Potassium Polymetaphosphate	216. Potassium Pyrophosphate
217. Potassium Stearate	218. Potassium Sulfate	219. Potassium Sulfite
220. Potassium Tripolyphosphate	221. Propyl Propionate	222. Propylene Glycol Diacetate
223. Propylene Glycol Mono- and Di-esters	224. Rice Bran Wax	225. Rosin
226. Silicone	227. Sodium Acid Pyrophosphate	228. Sodium Aluminosilicate ***(Received)***
229. Sodium Aluminum Phosphate Acidic	230. Sodium Aluminum Phosphate Basic	231. Sodium Aspartate
232. Sodium Bisulfate	233. Sodium Bisulfite	234. Sodium Carbonate Hydrate
235. Sodium Carboxymethyl Betaglucan	236. Sodium Caseinate	237. Sodium Chlorate
238. Sodium Citrate, Dibasic	239. Sodium Citrate, Monobasic	240. Sodium Dehydroacetate
241. Sodium Diacetate	242. Sodium Erythorbate	243. Sodium Ferric Pyrophosphate
244. Sodium Ferrocyanide	245. Sodium Hypophosphite ***(Received)***	246. Sodium Laureth Sulfate
247. Sodium Lauroyl Sarcosinate	248. Sodium Lauryl Sulfoacetate	249. Sodium Magnesium Aluminosilicate
250. Sodium Magnesium Silicate	251. Sodium Malate	252. Sodium Metaphosphate, Insoluble
253. Sodium Metasilicate	254. Sodium Methylate	255. Sodium Polyphosphates Glassy
256. Sodium Potassium Tripolyphosphate	257. Sodium Pyrophosphate	258. Sodium Pyrrolidone Carboxylate
259. Sodium Sesquicarbonate	260. Sodium Sesquinoleate	261. Sodium Stearoyl Lactylate
262. Sodium Thiomalate	263. Sodium Trimetaphosphate	264. Sodium Trioleate
265. Sodium Tripolyphosphate	266. Soy Polysaccharides	267. Stannous Tartrate
268. Starch, Pregelatinized Corn	269. Starch, Pregelatinized Tapioca	270. Stearalkonium Chloride
271. Stearyl Citrate	272. Stearyl Monoglyceridyl Citrate	273. Succinylated Monoglycerides
274. Sucrose Acetate Isobutyrate	275. Sucrose Fatty Acid Esters	276. Sucrose Stearate ***(Received)***
277. Sugar Fruit Fine	278. Sulfobutyl Ether Beta Cyclodextrin ***(Received)***	279. Tallow
280. Tallow Glycerides	281. Tallow Oil	282. Tetrafluoroethane
283. Thioglycerol	284. Thyme Oil	285. Tribehenin
286. Tricetearet-4 Phosphate	287. Trichloroethylene	288. Trimyristin
289. Trisodium Citrate	290. Trolamine Lauryl Sulfate	291. Vegetable Oil
292. Wheat Flour	293. Wheat Germ Oil	294. Wheat Gluten ***(Received)***
295. Whey		

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:
- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become effective before the effective date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The effective date for these revisions is stated on the next page.)

Readers should review this section to determine if they are affected by any of the changes.

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

Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the issue of a given *PF* volume.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements,* that does not accurately reflect the intended official requirements of the Council of Experts. Beginning with *PF* 35(2), Errata will be published both in the *Pharmacopeial Forum* and on the usp.org website. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to *USP 32–NF 27*. The page number indicates where the item is found in *USP–NF.* Errata are updated as necessary in each *Pharmacopeial Forum* issue and monthly on the usp.org website. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF.* The list of Errata has been relocated to www.usp.org, where updates will be posted monthly.

INTERIM REVISION ANNOUNCEMENT to *USP 32* and to *NF 27*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

John W. Mauger, *Chair*
USP Board of Trustees

Roger L. Williams, M.D., *Executive Vice President, CEO,* and *Chairman, USP Council of Experts*

Darrell R. Abernethy, M.D., Ph.D., *Chief Science Officer*
William F. Koch, Ph.D., FACB, *Chief Metrology Officer*

Released May 1, 2009 **Official June 1, 2009**

All inquiries and comments regarding *USP 32* text and *NF 27* text should be addressed to the Executive Secretariat, *USP–NF*, 12601 Twinbrook Parkway, Rockville, MD 20852 (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 32* or *NF 27* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard.

USP 23-Epi-26-deoxyactein RS (January 1, 2009)
USP Actein RS (January 1, 2009)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 32* or *NF 27* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of February 6, 2009. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at http://www.uspcatalog.com.

USP Acarbose RS
USP Acarbose System Suitability Mixture RS
USP *S*-Adenosyl-L-homocysteine RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Fludeoxyglucose Related Compound B RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Alpha Lipoic Acid RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Propylene Glycol Dilaurate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin Related Compound A RS
USP Vasopressin RS

MONOGRAPHS (USP)

Amantadine Hydrochloride Capsules

Add the following:

•**Labeling—**When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.•$_3$

Change to read:

Dissolution •$_3$⟨711⟩•—

•TEST 1—*Procedure for a Pooled Sample*.•$_3$
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Internal standard solution—Dissolve an accurately weighed quantity of naphthalene in hexane to obtain a solution having a known concentration of about 0.054 mg per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Amantadine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.1 mg per mL. Pipet 15.0 mL of this solution into a 50-mL screw-capped test tube, add 5.0 mL of 5 N sodium hydroxide and 10.0 mL of *Internal standard solution*, and shake for 60 minutes. Collect the hexane layer.
Test solution—Filter 15.0 mL of the solution under test and place into a 50-mL screw-capped test tube. Pipet 5.0 mL of 5 N sodium hydroxide and 10.0 mL of the *Internal standard solution* into the test tube, and shake for 60 minutes. Collect the hexane layer (*Test solution*).
Chromatographic system—Proceed as directed in the *Assay*.
Procedure—Separately inject equal volumes (about 2.5 µL) of the *Standard solution* and the *Test solution*. Record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{10}H_{17}N \cdot HCl$ dissolved.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{17}N \cdot HCl$ is dissolved in 45 minutes.
•TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: water; 900 mL.
Apparatus 2: 75 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.QLA-LLC.com or www.tabletdissolution.com or www.labhut.com.]
Time: 45 minutes.
Standard stock solution—Dissolve an accurately weighed quantity of USP Amantadine Hydrochloride RS in *Medium* to obtain a solution with a final concentration of about 0.12 mg per mL.
Internal standard solution—Dissolve an accurately weighed quantity of naphthalene in hexanes to obtain a solution having a final concentration of about 0.06 mg per mL.
Working standard solution—Transfer 60.0 mL of the *Standard stock solution* to a 200-mL volumetric flask. Add 20 mL of 5 N sodium hydroxide and 40.0 mL of *Internal standard solution*. Shake the flask for approximately 10 minutes, and allow the layers to separate. Use the top layer for injection. The final concentration is about 0.18 mg per mL.
Test solution—Transfer 3.0 mL of the solution under test to a centrifuge tube. Add 1.0 mL of 5 N sodium hydroxide and 2.0 mL of *Internal standard solution*. Shake the tube for approximately 10 minutes, and allow the layers to separate. Use the top layer for injection.
Chromatographic system (see *Chromatography* ⟨621⟩)— The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m column that contains 0.25-µm film of phase G1. The oven temperature is set at 100° for 3 minutes, then increased at a rate of 10° per minute to 200°, and held at 200° for 2 minutes. The injector temperature is maintained at 250°, and the detector at 300°. The carrier gas is helium flowing at a rate of 1.4 mL per minute, and the split flow rate is 20 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between naphthalene and amantadine hydrochloride is not less than 2; the tailing factor for the amantadine hydrochloride peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 2 µL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for all the peaks. Calculate the percentage of $C_{10}H_{17}N \cdot HCl$ dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times L}$$

in which A_U is the ratio of the peak areas obtained from the *Test solution*; C_S is the concentration, in mg per mL, of amantadine hydrochloride in the *Standard stock solution*; A_S is the average ratio of the peak areas obtained from the *Working standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and *L* is the Capsule label claim in mg.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{10}H_{17}N \cdot HCl$ is dissolved in 45 minutes.•$_3$

Bupropion Hydrochloride Extended-Release Tablets

Change to read:

Dissolution ⟨711⟩—

FOR PRODUCTS LABELED FOR DOSING EVERY 12 HOURS—
TEST 1—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Times: 1, 4, and 8 hours.
Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm, using a 1.0-cm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 45%
4	between 60% and 85%
8	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring about 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding about 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5 ± 0.05); 900 mL, deaerated.
Apparatus 1: 50 rpm.

Times: 1, 2, 4, and 6 hours.

Determine the percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing the following method.

Buffer solution—Dissolve 3.45 g of monobasic sodium phosphate monohydrate in 996 mL of water, add 4.0 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 2.80 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (65 : 35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Bupropion Hydrochloride RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration similar to the one expected in the *Test solution.*

Test solution—Use portions of the solution under test, and pass through a 0.45-μm nylon filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 298-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of bupropion hydrochloride dissolved at each time point.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 50%
2	between 40% and 65%
4	between 65% and 90%
6	not less than 80%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3.*

Medium, Apparatus, and *Procedure*—Proceed as directed for *Test 1,* except using the wavelength of about 250 nm.

Times: 1, 2, 4, and 6 hours.

Tolerances: The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 30% and 55%
2	between 50% and 75%
4	between 70% and 90%
6	not less than 80%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5.*

Medium and *Procedure*—Proceed as directed for *Test 1.*

Apparatus—Proceed as directed for *Test 1,* except to use a 0.5-cm cell.

Times: 1, 3, and 6 hours.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 35% and 55%
3	between 65% and 85%
6	not less than 80%

•TEST 7—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7.*

Medium, Apparatus 1, and *Times*—Proceed as directed for *Test 2,* including the quantitative chromatographic method, but using as the *Mobile phase* a mixture of *Buffer solution* with methanol (55 : 45).

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 50%
2	between 45% and 70%
4	not less than 70%
6	not less than 80%

•5

FOR PRODUCTS LABELED FOR DOSING EVERY 24 HOURS—

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.

Apparatus 1: 75 rpm.

Time: 2, 4, 8, and 16 hours.

Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 252 nm, using a 1.0-mm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	not more than 20%
4	between 20% and 45%
8	between 65% and 90%
16	not less than 80%

TEST 6—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium and *Apparatus:* Proceed as directed for *Test 4*.

Times: 1, 2, 4, 8, and 12 hours.

Procedure—Determine the amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm, using a 1.0-cm cell, on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Bupropion Hydrochloride RS in the same *Medium.*

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 15% and 35%
2	between 25% and 50%
4	between 40% and 65%
8	between 65% and 90%
12	not less than 80%

•TEST 8—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 75 rpm.

Times: 2, 4, 8, and 16 hours.

Standard solution—

FOR TABLETS LABELED TO CONTAIN 150 MG—Prepare a solution containing about 0.1667 mg of USP Buproprion Hydrochloride RS per mL in *Medium*.

FOR TABLETS LABELED TO CONTAIN 300 MG—Prepare a solution containing about 0.3333 mg of USP Bupropion Hydrochloride RS per mL in *Medium*.

Test solution—Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 μm.

Procedure—Determine the percentage of bupropion hydrochloride dissolved by employing UV absorption at the wavelength of maximum absorbance at about 298 nm on portions of the *Test solution* in comparison with the *Standard solution*, using *Medium* as the blank.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	not more than 10%
4	between 10% and 35%
8	between 45% and 75%
16	not less than 80%

•3

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

***USP32–NF27* Page**	**Title**	**Section**	**Description**
900	*Description and Solubility*	*Citalopram Hydrobromide*	Line 2: Change ''Freely soluble in water, in alcohol, and in chloroform.'' to: Soluble in alcohol and sparingly soluble in water.
969	*Calcium and Vitamin D with Minerals Tablets*	*Assay for copper*	Line 3 under *Assay preparation*: Replace ''Proceed as directed for *Assay preparation* in the *Assay for calcium* under *Calcium with Vitamin D Tablets*, except to prepare the *Assay preparation* to contain 2 μg of copper per mL and to omit the use of the *Lanthanum chloride solution*.'' with: Heat for 6 to 12 hours in a muffle furnace maintained at about 550°, and cool. Add about 15 mL of hydrochloric acid, and boil gently on a hot plate or a steam bath for about 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask, rinsing the crucible with portions of 6 N hydrochloric acid. Dilute the contents of the flask with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute the filtrate quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution having a concentration of about 2 μg of copper per mL.
		Assay for manganese	Line 4 under *Assay preparation*: Replace ''Proceed as directed for *Assay preparation* in the *Assay for calcium* under *Calcium with Vitamin D Tablets*, except to prepare the *Assay preparation* to contain 1 μg of manganese per mL and to omit the use of the *Lanthanum chloride solution*.'' with: Heat for 6 to 12 hours in a muffle furnace maintained at about 550°, and cool. Add about 15 mL of hydrochloric acid, and boil gently on a hot plate or a steam bath for about 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask, rinsing the crucible with portions of 6 N hydrochloric acid. Dilute the contents of the flask with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute the filtrate quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution having a concentration of about 1 μg of manganese per mL.

***USP32–NF27* Page**	**Title**	**Section**	**Description**
		Assay for zinc	Line 3 under *Assay preparation*: Replace ''Proceed as directed for *Assay preparation* in the *Assay for calcium* under *Calcium with Vitamin D Tablets*, except to prepare the *Assay preparation* to contain 2 μg of zinc per mL and to omit the use of the *Lanthanum chloride solution*.'' with: Heat for 6 to 12 hours in a muffle furnace maintained at about 550°, and cool. Add about 15 mL of hydrochloric acid, and boil gently on a hot plate or a steam bath for about 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask, rinsing the crucible with portions of 6 N hydrochloric acid. Dilute the contents of the flask with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute the filtrate quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution having a concentration of about 2 μg of zinc per mL.
1069	*Saw Palmetto*	*Content of fatty acids*	Line 17 under *Procedure*: Change ''0.5% of linolenic acid,'' to: 0.05% of linolenic acid,
1620	*Bacitracin*	*Composition*	Line 3 under *Peak identification solution*: Change ''sodium edetate (pH adjusted to 7.0'' to: edetate disodium (pH adjusted to 7.0
1623	*Bacitracin Zinc*	*Composition*	Line 1 under *Diluent*: Change ''Dissolve 40 g of sodium edetate'' to: Dissolve 40 g of edetate disodium
1807	*Carprofen Tablets*	*Chromatographic purity*	Line 6 under *Procedure*: Change ''in which C_S is the concentration, in mg per mL,'' to: in which C_S is the concentration, in μg per mL,
1845	*Cefotaxime for Injection*	*Uniformity of dosage units*	Line 1: Change ''**Uniformity of dosage units** ⟨905⟩— *Procedure*—Perform the *Assay* on individual containers using *Assay preparation 2, Assay preparation 3,* or *Assay preparation 4*, as appropriate.'' to: **Uniformity of dosage units** ⟨905⟩—meets the requirements.
2352	*Fenofibrate Capsules*	*Assay*	Line 1 under *Buffer solution pH 2.9*: Change ''Dissolve 136 g'' to: Dissolve 136 mg
2434	*Fluticasone Propionate*	*Limit of acetone*	Line 3 under *Chromatographic system*: Change the specified column from ''G15.'' to: G16.
3139	*Ondansetron Hydrochloride Oral Suspension*	*Definition*	Row 2 in the table: Change ''Vehicle: a mixture of Vehicle for Oral Suspension, (regular or sugar-free), *NF*, and Vehicle for Oral Solution, *NF* (1:1),'' to: Vehicle: a mixture of Vehicle for Oral Suspension, *NF* and Vehicle for Oral Solution (regular or sugar-free), *NF* (1:1),
3871	*Sterile Water for Irrigation*	*Bacterial endotoxins* ⟨85⟩	Line 1: Change ''not more than 0.25 Endotoxin Unit per mL.'' to: It contains less than 0.25 USP Endotoxin Unit per mL.

***USP32–NF27* Page**	**Title**	**Section**	**Description**
First Supplement to USP32–NF27			
3924	⟨11⟩ *USP Reference Standards*	*USP Estradiol Related Compound B RS*	*USP Estradiol Related Compound B RS*, along with *USP Estradiol Related Compound C RS*, was intended to be published in the *First Supplement to USP32–NF27*, but did not appear. *USP Estradiol Related Compound B RS* is now being added to the *First Supplement to USP 32–NF 27*.
4059	*Fosinopril Sodium*	*Related Compounds*	*Table 1, Relative Retention Time* for *Impurity 1*, under TEST 1: Change ''0.12'' to: 0.53 *Table 1, Relative Retention Time* for *Impurity 2*, under TEST 1: Change ''0.24'' to: 0.67

PROPOSED INTERIM REVISION ANNOUNCEMENTS

This section includes proposals for *Interim Revision Announcements* (*IRAs*) that will be published as official *USP* or *NF* standards. There is a 60-day comment period for these proposals, beginning on the 15th of the first month of this *Pharmacopeial Forum*. The approved official text will be published in a future *Pharmacopeial Forum* and additionally in the ''New Official Text'' section of USP's web site (www.usp.org). Readers should review material in this section and provide comments to the Scientific Liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Expert Committee members can consider reader's input as they are deciding whether to advance standards to official status.

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

MONOGRAPHS (USP)

BRIEFING

Budesonide, *USP 32* page 1714. On the basis of comments received, it is proposed to make the following changes under the test for *Limit of 11-ketobudesonide*:

1. Remove the resolution requirements as part of the system suitability. Comments were received indicating that the resolution cannot be established due to the absence of signal for 14,15-dehydrobudesonide and 21-dehydrobudesonide.
2. Add a requirement that the *Mobile phase* be pre-heated in order to stabilize the retention times. Subject to consideration of comments received, it is proposed to implement this change via the *Fifth Interim Revision Announcement* pertaining to *USP 32–NF 27* in *PF* 35(5) [Sept.–Oct. 2009] with an official date of October 1, 2009. Comments regarding this proposal should be received by July 15, 2009.

(MD-PS: D. Vicchio) RTS—C43834

Change to read:

Limit of 11-ketobudesonide—

Buffer solution—Proceed as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and isopropanol (65 : 26 : 9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*

Test solution—Proceed as directed for the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 3.5-µm packing L1. The flow rate is about 1.5 mL per minute.

•Pre-heat the *Mobile phase* to 50° and•$_{5}$ maintain the column at 50°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.73 and 0.78, respectively, for the two epimers of 11-ketobudesonide, about 0.68 for 21-dehydrobudesonide, about 0.84 for 14,15-dehydrobudesonide, and 1.0 for the first eluted epimer of budesonide (epimer B). ~~; the resolution, *R,* between the first epimer of 11-ketobudesonide and 21-dehydrobudesonide is not less than 1.0 and between the second epimer of 11-ketobudesonide and 14,15-dehydrobudesonide is not less than 1.2; and~~

••$_{5}$ The column efficiency is not less than 5500 theoretical plates, determined from the budesonide epimer B peak.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of 11-ketobudesonide in the portion of Budesonide taken by the formula:

$$100(r_i / r_U)$$

in which r_i is the sum of the areas of the two ketobudesonide peaks; and r_U is the sum of the areas of the two budesonide peaks; not more than 0.2% of 11-ketobudesonide is found.

Change to read:

Related compounds—

Buffer solution and *Mobile phase*—Proceed as directed in the *Assay.*

Standard solution—Prepare as directed for the *Standard preparation* in the *Assay.*

Test solution—Proceed as directed for the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 5500 theoretical plates, determined from the budesonide epimer B peak.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Budesonide taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak area response for each impurity; and r_s is the sum of the responses of all the peaks: the impurities meet the requirements listed in the *Table* below.

Compound Name	Relative Retention Time	Limit (%)
16α-Hydroxylprednisolone[1]	0.11	0.2
D-Homobudesonide[2]	0.36	0.10
21-Dehydrobudesonide (epimers)[3]	0.61; 0.66	0.07
14,15-Dehydrobudesonide[4]	0.86	■*■$_{2S}$ *(USP32)* 0.10
Total specified impurities	—	0.4
Any other individual impurity	—	■^{5}■$_{2S}$ *(USP32)* 0.10
Total unspecified impurities	—	0.4

[1] 11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione.

[2] ~~16α,17-[(1*RS*)-ethylidenebis(oxo)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione~~ ■16α,17-[(1*RS*-Butylidenebis(oxy)-11b-hydroxy-17-(hydroxymethyl)-D-homoandrosta-1,4-diene-3,17α-dione.■$_{2S}$ *(USP32)*

[3] 16α,17-[(1*RS*)-Butylidenebis(oxo)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al.

■* Limit includes both epimers.■$_{2S}$ *(USP32)*

[4] 16α,17-[(1*RS*)-Butylidenebis(oxo)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione.

■^{5} Total specified impurities includes 11-ketobudesonide obtained in the test for *Limit of 11-ketobudenoside* and the impurities listed above.■$_{2S}$ *(USP32)*

Proposed IRA

BRIEFING

Nefazodone Hydrochloride, *USP 32* page 3043. On the basis of comments received, it is proposed to revise *Identification* test *B* to change the solvent from water to methanol because of the slightly soluble nature of the drug substance in water. Subject to consideration of comments received during the comment period, it is proposed to implement this revision via an *Interim Revision Announcement* pertaining to *USP 32–NF 27* in *PF* 35(5) [Sept.–Oct. 2009], with an official date of October 1, 2009. Comments regarding this proposal should be received by July 15, 2009.

(MD-PP: R. Ravichandran) RTS—C72911

Change to read:

Identification—

A: *Infrared Absorption* ⟨197K⟩.
B: A solution of 10 mg per mL •in methanol•$_S$ meets the requirements of the test for *Chloride* ⟨191⟩.

Proposed IRA

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) items that previously appeared under *Pharmacopeial Previews* and are now formally proposed as revisions, (2) proposed revisions placed directly under *In-Process Revision,* or (3) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

> **Name of Item**, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:
>
> (DSN: L. Evans.) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. New text (if any) follows, and is enclosed in symbols and set off from the current official text by a paragraph break and by larger type (print edition only), as shown in the examples below:

•new text•

if slated for an *Interim Revision Announcement to USP 30–NF 25* (*IRA*);

▲new text▲$_{USP31}$

if slated for *USP 31–NF 26*; and

■new text■

if slated for a *Supplement to USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as •• or ■■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •$_2$ indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S ($_{USP\ 30}$) indicates that the proposed revision is slated for the *Second Supplement to USP 30*, and ▲$_{USP31}$ and ▲$_{NF26}$ indicate that the revisions are proposed for *USP 31* and *NF 26*, respectively.

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

MONOGRAPHS (USP)

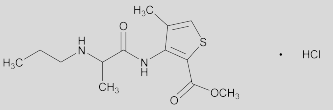
BRIEFING

Articaine Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on the current *European Pharmacopoeia*, version 6.2, 6th edition, is being proposed. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with a Symmetry C18, 4.6-mm × 25-cm, brand of L1 column. The typical retention time for articaine under these conditions is about 9.3 min.

(MD-PS: H. Ramanathan.) RTS—C57726

Add the following:

▪Articaine Hydrochloride



$C_{13}H_{20}N_2O_3S \cdot HCl$ 320.84

2-Thiophenecarboxylic acid, 4-methyl-3-[[1-oxo-2-(propylamino)propyl]amino]-, methyl ester, monohydrochloride;
Methyl 4-methyl-3-[2-(propylamino)propionamido]-2-thiophenecarboxylate, monohydrochloride [23964-57-0].

DEFINITION

Articaine Hydrochloride contains NLT 98.5% and NMT 101.0% of $C_{13}H_{20}N_2O_3 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197⟩**
  Standard solution: 12 mg/mL of USP Articaine RS in methylene chloride. Transfer 20 µL of this solution onto a 300-mg disk.
  Sample solution: Dissolve 100 mg of Articaine Hydrochloride in 5 mL of water. Add 3 mL of a saturated solution of sodium bicarbonate, and shake twice with 2 mL of methylene chloride. Combine the methylene chloride layers, dilute with methylene chloride to 5.0 mL, and dry over anhydrous sodium sulphate. Transfer 20 µL of this solution onto a 300-mg disk.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

- **PROCEDURE**
  Sample solution: 250 mg of Articaine Hydrochloride to a 250-mL conical flask. Add 5.0 mL of 0.01 M hydrochloride acid and 50 mL of alcohol. Stir to dissolve.
  Analysis: Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically, using a glass electrode. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 M sodium hydroxide is equivalent to 32.08 mg of $C_{13}H_{20}N_2O_3 \cdot HCl$.

Acceptance criteria: 98.5%–101.0%

IMPURITIES

Inorganic Impurities

- **HEAVY METALS,** *Method I* ⟨231⟩
  Sample solution: 200 mg/mL of Articaine Hydrochloride
  Acceptance criteria: NMT 5 ppm
- **RESIDUE ON IGNITION ⟨281⟩**
  Sample: 1 g
  Acceptance criteria: NMT 0.1%

Organic Impurities

- **PROCEDURE**
  Buffer solution: 2.02 g of sodium 1-heptanesulfonate and 4.08 g of potassium dihydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.0.
  Mobile phase: Acetonitrile and *Buffer solution* (1:3)
  System suitability solution: 1.0 mg/mL of USP Articaine Hydrochloride RS, 2 µg/mL of USP Articane Related Compound A RS, and 1 µg/mL of USP Articaine Related Compound E RS, in *Mobile phase*
  Standard solution: 2 µg/mL of USP Articaine Related Compound A , and 1 µg/mL each of USP Articaine Related Compound E RS and USP Articaine Hydrochloride RS in *Mobile phase.* [NOTE—This solution is also used to determine the reporting threshold limit.]
  Sample solution: 1.0 mg/mL of Articaine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩.)
  Mode: LC
  Detector: UV 276 nm
  Column: 4.6-mm × 25-cm column; packing L1
  Temperature: 45°
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 1.2 between articaine related compound A and articaine related compound E
  Analysis
  Samples: *Standard solution* and *Sample solution* [NOTE—Run time is 5 times the retention time of articaine.]
  Calculate the percentage of any articaine related compound A in the portion of Articaine Hydrochloride taken:

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

r_U = response of articaine related compound A from the *Sample solution*
r_S = response of articaine related compound A from the *Standard solution*
C_S = concentration of articaine related compound A in the *Standard solution* (mg/mL)
C_U = concentration of Articaine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each individual impurity in the portion of Articaine Hydrochloride taken:

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

r_U = response of each individual impurity from the *Sample solution*
r_S = response of articaine hydrochloride from the *Standard solution*
C_S = concentration of USP Articaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Articaine Hydrochloride in the *Sample solution* (mg/mL)

In-Process Revision

[NOTE— Disregard any peak below 0.05%.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine acid[1]	0.6	0.1
Ethylarticaine[2]	0.7	0.1
Articaine related compound A[3]	0.8	0.2
Articaine related compound E[4]	0.86	0.1
Articaine acid-propionamide[5]	0.9	0.1
Articaine	1.0	—
Butylarticaine[6]	1.7	0.1
Dipropylarticaine[7]	2.1	0.1
3-Aminoarticaine[8]	2.6	0.1
Articaine isopropyl ester[9]	3.6	0.1
Bromo compound[10]	4.0	0.1
Any other individual impurity	—	0.1

[1]4-Methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid.
[2]Methyl 3-[[(2*RS*)-2-(ethylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[3]Methyl 4-methyl-3-[2-(propylamino)acetamido]thiophene-2-carboxylate.
[4]Methyl 3-[2-(isopropylamino)propanamido]-4-methylthiophene-2-carboxylate.
[5]4-Methyl-*N*-propyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxamide.
[6]Methyl 3-[[(2*RS*)-2-(butylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[7]Methyl 3-[[(2*RS*)-2-(dipropylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[8]Methyl 3-amino-4-methylthiophene-2-carboxylate.
[9]1-Methylethyl 4-methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]-thiophene-2-carboxylate.
[10]Methyl 3-[[(2*RS*)-2-bromopropanoyl]amino]-4-methylthiophene-2-carboxylate.

SPECIFIC TESTS
- **LOSS ON DRYING ⟨731⟩:** Dry at 105° for 5 h: it loses NMT 0.5% of its weight.
- **pH ⟨791⟩**
  Sample solution: 10 mg/mL
  Acceptance criteria: 4.2–5.2

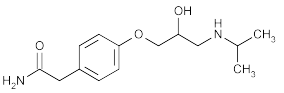
ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Articaine RS
  USP Articaine Hydrochloride RS
  USP Articaine Related Compound A RS
  USP Articaine Related Compound E RS■1S *(USP33)*

BRIEFING

Atenolol, *USP 32* page 1596. On the basis of comments received, it is proposed to revise the concentration of the *Sample solution* in *Identification* test *B*. The absorption of the sample is observed to be very high at the currently used concentration of 50 µg/mL.

(MD-CV: S. Ramakrishna.) RTS—C73266

Atenolol



$C_{14}H_{22}N_2O_3$ 266.34
Benzeneacetamide, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-;
2-[*p*-[2-Hydroxy-3-(isopropylamino)propoxy]-phenyl]acetamide [29122-68-7].

DEFINITION
Atenolol contains NLT 98.0% and NMT 102.0% of $C_{14}H_{22}N_2O_3$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**

Change to read:

- **B. ULTRAVIOLET ABSORPTION ⟨197U⟩**
  Sample solution: ~~50 µg/mL~~■20 µg/mL■1S *(USP33)* in methanol

ASSAY
- **PROCEDURE**
  Mobile phase: 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, mix, and pass through a filter having a 0.5-µm or finer porosity. Degas this solution before use.
  Standard solution: 0.01 mg/mL of USP Atenolol RS in *Mobile phase*
  Sample solution: 0.01 mg/mL of Atenolol in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 226 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 0.6 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 5000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{22}N_2O_3$ in the portion of Atenolol taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

In-Process Revision

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

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **CHLORIDE AND SULFATE,** *Chloride* **⟨221⟩**

Sample solution: 10 mg/mL in 0.15 N nitric acid, made to 100 mL

Acceptance criteria: Shows no more turbidity with 1 mL of silver nitrate TS than 1.4 mL of 0.020 N hydrochloric acid in 100 mL of 0.15 N nitric acid (0.1%)

Organic Impurities

- **PROCEDURE**

Mobile phase: Prepare as directed in the *Assay*.

Sample solution 1: 0.1 mg/mL of Atenolol in *Mobile phase*

Sample solution 2: 0.005 mg/mL Atenolol, made from *Sample solution 1* diluted with *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except use the injection size listed below.

Injection size: 50 µL

Analysis

Samples: *Sample solution 1* and *Sample solution 2*

[NOTE—Chromatograph *Sample solution 1* for a period of time that is 6 times the retention time of the atenolol peak.]

Calculate the percentage of each impurity observed in the chromatogram obtained from the *Sample solution 1*:

$$\text{Result} = 0.5(r_i/r_A)$$

r_i = peak response of any individual impurity in *Sample solution 1*

r_A = peak response of Atenolol in *Sample solution 2*

Acceptance criteria

Individual impurities: NMT 0.25%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE,** *Class I* **⟨741⟩:** 152°–156.5°
- **LOSS ON DRYING ⟨731⟩:** Dry it at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Atenolol RS

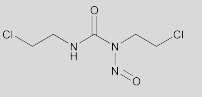
BRIEFING

Carmustine. Because there is no existing *USP* monograph for this drug substance, a new monograph based on the validated methods of analysis is being proposed. The liquid chromatographic procedures used in the test for *Procedure 2: Limit of Carmustine Related Compound A* and in the *Assay* are based on analyses performed with a Waters Spherisorb ODS2, 5-µm brand of L1 column. The typical retention times reported for carmustine related compound A and carmustine are about 2 and 8 min, respectively. The thin-layer chromatographic procedure in the test for *Procedure 3: Limit of 2-Chloroethylamine* is based on the analysis performed with E. Merck analytical glass plate coated with silica gel 60. The typical R_f values for 2-chloroethylamine monohydrochloride and carmustine are about 0.2 and 0.9, respectively. The gas chromatographic procedure in the test for the *Procedure 4: Limit of 2-Chloroethanol* is based on analysis performed with the J & W DB-WAX brand of G14 column. The typical retention time reported for 2-chloroethanol is about 15 min. The gas chromatographic procedure in the test for the *Procedure 5: Limit of Acetaldehyde* is based on analysis performed with the J & W DB-1 brand of G1 column. The typical retention time reported for acetaldehyde is about 15 min.

(MD-OOD: F. Mao.) RTS—C44603

Add the following:

▪Carmustine



$C_5H_9Cl_2N_3O_2$ 214.05

Urea, *N*,*N*′-bis(2-chloroethyl)-*N*-nitroso-;
1,3-Bis(2-chloroethyl)-1-nitrosourea [154-93-8].

DEFINITION

Carmustine contains NLT 98.0% and NMT 102.0% of $C_5H_9Cl_2N_3O_2$, calculated on the anhydrous and solvent-free basis. [**CAUTION—**Use disposable latex surgical gloves, arm covers, and a dusk mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197F⟩**

Sample: Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set temperature between 33° and 40°.

Standard: A similar preparation of USP Carmustine RS

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Prepare solutions in low-actinic glassware and keep them refrigerated until use.]

Mobile phase: Acetonitrile and water (3:7)

Standard solution: 1.5 mg/mL of USP Carmustine RS in acetonitrile

Sample solution: 1.5 mg/mL of Carmustine in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Refrigerated autosampler temperature: 4°–5°

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.9

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_5H_9Cl_2N_3O_2$ in the portion of Carmustine taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Carmustine RS in the *Standard solution* (mg/mL)

In-Process Revision

C_U = nominal concentration of Carmustine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

• **PROCEDURE 1: LIMIT OF ETHER INSOLUBLE SUBSTANCES**

[NOTE—Perform in a well-ventilated fume hood.]

Analysis: Transfer 1.0 g of sample to a suitable container containing 10 mL of anhydrous ether, stir for 5 min, and immediately filter through a tared glass filtering crucible of medium porosity. Wash the container with an additional 10 mL of ether, and filter through the same glass filtering crucible. Dry the crucible at 105° for 1 h. Cool in a desiccator and weigh.

Acceptance criteria: The weight of residue does not exceed 0.1%.

• **PROCEDURE 2: LIMIT OF CARMUSTINE RELATED COMPOUND A**

[NOTE—Prepare solutions in low-actinic glassware and keep them refrigerated until use.]

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed under *Assay*.

Carmustine standard solution: Use *Standard solution* under *Assay*.

Standard stock solution: 0.75 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

Standard solution: 0.0075 mg/mL of USP Carmustine Related Compound A RS in acetonitrile, from *Standard stock solution*

System suitability solution: 0.75 g/mL of USP Carmustine Related Compound A RS in acetonitrile, from *Standard solution*

Resolution solution: Transfer 5.0 mL of *Carmustine standard solution* and 10.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 5.0 mL of this solution into a 50-mL volumetric flask, and dilute with acetonitrile to volume.

System suitability

Samples: *Resolution solution*, *Carmustine standard solution*, and *System suitability solution*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10 between carmustine related compound A and carmustine, *Resolution solution*

Relative standard deviation: NMT 5%, *System suitability solution*

Tailing factor: NMT 1.9, *Carmustine standard solution*

Analysis

[NOTE—Run *Sample solution* at least 1.5 times of carmustine retention time.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine taken:

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

r_U = peak response of carmustine related compound A from the *Sample solution*

r_S = peak response of carmustine related compound A from the *Standard solution*

C_S = concentration of carmustine related compound A in the *Standard solution* (mg/mL)

C_U = nominal concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria

Carmustine related compound A: NMT 0.5%.

Any unspecified impurity: NMT 0.1%

• **PROCEDURE 3: LIMIT OF 2-CHLOROETHYLAMINE**

[NOTE—Prepare solutions in low-actinic glassware and keep them refrigerated until use.]

Standard solution 1 (0.2%): 1.2 mg/mL of 2-chloroethylamine monohydrochloride in methanol. [NOTE—1.2 mg/mL of 2-chloroethylamine monohydrochloride is equivalent to 0.8 mg/mL of 2-chloroethylamine.]

Standard solution 2 (0.1%): 0.4 mg/mL of USP Carmustine RS in methanol

Sample solution: 0.4 g/mL of Carmustine in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic plate (20 cm x 20 cm) coated with silica gel 60

Application volume: 1 µL

Developing solvent system 1: Ethyl acetate

Developing solvent system 2: Ethyl acetate and methanol (7:3)

Spray reagent 1: Diethylamine

Spray reagent 2: 0.1 N silver nitrate solution

Analysis

Samples: *Standard solution 1 (0.2%)*, *Standard solution 2 (0.1%)*, and *Sample solution*

Develop with *Developing solvent system 1* for 27 min, followed by air drying for 5 min. Develop again in *Developing solvent system 2* for 8 min, following by air drying for 10 min. Spray the plate with *Spray reagent 1*, and heat the plate for 20 min in an oven at 100°. Allow the plates to cool to the room temperature, and spray the plate with *Spray reagent 2*. Allow the plate to be exposed to the UV light at 365 nm for 15 min. Examine the plate under UV light.

Acceptance criteria

2-Chloroethylamine: The spot of 2-chloroethylamine in the *Sample solution* is not more intense than the principal spot obtained from *Standard solution 1 (0.2%)*.

Any unspecified impurity: Any spot if present in the chromatogram from the *Sample solution*, except the principle spot of Carmustine and spot of 2-chloroethylamine, is not more intense than the principal spot obtained from *Standard solution 2 (0.1%)*.

• **PROCEDURE 4: LIMIT OF 2-CHLOROETHANOL**

Standard solution: 0.02 mg/L of 2-chloroethanol in acetonitrile

System suitability solution: 0.01 mg/L of 2-chloroethanol in acetonitrile, diluted from *Standard solution*

Sample solution: 10 mg/mL of Carmustine in acetonitrile

[NOTE—Prepare in low-actinic glassware and keep refrigerated until use.]

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 1-µm film of phase G14

Column temperature: See the column temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	80	14
80	30	200	3

In-Process Revision

Injection port temperature: 90°
Detector temperature: 260°
Carrier gas: Helium
Flow rate: 7 mL/min
Injection size: 5 µL
Run time: 1.5 times of carmustine retention time
System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 2-chloroethanol in the portion of Carmustine taken:

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

r_U = peak response of 2-chloroethanol from the *Sample solution*
r_S = peak response of 2-chloroethanol from the *Standard solution*
C_S = concentration of 2-chloroethanol in the *Standard solution* (mg/mL)
C_U = nominal concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria
2-Chloroethanol: NMT 0.1%
Any unspecified impurity: NMT 0.1%

- **PROCEDURE 5: LIMIT OF ACETALDEHYDE**

Standard solution: 10 µg/mL of acetaldehyde in acetonitrile
Sample solution: 10 mg/mL of Carmustine in acetonitrile [NOTE—Prepare in low-actinic glassware and keep refrigerated until use.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 30-m × 0.53-mm column bonded with a 5-µm film of phase G1
Column temperature: See the column temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	210	3

Injection port temperature: 70°
Injector split ratio: 15:1
Detector temperature: 260°
Carrier gas: Helium
Flow rate: 3 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetaldehyde in the portion of Carmustine taken:

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

r_U = peak response of acetaldehyde from the *Sample solution*
r_S = peak response of acetaldehyde from the *Standard solution*
C_S = concentration of acetaldehyde in the *Standard solution* (mg/mL)
C_U = nominal concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria
Acetaldehyde: NMT 0.1%
Any unspecified impurity: NMT 0.1%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature between 2° and 8°.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Carmustine RS
USP Carmustine Related Compound A RS■1S *(USP33)*

BRIEFING

Carmustine for Injection. Because there is no existing *USP* monograph for this dosage form, a new monograph based on the validated methods of analysis is being proposed. The liquid chromatographic procedures used in the test for *Limit of Carmustine Related Compound A* and the *Assay* are based on analyses performed with a Beckman Ultrasphere C-18, 3-µm brand of L1 column. The typical retention times reported for carmustine related compound A and carmustine are about 3 and 6 min, respectively.

(MDOOD: F. Mao. MSA: R. Tirumalai.) RTS—C44782

Add the following:

■Carmustine for Injection

DEFINITION

Carmustine for Injection is a sterile lyophilized preparation of carmustine. It contains NLT 90.0% and NMT 110.0% of labeled amount of $C_5H_9Cl_2N_3O_2$.
[**CAUTION—**Use disposable latex surgical gloves, arm covers, and a dusk mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197F**⟩

Sample: Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

In-Process Revision

Standard: A similar preparation of USP Carmustin RS

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Mobile phase: See the gradient table below.

Time (min)	Water (%)	Acetonitrile (%)
0	90	10
2	90	10
6.5	40	60
8	90	10
10	90	10

Diluent: Acetonitrile and water (1:3)

Standard stock solution: 2.0 mg/mL of USP Carmustine RS in acetonitrile

Standard solution: 0.2 mg/mL of USP Carmustine RS in *Diluent*, from *Standard stock solution*

Impurity standard stock solution: 0.1 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

System suitability solution: 0.2 mg/mL of USP Carmustine RS and 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from *Standard stock solution* and *Impurity standard stock solution*

Sample stock solution: 2.0 mg/mL of carmustine in acetonitrile [NOTE—Allow test vials to warm to room temperature in a desiccator for 1 h.]

Sample solution: 0.2 mg/mL of carmustine in *Diluent*, diluted from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Refrigerated autosampler temperature: 5°

Column: 4.6-mm × 7.5-cm column; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between carmustine related compound A and carmustine

Relative standard deviation: NMT 2.0% for carmustine related compound A and carmustine peaks

Tailing factor: NMT 1.5 for carmustine related compound A and carmustine peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_5H_9Cl_2N_3O_2$ in the portion of Carmustine for Injection taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of carmustine in the *Standard solution* (mg/mL)
C_U = nominal concentration of carmustine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE: LIMIT OF CARMUSTINE RELATED COMPOUND A**

Diluent, Impurity standard stock solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed under *Assay*.

Standard solution: 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, diluted from *Impurity standard stock solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine for Injection taken:

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

r_U = peak response of carmustine related compound A from the *Sample solution*
r_S = peak response of carmustine related compound A from the *Standard solution*
C_S = concentration of carmustine related compound A in the *Standard solution* (mg/mL)
C_U = concentration of carmustine in the *Sample solution* (mg/mL) [NOTE—Concentration of carmustine is the nominal concentration, based on the label claim and mutiplied by assay, in percent, obtained under *Assay*.]

Acceptance criteria

Carmustine related compound A: NMT 0.5%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 0.95 USP Endotoxin Unit/mg of carmustine

• **STERILITY TESTS ⟨71⟩:** Meets the requirements

• **pH ⟨791⟩:** Between 4.0 and 6.8 in a constituted solution prepared as directed in the labeling

• **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩ at a temperature between 2° and 8°.

• **LABELING:** It meets the requirements for *Labeling* under *Injections* ⟨1⟩.

• **CONSTITUTED SOLUTION:** At time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Carmustine RS
USP Carmustine Related Compound A RS■1S *(USP33)*

BRIEFING

Dronabinol Capsules, *USP 32* page 2218. Because of the unavailability of the USP Δ^8-Tetrahydrocannabinol RS, it is proposed to replace it with the commercially available reagent-grade Δ^8-tetrahydrocannabinol. This change affects the *System suitability solution* in the *Assay*. It is also proposed to add a clarification to the *Definition*.

(MD-GRE: E. Gonikberg.) RTS—C72721

In-Process Revision

Dronabinol Capsules

DEFINITION

Change to read:

Dronabinol Capsules contain dronabinol in sesame oil. Dronabinol Capsules contain NLT 90.0% and NMT 110.0% of ■the labeled amount of■1S *(USP33)* dronabinol ($C_{21}H_{30}O_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (71:5:24)

■**System suitability stock solution:** 1.0 mg/mL of Δ^8-tetrahydrocannabinol in methanol■1S *(USP33)*

System suitability solution: 0.5 mg/mL of USP Δ^9-Tetrahydrocannabinol RS and 0.5 mg/mL of ~~USP Δ^8-Tetrahydrocannabinol RS in dehydrated alcohol~~■Δ^8-tetrahydrocannabinol. Mix equal volumes of USP Δ^9-Tetrahydrocannabinol RS and *System suitability stock solution.*■1S *(USP33)*

Standard solution: 0.2 mg/mL of USP Δ^9-Tetrahydrocannabinol RS in dehydrated alcohol

Sample solution: Equivalent to 0.2 mg/mL of dronabinol, from Capsule contents (NLT 20) in dehydrated alcohol

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Guard column: 4.6-mm × 30-mm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

[NOTE—The relative retention times for Δ^9-tetrahydrocannabinol and Δ^8-tetrahydrocannabinol are about 1.0 and 1.14, respectively.]

Suitability requirements

Sample: *System suitability solution* and *Standard solution*

Resolution: NLT 2.0 between dronabinol and Δ^8-tetrahydrocannabinol, *System suitability solution*

Tailing factor: NMT 2.0 of Δ^9-tetrahydrocannabinol, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{30}O_2$ in the portion of Capsules taken:

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

r_U = peak response of dronabinol from the *Sample solution*

r_S = peak response of dronabinol from the *Standard solution*

C_S = concentration of USP Δ^9-Tetrahydrocannabinol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dronabinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in NLT 15 but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in NLT 15 but NMT 30 min.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, in a cool place.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Δ^9-Tetrahydrocannabinol RS

~~USP Δ^8-Tetrahydrocannabinol RS~~

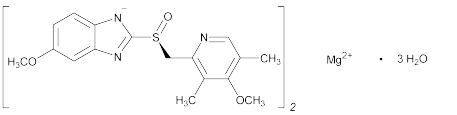
■1S *(USP33)*

BRIEFING

Esomeprazole Magnesium, *USP 32* page 2298. On the basis of comments received, it is proposed to delete the test for *Optical Rotation, Specific Rotation*. This test is redundant and lacks the specificity of the chromatographic methods, and the chiral purity of the Esomeprazole Magnesium is already controlled by the test for *Procedure 2: Enantiomeric Purity.* It is also proposed to correct the description of the analytical wavelength in *Identification* test *B* and in the test for *Content of Magnesium*.

(MD-GRE: E. Gonikberg.) RTS—C72585

Esomeprazole Magnesium



$C_{34}H_{36}MgN_6O_6S_2 \cdot 3H_2O$ Trihydrate: 767.17

$C_{34}H_{36}MgN_6O_6S_2$ Anhydrous: 713.12

1*H*-Benzimidazole,5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl], magnesium salt (2:1), trihydrate;

5-Methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl]benzimidazole, magnesium salt (2:1), trihydrate [217087-09-7].

DEFINITION

Esomeprazole Magnesium contains NLT 98.0% and NMT 102.0% of $C_{34}H_{36}MgN_6O_6S_2$, calculated on the anhydrous basis.

In-Process Revision

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Change to read:

• **B.** The *Sample solution*, prepared and tested as directed in the test for *Content of Magnesium*, exhibits a significant absorption ~~at the magnesium emission line~~■1S *(USP33)* at 285.2 nm.

ASSAY

• **PROCEDURE**

Solution A: 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Solution B: Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

Mobile phase: Acetonitrile and *Solution A* (7:13)

Standard solution: 10 mg of USP Omeprazole RS to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [NOTE—This solution contains 0.05 mg/mL of omeprazole.]

Sample solution: 10 mg of Esomeprazole Magnesium to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [NOTE—This solution contains 0.05 mg/mL of esomeprazole magnesium.]

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L7. [NOTE—Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.]

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{34}H_{36}MgN_6O_6S_2$ in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M_{r1}/(2 \times M_{r2})] \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of omeprazole in the *Standard solution* (mg/mL)
C_U = concentration of Esomeprazole Magnesium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of esomeprazole magnesium, 713.12
M_{r2} = molecular weight of omeprazole, 345.42

Acceptance criteria: 98.0%–102.0%

OTHER COMPONENTS

Change to read:

• **CONTENT OF MAGNESIUM**

Solution A: 58.7 g of lanthanum oxide into a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between the additions. Add water while stirring, cool to room temperature, and dilute with water to volume. [NOTE—Store the solution in a plastic bottle.]

Standard stock solution: 1000 µg/mL of magnesium, from a commercially prepared atomic absorption standard solution. [NOTE—Store the solution in a plastic bottle.]

Standard solution A: Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. [NOTE—This solution contains 2 µg/mL of magnesium.]

Standard solution B: Combine 5.0 mL of *Standard solution A* and 4.0 mL of *Solution A*, and dilute with water to 100.0 mL (0.1 µg/mL).

Standard solution C: Combine 10.0 mL of *Standard solution A* and 4.0 mL of *Solution A*, and dilute with water to 100.0 mL (0.2 µg/mL).

Standard solution D: Combine 15.0 mL of *Standard solution A* and 4.0 mL of *Solution A*, and dilute with water to 100.0 mL (0.3 µg/mL).

Standard solution E: Combine 20.0 mL of *Standard solution A* and 4.0 mL of *Solution A*, and dilute with water to 100.0 mL (0.4 µg/mL).

Standard solution F: Combine 25.0 mL of *Standard solution A* and 4.0 mL of *Solution A*, and dilute with water to 100.0 mL (0.5 µg/mL).

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of *Standard solutions* can be reduced.]

Blank solution: Transfer 4.0 mL of *Solution A* to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: 250 mg of Esomeprazole Magnesium to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 min. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution so obtained to another 100-mL volumetric flask, add 4.0 mL of *Solution A*, and dilute with water to volume.

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: Atomic absorption spectrophotometer using an air–acetylene flame

Analytical wavelength: 285.2 nm ~~at the magnesium emission line~~■1S *(USP33)*

Analysis

Samples: *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, *Standard solution F*, *Blank solution*, and *Sample solution*

Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of magnesium, and draw the straight line between the plotted points. From the graph so obtained, determine the concentration, C_S, in µg/mL, of magnesium in the *Sample solution*.

Calculate the content of magnesium (%) in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (C_S/C_U) \times (100/(100 - F)) \times 100$$

C_S = content of magnesium in the *Sample solution* as calculated above (µg/mL)
C_U = concentration of Esomeprazole Magnesium in the *Sample solution* (µg/mL)
F = content of water in Esomeprazole Magnesium, as determined in *Specific Tests, Water Determination* (%)

Acceptance criteria: 3.30%–3.55%

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

Solution A: 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL

In-Process Revision

of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.
Mobile phase: Acetonitrile and *Solution A* (27.5:72.5) [NOTE—To improve the resolution, the composition may be changed to 25:75, if necessary.]
System suitability solution: 1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A RS in 25 mL of *Mobile phase*. [NOTE—Omeprazole related compound A is omeprazole sulfone.]
Sample solution: 4 mg of Esomeprazole Magnesium in 25 mL of *Mobile phase*. [NOTE—Prepare this solution fresh.]
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L7. [NOTE—Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.]
Flow rate: 0.8–1 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—For relative retention times, see *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Analysis
Sample: *Sample solution*
Record the chromatogram for at least 4.5 times the retention time of the omeprazole peak, and measure the peak responses. Identify the impurities based on the relative retention times shown in *Impurity Table 1*.
Calculate the percentage of any individual impurity in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all peak responses
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole *N*-oxide[a]	0.45	0.1
Omeprazole sulfone[b] (related compound A)	0.8	0.2
Any other individual impurities	—	0.1
Omeprazole	1.0	—

[a]4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.
[b]5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.

- **PROCEDURE 2: ENANTIOMERIC PURITY**
Solution A: Mix 70 mL of 1 M monobasic sodium phosphate with 20 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL.
Diluent: Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (3:17)
System suitability solution: 2 mg of USP Omeprazole RS in 10 mL of *Diluent*. Dilute 1.0 mL of this solution with *Diluent* to 50 mL.
Sample solution: 40 mg of Esomeprazole Magnesium in 5 mL of methanol, and dilute with *Diluent* to 25 mL. Dilute 1 mL of this solution with *Diluent* to 50 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.0-mm × 10-cm; packing L41
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3 between the enantiomer peaks
[NOTE—The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]
Analysis
Sample: *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for the *R*-enantiomer
r_T = sum of the responses of both the esomeprazole and *R*-enantiomer peaks
Acceptance criteria: NMT 0.2% of the *R*-enantiomer is found.

SPECIFIC TESTS

Delete the following:

~~• **OPTICAL ROTATION,** *Specific Rotation* ⟨**7815**⟩~~
~~**Sample solution:** 10 mg/mL, in methanol~~
~~**Acceptance criteria:** −137° to −142°, at 20°~~ ■1S (*USP33*)

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** 6.0%–8.0%
- **COLOR OF SOLUTION**
Sample solution: 20 mg/mL of Esomeprazole Magnesium in methanol, and filter
Analysis: Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank.
Acceptance criteria: NMT 0.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Esomeprazole Magnesium RS
USP Omeprazole RS
USP Omeprazole Related Compound A RS

BRIEFING

Fluconazole Injection, page 266 of *PF* 34(2) [Mar.–Apr. 2008]. On the basis of comments received and also in line with USP's flexible monograph policy, it is proposed to add an impurity test to the *Organic Impurities* section. Users are to perform either (a) *Procedure 1* and *Procedure 2* or (b) *Procedure 3*. It is also proposed to add a *Labeling* requirement for *Organic Impurity* tests other than *Procedure 1* and *Procedure 2*. The liquid chromatographic procedure in *Organic Impurities: Procedure 3* was validated with a Symmetry brand column that contains 3.5-µm packing L1. The retention time of fluconazole under the specified conditions is 12–14 min. Also, on the basis of comments received, in *Organic Impurities: Procedure 2*, it is proposed to add acceptance criteria for the total impurities as a sum of the results from *Procedure 1* and *Procedure 2*.

(MD-AA: M. Puderbaugh; B. Davani.) RTS—C67034

In-Process Revision

Add the following:

■Fluconazole Injection

DEFINITION
Fluconazole Injection is a sterile solution of Fluconazole in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$).

IDENTIFICATION
- The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 0.82 mg/mL of sodium acetate in water. Adjust with 1 N acetic acid solution to a pH of 5.0.
Diluent: Methanol and *Buffer* (20:80)
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See the *gradient table* below.

Time (min)	*Solution A* (%)	*Solution B* (%)
0	80	20
9	80	20
15	15	85
18	80	20
25	80	20

System suitability solution: 0.04 mg/mL each of benzyl alcohol and USP Fluconazole RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Equivalent to 0.2 mg/mL from the Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution.*]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Column efficiency: NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{13}H_{12}F_2N_6O$ in the portion of Injection taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

Organic Impurities ■[NOTE—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2* or (b) *Procedure 3*. *Procedure 3* is recommended if Impurity G (see *Impurity Table 2*) is a potential related compound.]■1S (*USP33*)
- **PROCEDURE 1: FOR NONPOLAR IMPURITIES**

Buffer and **Diluent:** Prepare as directed in the *Assay*.
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See the *gradient table* below.

Time (min)	*Solution A* (%)	*Solution B* (%)
0	77	23
5	77	23
30	40	60
43	77	23
50	77	23

System suitability solution: 2.4 µg/mL and 20 µg/mL, respectively of 1,4-benzoquinone and USP Fluconazole RS in *Diluent*
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Diluted standard solution: 0.2 µg/mL of USP Fluconazole RS in *Diluent*, from the *Standard solution*
Sample solution: Equivalent to 1.0 mg/mL from the Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 100 µL
System suitability
Samples: *System suitability solution, Standard solution,* and *Diluted standard solution*
[NOTE—The relative retention times for 1,4-benzoquinone and fluconazole are about 0.5 and 1.0, respectively, *System suitability solution.*]
Suitability requirements
Resolution: NLT 5.0 between 1,4-benzoquinone and fluconazole, *System suitability solution*
Column efficiency: NLT 2500 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Ratio of average peak area: Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—For the following calculations, do not include peaks eluting before fluconazole and do not include impurities at relative retention times of 2.00–2.12 and 3.14–3.26. The disregarded impurities at the specified relative retention times are process impurities monitored in the drug substance. Furthermore, disregard any peak due to an excipient or any peak less than 0.02%. This test is for determination of the late-eluting peaks, and hence the early-eluting peaks are not quantitated using this procedure.]

Calculate the percentage of the largest unknown nonpolar impurity in the portion of Injection taken:

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

r_U = peak response for the largest impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole based on the label claim and the extent of dilution in the *Sample solution* (mg/mL)

Calculate the percentage of unknown nonpolar impurities in the portion of Injection taken:

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

r_U = peak response for the sum of peak areas of unknown peaks from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of fluconazole based on the label claim and the extent of dilution in the *Sample solution* (mg/mL)

Acceptance criteria

Largest unknown nonpolar impurity: NMT 0.1%
Total unknown nonpolar impurities: NMT 0.5%

• **PROCEDURE 2: FOR POLAR IMPURITIES**

Buffer, Diluent, Mobile phase, System suitability solution, and **Chromatographic system:** Prepare as directed in the *Assay*.

Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*

Diluted standard solution: 0.2 µg/mL of USP Fluconazole RS in *Diluent*, from the *Standard solution*

Sample solution: Equivalent to 0.2 mg/mL from the Injection in *Diluent*

System suitability

Samples: *System suitability solution, Standard solution*, and *Diluted standard solution*

[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*

Column efficiency: NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*

Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Ratio of average peak area: Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

[NOTE—The relative retention times of known related compounds versus fluconazole are included in *Impurity Table 1*.]

Impurity Table 1

Name	Approximate RRT*
Impurity A (if dextrose is present)[a]	0.22–0.28
Impurity B[b]	0.30–0.36
Impurity C (if dextrose is present)[c]	0.37–0.43
Impurity D[d]	0.47–0.59
Impurity E[e]	0.68–0.74
Impurity F[f]	0.77–0.83

*RRT, relative retention time.
[a]5-Hydroxymethylfurfural.
[b]4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4*H*-1,2,4-triazolium bromide.
[c]Dextrose-related compound.
[d]2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl)propan-2-ol.
[e]2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[f]Cyclohexanone.

[NOTE—Disregard the known related impurities from *Impurity Table 1* in the following calculation because these are process impurities that are monitored in the drug substance.]

Calculate the percentage of the single largest unknown polar impurity in the portion of Injection taken:

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

r_U = peak response for the largest impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole based on the label claim and the extent of dilution in the *Sample solution* (mg/mL)

Calculate the quantity, as a percentage, of the unknown polar impurities in the portion of Injection taken:

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

r_U = peak response for the total of peak areas of all unknown peaks from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole based on the label claim and the extent of dilution in the *Sample solution* (mg/mL)

Acceptance criteria

Largest unknown polar impurity: NMT 0.1%
Total unknown polar impurities: NMT 0.5%
■**Total unknown polar and nonpolar impurities:** NMT 1.0% (sum of results from *Procedure 1* and *Procedure 2*)■1S (*USP33*)

[NOTE—Disregard any peak due to an excipient or any peak less than 0.03%.]

■• **PROCEDURE 3**

Buffer: 0.63 mg/mL of ammonium formate in water
Mobile phase: Acetonitrile and *Buffer* (14:86)
Standard solution: 2 mg/mL of USP Fluconazole RS in *Buffer*

[NOTE—Use approximately 14% of the total volume as acetonitrile and sonicate if necessary, to facilitate dissolution.]

Sensitivity solution: 1 µg/mL USP Fluconazole RS in *Mobile phase* from the *Standard solution*
Sample solution: Use the injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Samples: *Sensitivity solution* and *Standard solution*
[NOTE—The retention time for fluconazole is between 12–14 min.]
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Column efficiency: NLT 3000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for fluconazole from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole based on the label claim in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Total impurities: NMT 0.5%

Impurity Table 2

Name	Relative Retention Time	Limit (%)
Impurity G[a]	0.13	0.2
Impurity D[b]	0.5	0.2
Impurity H[c]	2.6	0.2
Any other impurity	—	0.2

[a]1,3-bis(1*H*-1,2,4-Triazol-1-yl)propan-2-one.
[b]2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl)propan-2-ol.
[c]1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1,2,4-triazol.

■1S (*USP33*)

SPECIFIC TESTS
- **STERILITY TESTS ⟨71⟩:** Meets the requirement
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 0.416 USP Endotoxin Unit/mg of fluconazole
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at controlled room temperature.

Change to read:

- **LABELING:** Label to indicate the vehicle used. ■If a test for *Organic Impurities* other than *Procedure 1* and *Procedure 2* is used, then the labeling states with which *Organic Impurities* test the article complies.■1S (*USP33*)
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Endotoxin RS
USP Fluconazole RS■1S (*USP32*)

BRIEFING

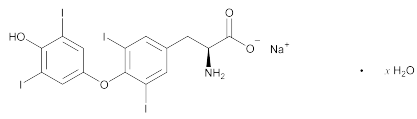
Levothyroxine Sodium, *USP 32* page 2777. On the basis of comments received, it is proposed to add a *Procedure 2* for *Organic Impurities*, using a flexible monograph approach, to capture the potential impurities by a different synthetic route. This test is based on an analytical method validated with the YMC C18 Pro brand of L1 column. The typical retention time for levothyroxine is about 16.5 min. This proposed revision also necessitates the addition of a *Labeling* statement and a revision of the section *USP Reference Standards* ⟨*11*⟩.

In addition, several changes are proposed for *Procedure 1* to make the impurity limits consistent throughout the monograph. The limit of T4-acetic acid is increased from NMT 0.15% to NMT 0.30%, and the limit of total impurities is increased from NMT 1.5% to NMT 2.0%. In addition, it is proposed to calculate the impurity limits on the as-is basis.

Finally, comments were received that for this material, different manufacturers have different storage conditions, which are supported by stability data. It is proposed to indicate that manufacturers can store the material in accordance with their labeling instructions.

(MD-GRE: E. Gonikberg.) RTS—C66966

Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85
L-Tyrosine, *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;
Monosodium L-thyroxine hydrate [25416-65-3].
Anhydrous [55-03-8].

DEFINITION
Levothyroxine Sodium is the sodium salt of L-3,3′,5,5′-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of $C_{15}H_{10}I_4NNaO_4$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. PROCEDURE**
Sample: 50 mg
Analysis: Ignite the *Sample* in a platinum dish over a flame.
Acceptance criteria: It decomposes and liberates iodine vapors. [NOTE—Cool the residue obtained, and reserve it for use in *Identification* test *D*.]
- **B. PROCEDURE**
Acid sodium chloride solution: Alcohol, 1 N sodium hydroxide, hydrochloric acid, and water (25:10:10:30)
Sample: 0.5 mg
Analysis: Add 7.5 mL of *Acid sodium chloride solution* and 1 mL of sodium nitrite solution (1:100) to the *Sample*. Allow to stand in the dark for 20 min, and add 1.25 mL of ammonium hydroxide.
Acceptance criteria: A pink color is produced.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.
- **D. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** The solution meets the requirements of the flame test.
Sample solution: To the residue retained from *Identification* test *A,* add a 1 N potassium hydroxide solution dropwise until the residue is dissolved.

In-Process Revision

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each 1000 mL

Solution A: 400 mg of sodium hydroxide in 500 mL of water. Cool, and add 500 mL of methanol.

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*

Liothyronine stock solution: 0.4 mg/mL of liothyronine from USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.

Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution*, in *Mobile phase*

Sample solution: Prepare a solution of Levothyroxine Sodium in *Mobile phase* having a known concentration of 10 µg/mL. [NOTE—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1.5 mL/min
Injection size: 100 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between liothyronine and levothyroxine
Relative standard deviation: NMT 2.0% of levothyroxine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{10}I_4NNaO_4$ in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Levothyroxine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levothyroxine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: 97.0%–103.0%

IMPURITIES

Change to read:

Organic Impurities

■[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when related compounds listed in *Impurity Table 2* may be present.]■1S *(USP33)*

- **PROCEDURE 1**

Diluent: Acetonitrile and water (1:1)

Solution A: Dilute 5 mL of phosphoric acid with *Diluent* to 100.0 mL.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in 200 mL of water. Add 200 mL of acetonitrile, 400 mL of methanol, and 1.0 mL of phosphoric acid. Dilute with water to 1 L.

Standard stock solution 1: Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard stock solution 2: Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

System suitability solution: Transfer 5.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard solution: Pipet 4.0 mL of the *System suitability solution* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Blank: Add 7 mL of *Solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Transfer 25 mg of Levothyroxine Sodium to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection size: 15 µL
Temperature: 35°

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between levothyroxine and liothyronine, *System suitability solution*
Relative standard deviation: NMT 2.0% for the levothyroxine peak in the *Standard solution*

Analysis
Samples: *Standard solution*, *Blank*, and *Sample solution*
[NOTE—Record the chromatograms for at least six times the retention time of the levothyroxine peak. Verify that no peaks elute in the *Blank solution* at the expected retention times for levothyroxine and related compounds.]
Calculate the area percentage of each related compound in the portion of Levothyroxine ■Sodium■1S *(USP33)* taken:

~~$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100/(1 - 0.01L)$~~

■$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$■1S *(USP33)*

r_U = peak area for each impurity from the *Sample solution*
r_S = peak area for levothyroxine from the *Standard solution*
C_S = concentration of levothyroxine in the *Standard solution* (mg/ml)
C_U = nominal concentration of levothyroxine sodium in the *Sample solution* (mg/ml)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87
~~L = percentage of water in Levothyroxine Sodium, as determined separately in the test for *Water Determination* ⟨921⟩~~

■■1S *(USP33)*

[NOTE—The relative response factor for the impurities listed in *Impurity Table 1* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]
Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

In-Process Revision

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT ~~1.5%~~ ■2.0%■1S (*USP33*)

Impurity Table 1

Name	Relative Retention Time	Limit (%)
Liothyronine	0.65–0.70	1.0
β-Hydroxy-T4[a]	0.71–0.76	0.15
Levothyroxine	1.0	N/A
T4-Hydroxyacetic acid[b]	1.13–1.28	0.15
N-Formyl-T4[c] and T4-Acetamide[d]	1.47–1.53	0.15
N-Acetyl-T4[e]	1.50–1.86	0.20
T4-Acetic acid[f]	2.42–2.51	~~0.15~~ ■0.30■1S (*USP33*)
T4-Aldehyde[g]	3.17–3.45	0.15
T4-Benzoic acid[h]	3.46–3.70	0.15
Individual unspecified impurity	N/A	0.10

[a] *O*-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-*β*-hydroxy-L-tyrosine.
[b] 2-Hydroxy-2-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.
[c] *N*-Formyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[d] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetamide.
[e] *N*-Acetyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[f] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.
[g] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.
[h] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

■• **PROCEDURE 2**
Solution A: Dissolve 9.7 g of sulfamic acid in 2000 mL of water. Add 1.5 g of sodium hydroxide, mix to dissolve, and adjust with 2 N sodium hydroxide to a pH of 2.0.
Solution B: Use acetonitrile.
Diluent 1: Methanol and *Solution A* (90:10)
Diluent 2: Acetonitrile and *Solution A* (30:70); mix (1:1) with *Diluent 1*.
Mobile phase: See the *Gradient Table* below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
40	20	80
50	20	80
53	70	30
75	70	30

Blank solution: Use *Diluent 2*.
Standard stock solution: 0.1 mg/mL of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*
Standard solution: 0.002 mg/mL of USP Levothyroxine RS and USP Liothyronine RS, prepared using *Standard stock solution* in *Diluent 2*
Sensitivity solution: 0.0002 mg/mL of USP Levothyroxine RS and USP Liothyronine RS, prepared using *Standard solution* in *Diluent 2*
Identification solution: Dissolve 5.0 mg of USP Levothyroxine for Peak Identification RS in 4.5 mL of methanol. Add 0.5 mL of *Solution A*. Further dilute a portion of this solution with *Diluent 2* to obtain a solution containing about 0.2 mg/mL.

Sample solution: Dissolve an accurately weighed amount of Levothyroxine Sodium in *Diluent 1* to obtain a solution having a known concentration of about 1.0 mg/mL. Further dilute a portion of this solution with *Diluent 2* to obtain a solution having a known concentration of about 0.2 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 25 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5 between levothyroxine and liothyronine, *Standard solution*
Signal-to-noise ratio: NLT 5 for each peak from the *Sensitivity solution*, calculated by:

$$(2H)/h$$

H = measured height of the peak
h = amplitude of the average measured baseline noise

Analysis
Samples: *Blank solution*, *Identification solution*, *Standard solution*, and *Sample solution*
[NOTE—Identify the components on the basis of their relative retention times as listed in *Impurity Table 2*.]
Calculate the percentage of liothyronine sodium in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of liothyronine from the *Sample solution*
r_S = peak area of liothyronine from the *Standard solution*
C_S = concentration of liothyronine in the *Standard solution* (mg/mL)
C_U = concentration of levothyroxine sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of liothyronine sodium, 672.96
M_{r2} = molecular weight of liothyronine, 650.98

Calculate the percentage of any other impurity in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of levothyroxine from the *Standard solution*
C_S = concentration of levothyroxine in the *Standard solution* (mg/mL)
C_U = concentration of levothyroxine sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in *Impurity Table 2* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]
Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Total impurities: NMT 2.0%

Impurity Table 2

Name	Relative Retention Time	Limit (%)
Liothyronine	0.65	1.0
Monochlorotriiodothyronine[a]	0.94	0.15
Levothyroxine	1.0	N/A
Triiodothyroacetic acid, or T3-Acetic acid[b]	1.57	0.15
O-(4-hydroxy-3,5-diiodophenyl)thyroxine, or T6[c]	1.61	0.50
O-methyl-tetraiodothyroethylamine, or T4-amine *O*-methyl[d]	1.76	0.30
T4-Acetic acid[e]	1.79	0.30
Individual unspecified impurity	N/A	0.10

[a] (*S*)-2-Amino-3-(3-chloro-4-(4-hydroxy-3,5-diiodophenoxy)-5-iodophenyl)propanoic acid.
[b] [4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid.
[c] (*S*)-2-Amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid.
[d] 2-[4-(3,5-Diiodo-4-methoxyphenoxy)-3,5-diiodophenyl]ethanamine.
[e] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

■1S (*USP33*)

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –5° to –6°
  Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium, in alcohol and 1 N sodium hydroxide (2:1)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 11.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. ■Store as stated in the labeling instructions.■1S (*USP33*)

Add the following:

■• **LABELING:** If a test for *Organic Impurities* other than *Test 1* is used, the labeling states the test with which the article complies.■1S (*USP33*)

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Levothyroxine RS
  USP Liothyronine RS
  ■USP Levothyroxine for Peak Identification RS■1S (*USP33*)

BRIEFING

Human Acellular Dermal Matrix. Because there is no existing *USP* monograph for this product, a new monograph is being proposed. The proposed procedure for tissue evaluation is based on histological analysis. No evidence of cell nuclei or cytoplasm is apparent in prepared histological sections.

(BB CGT: F. Atouf.) RTS—C60477

Add the following:

■Human Acellular Dermal Matrix

DEFINITION

Human Acellular Dermal Matrix is derived from donated allograft human dermis that is processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. It is biocompatible and supports remodeling by the recipient's own tissue. The matrix is comprised of native human dermal architecture, consisting of collagen (mainly collagen Type I, with additional collagen Type III and IV components), chondroitin sulfate and hyaluronic acid glycosaminoglycans, and elastin.

The donated human skin is processed in a manner that removes all cellular components including the epidermal layer and dermal cells. Human Acellular Dermal Matrix does not contain intact cells and cell nuclei. Human skin used to produce Human Acellular Dermal Matrix is obtained from sources that have passed applicable donor eligibility requirements for relevant communicable diseases. Human Acellular Dermal Matrix is manufactured using sterile solutions and equipment under aseptic conditions. The final product is inspected and tested to ensure that the product meets the specifications.

SPECIFIC TESTS

- **HISTOLOGICAL EVALUATION**
  Sucrose solution: Dissolve 20 g of sucrose in 100 mL of water.
  Tissue preparation: Submerge a 1.4-cm or greater size piece of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 hr. Cut three 1.5-cm × 0.5-cm pieces of Human Acellular Dermal Matrix, and place each piece from the same donor lot into a suitable histological tissue cassette for routine histology processing and paraffin embedding. Place the cassettes in 10% neutral buffered formalin[1] at room temperature for a minimum of 12 h. [NOTE—the cassettes may be kept at room temperature for 1 month.] From the same donor lot, cut a minimum of three 1.0-cm × 0.5-cm pieces of Human Acellular Dermal Matrix for immunohistochemical (IHC) staining and place in a vial containing *Sucrose solution* and store at 2°–8° for a minimum of 4 h, but the product can be held in the solution for up to 2 weeks.
  Embedding and sectioning of histological evaluation samples: Place all cassettes to be processed into a suitable histological cassette basket including one cassette containing formalin-fixed normal skin to serve as a process control. The following steps can be performed using a vacuum infiltration tissue processor.[2] At room temperature (37° ± 2°), tissue samples continue to fix in 10% neutral buffered formalin, are dehydrated through graded reagent alcohol (70%, 80%, 95%

[1] 10% Neutral buffered formalin can be purchased from Statlab Medical Products, Inc., Lewisville, TX.
[2] A suitable processor is the Tissue Tek V.I.P. Model #1000 vacuum infiltration tissue processor or the Leica ASP300 tissue processor.

In-Process Revision

and 100%).[3] The tissue samples are cleared in a xylene substitute.[4]

The tissue is infiltrated with paraffin.[5] at 60°. The following embedding procedure can be performed with the use of a histology tissue embedding unit, consisting of a thermal console, dispensing console, and cryo-console. Open the histological cassette. Partially fill embedding molds with molten paraffin (60°). While keeping the paraffin heated, position the three tissue pieces on edge in the paraffin so that cross-sections of the Human Acellular Dermal Matrix can be cut once completely embedded. Rapidly cool the paraffin so that it partially solidifies. Completely fill the embedding mold with additional molten paraffin (60°), and cool until completely solidified.

Remove solidified paraffin blocks from embedding molds for sectioning. Sectioning of paraffin-embedded samples can be performed on a suitable microtome[6]. Clamp the tissue block into the holder of the microtome. Fill a histological water bath with fresh water [NOTE—A quarter teaspoon of USP gelatin may be added to the water bath for section adhesion.], and maintain the water bath at 39°± 2°. Cut sections to a thickness of 3–5 µm to form a ribbon, and gently lay the ribbon on the water bath. Place two consecutive sections on each glass microscope slide.

Embedding and sectioning of immunohistochemical samples: Fill histology embedding molds with a cryo-protectant embedding media. Transfer tissue pieces from *Sucrose solution* to the embedding media in the mold, orient so that cross-sections can be cut, and let sit for 1–30 min. Freeze the molds with liquid nitrogen and store at –80°. Remove frozen blocks from molds for sectioning. Cryo-section each block to 4–7 µm in thickness and place sections on glass microscope slides. Place at least two sections on each slide. Slides can be stored at –70° to –85°.

Hematoxylin and eosin sample staining: [NOTE—Store all solutions at room temperature.]

1% Acid alcohol solution: Alcohol, hydrochloric acid, and water (1309:20:509)

95% Reagent alcohol solution: Alcohol and water (190:10)

80% Reagent alcohol solution: Alcohol and water (160:40)

Saturated lithium carbonate solution: Water, lithium carbonate (200:2), (v:w)

Analysis: The slide with affixed tissue (sections) is deparaffinized in three changes of xylene substitute, rehydrated in graded reagent alcohols (100% and 95%) and rinsed in tap water. The sections are stained with hematoxylin, rinsed, decolorized with *1% Acid alcohol solution*, and neutralized with *Saturated lithium carbonate solution*. The sections are counterstained with 1% alcoholic eosin Y, dehydrated through graded reagent alcohols (95% and 100%) and xylene substitute, and then coverslipped. A positive control is stained with each batch to ensure successful staining.

Acceptance criteria: Adequate staining is defined by a blue nuclear stain and a pink-red cytoplasm. Sections are assessed for tissue matrix integrity and are acceptable if they pass the following criteria: they must not show evidence of intact epidermal or dermal cells, and must not show evidence of widespread collagen damage (broken, condensed, or distorted fibers) or unrecognizable papillary layer, as shown in the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance.

Immunohistochemical sample staining

0.5 M phosphate-buffered saline, pH 7.4 (PBS): Prepare by combining 8.50 g of sodium chloride, 0.85 g of dibasic sodium phosphate, and 0.54 g of monobasic potassium phosphate in 1 L of water. Adjust the pH to 7.4 with 1.0–3.0 M sodium hydroxide. Store at room temperature.

Diluent PBS: Combine 0.250 g of bovine serum albumin, 25 mL of *PBS*, and 0.02–0.03 g of thimerosal. Store at between 2° and 8° for up to 1 year.

Primary and secondary antibodies: Perform a titer assay to determine optimal dilution for each antibody when a new lot is used. Dilute in *Diluent PBS* as per titer assay.

Human monoclonal anti-type IV collagen[7]: 25–100 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Human monoclonal anti-MHC class I[8]: 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Human monoclonal anti-MHC class II[9]: 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Goat anti-mouse IgG (Fab-specific) peroxidase conjugate[10]: 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

3,3 Diaminobenzidine staining kit or stable diaminobenzidine (DAB)[11]: To make the working stain, consult kit instructions. Store kit at between 2° and 8°. 3,3 Diaminobenzidine is light-sensitive, minimum exposure to light is advisable. Stable DAB is stored at between 0° and –20°.

3% Hydrogen peroxide: Store at room temperature.

Acetone: Store at between –10° and –20°.

Analysis for monoclonal anti-MHC class I and class II: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. A cocktail of *Human monoclonal anti-MHC class I, Human monoclonal anti-MHC Class II,* and bovine serum albumin are applied to the sections. Sections are rinsed with *PBS* and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS* and the *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives the same exact treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved the stain is repeated.

Acceptance criteria: Positive staining of sections will appear brown in color, focal, cell-associated, and be clearly distinct from the background, as shown in the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance for staining with MHC I and II antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

Analysis for monoclonal anti-type IV collagen: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. *Human monoclonal anti-type IV collagen* along with bovine serum albumin are applied to the sections. Sections are rinsed with *PBS* and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS* and the *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives the same exact treatment as the test samples. The negative control is the same tissue section as the positive. The difference

[3]Graded alcohols: 70%, 80%, and 95% are prepared using 100% Reagent Alcohol such as that purchased from Statlab Medical Products, Inc., Lewisville, TX.
[4]Such as a citrus clearing solvent from Richard-Allan Scientific, Kalamazoo, MI.
[5]Such as Paraplast, Extra, manufactured by McCormick Scientific, St. Louis, MO.
[6]Such as Leica RM 2155 or RM 2255 Rotary Microtome.
[7]Suitable primary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, cat #C1926.
[8]Suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, cat #H58A.
[9]Suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, cat #TH14B.
[10]Suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, cat #A9917.
[11]DAB can be sourced from Invitrogen/Zymed, Serotec, or Dako.

is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved the stain is repeated.

Acceptance criteria: Positive stain results will reveal a brown stain in the location of basement membrane, as shown in the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance for staining with collagen type IV antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

• **BIOCHEMICAL ANALYSIS**

Sample solution: Submerge 20 mg of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Freeze the sample and mill using a freezer mill.[12] Suspend in 10 mL of 4 M guanidine hydrochloride, 50 mM sodium acetate, 5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM *N*-ethylmaleimide (NEM), 0.2% Triton X-100, pH 5.8, and extract for 48 h at 4°, with agitation. Centrifuge for 10 min at 4° and 23,500 *g*. Remove the supernatant and digest with 20 µL chondroitinase ABC,[13] 1 milli Unit/µL. Analyze for *Glycosaminoglycan (GAG) content* and *Immunochemical analysis of decorin* (below). For *Collagen content* analysis, wash the pellet three times with 10 mL of deionized water, centrifuging for 10 min at 4° and 23,500 *g* after each wash. Resuspend in a minimal amount of water, freeze at –80°, and lyophilize. Resuspend 20 mg of the lyophilized pellet in 20 mL of 0.5 M acetic acid containing 100 µg of pepsin/mg of tissue, and incubate overnight at 4° with agitation. Remove undigested material by centrifugation at 23,500 *g* for 20 min.

Collagen content

Sample buffer: Prepare as directed for *Sample Buffer 1* in *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩*, Electrophoretic Separation*.

Sample collagen solution: Add 20 µL of the supernatant from the *Sample solution* to 20 µL of *Sample buffer,* mix, and incubate at 60° for 15 min.

Control samples: Prepare suitable positive control samples (human placental collagen types I[14] and III[15]) similar to *Sample collagen solution.*

Gel electrophoresis: Load 40 µL of each of *Sample collagen solution* and the *Control samples* onto a 6% acrylamide gel, perform the electrophoretic separation, and stain with Coomassie brilliant blue as directed under *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.

Acceptance criteria: The bands obtained from Human Acellular Dermal Matrix are distinct and clearly visible at a mobility similar to the bands obtained from the collagen types I and III control samples. No smears corresponding to degraded collagen types I and III are found on the gel.

Glycosaminoglycan (GAG) content

Sample GAG solution: Use 15 µL of the supernatant prepared as directed under the *Sample solution.*

DMMB reagent: Dissolve 16 mg of 1,9 dimethyl-methylene blue in 5 mL of ethanol. Add 3.24 mL of formic acid and 2.94 mL of 10 M sodium hydroxide. Bring to 1000 mL with water.

50 mM sodium acetate: Dissolve 1.03 g of sodium acetate in 250 mL of water.

Analysis: Add 15 µL of *Sample GAG solution* to each well of a 96-well microtiter plate. To each sample well, add 200 µL of *DMMB reagent* and 35 µL of *50 mM sodium acetate solution*. Read the samples on a spectrophotometric microplate reader at 540 nm, using 595 nm as a reference. Perform the same procedure on a sample of bovine collagen type I[16] negative control and positive controls hyaluronic acid[17] and chondroitin sulfate,[18] using 15 µL each of three mg/mL solutions.

Acceptance criteria: Human Acellular Dermal Matrix will contain glycosaminoglycans (GAG) as represented by positive control standards. GAG concentration can be determined by the preferential binding of DMMB dye to negatively charged ions such as sulfated GAGs.

Immunochemical analysis of decorin

Sample decorin solution: Submerge 100–200 mg of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Using a freezer mill as outlined above, extract the tissue in a solution of 4 M guanidine hydrochloride, 50 mM sodium acetate (pH 5.8) containing 5 mM EDTA, 0.1 mM PMSF, and 10 mM NEM for 24–48 h with agitation at 4°. Centrifuge the extracted tissue at 23,500 *g* for 10 min, and remove the supernatant.

Nitro blue tetrazolium (NBT): Dissolve 0.5 g in 10 mL of 70% dimethyl sulfoxide (DMSO). Store in the dark at room temperature.

5–Bromo-4-chloro-3-indolyl phosphate (BCIP): Dissolve 0.5 g in 10 mL of 100% DMSO. Store in the dark at room temperature.

Alkaline phosphatase buffer: 100 mM sodium chloride, 5 mM magnesium chloride, and 100 mM Tris(hydroxymethyl)aminomethane hydrochloride, pH 9.5. Filter and store at 4°.

Chromogenic substrate mixture: Mix 66 µL of *NBT* and 33 µL of *BCIP* in 10 mL of *Alkaline phosphatase buffer*.

Blotting buffer 1: 50 mM Tris buffered saline, pH 7.4, with 0.1% Tween 20 (TBST)

Blotting buffer 2: 5% blotting grade milk in TBST

Blotting buffer 3: 0.1% blotting grade milk in TBST

Analysis: Run an aliquot of *Sample decorin solution* on an 8% polyacrylamide gel at 100 V for approximately 60 min along with 100 µg recombinant decorin[19] positive control, bovine type I collagen[20] negative control, and prestained molecular weight marker, and transfer to an polyvinylidene fluoride (PVDF) membrane according to the following procedure. In a shallow tray, soak two sheets of Whatman paper and two pieces of sponge in transfer buffer (200 mM glycine, 25 mM tris, 20% methanol, pH 8.3). Soak the transfer membrane in methanol prior to soaking in the transfer buffer. Equilibrate the gel in transfer buffer for 10 min. Assemble the tank transfer apparatus according to the manufacturer's instructions. Layer the following items within the apparatus in proper order: sponge, paper, gel, PVDF membrane, paper, and sponge. Clamp the apparatus closed. Connect the electrodes, and transfer the gel bands to the membrane at about 100 V at 4° for about 1 h. The use of prestained protein molecular weight marker and staining the gel after electro-blotting allows for visual determination of transfer completion. When transfer is complete, disconnect the transfer apparatus and carefully remove the membrane. Block the membrane for at least 1 h with *Blotting buffer 2*, cover, and shake gently for 30 min. Wash the membrane three times for 5 min each with *Blotting buffer 1*. Add rabbit anti-human decorin primary antibody,[21] diluted to 2 µg/mL in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*.

[12]Such as SPEX/CertiPrep Freezer/Mill.

[13]Suitable chondroitinase is available from Seikagaku, Tokyo, Japan, catalog #100330.

[14]Suitable type I collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C7774.

[15]Suitable type III collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4407.

[16]Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave, PO Box 6482, Carlsbad, CA 92008, catalog #100.

[17]Suitable hyaluronic acid is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #H1876.

[18]Suitable chondroitin sulfate is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4384.

[19]Suitable decorin is available from R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, catalog #143DE100.

[20]Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave, PO Box 6482 Carlsbad, CA 92008.

[21]Suitable primary antibody is available from BioVision, 980 Linda Vista Ave., Mountain View, CA 94043, catalog #3645100.

In-Process Revision

Add goat anti-rabbit IgG secondary antibody conjugated[22] to alkaline phosphatase, diluted 1:3000 in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add 10 mL of *Chromogenic substrate mixture*, and incubate until color has developed. When the bands are clearly visible, stop the development reaction by rinsing the membrane with water.

Acceptance criteria: The decorin band obtained from Human Acellular Dermal Matrix is the only band on the gel and is clearly visible at mobility similar to the band obtained from the control sample recombinant decorin.

- **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Human Acellular Dermal Matrix meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **LABELING:** The package label indicates the length, width, and thickness of the Human Acellular Dermal Matrix. The label also contains the lot number, the expiration date, the required storage conditions, cautionary statements pertaining to package handling, and manufacture and/or distributor contact information. "Instructions for Use", which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each unit.
- **PACKAGING AND STORAGE:** Human Acellular Dermal Matrix is aseptically packaged and supplied in a freeze-dried configuration in a double pouch configuration. Human Acellular Dermal Matrix is supplied in various sizes and thicknesses. Human Acellular Dermal Matrix is stored at between 1° and 10° and labeled with an expiration date.
- **USP AUTHENTIC VISUAL REFERENCES**

USP Human Acellular Dermal Matrix Reference Photomicrographs. The samples were prepared as directed in the test for *Histological Evaluation*. These photomicrographs show the histological appearance of passing histological evaluation with no evidence of intact epidermal or dermal cells, and no evidence of collagen damage (photomicrographs 2–6) and of failed material with collagen damage and condensed papillary dermis or attached epidermal remnant (photomicrographs 7–10). Passing and failed material are compared to photomicrograph 1 showing the staining of unprocessed normal human skin with intact epidermal or dermal cells. Photomicrographs 11 and 12 are negative and positive controls for the analysis of immunostaining with MHC I and II antibodies. Photomicrographs 13 and 14 are negative and positive controls for the analysis of immunostaining with collagen type IV antibodies. For both types of staining, a passing positive stain will appear brown in color, focal, and cell-associated while a failed negative stain will appear clear with no brown staining.■1S (*USP33*)

BRIEFING

Microsized Human Acellular Dermal Matrix. Because there is no existing *USP* monograph for this product, a new monograph is being proposed. The procedure for tissue evaluation is based on histological analysis. No evidence of cell nuclei or cytoplasm is apparent in prepared histological sections.

(BB CGT: F. Atouf.) RTS—C52657

[22] Suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, cat #A9917.

Add the following:

■Microsized Human Acellular Dermal Matrix

DEFINITION

Microsized Human Acellular Dermal Matrix is derived from donated allograft human dermis that is processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. It is biocompatible and supports remodeling by the recipient's own tissue. The matrix is comprised of native human dermal architecture, consisting of collagen (mainly collagen Type I, with additional collagen Type III and IV components), chondroitin sulfate and hyaluronic acid glycosaminoglycans, and elastin.

The donated human skin is processed in a manner that removes all cellular components, including the epidermal layer and dermal cells. The resulting acellular dermal matrix is then rendered into a particulate form (microsized), with a mean particle size of less than 100 μm, by processing with a freezer mill. Microsized Human Acellular Dermal Matrix does not contain intact cells, cell nuclei, or chemically-induced crosslinks. Human skin used to produce Microsized Human Acellular Dermal Matrix is obtained from sources that have passed applicable donor eligibility requirements for relevant communicable diseases. Microsized Human Acellular Dermal Matrix is manufactured using sterile solutions and equipment under aseptic conditions. The final product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

- **HISTOLOGICAL EVALUATION**

[NOTE—Histological evaluation of Microsized Human Acellular Dermal Matrix is performed on the Human Acellular Dermal Matrix prior to micronization.]

Sucrose solution: Dissolve 20 g of sucrose in 100 mL of water.

Tissue preparation: Submerge a 1.4-cm or greater size piece of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Cut three 1.5-cm × 0.5-cm pieces of Human Acellular Dermal Matrix, and place each piece from the same donor lot into a suitable histological tissue cassette for routine histology processing and paraffin embedding. Place the cassettes in 10% neutral buffered formalin[1] at room temperature for a minimum of 12 h. [NOTE—the cassettes may be kept at room temperature for 1 month.] From the same donor lot, cut a minimum of three 1.0-cm × 0.5-cm pieces of Human Acellular Dermal Matrix for immunohistochemical (IHC) staining, and place in a vial containing the *Sucrose solution*. Store at between 2° and 8° for a minimum of 4 h, but the product can be held in the solution for up to 2 weeks.

Embedding and sectioning of histological samples: Place all cassettes to be processed into a suitable histological cassette basket including one cassette containing formalin-fixed normal skin to serve as a process control. The following steps can be performed using a vacuum infiltration tissue processor.[2] At room temperature (37° ± 2°), tissue samples that continue to fix in 10% neutral buffered formalin, are dehydrated through graded reagent alcohol (70%, 80%, 95% and 100%).[3] The tissue samples are cleared in a xylene substitute.[4]

The tissue is infiltrated with paraffin[5] at 60°. The following embedding procedure can be performed with the use of a

[1] 10% Neutral buffered formalin can be purchased through Statlab Medical Products, Inc. Lewisville, TX.

[2] A suitable processor can be the Tissue Tek V.I.P. Model #1000 vacuum infiltration tissue processor or the Leica ASP300 tissue processor.

[3] Graded alcohols: 70%, 80%, and 95% are prepared using 100% reagent alcohol, such as that purchased from Statlab Medical Products, Inc. Lewisville, TX.

[4] Such as a citrus clearing solvent from Richard-Allan Scientific, Kalamazoo, MI.

[5] Such as Paraplast, Extra, manufactured by McCormick Scientific, St. Louis, MO.

histology tissue embedding unit, consisting of a thermal console, dispensing console, and cryo-console. Open the histological cassette. Partially fill embedding molds with molten paraffin (60°). While keeping the paraffin heated, position the three tissue pieces on edge in the paraffin so that cross-sections of the Human Acellular Dermal Matrix can be cut once completely embedded. Rapidly cool the paraffin so that it partially solidifies. Completely fill the embedding mold with additional molten paraffin (60°), and cool until completely solidified.

Remove solidified paraffin blocks from embedding molds for sectioning. Sectioning of paraffin-embedded samples can be performed on a suitable microtome.[6] Clamp the tissue block into the holder of the microtome. Fill a histological water bath with fresh water. [NOTE—A quarter teaspoon of gelatin may be added to the water bath for section adhesion.] Maintain the water bath at 39°± 2°. Cut sections to a thickness of 3–5 µm to form a ribbon, and gently lay the ribbon on the water bath. Place two consecutive sections on each glass microscope slide.

Embedding and sectioning of immunohistochemical samples: Fill histology embedding molds with a cryo-protectant embedding media. Transfer tissue pieces from the *Sucrose solution* to the embedding media in the mold, orient so that cross-sections can be cut, and let sit for 1–30 min. Freeze the molds with liquid nitrogen and store at –80°. Remove the frozen blocks from the molds for sectioning. Cryo-section each block to 4–7 µm in thickness, and place the sections on glass microscope slides. Place at least two sections on each slide. Slides can be stored at between –70° and –85°.

Hematoxylin and eosin sample staining: [NOTE—Store all solutions at room temperature.]

1% Acid alcohol solution: Alcohol, hydrochloric acid, and water (1309:20:509)

95% Reagent alcohol solution: Alcohol and water (190:10)

80% Reagent alcohol solution: Alcohol and water (160:40)

Saturated lithium carbonate solution: Water, lithium carbonate (200:2), (v:w)

Analysis: The slide with affixed tissue (sections) is deparaffinized in three changes of xylene substitute, rehydrated in graded reagent alcohols (100% and 95%), and rinsed in tap water. The sections are stained with hematoxylin, rinsed, decolorized with *1% Acid alcohol solution*, and neutralized with *Saturated lithium carbonate solution*. The sections are counterstained with 1% alcoholic eosin Y, dehydrated through graded reagent alcohols (95% and 100%) and xylene substitute, and then coverslipped. A positive control is stained with each batch to ensure successful staining.

Acceptance criteria: Adequate staining is defined by a blue nuclear stain and a pink-red cytoplasm. Sections are assessed for tissue matrix integrity and are acceptable if they pass the following criteria: they must not show evidence of intact epidermal or dermal cells, and must not show evidence of widespread collagen damage (broken, condensed, or distorted fibers) or unrecognizable papillary layer, as shown in the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance.

Immunohistochemical sample staining

0.5 M phosphate-buffered saline, pH 7.4 (PBS): Prepare by combining 8.50 g of sodium chloride, 0.85 g of dibasic sodium phosphate, and 0.54 g of monobasic potassium phosphate in 1 L water. Adjust the pH to 7.4 with 1.0–3.0 M sodium hydroxide. Store at room temperature.

Diluent PBS: Combine 0.250 g of bovine serum albumin, 25 mL of *PBS*, and 0.02–0.03 g of thimerosal. Store at between 2° and 8° for up to 1 year.

Primary and secondary antibodies: Perform a titer assay to determine the optimal dilution for each antibody when a new lot is used. Dilute in *Diluent PBS* as per titer assay.

Human monoclonal anti-type IV collagen:[7] 25–100 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

[6]Such as Leica RM 2155 or RM 2255 Rotary Microtome.

[7]A suitable primary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C1926.

Human monoclonal anti-MHC class I:[8] 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Human monoclonal anti-MHC class II:[9] 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Goat anti-mouse IgG (Fab-specific) peroxidase conjugate:[10] 100–200 µL aliquots of concentrated antibody may be stored at between –70° and –85°

3,3 Diaminobenzidine staining kit or stable diaminobenzidine (DAB):[11] To make the working stain, consult kit instructions. Store the kit at between 2° and 8°. 3,3 Diaminobenzidine is light sensitive, minimum exposure to light is advisable. Stable DAB is stored at between 0° and –20°.

3% Hydrogen peroxide: Store at room temperature.

Acetone: Store at between –10° and –20°.

Analysis for monoclonal anti-MHC class I and class II: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. A cocktail of *Human monoclonal anti-MHC class I*, *Human monoclonal anti-MHC class II*, and bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS* and the *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives the same exact treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Positive staining of sections will appear brown in color, focal, cell-associated, and be clearly distinct from the background, as shown in the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance for staining with MHC I and II antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

Analysis for monoclonal anti-type IV collagen: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. *Human monoclonal anti-type IV collagen* along with bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS* and the *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives the same exact treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Positive stain results will reveal a brown stain in the location of the basement membrane, as shown in

[8]A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #H58A.

[9]A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #TH14B.

[10]A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103 cat #A9917.

[11]DAB can be sourced from Invitrogen/Zymed, Serotec, or Dako.

In-Process Revision

the USP Human Acellular Dermal Matrix Reference Photomicrographs of products with acceptable appearance for staining with collagen type IV antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

• **BIOCHEMICAL ANALYSIS**

Sample solution: Submerge 20 mg of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Freeze the sample and mill using a freezer mill.[12] Suspend in 10 mL of 4 M guanidine hydrochloride, 50 mM sodium acetate, 5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM *N*-ethylmaleimide (NEM), 0.2% Triton X-100, pH 5.8, and extract for 48 h at 4°, with agitation. Centrifuge for 10 min at 4° and 23,500 *g*. Remove the supernatant and digest with 20 µL chondroitinase ABC,[13] 1 milli Unit/µL. Analyze for *Glycosaminoglycan (GAG) content* and *Immunochemical analysis of decorin* (below). For *Collagen content* analysis, wash the pellet three times with 10 mL of deionized water, centrifuging for 10 min at 4° and 23,500 *g* after each wash. Resuspend in a minimal amount of water, freeze at –80°, and lyophilize. Resuspend 20 mg of the lyophilized pellet in 20 mL of 0.5 M acetic acid containing 100 µg of pepsin/mg of tissue, and incubate overnight at 4° with agitation. Remove undigested material by centrifugation at 23,500 *g* for 20 min.

Collagen content

Sample buffer: Prepare as directed for *Sample Buffer 1* in *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩, *Electrophoretic Separation*.

Sample collagen solution: Add 20 µL of the supernatant from the *Sample solution* to 20 µL of *Sample buffer,* mix, and incubate at 60° for 15 min.

Control samples: Prepare suitable positive control samples (human placental collagen types I[14] and III[15]) similar to *Sample collagen solution.*

Gel electrophoresis: Load 40 µL of each of *Sample collagen solution* and the *Control samples* onto a 6% acrylamide gel, perform the electrophoretic separation, and stain with Coomassie brilliant blue as directed under *Biotechnology Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.

Acceptance criteria: The bands obtained from Human Acellular Dermal Matrix are distinct and clearly visible at a mobility similar to the bands obtained from the collagen types I and III control samples. No smears corresponding to degraded collagen types I and III are found on the gel.

Glycosaminoglycan (GAG) content

Sample GAG solution: Use 15 µL of the supernatant prepared as directed under the *Sample solution.*

DMMB reagent: Dissolve 16 mg 1,9 dimethyl-methylene blue (DMMB) in 5 mL ethanol. Add 3.24 mL of formic acid and 2.94 mL of 10 M sodium hydroxide. Bring to 1000 mL with water.

50 mM Sodium acetate solution: Dissolve 1.03 g sodium acetate in 250 mL water.

Analysis: Add 15 µL of *Sample GAG solution* to each well of a 96-well microtiter plate. To each sample well, add 200 µL *DMMB reagent* and 35 µL of *50 mM Sodium acetate solution*. Read the samples on a spectrophotometric microplate reader at 540 nm, using 595 nm as a reference. Perform the same procedure on a sample of bovine collagen type I[16] negative control and positive controls hyaluronic acid[17] and chondroitin sulfate,[18] using 15 µL each of three mg/mL solutions.

Acceptance criteria: Microsized Human Acellular Dermal Matrix will contain glycosaminoglycans (GAG) as represented by positive control standards. GAG concentration can be determined by the preferential binding of DMMB dye to negatively charged ions such as sulfated GAGs.

Immunochemical analysis of decorin

Sample decorin solution: Submerge 100–200 mg of Human Acellular Dermal Matrix in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Using a freezer mill as outlined above, extract the tissue in a solution of 4 M guanidine hydrochloride, 50 mM sodium acetate (pH 5.8) containing 5 mM EDTA, 0.1 mM PMSF, 10 mM NEM for 24–48 h with agitation at 4°. Centrifuge the extracted tissue at 23,500 *g* for 10 min, and remove the supernatant.

Nitro blue tetrazolium (NBT): Dissolve 0.5 g in 10 mL of 70% dimethyl sulfoxide (DMSO). Store in the dark at room temperature.

5-Bromo-4-chloro-3-indolyl phosphate (BCIP): Dissolve 0.5 g in 10 mL of 100% DMSO. Store in the dark at room temperature.

Alkaline phosphatase buffer: 100 mM sodium chloride, 5 mM magnesium chloride, 100 mM Tris(hydroxymethyl)aminomethane hydrochloride, pH 9.5. Filter and store at 4°.

Chromogenic substrate mixture: Mix 66 µL of *NBT* and 33 µL of *BCIP* in 10 mL *Alkaline phosphatase buffer*.

Blotting buffer 1: 50 mM Tris buffered saline, pH 7.4, with 0.1% Tween 20 (TBST)

Blotting buffer 2: 5% blotting grade milk in TBST

Blotting buffer 3: 0.1% blotting grade milk in TBST

Analysis: Run an aliquot of *Sample decorin solution* on an 8% polyacrylamide gel at 100 V for approximately 60 min along with 100 µg recombinant decorin[19] positive control, bovine type I collagen[20] negative control, and prestained molecular weight marker, and transfer to an polyvinylidene fluoride (PVDF) membrane according to the following procedure. In a shallow tray, soak two sheets of Whatman paper and two pieces of sponge in transfer buffer (200 mM glycine, 25 mM tris, 20% methanol, pH 8.3). Soak the transfer membrane in methanol prior to soaking in the transfer buffer. Equilibrate the gel in transfer buffer for 10 min. Assemble the tank transfer apparatus according to manufacturer's instructions. Layer the following items within the apparatus in proper order: sponge, paper, gel, PVDF membrane, paper, and sponge. Clamp the apparatus closed. Connect the electrodes, and transfer the gel bands to the membrane at about 100 V at 4° for about 1 h. The use of prestained protein molecular weight marker, and staining the gel after electro-blotting, allows for visual determination of transfer completion. When transfer is complete, disconnect the transfer apparatus and carefully remove the membrane. Block the membrane for at least 1 h with *Blotting buffer 2*, cover, and shake gently for 30 min. Wash the membrane three times for 5 min each with *Blotting buffer 1*. Add rabbit anti-human decorin primary antibody,[21] diluted to 2 µg/mL in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add goat anti-rabbit IgG secondary antibody conjugated[22] to alkaline phosphatase, diluted 1:3000 in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add 10 mL of *Chromogenic substrate mixture*, and incubate until color has developed. When the bands are clearly visible, stop the development reaction by rinsing the membrane with water.

Acceptance criteria: The decorin band obtained from Human Acellular Dermal Matrix is the only band on the gel

[12]Such as SPEX/CertiPrep Freezer/Mill.

[13]Suitable chondroitinase is available from Seikagaku, Tokyo Japan, catalog #100330.

[14]Suitable type I collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C7774.

[15]Suitable type III collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4407.

[16]Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave, PO Box 6482 Carlsbad, CA 92008 catalog #100.

[17]Suitable hyaluronic acid is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #H1876.

[18]Suitable chondroitin sulfate is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4384.

[19]Suitable decorin is available from R&D Systems, 614 McKinley Place NE Minneapolis, MN 55413 catalog #143DE100.

[20]Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave, PO Box 6482 Carlsbad, CA 92008.

[21]Suitable primary antibody available from BioVision, 980 Linda Vista Ave, Mountain View, CA 94043 catalog #3645100.

[22] A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103 cat #A9917.

and is clearly visible at a mobility similar to the band obtained from the control sample recombinant decorin.

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Microsized Human Acellular Dermal Matrix meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PARTICLE SIZE ANALYSIS**

(See *Particulate Matter in Injections* ⟨788⟩.)

Sample: 100 mg of Microsized Human Acellular Dermal Matrix material

Analysis: Add the *Sample* to 25 mL of saline in a clean 50-mL conical tube. Rehydrate and disperse the particles by probe sonication at 20 W for 60 s. Place an appropriate aliquot of sample suspension in an appropriate liquid particle counter (LPC) for analysis by laser light obscuration (light extinction).

Acceptance criteria: The mean particle size is NMT 100 µm.

ADDITIONAL REQUIREMENTS

- **LABELING:** The package label indicates the weight of the Microsized Human Acellular Dermal Matrix material enclosed. The label also contains the lot number, the expiration date, the trade name, the required storage conditions, the dosage information, and manufacturer and/or distributor contact information. "Instructions for Use," which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each matrix.
- **PACKAGING AND STORAGE:** Microsized Human Acellular Dermal Matrix is aseptically packaged and supplied in a freeze-dried configuration in a single-use delivery syringe. The syringe is packaged in a hermetically sealed pouch. Microsized Human Acellular Dermal Matrix is stored at between 1° and 10° and labeled with an expiration date.
- **USP AUTHENTIC VISUAL REFERENCES** ⟨**11**⟩

USP Human Acellular Dermal Matrix Reference Photomicrographs. The samples were prepared as directed in the test for *Histological Evaluation*. These photomicrographs show the histological appearance of passing histological evaluation with no evidence of intact epidermal or dermal cells, and no evidence of collagen damage (photomicrographs 2–6) and of failed material with collagen damage and condensed papillary dermis or attached epidermal remnant (photomicrographs 7–10). Passing and failed material are compared to photomicrograph 1 showing the staining of unprocessed normal human skin with intact epidermal or dermal cells. Photomicrographs 11 and 12 are negative and positive controls for the analysis of immunostaining with MHC I and II antibodies. Photomicrographs 13 and 14 are negative and positive controls for the analysis of immunostaining with collagen type IV antibodies. For both types of staining, a passing positive stain will appear brown in color, focal, and cell-associated, while a failed negative stain will appear clear with no brown staining.■1S (*USP33*)

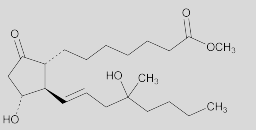
BRIEFING

Misoprostol. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedures in the test for *Organic Impurities, Procedure 1* and in the *Assay* are based on analyses performed with the Zorbax Sil brand of L3 column. The typical retention time for misoprostol is about 15 min. The liquid chromatographic procedure in the test for *Content of Diastereomers* is based on analyses performed with the Spherisorb Silica brand of L3 column. The typical retention times for the two diastereomers are about 23 and 25 min, respectively. An alternative suitable column is Kromasil Si-60, with the typical retention times for the two diastereomers of about 19 and 21 min, respectively.

(MD-GRE: E. Gonikberg.) RTS—C68834

Add the following:

■Misoprostol



$C_{22}H_{38}O_5$ 382.53

Prost-13-en-1-oic acid, 11,16-dihydroxy-16-methyl-9-oxo-, methyl ester, (1*R**,2*R**,3*R**,*E*)-;

(±)-Methyl (1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(4*RS*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate [59122-46-2].

DEFINITION

Misoprostol contains NLT 97.0% and NMT 102.0% of $C_{22}H_{38}O_5$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197S**⟩

Sample solution: 30 mg/mL

Medium: Chloroform

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 2,2,4-Trimethylpentane, dioxane, and acetonitrile (78:21.5:0.5)

Standard solution: 5.0 mg/mL of USP Misoprostol RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Misoprostol in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L3

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution* [NOTE—Identify the impurities based on the retention times shown in *Impurity Table 1*.]

Suitability requirements

Resolution: NLT 1.4, between the second diastereomer peak for 12-epimisoprostol and the misoprostol peak

Relative standard deviation: NMT 1.0%, for three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{38}O_5$ in the portion of Misoprostol taken:

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

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

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

In-Process Revision

Acceptance criteria: 97.0%–102.0%

IMPURITIES
Organic Impurities
• **PROCEDURE 1**
Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatogram for at least 3 times the retention time of the misoprostol peak, and measure the peak responses. Identify the impurities based on the retention times shown in *Impurity Table 1*.
Calculate the percentage of each impurity in the portion of Misoprostol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response for each impurity from the *Sample solution*
r_S = response of the *Standard solution*
C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL).
C_U = concentration of Misoprostol in the *Sample solution* (mg/mL).
F = relative response factor (see *Impurity Table 1*)
Acceptance criteria
Individual impurities: See *Impurity Table 1*
Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
A-Type misoprostol[a]	0.22	7.8	0.1
B-Type misoprostol[b]	0.33	0.80	0.1
Norprostol[c]	0.51	8.4	0.1
8-Epimisoprostol[d]	0.71	1.05	0.3
12-Epimisoprostol[e]	0.86 and 0.92[f]	1.08	1.0[f]
Misoprostol	1.0	—	—
Any other individual impurity	—	1.0	0.1

[a]Methyl 7-{(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl}heptanoate.
[b](*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl]heptanoate.
[c]Methyl 7-(3-hydroxy-5-oxocyclopent-1-enyl)heptanoate.
[d]Methyl (1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.
[e]Methyl (1*S**,2*R**,3*S**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.
[f]12-Epimisoprostol consists of two diastereomers that are separated under these conditions; integrate both peaks together for the impurity calculations.

• **PROCEDURE 2: CONTENT OF DIASTEREOMERS**
Mobile phase: Hexane, ethanol, and isopropyl alcohol (94:4:2)
Sample solution: 1.0 mg/mL of Misoprostol in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L3
Temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Sample solution* [NOTE—Identify the components based on their relative retention times which are about 0.92 for the first diastereomer peak and 1.0 for the second diastereomer peak.]
Suitability requirements
Resolution: NLT 2.0, between the two diastereomer peaks
Relative standard deviation: NMT 2.0% from the area of the first diastereomer peak
Analysis
Sample: *Sample solution*
Calculate the fraction of the first diastereomer in the portion of Misoprostol taken:

$$\text{Result} = r_1/(r_1 + r_2)$$

r_1 = response for the first diastereomer
r_2 = response for the second diastereomer
Acceptance criteria
Fraction of the first diastereomer: 0.51–0.56

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a freezer.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Misoprostol RS■1S *(USP33)*

BRIEFING

Morphine Sulfate Extended-Release Capsules, *USP 32* page 3008. On the basis of comments received, it is proposed to revise the *Assay* and *Organic Impurities* methods and introduce new specifications for specified and unspecified impurities.

(MD-CCA: C. Anthony.) RTS—C46368

Morphine Sulfate Extended-Release Capsules

DEFINITION
Morphine Sulfate Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Diluent: Water. Adjust with phosphoric acid to a pH of 3.60.
Buffer solution: 13.8 mg/mL of monobasic sodium phosphate
~~**Mobile phase**~~■**Solution A:**■1S *(USP33)* Acetonitrile, triethylamine, *Buffer solution*, and water (25:0.5:100:874.5)■Adjust with phosphoric acid to a pH of 3.60.

In-Process Revision

Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
33	100	0
44	85	15
54	85	15
55	100	0
65	100	0

■1S (*USP33*)

System suitability solution: ~~Prepare a solution containing 10 mg/mL of USP Morphine Sulfate RS in *Diluent*. Mix 1.0 mL of this solution with 2.0 mL of 30% hydrogen peroxide. Heat, with stirring, in a water bath at a temperature of 80° for 30 min. Cool to room temperature, and dilute with *Diluent* to 10 mL.~~■400 µg/mL USP Morphine Sulfate RS and 10 µg/mL USP Morphine Related Compound A RS and 10 µg/mL USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*■1S (*USP33*)

Standard solution: 1.0 mg/mL of USP Morphine Sulfate RS in *Diluent*

■**Sample stock solution:** Transfer a weighed portion of the contents from NLT 20 Capsules, equivalent to 250 mg of morphine sulfate, to a 100-mL volumetric flask. Add 5 mL of methanol, and mix well for 30 min with gently swirling every 5 min. Add *Diluent* up to half of the flask volume, and sonicate for 5 min to dissolve. Dilute with *Diluent* to volume.■1S (*USP33*)

Sample solution: ~~Transfer the contents of 10 Capsules to a suitable volumetric flask to obtain a solution having a final concentration of 1 mg/mL of morphine sulfate. Add a quantity of methanol equivalent to 4.5% of the flask volume. Mix for 30 min, gently swirling every 5 min. Add *Diluent* up to half of the flask volume, and sonicate for 5 min to dissolve. Rinse the inner wall and neck of the flask with a quantity of methanol equivalent to about 0.5% of the flask volume, and dilute with *Diluent* to volume. Pass through a suitable filter, and use the clear filtrate.~~■1.0 mg/mL of morphine sulfate from *Sample stock solution* in *Diluent*. Pass through a suitable filter, and use the clear filtrate.■1S (*USP33*)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Guard column: Packing L1
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection size: ~~30 µL~~■40 µL■1S (*USP33*)

System suitability
Samples: *System suitability solution* and *Standard solution*
~~[NOTE—The relative retention times for morphine *N*-oxide and pseudomorphine are between 1.2 and 1.4 and 2.2 and 2.8, respectively.]~~■■1S (*USP33*)

Suitability requirements
Resolution: NLT 2.0 between the ~~morphine *N*-oxide~~■morphine related compound A■1S (*USP33*) and morphine sulfate peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
pH 7.5 Phosphate buffer: 6.8 mg/mL of monobasic potassium phosphate and 1.6 mg/mL of sodium hydroxide. Adjust with phosphoric acid or 2 N sodium hydroxide to a pH of 7.5.
Medium: Proceed as directed for *Procedure* for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*, observing the following exceptions. Perform *Acid Stage* testing, using 500 mL of 0.1 N hydrochloric acid for 1 h, and perform *Buffer Stage* testing, using 500 mL of *pH 7.5 Phosphate buffer* for NLT 8 h.
Apparatus 1: 100 rpm
Times: 1, 4, 6, and 9 h
Determine the amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved by employing the following method.
Mobile phase: Methanol, glacial acetic acid, and water (28:1:72), containing 0.73 g of sodium 1-heptanesulfonate
System suitability solution: 0.1 mg/mL each of phenol and USP Morphine Sulfate RS in *Mobile phase*
Standard solution: USP Morphine Sulfate RS in *pH 7.5 Phosphate buffer* to obtain a solution having a known concentration corresponding to that of the *Sample solution*
Sample solution: Sample per *Dissolution* ⟨711⟩.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 284 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection size: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for phenol and morphine sulfate are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the phenol and morphine sulfate peaks
Tailing factor: NMT 2.0 for the morphine sulfate peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: The percentage of the labeled amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved in 1 h conforms to *Acceptance Table 3*. The percentages of the labeled amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved at the other times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved (%)
1	NMT 10
4	25–50
6	50–90
9	NLT 85

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**
Diluent, Chromatographic system, ~~Buffer solution,~~■■1S (*USP33*)
Mobile phase, System suitability solution, Standard solution, ~~Sample solution, and System suitability~~■and Sample solution:■1S (*USP33*) Proceed as directed in the *Assay*.

In-Process Revision

■**Sensitivity solution:** 0.5 µg/mL of USP Morphine Sulfate RS in *Diluent*

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*

Sensitivity: Morphine peak is detectable, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

■1S (*USP33*)

Analysis

Samples: ~~Inject equal volumes (about 30 µL) of~~ ■■1S (*USP33*)*Diluent*, *Sample solution*, and *Sensitivity solution*

[NOTE—Disregard the peaks corresponding to those of the *Diluent*.]

Calculate the percentage of each impurity in the portion of Capsules taken:

~~Result = (100/F) × (r_i/r_M)~~

■

$$\text{Result} = (1/F) \times 100 \times (r_U/r_M)$$ ■1S (*USP33*)

F = relative response factor ~~(2.1 for morphine sulfate related compound B (pseudomorphine) and 1.0 for mophine sulfate related compound A (morphine-N-oxide) and any unspecified impurity peak~~■from *Impurity Table 1*■1S (*USP33*).

r_U = peak response for each impurity from the *Sample solution*

r_M = peak response for morphine sulfate from the *Sample solution*

Acceptance criteria

Individual impurities: ~~NMT 1.0%~~■See *Impurity Table 1*.■1S (*USP33*)

~~Total impurities:~~ ~~NMT 2.0%~~

■

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Morphine related compound A[a]	1.4	1.0	0.5
Morphine sulphate	1.0	—	—
Morphine related compound B[b]	2.3	2.1	0.5
Any unspecified impurity	—	—	0.2
Total impurities	—	—	1.5

[a]7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol, *N*-oxide.
[b]2,2′-Bimorphine.

■1S (*USP33*)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Morphine Sulfate RS
  ■USP Morphine Related Compound A RS
  USP Morphine Related Compound B RS■1S (*USP33*)

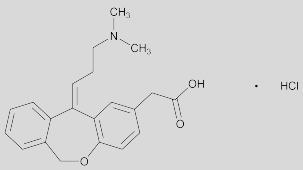
BRIEFING

Olopatadine Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with the Ultracarb C8, 5-µm brand of L7 column. The typical retention time for olopatadine is between 7 and 10 min. See also *Olopatadine Hydrochloride Ophthalmic Solution* appearing elsewhere in this issue of *PF.*

(MD-OOD: F. Mao.) RTS—C62481

Add the following:

■Olopatadine Hydrochloride



$C_{21}H_{23}NO_3 \cdot HCl$ 373.87

Dibenz[*b,e*]oxepin-2-acetic acid, 11-[3-(dimethylamino)propylidene]-6,11-dihydro-, hydrochloride, (*Z*)-;
11-[(*Z*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid, hydrochloride [140462-76-6].

DEFINITION

Olopatadine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{21}H_{23}NO_3 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **PROCEDURE** [NOTE—Protect solutions from light.]

Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (7:18)

Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Olopatadine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{23}NO_3 \cdot HCl$ in the portion of Olopatadine Hydrochloride taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of olopatadine hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Heavy Metals** *Method II* **⟨231⟩:** NMT 10 ppm

Organic Impurities

- **Procedure** [Note—Protect solutions from light.]

Mobile phase: Proceed as directed in the *Assay*.
Blank solution: *Mobile phase*
System suitability solution: 0.2 mg/mL of Olopatadine Hydrochloride and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Olopatadine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
Run time: At least 2.5 times the retention time of the major peak
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for olopatadine and olopatadine related compound B are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between olopatadine and olopatadine related compound B
Column efficiency: NLT 2000, olopatadine peak
Tailing factor: NMT 2.0, olopatadine peak
Relative standard deviation: NMT 2.0%, olopatadine peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor for each individual impurity (see *Impurity Table 1*)

[Note—Disregard any peaks corresponding to those of the *Blank solution*.]

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.25%

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
α-Hydroxy Olopatadine [a]	0.4	1.0	0.2
Olopatadine *E*-isomer [b]	0.7	1.3	0.1
Olopatadine	1.0	—	—
Any other individual impurity	—	1.0	0.1

[a](*Z*)-2-{11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-yl}-2-hydroxyacetic acid
[b]11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid

SPECIFIC TESTS

- **pH ⟨791⟩:** Between 2.0 and 4.0, in a solution (1 in 100)
- **Loss on Drying ⟨731⟩:** Dry it at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP Reference Standards ⟨11⟩**
USP Olopatadine Hydrochloride RS
USP Olopatadine Related Compound B RS■1S *(USP33)*

Briefing

Olopatadine Hydrochloride Ophthalmic Solution. See briefing under *Olopatadine Hydrochloride*. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with the Ultracarb C8, 5-µm brand of L7 column. The typical retention time for olopatadine is between 7 and 10 min in the test for *Limit of Early Eluting Impurities* and in the *Assay*. The typical retention time for olopatadine related compound C in the test for *Limit of Late Eluting Impurities* is about 5 min.

(MD-OOD: F. Mao. MSA: R. Tirumalai.) RTS—C62481

Add the following:

■Olopatadine Hydrochloride Ophthalmic Solution

DEFINITION

Olopatadine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Olopatadine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$). It may contain suitable antimicrobial agents.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE** [NOTE—Protect solutions from light.]

Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (7:18)

Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: Equivalent to 0.1 mg/mL of olopatadine in *Mobile phase*, from Olopatadine Hydrochloride Ophthalmic Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L7

Flow rate: 1 mL/min

Injection size: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{23}NO_3$ in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of olopatadine hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of olopatadine, 337.41

M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities [NOTE—Protect solutions from light.]

• **PROCEDURE 1: LIMIT OF EARLY ELUTING IMPURITIES**

Mobile phase: Proceed as directed in the *Assay*.

Blank solution: *Mobile phase*

System suitability solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: Equivalent to 0.2 mg/mL of olopatadine in *Mobile phase*, from Olopatadine Hydrochloride Ophthalmic Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L7

Flow rate: 1 mL/min

Injection size: 30 µL

Run time: At least 1.6 times the retention time of the major peak

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between olopatadine and olopatadine related compound B, *System suitability solution*

Column efficiency: NLT 2000, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = response of each impurity from the *Sample solution*

r_S = response of olopatadine from the *Standard solution*

C_S = concentration of olopatadine hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of olopatadine, 337.41

M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

F = relative response factor for each individual impurity (See *Impurity Table 1*.)

[NOTE—Disregard any peaks corresponding to those of the *Blank solution* and any peaks with relative retention time, measured with respect to olopatadine, greater than 1.5.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Olopatadine *E*-isomer [a]	0.7	1.3	0.5
Olopatadine	1.0	—	—
Olopatadine related compound B	1.2	1.0	2
Olopatadine carbaldehyde [b]	1.3	4.5	0.5
Any Unspecified Impurity	—	1.0	0.5

[a] 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic acid

[b] (*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenzo[*b*,*e*]oxepine-2-carbaldehyde

• **PROCEDURE 2: LIMIT OF LATE ELUTING IMPURITIES**

Buffer: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Buffer* (1:1)

Blank solution: *Mobile phase*

System suitability solution: 0.02 mg/mL of olopatadine hydrochloride and 0.01 mg/mL of olopatadine related compound C in *Mobile phase*

Standard solution: 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*

Sample solution: Use *Sample solution* from the test for *Limit of Early Eluting Impurities*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L7

Flow rate: 1 mL/min

Injection size: 30 µL

Run time: At least 3 times the retention time of the olopatadine related compound C peak

System suitability

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

[NOTE—The relative retention times for olopatadine and olopatadine related compound C are 0.3 and 1.0, respectively.]

In-Process Revision

Suitability requirements
Resolution: NLT 7.0 between olopatadine and olopatadine related compound C, *System suitability solution*
Column efficiency: NLT 2000, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of olopatadine related compound C from the *Standard solution*
C_S = concentration of USP Olopatadine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

[NOTE—Disregard any peaks corresponding to those of the *Blank solution* and any peaks with relative retention time, measured with respect to olopatadine related compound C, less than 0.7.]

Acceptance criteria
Individual impurities: NMT 1% of olopatadine related compound C is found; and NMT 0.5% of any other individual impurity is found.
Total impurities: NMT 3% [NOTE—Total impurities are the sum of olopatadine related compound B, olopatadine related compound C, Olopatadine *E*-isomer, Olopatadine carbaldehyde, and all other impurities found in the tests for *Limit of Early Eluting Impurities* and *Limit of Late Eluting Impurities*.]

SPECIFIC TESTS
- **STERILITY TESTS ⟨71⟩:** Meets the requirements
- **pH ⟨791⟩:** Between 5.0 and 8.0
- **OSMOLALITY AND OSMOLARITY ⟨785⟩:** Between 260 and 320 mOsmol/kg

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 25°.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Olopatadine Hydrochloride RS
  USP Olopatadine Related Compound B RS
  USP Olopatadine Related Compound C RS■1S *(USP33)*

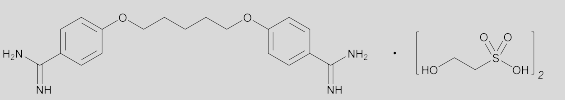
BRIEFING

Pentamidine Isethionate. Because there is no existing *USP* monograph for this drug substance, a new monograph, adopted from the official *European Pharmacopoeia* 6.0 monograph, is being proposed. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with a Symmetry C-18 brand of L1 column. The typical retention time of pentamidine is about 3 min.

(MD-AA: L. Santos, B. Davani.) RTS—C49314

Add the following:

■Pentamidine Isethionate



$C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$ 592.70
Ethanesulfonic acid, 2-hydroxy-, compd. with 4,4′-[1,5-pentanediylbis(oxy)]bis [benzenecarboximidamide];
4,4′-(Pentane-1,5-diylbis(oxy))dibenzimidamide bis(2-hydroxyethanesulfonate) [140-64-7].

DEFINITION
Pentamidine Isethionate contains NLT 98.5% and NMT 101.5% of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B. OXYGEN-FLASK COMBUSTION ⟨471⟩**
  Barium chloride solution: 60 mg/mL of barium chloride in water.
  Analysis: Burn 150 mg, using 10 mL of 3% hydrogen peroxide as the absorbing liquid. When the process is complete, acidify with 1 mL of diluted hydrochloric acid, and add 1 mL of *Barium chloride solution*. A white precipitate forms.
- **C.** The retention time of the pentamidine isethionate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Sample solution: 5 mg/mL in dimethylformamide. Add 0.25 mL of thymol blue TS.
  Analysis: Titrate under a stream of nitrogen with 0.1M tetrabutylammonium hydroxide VS, determining the endpoint until the color changes to intense blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1M tetrabutylammonium hydroxide is equivalent to 29.63 mg of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$.
  Acceptance criteria: 98.5%–101.5%

IMPURITIES
Inorganic Impurities
- **HEAVY METALS,** *Method I* **⟨231⟩:** NMT 20 ppm
- **RESIDUE ON IGNITION ⟨281⟩**
  Acceptance criteria: NMT 0.1% on a 1 g sample

Organic Impurities
- **PROCEDURE**
  Buffer: 30 mg/mL of ammonium acetate in water, adjusted with triethylamine to a pH of 7.5
  Mobile phase: Methanol and *Buffer* (65:35)
  System suitability solution: Prepare 40.0 mL of a 2.5 mg/mL solution of USP Pentamidine Isethionate RS in water. Adjust with 0.2 M sodium hydroxide to a pH of 10.5, and boil under reflux for 20 min. Cool, and dilute with water to 50.0 mL. Transfer quantitatively 1 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Standard solution: 2 µg/mL of USP Pentamidine Isethionate RS in *Mobile phase*
  Sample solution: 1.0 mg/mL of Pentamidine Isethionate in *Mobile phase*. [NOTE—It must be demonstrated that the final product does not contain a detectable amount of alkyl 2-hydroxyethanesulphonates, a potential in-process impurity.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 3.5 times the retention time of pentamidine
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2 between the two major peaks. [NOTE—The chromatogram obtained shows two major peaks.]
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria
Individual impurities: NMT 0.4% [NOTE—Exclude any other peak producing a response of less than 0.02%.]
Total impurities: NMT 0.7%

SPECIFIC TESTS
- **pH ⟨791⟩:** 4.5–6.5, in a carbon dioxide-free aqueous solution containing 50 mg/mL of pentamidine isethionate
- **Loss on Drying ⟨731⟩:** Dry at 105°: it loses NMT 4.0% of its weight.

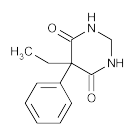
ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **USP Reference Standards ⟨11⟩**
USP Pentamidine Isethionate RS■1S *(USP33)*

BRIEFING

Primidone. *USP* 32 page 3382. It is proposed to make the following changes to the monograph:
1. Replace the UV assay with a validated HPLC procedure. The HPLC method was validated for tablets using a Waters Atlantis C18 brand of L1 column. The typical retention time for Primidone is about 4 min.
2. Replace the TLC ordinary impurities method with the HPLC method described in the current *European Pharmacopoeia*. The current *USP* method is nonselective and uses chlorine gas which can pose a safety hazard. The proposed method uses a Merck KGaA Chromolith RP-18E brand of L1 column from which primidone typically elutes at about 2 min.
3. Replace *Identification* tests *B* and *C* with an HPLC retention time comparison of the major peaks in the chromatograms of the *Standard solution* and *Sample solution* in the *Assay*.
4. Delete the test for *Melting Range or Temperature*. Because a selective HPLC impurity test is being added, the test for *Melting Range or Temperature* contributes no additional value in establishing the quality of the drug substance.

(MD-PP: D. Vicchio, R. Ravichandran.) RTS—C57472

Primidone



$C_{12}H_{14}N_2O_2$ 218.25
4,6(1*H*,5*H*)-Pyrimidinedione, 5-ethyldihydro-5-phenyl-;
5-Ethyldihydro-5-phenyl-4,6(1*H*,5*H*)-pyrimidinedione [125-33-7].

DEFINITION
Primidone contains NLT 98.0% and NMT 102.0% of $C_{12}H_{14}N_2O_2$, calculated on the dried basis.

IDENTIFICATION
- **A. Infrared Absorption ⟨197K⟩:** If a difference appears, dissolve portions of both the sample and the Reference Standard in alcohol, evaporate the solutions to dryness, and repeat the tests.

Change to read:

- **B. ~~Ultraviolet Absorption ⟨197U⟩~~**
~~Sample solution:~~ ~~400 µg/mL in alcohol~~
~~Analytical wavelength:~~ ~~257 nm~~
~~Acceptance criteria:~~ ~~Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.~~
■The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.■1S *(USP33)*

Delete the following:

■**~~C. Procedure:~~** ~~Fuse 0.20 g with 0.20 g of anhydrous sodium carbonate: ammonia is evolved.~~■1S *(USP33)*

ASSAY

Change to read:

- **Procedure**
~~Sample solution:~~ ~~40 mg of Primidone in 70 mL of alcohol, and boil gently to dissolve. Cool, and add alcohol to make 100 mL.~~
~~Standard solution:~~ ~~400 µg/mL of USP Primidone RS in alcohol~~
~~Spectrometric conditions~~
~~Analytical wavelengths~~
~~Minima:~~ ~~254 nm and 261 nm~~
~~Maxima:~~ ~~257 nm~~
~~Cell:~~ ~~2 cm~~
~~Blank:~~ ~~Alcohol~~
~~Analysis:~~ ~~Determine the absorbances of the *Sample solution* and the *Standard solution*.~~
~~Calculate the percentage of $C_{12}H_{14}N_2O_2$ in the Primidone taken:~~

$$\text{Result} = [(2A_{257,U} - A_{254,U} - A_{261,U})/(2A_{257,S} - A_{254,S} - A_{261,S})] \times (C_S/C_U) \times 100$$

C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Primidone in the *Sample solution* (mg/mL)
$A_{257,U}$ = absorbance of the *Sample solution* at 257 nm
$A_{254,U}$ = absorbance of the *Sample solution* at 254 nm
$A_{261,U}$ = absorbance of the *Sample solution* at 261 nm
$A_{257,S}$ = absorbance of the *Standard solution* at 257 nm
$A_{254,S}$ = absorbance of the *Standard solution* at 254 nm
$A_{261,S}$ = absorbance of the *Standard solution* at 261 nm

■**Solution A:** 6.8 g/L of monobasic potassium phosphate
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (35:0.5:65)
Diluent: Methanol and water (35:65)
Standard stock solution: 0.5 mg/mL of USP Primidone RS in methanol
Standard solution: 0.05 mg/mL of USP Primidone RS in *Diluent*, prepared from the *Standard stock solution*
Sample stock solution: 0.5 mg/mL of Primidone in methanol
Sample solution: 0.05 mg/mL of Primidone from the *Sample stock solution* diluted with *Diluent*

In-Process Revision

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.3 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_{14}N_2O_2$ in the portion of Primidone taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Primidone in the *Sample solution* (mg/mL)

■1S (*USP33*)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

• **Residue on Ignition ⟨281⟩:** NMT 0.2%

Change to read:

Organic Impurities

• **~~Procedure: Ordinary Impurities ⟨466⟩~~**
~~Standard solution A:~~ ~~2 µg/mL of USP Primidone RS in methanol~~
~~Standard solution B:~~ ~~10 µg/mL of USP Primidone RS in methanol~~
~~Standard solution C:~~ ~~20 µg/mL of USP Primidone RS in methanol~~
~~Standard solution D:~~ ~~40 µg/mL of USP Primidone RS in methanol~~
~~Sample solution:~~ ~~2 mg/mL in methanol~~
~~Eluant:~~ ~~Butyl alcohol, glacial acetic acid, and water (5:3:2)~~
~~Visualization:~~ ~~Expose the plate to chlorine gas for 15 min, air-dry until the chlorine has dissipated (15 min), and follow with Visualization Technique 20.~~

■• **Procedure**
Solution A: 1.36 g/L of monobasic potassium phosphate
Solution B: Methanol
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1.0	75	25
6.0	40	60
8.0	40	60
8.5	75	25
10.0	75	25

Standard stock solution: 1000 µg/mL of USP Primidone RS in methanol
Standard solution: 1 µg/mL of USP Primidone RS in methanol, prepared from the *Standard stock solution* in methanol.
System suitability solution: 1000 µg/mL of USP Primidone RS, 10 µg/mL each of USP Phenobarbital RS and USP Primidone Related Compound C RS. Prepared by diluting weighed quantities of USP Phenobarbital RS and USP Primidone Related Compound C RS with *Standard stock solution*.
Sample solution: 1000 µg/mL of Primidone in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 10-cm; monolithic packing L1
Flow rate: 3.2 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between phenobarbital and primidone related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for primidone, *Standard solution*
Relative standard deviation: NMT 5.0% for primidone, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the impurities using the relative retention times listed in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of sample taken:

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

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (µg/mL)
C_U = concentration of Primidone in the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%
[Note—Disregard impurity peaks below 0.05%.]

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Primidone related compound A[a]	0.5	0.67	0.1
Phenobarbital	1.4	1.0	0.1
Primidone related compound C[b]	1.6	0.67	0.1
2-Cyano-2-phenylbutyramide	1.8	0.71	0.1
2-Phenylbutyric acid	2.0	0.77	0.1
Phenylpropylprimidone[c]	2.8	1.0	0.3
Any unspecified impurity	—	1.0	0.1

[a]2-Ethyl-2-phenylmalonamide (2-ethyl-2-phenylpropanediamide).
[b]2-Phenylbutyramide.
[c]5-Ethyl-5-phenyl-2-(1-phenylpropyl)dihydropyrimidine-4,6(1*H*,5*H*)-dione.

■1S (*USP33*)

SPECIFIC TESTS

Delete the following:

■• **~~Melting Range or Temperature ⟨741⟩:~~** ~~279°–284°~~ ■1S (*USP33*)

• **Loss on Drying ⟨731⟩:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

In-Process Revision

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  ■USP Phenobarbital RS■1S *(USP33)*
  USP Primidone RS
  ■USP Primidone Related Compound C RS■1S *(USP33)*

BRIEFING

Primidone Tablets, *USP 32* page 3383. It is proposed to replace the packed column GC assay method with a validated HPLC method. The HPLC method was validated using a Waters Atlantis C18 brand of L1 column. The typical retention time for primidone is about 3.5–4.5 min. It is also proposed to add an *Organic Impurities* section using the assay method to quantify any impurities present.

(MD-PP: D. Vicchio, R. Ravichandran.) RTS—C57472

Primidone Tablets

DEFINITION
Primidone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of $C_{12}H_{14}N_2O_2$.

IDENTIFICATION
- The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  ~~**Internal standard solution:** 10 mg/mL of androsterone in alcohol~~
  ~~**Standard solution:** 100 mg of USP Primidone RS in 65 mL of alcohol, and boil for 1 h. Allow to cool to ambient temperature, add 10.0 mL of *Internal standard solution*, dilute with alcohol to 100 mL, and filter.~~
  ~~**Sample solution:** Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of primidone, to a 50-mL volumetric flask. Add 35 mL of alcohol, and boil for 1 h. Allow to cool to ambient temperature, add 5.0 mL of *Internal standard solution*, dilute with alcohol to volume, mix, and filter.~~
  ~~**Chromatographic system**~~
  ~~(See *Chromatography* ⟨621⟩*, System Suitability*.)~~
  ~~**Mode:** GC~~
  ~~**Detector:** Flame ionization~~
  ~~**Column:** 4.0-mm × 120-cm; packed with 10% liquid phase G3 on support S1AB~~
  ~~**Carrier gas:** Helium~~
  ~~**Temperature**~~
  ~~**Detector:** 310°~~
  ~~**Injection port:** 310°~~
  ~~**Column:** 260°~~
  ~~**Flow rate:** 40 mL/min~~
  ~~**Injection size:** 3 µL~~
  ~~**System suitability**~~
  ~~**Sample:** *Standard solution* (three replicate injections)~~
  ~~[NOTE—The relative retention times for primidone and androsterone are about 0.8 and 1.0, respectively.]~~
  ~~**Suitability requirements**~~
  ~~**Resolution:** NLT 1.5 between primidone and androsterone~~
  ~~**Relative standard deviation:** NMT 2.0%~~
  ~~**Analysis**~~
  ~~**Samples:** *Standard solution* and *Sample solution*~~
  ~~Calculate the percentage of $C_{12}H_{14}N_2O_2$ in the Tablets taken:~~

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$~~

~~R_U = peak response ratio from the *Sample solution*~~
~~R_S = peak response ratio from the *Standard solution*~~
~~C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)~~
~~C_U = nominal concentration of Primidone in the *Sample solution* (mg/mL)~~

■**Solution A:** 6.8 g/L of monobasic potassium phosphate
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (35:0.5:65)
Diluent: Methanol and water (35:65)
Standard stock solution: 0.5 mg/mL of USP Primidone RS in methanol
Standard solution: 0.05 mg/mL of USP Primidone RS in *Diluent*, prepared from the *Standard stock solution*
Sample stock solution: Transfer an equivalent to about 250 mg of primidone, from finely powdered Tablets (NLT 20), based on the label claim, to a 250-mL volumetric flask, add about 125 mL of methanol, sonicate for about 15 min, and shake by mechanical means until all the solid is dispersed, usually 20 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Pass a portion of this solution through a PTFE (or equivalent) filter having a 0.45-µm or finer porosity, discard the first 5 mL of filtrate.
Sample solution: 0.05 mg/mL of primidone from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm x 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.3 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_{14}N_2O_2$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = concentration of primidone in the *Sample solution* (mg/mL)

■1S *(USP33)*

In-Process Revision

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩**
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Detector: UV 257 nm
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Standard solution: USP Primidone RS in *Medium*. [NOTE—Perform baseline corrections, if necessary, in determining the absorbance by extrapolating the baseline through the absorbance minima at 300 and 280 nm and beyond 257 nm.]
Tolerances: NLT 75% (Q) of the labeled amount of $C_{12}H_{14}N_2O_2$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Add the following:

■**Organic Impurities**

• **PROCEDURE**
Solution A, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Primidone stock solution: 0.05 mg/mL of USP Primidone RS in methanol
Primidone related compound A stock solution: 200 µg/mL of USP Primidone Related Compound A RS in methanol
Primidone related compound A solution: 20 µg/mL of USP Primidone Related Compound A RS in *Diluent*, from *Primidone related compound A stock solution*
System suitability solution: 1 µg/mL of USP Primidone Related Compound A RS and 2 µg/mL of USP Primidone RS in *Diluent*, from *Primidone related compound A solution* and *Primidone stock solution*
Standard solution: 2 µg/mL of USP Primidone RS in *Diluent*, from *Primidone stock solution*
Sample solution: Transfer an equivalent to about 250 mg of primidone, from finely powdered Tablets (NLT 20), based on the label claim, to a 250-mL volumetric flask, add about 90 mL of methanol, sonicate for about 15 min, and shake by mechanical means until all the solid is dispersed, usually 20 min. Allow the solution to cool to room temperature, and dilute with water to volume. Pass a portion of this solution through a PTFE (or equivalent) filter having a 0.45-µm or finer porosity, discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for primidone related compound A and primidone are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between primidone and primidone related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0% for primidone, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (µg/mL)
C_U = concentration of primidone in the *Sample solution* (µg/mL)
F = relative response factor

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
[NOTE—Disregard impurity peaks that are less than 0.025%.]
Total impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (%)
Primidone related compound A[a]	0.5	0.76	0.1
Primidone	1.0	—	—
Phenobarbital	1.6	1.4	0.1
Primidone related compound C[b]	1.9	0.92	0.1
2-Phenylbutyric acid	4.1	0.91	0.1
Any unspecified impurity	—	1.0	0.1

[a] 2-Ethyl-2-phenylmalonamide.
[b] 2-Phenylbutyramide.

■1S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Tablets intended solely for veterinary use are so labeled.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**
USP Primidone RS
■USP Primidone Related Compound A RS■1S (*USP33*)

BRIEFING

Propoxyphene Hydrochloride Capsules, *USP 32* page 3418. On the basis of comments received, it is proposed to delete *Identification* test *B* because the optical rotation is not needed in the monograph as the test is already performed in the drug substance monograph. In addition, the *Identification* section already has two orthogonal tests.

(MD-CCA: C. Anthony.) RTS—C42520

Propoxyphene Hydrochloride Capsules

DEFINITION
Propoxyphene Hydrochloride Capsules contain NLT 92.5% and NMT 107.5% of the labeled amount of $C_{22}H_{29}NO_2 \cdot HCl$.

IDENTIFICATION
[NOTE—Use the following solutions in the tests below.]
Standard solution: Transfer 125 mg of USP Propoxyphene Hydrochloride RS to a 125-mL separator containing 8 mL of acetone, 32 mL of water, and 20 mL of 100 mg/mL of sodium carbonate solution, and swirl the mixture for 3 min. Add

In-Process Revision

25.0 mL of chloroform, insert the stopper, and shake the mixture by mechanical means for 1 h. Filter the chloroform extract through a layer of anhydrous sodium sulfate into a suitable beaker.

Sample solution: Transfer a quantity of Capsule contents, remaining from the preparation of the *Sample solution* in the *Assay*, equivalent to 320 mg of propoxyphene hydrochloride to a 100-mL volumetric flask. Add 20 mL of acetone, and sonicate for 1 min. Dilute the solution with water to 100 mL. Allow to stand until the excipients have settled, usually 15–20 min. Transfer 40.0 mL of this solution to a 125-mL separator containing 20 mL of 100 mg/mL of sodium carbonate solution, and swirl the mixture for 3 min. Add 25.0 mL of chloroform, insert the stopper, and shake the mixture by mechanical means for 1 h. Filter the chloroform extract through a layer of anhydrous sodium sulfate into a suitable beaker. Use the *Standard solution* and *Sample solution* for the following tests.

- **A.** The IR absorption spectrum of the *Sample solution*, concentrated if necessary by evaporating a portion on a steam bath with the aid of a current of air to one-fifth of its volume, exhibits maxima only at the same wavelengths as that of the *Standard solution*.

Delete the following:

~~- **B. OPTICAL ROTATION** ⟨781⟩~~

~~**Analysis:** Transfer 20.0 mL of each *Sample solution* and *Standard solution* to separate beakers, and evaporate on a steam bath with the aid of a current of air to 5 mL. Remove the beakers from the steam bath, and continue evaporation with the aid of a current of air until the chloroform is completely evaporated. Add 5.0 mL of 0.1 N hydrochloric acid to each, and dissolve the residue.~~

~~Using the solution of the *Sample solution*, determine the specific rotation, in which C, the concentration of propoxyphene hydrochloride/100 mL of solution, is calculated as follows:~~

~~$$\text{Result} = A \times (W_U/W_T) \times 0.0064$$~~

~~A = quantity of propoxyphene hydrochloride/Capsule, as obtained in the *Assay* (mg)~~
~~W_U = weight of the portion of Capsule contents taken (mg)~~
~~W_T = average weight of the contents of each Capsule (mg)~~

~~Using the solution of the *Standard solution*, determine the specific rotation, C, being calculated by multiplying the weight, in mg, of USP Propoxyphene Hydrochloride RS taken by 0.016.~~

~~**Acceptance criteria:** The specific rotation of the *Sample solution* is NLT 95.0% of that of the *Standard solution*.~~ 1S (*USP33*)

Change to read:

- **C. B.** 1S (*USP33*) The chromatogram of the *Sample solution* obtained as directed in the *Assay* exhibits a major peak for propoxyphene hydrochloride, the retention time of which corresponds to that exhibited for the *Standard solution* in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 6.8 g of monobasic potassium phosphate in 900 mL of water. Add 2.0 mL of triethylamine and adjust by the addition of phosphoric acid to a pH of 3.0 ± 0.1. Dilute with water to 1000 mL (phosphate buffer).

Diluent: Acetonitrile and water (2:3)
Mobile phase: Acetonitrile and *Solution A* (2:3)
Standard solution: 6.5 µg/mL of USP Propoxyphene Hydrochloride RS in *Diluent*. [NOTE—Sonicate as needed to aid dissolution.]
Sample stock solution: Remove, as completely as possible, the contents of NLT 20 Capsules. Weigh the contents, and determine the average weight/Capsule. Transfer the mixed combined contents of Capsules nominally equivalent to 65 mg of propoxyphene hydrochloride to 50 mL of *Diluent*, sonicate for 5 min, and shake by mechanical means for 15 min. Dilute with *Diluent* to 200 mL, and filter, discarding the first 20 mL of the filtrate.
Sample solution: Nominally equivalent to 6.5 µg/mL from the *Sample stock solution* diluted with *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 3.3-cm; 3-µm packing L1 that is base-deactivated. [NOTE—Precondition the column for at least 30 min with *Mobile phase*.]
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2 for propoxyphene hydrochloride peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{29}NO_2 \cdot HCl$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propoxyphene Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of propoxyphene hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Solution A: 2.99 g of sodium acetate trihydrate in 200 mL of water. Add 1.66 mL of glacial acetic acid, and dilute with water to 1000 mL (pH 4.5 acetate buffer).
Medium: *Solution A*; 500 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: USP Propoxyphene Hydrochloride RS in *Diluent*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Diluent* to a concentration that is similar to the *Standard solution*.
Analysis: Proceed as directed in the *Assay*, using a filtered portion of the *Sample solution* and a *Standard solution*.
Tolerances: NLT 85% (Q) of the labeled amount of $C_{22}H_{29}NO_2 \cdot HCl$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Propoxyphene Hydrochloride RS

In-Process Revision

BRIEFING

Ribavirin Capsules. Because there is no existing *USP* monograph for this dosage form, a new monograph is being proposed. The liquid chromatographic procedure in the tests for *Organic Impurities* and in the *Assay* is based on analysis performed with the Waters IC-PAK brand of column containing 7-µm packing L17. The typical retention time for the ribavirin peak is about 5.1 min. The chromatographic procedure in the test for *Dissolution* was validated using an Aminex HPX-87H column with packing L17. With this column, the retention time of ribavirin is about 6.6 min.

(MD-AA: L. Santos, B. Davani. BPC: M. Marques.) RTS—C64170

Add the following:

▪Ribavirin Capsules

DEFINITION

Ribavirin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$).

IDENTIFICATION

• The retention time of the ribavirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Water. Adjust with sulfuric acid to a pH of 2.5 ± 0.1.

Diluent: *Mobile phase*

Standard solution: 0.025 mg/mL of USP Ribavirin RS in *Diluent*

Sample stock solution: Transfer an equivalent to 50 mg of ribavirin, from NLT 20 finely powdered Capsules, to a 100-mL volumetric flask. Add about 50 mL of *Diluent*, and sonicate with occasional shaking for about 20 min. Cool to room temperature, dilute with *Diluent* to volume, and mix.

Sample solution: 0.025 mg/mL of ribavirin in *Diluent*. Pass the solution through a suitable filter (PTFE, PVDF, or equivalent) having a porosity of 0.45 µm.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 7.8-mm × 15-cm column; packing L17

Temperature: 65°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.7–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_8H_{12}N_4O_5$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: 0.0225 mg/mL of USP Ribavirin RS in *Medium*

Sample solution: Pass the solution through a suitable filter (PTFE, PVDF, or equivalent) having a porosity of 0.45 µm. Transfer 5.0 mL of the filtrate to a 50.0-mL volumetric flask, and dilute with *Medium* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 7.8-mm × 30-cm, 9-µm packing L17

Temperature: 65°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_8H_{12}N_4O_5$ dissolved in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium* (mL)
D = dilution factor of the solution under test

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Mobile phase, Diluent, Standard solution, and **Chromatographic system:** Proceed as directed for the *Assay*.

Sample solution: Count NLT 20 Capsules, and remove their content as completely as possible. Calculate the average net content. Transfer an equivalent to 50 mg of ribavirin from NLT 20 finely powdered Capsules to a 100-mL volumetric flask. Add about 50 mL of *Diluent*, and sonicate with occasional shaking for about 20 min. Cool to room temperature, dilute with *Diluent* to volume, and mix. Pass the solution through a suitable filter (PTFE, PVDF, or equivalent) having a porosity of 0.45 µm.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ribose triazolole carboxylic acid and any other unknown impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response of ribose triazolole carboxylic acid or any other unknown impurity from the *Sample solution*
r_S = response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)
F = relative response factor (See *Impurity Table 1*.)

In-Process Revision

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Ribose triazolole carboxylic acid	0.7	0.7	0.25
Ribavirin	1.0	—	—
Any individual unknown impurity	—	1.0	0.10

Acceptance criteria
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Ribavirin RS■1S *(USP33)*

BRIEFING

Sulfadiazine Tablets, *USP 32* page 3618. It is proposed to make some modifications in the quantitation step in the *Dissolution* test to simplify it.

(BPC: M. Marques.) RTS—C73273

Sulfadiazine Tablets

DEFINITION
Sulfadiazine Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of $C_{10}H_{10}N_4O_2S$.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B. MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨**741**⟩
Sample solution: Equivalent to 100 mg/mL of sulfadiazine from finely powdered Tablets in chloroform
Analysis: Transfer the *Sample solution* to a small filter. Wash with 5 mL of chloroform, and discard the filtrate. Triturate the residue with 10 mL of 6 N ammonium hydroxide for 5 min, add 10 mL of water, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and add 6 N acetic acid until the reaction is distinctly acid: a precipitate of sulfadiazine is formed. Collect the precipitate on a filter, wash it with cold water, and dry at 105° for 1 h.
Acceptance criteria: The sulfadiazine so obtained melts at 250°–254°.

ASSAY
- **PROCEDURE**
Mobile phase: Acetonitrile, glacial acetic acid, and water (12:1:87)
Standard solution: 1 mg/mL of USP Sulfadiazine RS in 0.025 N sodium hydroxide
Sample solution: 1 mg/mL of sulfadiazine from powdered Tablets in 0.025 N sodium hydroxide. [NOTE—Weigh NLT 20 Tablets, shake the solution for 30 min, and centrifuge, if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for sulfadiazine
Relative standard deviation: NMT 2.0% (five replicate injections)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{10}H_{10}N_4O_2S$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfadiazine in the *Sample solution* (mg/mL)
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 90 min
Detector: UV ~~254 nm~~■242 nm■1S *(USP33)*
Analysis: Determine the quantity of $C_{10}H_{10}N_4O_2S$ dissolved by using UV absorption at the wavelength of maximum absorbance at about ~~254 nm~~■242 nm■1S *(USP33)* on filtered portions of the solution under test, ~~suitably diluted with 0.01 N sodium hydroxide~~■1S *(USP33)* in comparison with a standard solution having a known concentration of USP Sulfadiazine RS in the same medium. ■Use *Medium* as the blank and cells with a path length of 0.1 cm.■1S *(USP33)*
Tolerances: NLT 70% (Q) of the labeled amount of $C_{10}H_{10}N_4O_2S$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Sulfadiazine RS

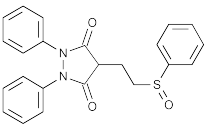
BRIEFING

Sulfinpyrazone, *USP 32* page 3634 and page 1483 of *PF* 34(6) [Nov.–Dec. 2008]. It is proposed to add an *Identification* test *B* that uses the retention time data obtained from the *Organic Impurities* test. Additionally, it is proposed to omit the test for *Melting Range or Temperature* because the *Organic Impurities* test by HPLC provides sufficient information for potential impurities.

(MD-AA: H. Ramanathan, B. Davani.) RTS—C73281

In-Process Revision

Sulfinpyrazone



$C_{23}H_{20}N_2O_3S$ 404.48
3,5-Pyrazolidinedione, 1,2-diphenyl-4-[2-(phenylsulfinyl)ethyl]-;
1,2-Diphenyl-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione [57-96-5].

DEFINITION
Sulfinpyrazone contains NLT 98.5% and NMT 101.5% of $C_{23}H_{20}N_2O_3S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• ■**A.**■1S *(USP33)* **INFRARED ABSORPTION** ⟨**197K**⟩

Add the following:

■• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Organic Impurities* test.■1S *(USP33)*

ASSAY
• **PROCEDURE**
Sample: 600 mg of Sulfinpyrazone
Analysis: Dissolve the *Sample* in 50 mL of neutralized alcohol, with slight heating. Add phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 40.45 mg of $C_{23}H_{20}N_2O_3S$.
Acceptance criteria: 98.5%–101.5%

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm
Organic Impurities
• **PROCEDURE**
Solution A: Acetonitrile and tetrahydrofuran (4:1)
Mobile phase: *Solution A* and dilute phosphoric acid (3 in 1000) (7:13)
Solution B: 0.1 N acetic acid, 0.1 N potassium hydroxide, and water (6:5:14). Adjust to a pH of 5.0.
Diluent: Acetonitrile and *Solution B* (4:1)
Standard solution: 10 µg/mL of USP Sulfinpyrazone RS in *Diluent*
Sample solution: 2 mg/mL of Sulfinpyrazone in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sulfinpyrazone is about 4.0–4.6 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The sum of the peak responses, other than that of the sulfinpyrazone peak, from the *Sample solution* is NMT four times the sulfinpyrazone peak response from the *Standard solution* (2.0%); and no single peak response is greater than twice the sulfinpyrazone peak response from the *Standard solution* (1.0%).

SPECIFIC TESTS

Delete the following:

■• ~~**MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** 130.5°–134.5°~~■1S *(USP33)*

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

Delete the following:

▲• ~~**SOLUBILITY IN ACETONE:** A 250-mg portion dissolves in 5.0 mL of acetone to yield a clear, practically colorless solution.~~▲*USP33*

Delete the following:

▲• ~~**SOLUBILITY IN 0.50 N SODIUM HYDROXIDE:** A 0.50-g portion dissolves in 10.0 mL of 0.50 N sodium hydroxide to yield a clear, practically colorless solution.~~▲*USP33*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Sulfinpyrazone RS

BRIEFING

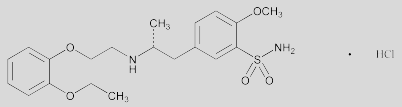
Tamsulosin Hydrochloride, *USP 32* page 3652. On the basis of comments received, it is proposed to report the limit of the *Residue on ignition* test rounded off to one decimal place. In addition, in the test for *Organic impurities*, it is proposed to revise the limit of the sum of two coeluting impurities, des-ethoxy and methoxy impurities, from NMT 0.20% to NMT 0.15%.

(MD-GRE: E. Gonikberg.) RTS—C67998

In-Process Revision

Add the following:

▲Tamsulosin Hydrochloride



$C_{20}H_{28}N_2O_5S \cdot HCl$ 444.97

Benzenesulfonamide, 5-[2-[[2-(2-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxy-, monohydrochloride, (*R*)-; (–)-(*R*)-5-[2-[[2-(*o*-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide monohydrochloride [106463-17-6].

DEFINITION

Tamsulosin Hydrochloride contains NLT 98.5% and NMT 101.0% of $C_{20}H_{28}N_2O_5S \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩
  Sample solution: 7.5 mg/mL in water. [NOTE—Heat to dissolve.] In an ice bath, cool 5 mL, and add 3 mL of diluted nitric acid. Allow to stand for 30 min at room temperature, and filter.

ASSAY

- **PROCEDURE**
  Sample: 350 mg, previously dried at 105° for 2 h
  Analysis: Dilute the *Sample* with 5 mL of formic acid, and add 75 mL of a mixture of glacial acetic acid and acetic anhydride (3:2). Titrate immediately with 0.1 N perchloric acid VS, and determine the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 44.50 mg of $C_{20}H_{28}N_2O_5S \cdot HCl$.

IMPURITIES

Inorganic Impurities

Change to read:

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT ~~0.10%~~■0.1%■1S *(USP33)*
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Change to read:

Organic Impurities

- **PROCEDURE 1**
  [NOTE—For impurities eluting before tamsulosin.]
  Buffer: Dissolve 8.7 mL of perchloric acid (70%) and 3.0 g of sodium hydroxide in 1900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 2.0, and add sufficient water to make 2000 mL.
  Mobile phase: Acetonitrile and *Buffer* (3:7)
  System suitability solution: 25 µg /mL of Tamsulosin Hydrochloride and 50 µg/mL of propylparaben in *Mobile phase*
  Standard solution: 10 µg/mL of Tamsulosin Hydrochloride from *Sample solution*, in *Mobile phase*
  Sample solution: 5.0 mg/mL of Tamsulosin Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm x 15-cm; 5-µm packing L1
  Temperature: 40°
  Flow rate: 1.3 mL/min
  Injection size: 10 µL
  [NOTE—Record the chromatogram for NLT 1.5 times the retention time of tamsulosin.]
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 12 between tamsulosin and propylparaben, *System suitability solution*. [NOTE—Tamsulosin elutes first.]
  Relative standard deviation: NMT 4% for 6 replicates, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of any individual impurity eluting before the tamsulosin peak in the portion of Tamsulosin Hydrochloride taken:

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

  r_U = peak response of each impurity eluting before tamsulosin from the *Sample solution*
  r_S = peak response of tamsulosin from the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  C_U = concentration of the *Sample solution* (mg/mL)
  Acceptance criteria
  Individual impurities: NMT 0.10% for any individual impurity. [NOTE—If present, the des-ethoxy impurity and the methoxy impurity eluting at the relative retention time of about 0.8 are not separated by this method and should be integrated together to determine conformance. (The des-ethoxy impurity is 2-methoxy-5-[(2*R*)-2-[(2-phenoxyethyl)amino]propyl]benzenesulfonamide, and the methoxy impurity is 2-methoxy-5-[(2*R*)-2-[[2-(2-methoxyphenoxy)ethyl]amino]propyl]benzenesulfonamide.) NMT ~~0.20%~~■0.15%■1S *(USP33)* of the sum of the des-ethoxy and methoxy impurities is found.]
  [NOTE—The reporting level for impurities is 0.05%.]
- **PROCEDURE 2**
  [NOTE—For impurities eluting after tamsulosin.]
  Buffer: Proceed as directed for *Procedure 1*.
  Mobile phase: Acetonitrile and *Buffer* (1:1)
  Standard solution: Proceed as directed for *Procedure 1*.
  [NOTE—Use *Mobile phase* from *Procedure 1* to prepare the solution.]
  Sample solution: Proceed as directed for *Procedure 1*.
  [NOTE—Use *Mobile phase* from *Procedure 1* to prepare the solution.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm x 15-cm; 5-µm packing L1
  Temperature: 40°
  Flow rate: 1.0 mL/min
  Injection size: 10 µL
  [NOTE—Record the chromatogram for NLT 5 times the retention time of tamsulosin.]
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: Use a column that meets the resolution requirements of *Procedure 1*.
  Relative standard deviation: 6 replicates, NMT 4%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of any individual impurity eluting after the tamsulosin peak in the portion of Tamsulosin Hydrochloride taken:

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

  r_U = peak response of each impurity eluting after tamsulosin from the *Sample solution*
  r_S = peak response of tamsulosin from the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  C_U = concentration of the *Sample solution* (mg/mL)

In-Process Revision

Acceptance criteria
Individual impurities: NMT 0.10% for any individual impurity. [NOTE—The reporting level for impurities is 0.05%.]
Total impurities: NMT 0.2%, including all impurities in *Procedure 1* and *Procedure 2*

SPECIFIC TESTS
- **ENANTIOMERIC PURITY**

Mobile phase: Hexane, dehydrated alcohol, methanol, and diethylamine (650:200:150:1)
System suitability solution: 40 µg/mL of USP Racemic Tamsulosin Hydrochloride RS in methanol
Sample solution: 2.0 mg/mL of Tamsulosin Hydrochloride in methanol
Standard solution: 2 µg/mL of Tamsulosin Hydrochloride, from *Sample solution*, in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L51
Temperature: 40°
Flow rate: 0.5 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for the optical isomer and tamsulosin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between optical isomer and tamsulosin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the optical isomer in the portion of Tamsulosin Hydrochloride taken:

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

r_U = peak response of optical isomer from the *Sample solution*
r_S = peak response of tamsulosin from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria
Individual impurities: NMT 0.3%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –17.5° to –20.5° at 20°

Sample solution: 7.5 mg/mL in water. [NOTE—Sample should be previously dried at 105° for 2 h; heat at 60°–70° to dissolve, and cool before using.]
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Racemic Tamsulosin Hydrochloride RS
USP Tamsulosin Hydrochloride RS▲*USP32*

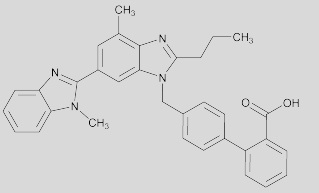
BRIEFING

Telmisartan. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed based on validated methods of analysis. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed using a Kromasil brand column of 5-µm packing L1. The typical retention time for the telmisartan peak is about 11 min, as per the specified conditions in the test for *Organic Impurities*.

(MD-CV: S. Ramakrishna.) RTS—C52047

Add the following:

▪Telmisartan



$C_{33}H_{30}N_4O_2$ 514.62
[1,1′-Biphenyl]-2-carboxylic acid, 4′-[(1,4′-dimethyl-2′-propyl[2,6′-bi-1*H*-benzimidazol]-1′-yl)methyl;
4′-[[4-Methyl-6-(1-methyl-2-benzimidazolyl)-2-propyl-1-benzimidazolyl]methyl]-2-biphenylcarboxylic acid
[144701-48-4].

DEFINITION
Telmisartan contains NLT 98.0% and NMT 101.0% of $C_{33}H_{30}N_4O_2$, calculated on dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197K**⟩**:** If the spectra obtained shows differences, proceed with the samples prepared as follows. Separately dissolve a quantity of USP Telmisartan RS and the Telmisartan sample in alcohol. [NOTE—Heating the solution may be necessary for complete dissolution.] Cool the solution in an ice bath, filter the crystals, and dry at 105°.
- **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Titrimetry ⟨**541**⟩
Sample solution: 190 mg of Telmisartan in 5 mL of anhydrous formic acid. Dilute with 75 mL of acetic anhydride.
Analysis: Titrate with 0.1 M perchloric acid versus a blank determination under the same conditions. Each mL of 0.1 M perchloric acid is equivalent to 25.73 mg of $C_{33}H_{30}N_4O_2$.
Acceptance criteria: 98%–101.0%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**231**⟩**:** NMT 0.1%. A 1 g sample is used.
- **HEAVY METALS** ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities
- **PROCEDURE**

[NOTE—Prepare sample solutions freshly and protect from light.]
Solution A: 2.0 g of monobasic potassium phosphate and 3.8 g of sodium pentanesulphonate monohydrate in 1 L water. Adjust the pH to 3.0 using 1 M phosphoric acid.

In-Process Revision

Solution B: Acetonitrile, methanol (4:1)
Mobile phase: See the gradient table below.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	70	30
2	70	30
27	20	80
32	20	80
32.1	70	30
37	70	30

System suitability solution: Dissolve USP Telmisartan RS and USP Telmisartan Related Compound B RS in methanol (0.2 mL/mg of USP Telmisartan RS) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. Final concentration is 2.5 mg/mL of the USP Telmisartan RS and 2.5 µg/mL of USP Telmisartan Related Compound B RS in methanol.
Standard solution: Dissolve USP Telmisartan RS in methanol (0.2 mL/mg of USP Telmisartan RS) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. The final concentration is 0.025 mg/mL.
Sample solution: Dissolve Telmisartan in methanol (0.2 mL/mg of Telmisartan) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. The final concentration is 2.5 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 12.5-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between telmisartan and telmisartan related compound B, *System suitability solution*
Tailing factor: Between 0.9–1.5 for telmisartan related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for the telmisartan peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Telmisartan taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of Telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution*
C_U = concentration of Telmisartan in the *Sample solution*

Acceptance criteria
Individual impurities: See *Table 1*.
Total impurities: See *Table 1*. [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

Table 1

Name	Approximate Relative Retention Time	Limit (%)
Telmisartan related compound A[1]	0.3	NMT 0.1
Telmisartan amide[2]	0.7	NMT 0.1
Telmisartan related compound B[3]	0.9	NMT 0.1
Telmisartan diacid[4]	1.1	NMT 0.1
Telmisartan *tert*-butyl ester[5]	1.7	NMT 0.2
Telmisartan unknown impurity	1.8	NMT 0.2
Any other individual impurity	N/A	NMT 0.1
Total impurities	N/A	NMT 1.0

[1]1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[d]imidazole.
[2]4′-[(1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[d]imidazol-3′-yl)methyl]biphenyl-2-carboxamide.
[3]4′-[(1,7′-Dimethyl-2′-propyl-1*H*,1′*H*-2,5′-bibenzo[d]imidazol-1′-yl)methyl]biphenyl-2-carboxylic acid.
[4]1-[(2′-carboxybiphenyl-4-yl)methyl-4-methyl-2-propyl]-1*H*-benzimidazole-6-carboxylic acid.
[5]*tert*-Butyl 4′-[(1,7′-dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[d]imidazol-3′-yl)methyl]biphenyl-2-carboxylate.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of sample at 105° to constant weight. It loses NMT 1.5%.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Telmisartan RS
  USP Telmisartan Related Compound B RS■1S *(USP33)*

BRIEFING

Telmisartan Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed based on the validated methods. The proposed liquid chromatographic procedures in the *Assay* and the test for *Procedure* under *Organic Impurities* are performed using a Nucleosil 100, 5-µm column of packing L1. The typical retention time for telmisartan is about 1.4 min under the specified conditions for the *Assay*.

(MDCV: S. Ramakrishna; . BPC: M. Marques .) RTS—C57642

Add the following:

■Telmisartan Tablets

DEFINITION
Telmisartan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$).

In-Process Revision

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩:** The spectrum of the solution under test corresponds to that of the *Standard solution*, as obtained in *Performance Tests* for *Dissolution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: 0.005 N methanolic solution of sodium hydroxide

Buffer: 2.0 g/L ammonium dihydrogen phosphate. Adjust with 1 M phosphoric acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (7:3)

Standard solution: 0.11 mg/mL of USP Telmisartan RS and 0.013 mg/mL of USP Telmisartan Related Compound A RS in *Diluent*. Pass the solution through a 0.45-µm membrane filter.

Sample solution: Transfer not fewer than 20 Tablets into a suitable volumetric flask, and add about 80% of the volume of *Diluent*. Swirl to disperse, and sonicate for about 10 min. Allow to cool to room temperature, dilute with *Diluent* to volume, and mix. Pass the resulting solution through a 0.45-µm membrane filter. Further dilute quantitatively in *Mobile phase* to obtain a solution having a concentration of 0.11 mg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 298 nm

Column: 4.0-mm × 4-cm column; 5-µm, packing L1

Temperature: 40°

Flow rate: 0.7 mL/min

Injection size: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3 between telmisartan and telmisartan related compound A

Tailing factor: NMT 2.0 for the telmisartan peak

Capacity factor: NLT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{33}H_{30}N_4O_2$ in the portion of Tablets taken:

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

r_U = response of telmisartan peak from the *Sample solution*

r_S = response of telmisartan peak from the *Standard solution*

C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of telmisartan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: pH 7.5 phosphate buffer (prepared by dissolving 13.61 g of potassium dihydrogen phosphate in about 800 mL of water, adjusting with 2 M sodium hydroxide to a pH of 7.5, and diluting with water to 1000 mL); 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution: Transfer about 44 mg of USP Telmisartan RS to a 100-mL volumetric flask. Add 1 mL of 0.1 M sodium hydroxide, and dilute with methanol to volume. Dilute this solution quantitatively with *Medium* to obtain a solution with a final concentration of about 0.011 mg/mL.

Sample solution: Dilute the solution under test 1:2 with *Medium*, and pass the final solution through a suitable 0.45-µm filter.

Detection: UV 296 nm

Blank: *Medium*

Determine the percentage of $C_{33}H_{30}N_4O_2$ dissolved:

$$\text{Result} = (A_U \times C_S \times V \times F)/(A_S \times D_U \times L)$$

A_U = absorbance of the *Sample solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium* (mL), 900

F = percentage conversion factor, 100

D_U = dilution factor of the *Sample solution*

L = Tablet label claim (mg)

Tolerances: Not less than 75% (Q) of the labeled amount of $C_{33}H_{30}N_4O_2$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

- **PROCEDURE**

Diluent, Buffer, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed under *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_s) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_s = response of telmisartan from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.2% of any individual impurity

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and stored at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Telmisartan RS

USP Telmisartan Related Compound A RS■1S *(USP33)*

BRIEFING

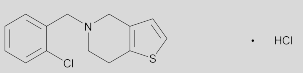
Ticlopidine Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph based on the validated methods is being proposed. The liquid chromatographic procedure for *Organic Impurities*, *Procedure 2* and *Assay* is based on analyses performed using a Lichrosorb RP8 10-µm column of packing L7. The typical retention time for the ticlopidine peak is about 8 min. The liquid chromatographic procedure in the test for *Limit of Formaldehyde* is based on analyses performed using a Spherisorb ODS II, 5-µm column of packing L1.

(MD-CV: S. Ramakrishna.) RTS—C49319

In-Process Revision

Add the following:

▪Ticlopidine Hydrochloride



$C_{14}H_{14}ClNS \cdot HCl$ 300.25

Thieno[3,2-*c*]pyridine, 5-[(2-chlorophenyl)methyl]-4,5,6,7-tetrahydro-, hydrochloride;
5-(*o*-Chlorobenzyl)-4,5,6,7-tetrahydrothieno-[3,2-*c*]pyridine hydrochloride [53885-35-1].

DEFINITION

Ticlopidine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{14}H_{14}ClNS \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨**197M**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C. Identification Tests—General,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **Procedure**

Buffer: 1.1 g of monobasic sodium phosphate and 0.28 g of dibasic sodium phosphate in 1000 mL of water. The pH of solution is between 6.1 and 6.6. If necessary, adjust to the required pH using phosphoric acid or sodium hydroxide.
Mobile phase: Acetonitrile, methanol, and *Buffer* (6:7:7)
System suitability solution: 0.2 mg/mL of USP Ticlopidine Hydrochloride RS and 0.2 mg/mL of USP Sulconazole Nitrate RS in *Mobile phase.* [NOTE—Sonication may be necessary for complete dissolution.]
Standard solution: 0.4 mg/mL of USP Ticlopidine Hydrochloride RS in *Mobile phase*
Sample solution: 0.4 mg/mL of Ticlopidine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm column; 10-µm packing L7
Flow rate: 2 mL/min
Column temperature: 40°
Run time: 1.5 × the retention time of the ticlopidine peak
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.6 between ticlopidine hydrochloride and sulconazole nitrate, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{14}ClNS \cdot HCl$ in the portion of Ticlopidine Hydrochloride taken:

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

r_U = peak response of ticlopidine from the *Sample solution*
r_S = peak response of ticlopidine from the *Standard solution*
C_S = concentration of ticlopidine in the *Standard solution*
C_U = concentration of ticlopidine in the *Sample solution*

IMPURITIES

Inorganic Impurities

- **Residue on Ignition** ⟨**231**⟩**:** NMT 0.1% on a 1-g sample
- **Heavy Metals,** *Method 1* ⟨**231**⟩**:** NMT 0.002%

Organic Impurities

- **Procedure 1**

Adsorbent: 0.25-mm thickness of silica
Developing solvent: Butanol, water, and glacial acetic acid (4:5:1). Shake well in a separatory funnel, allow it to settle, and discard the lower aqueous layer and use the upper organic layer.
Diluent: Methylene chloride and methanol (1:2)
Iodine–methanol reagent: Iodine TS and methanol (1:1)
Standard solution A: 15 mg/mL of USP Ticlopidine Hydrochloride RS in *Diluent*
Standard solution B: 2.5 mg/mL of each of USP Ticlopidine Related Compound A RS and USP Ticlopidine Related Compound B RS in *Diluent*
Sample solution: 15 mg/mL of Ticlopidine Hydrochloride in *Diluent*
Combined standard solution: 1.5 mL of *Standard solution B* and 250 µL of *Sample solution* in a 25-mL volumetric flask in *Diluent*
Application size: 2, 5, and 10 µL of *Combined standard solution* and 20 µL of *Sample solution*
Analysis
Samples: *Sample solution* and *Combined standard solution*
Develop the plate to a distance of at least 15 cm from the origin, and remove the plate and air dry for at least 1 h. Analyze visually under UV light. Estimate the amounts of ticlopidine related compound A and ticlopidine related compound B. Spray the plate with the *Iodine–methanol reagent*, and estimate any other impurities by comparing to ticlopidine hydrochloride spots in the *Combined standard solution.*
Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Related Compound	Approximate Relative Retention Factor	Acceptance Criteria, NMT (%)
Ticlopidine hydrochloride	1.00	—
Ticlopidine hydrochloride related compound A[a]	1.26	0.5
Ticlopidine hydrochloride related compound B[b]	1.41	0.5

[a] 4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazole.
[b] 4′-[[7-Methyl-5-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxylic acid.

- **Procedure 2**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *N*-methyl ticlopidine in the portion of Ticlopidine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of *N*-methyl ticlopidine in the *Sample solution*
r_T = sum of responses of all the peaks in the *Sample solution*

Calculate the percentage of any individual impurity in the portion of the Ticlopidine Hydrochloride taken:

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

r_U = response of any impurity in the *Sample solution*
r_S = response of ticlopidine in the *Standard solution*

In-Process Revision

Acceptance criteria
Individual impurities: See *Impurity Table 2.*
Total impurities: NMT 1.0%

Impurity Table 2

Component	Relative Retention Time (min)	Acceptance Criteria, NMT (%)
Ticlopidine hydrochloride	1	—
N-Methyl ticlopidine[a]	1.18	0.5
Any other individual impurity	—	0.10
Total impurities[b]	—	1.0

[a]*N*-(2-Chlorobenzyl)-2-(thiophen-2-yl)ethanamine.
[b]Total of *N*-methyl ticlopidine and sum of % individual impurities from *Procedure 1.*

SPECIFIC TESTS
- **Loss on Drying ⟨731⟩:** NMT 1.0%. 1.0-g sample size at 80° for 5 h.
- **Limit of Formaldehyde**

Mobile phase: Acetonitrile, water, and hydrochloric acid (3:2:0.004)
2,4 Dintirophenyl hydrazine solution: 1.65 mg/mL of 2,4-dinitrophenylhydrazine in acetonitrile
Standard stock solution: Transfer a known amount of formaldehyde solution, equivalent to 37 mg of formaldehyde, into a 100-mL volumetric flask. Dilute with methanol to volume.
Standard solution: Dilute *Standard stock solution* with methanol to prepare a 1.85 µg/mL solution.
Sample solution: 50 mg/mL of Ticlopidine Hydrochloride in methanol (sonication may be necessary for complete dissolution)
Derivatized Standard and Sample solutions: Transfer 2.0 mL of *2,4-Dinitrophenyl hydrazine solution* to five different 10-mL volumetric flasks; 50 µL of 2 N hydrochloric acid and 150, 250, and 500 µL of *Standard solution* to the first three flasks; 500 µL of *Sample solution* to the fourth; and 500 µL of methanol to the fifth flask. Mix each solution well and allow the solutions to react for at least 30 min at ambient temperature. Dilute each flask with *Mobile phase* to volume and mix well. The solutions should be analyzed within 4 h.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: LC
Detector: UV 365 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10.0% for the peak obtained from derivatized *Standard solution* prepared from 500 µL
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The approximate retention time for 2,4 dinitrophenylhydrazine is about 3.5 min and for the formaldehyde and 2,4 dinitrophenylhydrazine derivative is about 3.8 min.]
Calculate the formaldehyde concentration in ppm in the derivatized *Sample solution* (prepared in flask 4 as stated under *Derivatized formaldehyde solutions*) as the concentration in the Ticlopidine Hydrochloride taken:

$$\text{Result} = (C \times D)/W$$

C = concentration of formaldehyde from the calibration curve generated from the peak areas of the derivatized methanol and the three *Standard solutions* (µg/mL)
D = dilution factor, 200
W = sample weight (g)
Acceptance criteria
Formaldehyde: NMT 20 ppm

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, and store at a temperature below 30°.
- **USP Reference Standards ⟨11⟩**
USP Sulconazole Nitrate RS
USP Ticlopidine Hydrochloride RS
USP Ticlopidine Hydrochloride Related Compound A RS
USP Ticlopidine Hydrochloride Related Compound B RS■1S *(USP33)*

BRIEFING

Ticlopidine Hydrochloride Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedure in the test for the *Assay* is validated using a Waters µBondapak column of packing L1. The typical retention time for ticlopidine hydrochloride is about 12 min under the conditions specified.

(MD-CV: S. Ramakrishna. BPC: M. Marques.) RTS—C40389

Add the following:

■Ticlopidine Hydrochloride Tablets

DEFINITION
Ticlopidine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ticlopidine hydrochloride $C_{14}H_{14}ClNS \cdot HCl$.

IDENTIFICATION
- **A. Ultraviolet Absorption ⟨197U⟩:** Place a portion of powdered Tablets, equivalent to 100 mg of ticlopidine hydrochloride, in a suitable flask. Add 1 mL of water, and allow to stand for 15 min with occasional shaking. Add about 60 mL of *Mobile phase*, sonicate for 15 min, and shake by mechanical means for an additional 15 min. Dilute with *Mobile phase* to volume, and mix. Dilute a portion of this solution with *Mobile phase* to prepare 0.1 mg/mL of Ticlopidine hydrochloride: the UV absorption spectra of the solution exhibit maxima at the same wavelength (232 nm) as that of a similar solution prepared from USP Ticlopidine Hydrochloride RS, concomitantly measured.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY
- **PROCEDURE**

Buffer: 3.55 g of dibasic sodium phosphate in 1000 mL of water
Mobile phase: Acetonitrile and *Buffer* (3:2). Adjust with phosphoric acid to a pH of 7.0 ± 0.1.
Standard solution: 0.1 mg/mL of USP Ticlopidine Hydrochloride RS in *Mobile phase*
Sample solution: Weigh and finely powder not fewer than 20 Tablets. Transfer a portion of the powder, equivalent to about 250 mg of ticlopidine hydrochloride to a 250-mL volumetric flask, add 150 mL of *Mobile phase*, sonicate for about 5 min, and shake mechanically for 10 additional min. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 0.45-µm filter, and use the filtrate after discarding the first few mL. Transfer 5 mL of the filtrate into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 3.9-mm × 3-cm column; 5-µm packing L1
Temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 15 µL
Run time: Twice the retention time of the ticlopidine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{14}ClNS \cdot HCl$ in the portion of Tablets taken:

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

r_U = peak response of ticlopidine from the *Sample solution*
r_S = peak response of ticlopidine from the *Standard solution*
C_S = concentration of USP Ticlopidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ticlopidine hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION**

Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.278 mg/mL of USP Ticlopidine Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Detection: UV 232 nm
Path length: 0.1 cm
Blank: *Medium*
Calculate the percentage of ticlopidine hydrochloride dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = Tablet label claim (mg)
V = 900 mL
Tolerances: NLT 75% (Q) of the labeled amount of ticlopidine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers and stored at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ticlopidine RS■1S (*USP33*)

BRIEFING

Tizanidine Tablets, *USP 32* page 3754. It is proposed to revise the *Tolerances* in *Dissolution Test 1* to express the results as percentage of tizanidine base dissolved rather than percentage of tizanidine hydrochloride dissolved.

(BPC: M. Marques.) RTS—C73274

Tizanidine Tablets

DEFINITION
Tizanidine Tablets contain Tizanidine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of tizanidine ($C_9H_8ClN_5S$).

IDENTIFICATION
- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Water and phosphoric acid (44:6)
Buffer: 3.5 mg/mL of sodium 1-pentanesulfonate. Adjust with *Solution A* or 1 N sodium hydroxide to a pH of 3.0 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Tizanidine related compound A solution: 0.1 mg/mL of USP Tizanidine Related Compound A RS in methanol
Tizanidine related compound B solution: 0.1 mg/mL of USP Tizanidine Related Compound B RS in methanol
Tizanidine related compound C solution: 0.1 mg/mL of USP Tizanidine Related Compound C RS in methanol
System suitability solution: Transfer 23 mg of USP Tizanidine Hydrochloride RS to a 100-mL volumetric flask, and add 20 mL of *Mobile phase* and 10 mL each of *Tizanidine related compound A solution, Tizanidine related compound B solution,* and *Tizanidine related compound C solution*. Sonicate to dissolve the USP Tizanidine Hydrochloride RS, and dilute with *Mobile phase* to volume.
Standard solution: 0.046 mg/mL of USP Tizanidine Hydrochloride RS in *Mobile phase*
Sample solution: Transfer a weighed portion of finely powdered Tablets (NLT 20), equivalent to 20 mg of tizanidine, to a 500-mL volumetric flask. Add 250 mL of *Buffer*, sonicate for 15 min with occasional shaking, and shake by mechanical means for 15 min. Add 100 mL of acetonitrile, and mix. Allow to cool, and dilute with *Buffer* to volume. Centrifuge a portion of this solution at 2000 rpm or higher for 10 min. Pass a portion of this solution through a filter having a 45-µm or finer porosity, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times are listed in *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 4.0 between tizanidine and tizanidine related compound C; NLT 4.0 between tizanidine and tizanidine related compound B, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_9H_8ClN_5S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tizanidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩
Test 1
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Time: 15 min
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Solution A, Buffer, and **Mobile phase:** Proceed as directed in the *Assay*.
Standard stock solution: 0.2 mg/mL of USP Tizanidine Hydrochloride RS in *Medium*
Standard solution
For Tablets labeled to contain 2 mg: Dilute 4.0 mL of the *Standard stock solution* with *Medium* to 200 mL.
For Tablets labeled to contain 4 mg: Dilute 4.0 mL of the *Standard stock solution* with *Medium* to 100 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_9H_8ClN_5S \cdot HCl$ dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times 1/L \times 100$$

r_U = peak response of tizanidine from the *Sample solution*
r_S = peak response of tizanidine from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL), 500
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17
L = tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_9H_8ClN_5S \cdot HCl$ is dissolved.

Test 2
[NOTE—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.]
Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
Apparatus 1: 100 rpm
Time: 30 min
Standard stock solution: 0.1 mg/mL of USP Tizanidine Hydrochloride RS in *Medium*
Standard solution
For Tablets labeled to contain 2 mg: Dilute 5.0 mL of the *Standard stock solution* with *Medium* to 100 mL.
For Tablets labeled to contain 4 mg: Dilute 10.0 mL of the *Standard stock solution* with *Medium* to 100 mL.
Sample solution: Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm.
Spectrometric conditions
Mode: UV
Analytical wavelength: 228 nm
Cell: 1.0 cm
Blank: *Medium*

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of $C_9H_8ClN_5S$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times 1/L \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL), 500
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17
L = tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_9H_8ClN_5S$ is dissolved.

IMPURITIES

Organic Impurities

• **PROCEDURE**
Solution A, Buffer, Mobile phase, and **Standard solution:** Proceed as directed in the *Assay*.
Sample solution: Transfer a weighed portion of finely powdered Tablets (NLT 20), equivalent to 20 mg of tizanidine, to

In-Process Revision

a 100-mL volumetric flask. Add about 50 mL of *Buffer*, sonicate for about 15 min with occasional shaking, and shake by mechanical means for 15 min. Add 20 mL of acetonitrile, and mix. Allow to cool, dilute with *Buffer* to volume, and mix. Centrifuge a portion of this solution at 2000 rpm or higher for 10 min. Pass a portion of this solution through a filter having a 45-µm or finer porosity, and use the filtrate.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

r_U = peak response of tizanidine from the *Sample solution*
r_S = peak response of tizanidine from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tizanidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17
F = relative response factor from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tizanidine related compound C	About 0.8	1.0	0.2
Tizanidine	1.0	—	—
Tizanidine related compound B	About 1.4	0.9	0.2
Tizanidine related compound A	About 10.2	0.9	0.2
Individual unidentified impurity	—	1.0	0.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Tizanidine Hydrochloride RS
  USP Tizanidine Related Compound A RS
  USP Tizanidine Related Compound B RS
  USP Tizanidine Related Compound C RS

BRIEFING

Tranylcypromine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure for the *Assay* was validated with a Phenomenex Synergi Polar RP brand of L11 column, in which tranylcypromine elutes at about 7 min. The *Dissolution* test was validated using an Xterra RP18 brand of L1 column, in which tranylcypromine elutes at about 4.6 min. The liquid chromatographic procedure for *Organic Impurities* was validated with an Atlantis brand of L1 column, in which tranylcypromine elutes at about 7 min.

(MD-PP: D. Vicchio, R. Ravichandran. BPC: M. Marques.) RTS—C62529

Add the following:

▪Tranylcypromine Tablets

DEFINITION

Tranylcypromine Tablets contain an amount of tranylcypromine sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of tranylcypromine ($C_9H_{11}N$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: Transfer 3.4 g of monobasic ammonium phosphate into a 1-L volumetric flask containing about 900 mL of water. Adjust the pH of the solution to 2.2 ± 0.1 with phosphoric acid. Dilute with water to volume, and mix well.

Mobile phase: Methanol and *Solution A* (3:7)

0.05 N sulfuric acid: Cautiously add 1.3 mL of sulfuric acid into 100 mL of water, cool to room temperature, and dilute to 1000 mL.

Diluent: Methanol, water, and *0.05 N sulfuric acid* (1:3:1)

Standard stock solution: Dissolve USP Tranylcypromine Sulfate RS in a 50:50 mixture of methanol and *0.05 N sulfuric acid* (about 60% of the final volume), using a sonicator, and dilute with *Diluent* to obtain a 0.4 mg/mL solution of USP Tranylcypromine Sulfate RS.

Standard solution: 0.04 mg/mL of USP Tranylcypromine Sulfate RS in *Diluent*, from the *Standard stock solution*

Sample stock solution: Transfer the number of Tablets equivalent to about 100 mg, based on the label claim, of tranylcypromine, to a 200-mL volumetric flask, add 60 mL of *0.05 N sulfuric acid*, and sonicate for 10 min with intermittent shaking. Add 60 mL of methanol, and sonicate for 10 min with intermittent shaking. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix to obtain a solution containing the equivalent of 0.5 mg/mL of tranylcypromine. Centrifuge a portion of the solution, and pass the supernatant through a 0.45-µm filter, discarding the first 2 mL.

Sample solution: 0.03 mg/mL of tranylcypromine in *Diluent*, from the *Sample stock solution* filtrate

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm column; 4-µm packing L11

Temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_9H_{11}N$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

In-Process Revision

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of tranylcypromine in the *Sample solution* (mg/mL)
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩**

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
Apparatus 2: 100 rpm
Time: 45 min
Solution A: Transfer 6.94 g of sodium perchlorate monohydrate to a 1000-mL volumetric flask containing 900 mL of water, and mix until dissolved. Adjust with perchloric acid to a pH of 2.50, and dilute with water to volume.
Mobile phase: Acetonitrile and *Solution A* (3:17)
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Standard stock solution: 0.54 mg/mL of USP Tranylcypromine Sulfate RS in water. [NOTE—Sonicate as needed.]
Standard solution: 0.027 mg/mL of USP Tranylcypromine Sulfate RS in *Medium*, from the *Standard stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector UV 220 nm
Column: 3.9 mm × 15 cm column, 5-µm packing L1
Temperature: 35°
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity dissolved as a percentage of the labeled amount of tranylcypromine:

$$\text{Result} = (r_U/r_S) \times (C_S \times V) \times (M \times M_{r1}/M_{r2}) \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of tranylcypromine is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Solution A, 0.05 N sulfuric acid, and Diluent: Prepare as directed in the *Assay*.
Solution B: Methanol and *Solution A* (3:17)
Solution C: Methanol and *Solution A* (3:7)
Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
20	100	0
25	0	100
37	0	100
39	100	0
45	100	0

Standard stock solution: 70 µg/mL of USP Tranylcypromine Sulfate RS and 280 µg/mL of USP Tranylcypromine Related Compound A RS in *Diluent*. [NOTE—Sonicate as needed.]
Standard solution: Transfer 2 mL of *Standard stock solution* to a 200-mL volumetric flask, add 60 mL each of *0.05 N sulfuric acid* and methanol, and dilute with *Diluent* to obtain a solution containing 0.7 µg/mL of USP Tranylcypromine RS and 2.8 µg/mL of USP Tranylcypromine Related Compound A RS.
Sample solution: Use the *Sample stock solution* in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm column; 3-µm packing L1
Temperature: 35°
Flow rate: 1.2 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Resolution: NLT 2.0 between tranylcypromine and tranylcypromine related compound A
Relative standard deviation: NMT 6.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of tranylcypromine in the *Sample solution* (mg/mL)
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 1.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**
USP Tranylcypromine Sulfate RS
USP Tranylcypromine Related Compound A RS■1S *(USP33)*

In-Process Revision

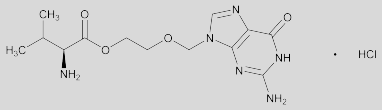
BRIEFING

Valacyclovir Hydrochloride. Because there is no existing monograph for this drug substance, a new monograph based on the validated methods is being proposed. The related compounds are detected using two different chromatographic techniques. The thin layer chromatography procedure in the test for *Organic Impurities*, *Procedure 1* uses a Merck silica gel 60F TLC plate (10 × 20 cm). The liquid chromatographic procedure in the test for *Organic Impurities*, *Procedure 2* employs a Luna Phenyl hexyl brand of column containing 5-µm packing L11. The liquid chromatographic procedure in the tests for *Organic Impurities*, *Procedure 3* and *Assay* employs a Daicel Crownpack CR (+) brand of column containing 5-µm packing L66. The typical retention time for valacyclovir is about 21 min.

(MD-AA: S. Ramakrishna, B. Davani.) RTS—C44129

Add the following:

▪Valacyclovir Hydrochloride



$C_{13}H_{20}N_6O_4 \cdot HCl$ 360.80

L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride;

L-Valine, ester with 9-[(2-hydroxyethoxy)methyl]guanine, monohydrochloride [124832-27-5].

DEFINITION

Valacyclovir Hydrochloride contains NLT 95.0% and NMT 102.0% of $C_{13}H_{20}N_6O_4$.HCl, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, and perchloric acid (1:19:0.1)

Standard solution: 0.5 mg/mL of USP Valacyclovir Hydrochloride RS in 0.05 M hydrochloric acid. [NOTE—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D valacyclovir.]

Sample solution: 0.5 mg/mL of Valacyclovir Hydrochloride in 0.05 M hydrochloric acid

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 15-cm column; 5-µm, packing L66

Flow rate: 0.75 mL/min

Column temperature: 10°

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between valacyclovir hydrochloride and D valacyclovir

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Valacyclovir Hydrochloride in the portion taken:

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

r_U = peak response of valacyclovir from the *Sample solution*

r_S = peak response of valacyclovir from the *Standard solution*

C_S = concentration of Valacyclovir Hydrochloride in the *Standard solution* (mg/mL)

C_U = concentration of Valacyclovir Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨231⟩:** NMT 0.1% on a 2-g sample

Organic Impurities

- **PROCEDURE 1 (FOR RELATED COMPOUNDS E, F, AND G)**

Developing solvent: Methylene chloride, methanol, tetrahydrofuran, and ammonia solution (54:34:12:3)

Standard stock solution: Transfer 5 mg of each of USP Valacyclovir Related Compound D RS, USP Valacyclovir Related Compound E RS, and USP Valacyclovir Related Compound G RS, and 8.4 mg of USP Valacyclovir Related Compound F RS into a 10-mL volumetric flask. Add 2 mL of water with swirling, followed by 6 mL of alcohol, and sonicate for 20 min. Allow to cool, and dilute with alcohol to volume.

Standard solutions: Transfer 1.0 mL and 0.5 mL of *Standard stock solution* into two separate 10-mL volumetric flasks. Dilute the solution in both flasks with alcohol to volume.

Sample solution: Transfer 250 mg of Valacyclovir Hydrochloride into a 5-mL volumetric flask. Add 2 mL of water and sonicate for 20 min to dissolve. Add alcohol to about 95% volume of the flask, cool, and dilute with the same to volume. Pass through a suitable 0.45-µm filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: TLC

Detector: UV, long and short wavelength

Plate: TLC plate coated with 0.25-mm layer of chromatographic silica gel mixture. Prewash the plate with methanol.

Developing distance: NLT 7 cm from origin

Application size: 4 µL

Analysis

Samples: *Standard solutions* and *Sample solution*

Develop the plate to the specified distance. Remove the plate from the solvent chamber and allow to dry. Examine the plate under short-wavelength UV light and visually estimate the valacyclovir related compound E and G in the sample using the appropriate standard spots. The chromatograms obtained with the *Standard solutions* each show three clearly separated spots due to valacyclovir related compounds D, E, and G. Spray the plate with 0.01% fluorescamine in ethylene dichloride, and examine the sprayed plate under long-wavelength UV to estimate the level of valacyclovir related compound F in the sample using the appropriate standard spot. The R_F values and limits for each impurity are provided in *Impurity Table 1.*

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Component	R_F Value	Relative R_F Value	Acceptance Criteria, NMT (%)
Valacyclovir hydrochloride	0.36	1	—
Valacyclovir related compound D[a]	0.41	1.1	—
Valacyclovir related compound E[b]	0.48	1.3	0.2
Valacyclovir related compound F[c]	0.66	1.8	0.1
Valacyclovir related compound G[d]	0.70	1.9	0.05

[a]This impurity is quantitated using *Procedure 2.*
[b]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-[(benzyloxy)carbonyl]-L-valinate.
[c]2-Hydroxyethyl-L-valinate.
[d] *N*,*N*-dimethylpyridin-4-amine.

- **PROCEDURE 2**

Solution A: 0.3% w/w trifluoroacetic acid solution in water
Solution B: 0.3% w/w trifluoroacetic acid solution in methanol
Diluent: Alcohol and water (1:4)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
35	60	40
35.01	90	10
45	90	10

System suitability solution: 0.4 mg/mL of USP Valacyclovir Hydrochloride RS, 0.0008 mg/mL of USP Valacyclovir Hydrochloride Related Compound C RS, and 0.0016 mg/mL of USP Acyclovir Related Compound A RS in *Diluent*
Sensitivity solution: Dilute 3.0 mL of the *System suitability solution* into a 100-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and make up to volume with *Diluent*.
Sample solution: 0.4 mg/mL of Valacyclovir Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm column; 5-µm, packing L11
Flow rate: 0.8 mL/min
Column temperature: 15°
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Resolution: NLT 1.5 between valacyclovir and the valacyclovir related compound C, and NLT 1.5 between valacyclovir related compound C and acyclovir related compound A, *System suitability solution*
Tailing factor: NMT 1.5% for the valacyclovir hydrochloride peak, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of any impurity in the *Sample solution*
r_T = sum of response of all the peaks in the *Sample solution*

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Total unspecified impurities: NMT 0.2%

Impurity Table 2

Component	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine (near solvent front)[a,b]	0.31	—	—
Acyclovir[a,c]	0.42	—	—
Acyclovir alaninate[d]	0.54	—	0.2
Valacyclovir	1.00	—	—
Valacyclovir related compound C[e]	1.06	—	0.3
Acyclovir related compound A[a,f]	1.09	—	—
Valacyclovir related compound D[g]	1.17	—	0.5
Acyclovir isoleucinate[h]	1.30	—	0.2
N formyl valacyclovir[i]	1.61	—	0.8
Guaninyl valacyclovir[j]	1.66	—	0.2
Bis valacyclovir[k]	2.0	—	0.3
Any other individual impurity	—	—	0.1

[a]This impurity is quantitated by the *Procedure 3* method.
[b]2-Amino-1,9-dihydro-6*H*-purin-6-one (guanine).
[c]2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*purin-6-one (Acyclovir).
[d]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-alaninate.
[e]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate.
[f]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[g]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate.
[h]2-[[[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methyoxy]ethyl isoleucinate.
[i]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-formyl-L-valinate.
[j]2-[[6-Oxo-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]amino]-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate.
[k]2,2′-[Methylenebis[imino(6-oxo-1,6-dihydro-9*H*-purine-9,2-diyl)methyleneoxy]]diethyl di(L-valinate.

- **PROCEDURE 3**

Mobile phase, Chromatographic system, Standard solution, and Sample solution: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

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

r_U = peak response of guanine plus acyclovir or acyclovir acetate or D valacyclovir in the *Sample solution*
r_S = peak response of valacyclovir in the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Valacyclovir Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor as given in *Impurity Table 3*

In-Process Revision

Acceptance criteria
Individual impurities: See *Impurity Table 3*.

Impurity Table 3

Component	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine and acyclovir[a, b]	0.18	1.51	2.0
Acyclovir related compound A[c]	0.42	1.12	0.2
D valacyclovir[d]	0.55	1.0	3.0
Valacyclovir	1.0	—	—

[a]2-Amino-1,9-dihydro-6*H*-purin-6-one (guanine).
[b]2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*purin-6-one (Acyclovir).
[c]2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[d]D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

Total impurities: [NOTE—Sum of all impurities from *Procedures 1*, *2*, and *3*.] NMT 5.0%

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** For anhydrous form: NMT 2.0% (200 mg of sample); if labeled as the hydrated form: between 5.0 and 9.0%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm
- **CONTENT OF CHLORIDE, TITRIMETRY** ⟨**541**⟩
  Sample solution: 350 mg of Valacyclovir Hydrochloride in 100 mL water and add 4 drops of nitric acid
  Analysis: Titrate with 0.1 M silver nitrate VS a blank determination under the same conditions. Each mL of 0.1 M silver nitrate is equivalent to 3.55 mg of chloride.
  Acceptance criteria: 9.4–9.9% on the anhydrous basis
- **LIMIT OF PALLADIUM** ⟨**730**⟩ **(***if present***)**
  Diluent: Dimethyl sulfoxide and hydrochloric acid (98:2)
  Blank solution: Use *Diluent.*
  Standard solutions: Dilute with *Diluent* any commercially available standard stock solution of 1 mg/mL of palladium [NOTE—The suggested sources for palladium stock solutions are NIST's SRM 3138 or Perkin elmer's PE PURE.] to prepare the following two solutions: 1 µg/mL of palladium and 10 µg/mL of palladium.
  Sample solution: 10 mg/mL of Valacyclovir Hydrochloride in *Diluent*
  Wavelength: 340.458 nm
  Suitable spectrophotometer system: Use a suitable standard Inductively Coupled Plasma-Optical Emission spectrophotometric system and construct a calibration curve.
  System suitability
  Samples: *Blank solution* and *Standard solutions*
  Suitability requirements
  Relative standard deviation: NMT 2.0%, *Standard solutions*
  Correlation coefficient: NLT 0.999, *Blank* and *Standard solutions*
  Analysis
  Samples: *Blank solution* and *Sample solution*
  Calculate the concentration of palladium using the calibration curve corrected for the emission response of the *Blank solution* and sample weight. Calculate the amount of palladium in the Valacyclovir Hydrochloride taken to prepare the *Sample solution*.
  Acceptance criteria: NMT 10 ppm

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature below 30°.
- **LABELING:** Where it is the hydrated form, the label so indicates.

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Acyclovir Related Compound A RS [NOTE—USP Acyclovir Related Compound AS is equivalent.]
  USP Valacyclovir Hydrochloride RS
  USP Valacyclovir Hydrochloride Related Compound C RS
  USP Valacyclovir Hydrochloride Related Compound D RS
  USP Valacyclovir Hydrochloride Related Compound E RS
  USP Valacyclovir Hydrochloride Related Compound F RS
  USP Valacyclovir Hydrochloride Related Compound G RS■1S (*USP33*)

BRIEFING

Valproic Acid Capsules, *USP 32* page 3839. It is proposed to delete the *Disintegration* test. When a revision was made to replace the *Disintegration* test with a *Dissolution* test, the *Disintegration* test was not deleted.

(BPC: M. Marques.) RTS—C73275

Valproic Acid Capsules

DEFINITION
Valproic Acid Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$).

IDENTIFICATION
- **A.** The retention time ratios of the valproic acid peak to the internal standard peak from the *Standard solution* and the *Sample solution*, as directed in the *Assay*, do not differ by more than 2.0%.
- **B. PROCEDURE**
  Sample: Equivalent to 250 mg of valproic acid
  Analysis: Place the *Sample* in a separator, add 20 mL of 1 N sodium hydroxide, shake, and allow the layers to separate. Transfer the aqueous layer to a second separator, add 4 mL of hydrochloric acid, mix, and extract with 40 mL of *n*-heptane. Filter the *n*-heptane layer through glass wool into a beaker, and evaporate the solvent completely on a steam bath with the aid of a current of air. Transfer 2 drops of the residue to a test tube containing 0.5 mL each of potassium iodide solution (1 in 50) and potassium iodate solution (1 in 25), and mix.
  Acceptance criteria: A yellow color is produced.

ASSAY
- **PROCEDURE**
  Internal standard solution: 5 mg/mL of biphenyl in *n*-heptane
  Standard stock solution: 2.5 mg/mL of USP Valproic Acid RS in *n*-heptane
  Standard solution: Combine 10.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution* in a container equipped with a closure.
  Sample stock solution: Transfer NLT 20 Capsules to a blender jar or other container. Add 150 mL of methylene chloride, and cool in a solid carbon dioxide–acetone mixture until the contents have solidified. If necessary, transfer the mixture of Capsules and methylene chloride to a blender jar, and blend with a high-speed blender until all the solids are reduced to fine particles. Transfer the mixture to a 500-mL volumetric flask, add *n*-heptane to volume, mix, and allow solids to settle. Dilute the solution to obtain a final concentration of 2.5 mg/mL in *n*-heptane, made up to 100 mL.
  Sample solution: Combine 10.0 mL of *Sample stock solution* and 4.0 mL of *Internal standard solution* in a container equipped with a closure.

In-Process Revision

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m glass column packed with 10% phase G34 on 80- to 100-mesh support S1A
Temperature
Column: 150°
Injector port: 250°
Detector: 250°
Carrier gas: Dry helium
Flow rate: 40 mL/min
Injection size: 2 µL
System suitability
[NOTE—The relative retention times for valproic acid and biphenyl acid are 0.5 and 1.0, respectively.]
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between valproic acid and biphenyl
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_8H_{16}O_2$ in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of valproic acid to internal standard obtained from the *Sample solution*
R_S = peak response ratio of valproic acid to internal standard obtained from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Delete the following:

■• **DISINTEGRATION** ⟨~~701~~⟩**:** ~~Determine as directed for *Soft Gelatin Capsules*.~~
~~**Time:** 15 min~~■1S (*USP33*)

• **DISSOLUTION** ⟨711⟩
Medium: 5 mg/mL of sodium lauryl sulfate in simulated intestinal fluid TS (prepared without the enzyme and with monobasic sodium phosphate instead of monobasic potassium phosphate), adjusted with 5 M sodium hydroxide to a pH of 7.5; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Internal standard solution: Proceed as directed in the *Assay*.
Standard solution: Prepare a solution of USP Valproic Acid RS having a concentration similar to that of the *Sample solution*. Transfer 10.0 mL to a suitable container, add 3.0 g of sodium chloride, and mix on a vortex mixer for 5 min. Add 1 mL of 6 N hydrochloric acid and 5.0 mL of *Internal standard solution*, and shake for 2 min. Allow the phases to separate, remove the *n*-heptane layer, and filter. Discard the aqueous layer.
Sample solution: Transfer 10.0 mL of the solution under test to a suitable container, add 3.0 g of sodium chloride, and mix on a vortex mixer for 5 min. Add 1 mL of 6 N hydrochloric acid and 5.0 mL of *Internal standard solution*, and shake for 2 min. Allow the phases to separate, remove the *n*-heptane layer, and filter. Discard the aqueous layer.
Chromatographic system: Proceed as directed in the *Assay*.
System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_8H_{16}O_2$ dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times (V/L) \times 100$$

R_U = peak response ratio of valproic acid to internal standard obtained from the *Sample solution*
R_S = peak response ratio of valproic acid to internal standard obtained from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium* in mL
L = label claim of Capsules (mg)
Acceptance criteria: NLT 85% (Q) of $C_8H_{16}O_2$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements, chloroform being used as the solvent in the procedure for Soft Capsules

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Valproic Acid RS

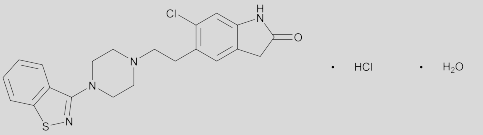
BRIEFING

Ziprasidone Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed based on validated methods of analysis. The liquid chromatographic procedures in the tests for *Procedure 2: Limit of Early Eluting Impurities*, *Procedure 3: Limit of Late Eluting Impurities*, and in the *Assay* are based on analyses performed with a Mac Mod Analytical Zorbax RX-C8, 5-µm brand of L7 column. The typical retention time for ziprasidone in the *Assay* is about 7.5 min. The gas chromatographic procedure in the test for *Procedure 1: Limit of Tetrahydrofuran* is based on analysis performed with the J & W DB-624 brand of G43 column. The typical retention time for tetrahydrofuran is about 12 min.

(MD-PP: R. Ravichandran, F. Mao.) RTS—C44606

Add the following:

■Ziprasidone Hydrochloride



$C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$ 467.41
2*H*-Indol-2-one, 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-, monohydrochloride, monohydrate;
5-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-2-indolinone monohydrochloride, monohydrate [138982-67-9].

DEFINITION

Ziprasidone Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{21}H_{21}ClN_4OS \cdot HCl$, calculated on the anhydrous and solvent-free basis.

In-Process Revision

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 85% phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (2:3)
Diluent: Methanol and water (3:2)
Standard solution: 0.23 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent*
Sample solution: 0.23 mg/mL of Ziprasidone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 229 nm
Refrigerated autosampler temperature: 5°
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{21}ClN_4OS \cdot HCl$ in the portion of Ziprasidone Hydrochloride taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: LIMIT OF TETRAHYDROFURAN**

Standard solution: 0.05 mg/mL in dimethyl sulfoxide. Transfer 4 mL of this solution to a 20-mL headspace vial, and seal.
Sample solution: Transfer 40 mg of Ziprasidone Hydrochloride and 4.0 mL of dimethyl sulfoxide to a 20-mL headspace vial, seal, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC with headspace injector
Detector: Flame ionization
Column: 30-m × 0.32-mm fused silica column coated with a 1.8-µm film of phase G43
Column temperature: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	2	90	0
90	30	225	2

Injection port temperature: 180°
Injector split ratio: 30:1
Detector temperature: 260°
Carrier gas: Helium
Flow rate: 1.6 mL/min
Injection size: 2 mL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis: [NOTE—The headspace vials are maintained at 105° for 60 min prior to headspace injection.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tetrahydrofuran in the portion of Ziprasidone Hydrochloride taken:

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

r_U = peak response of tetrahydrofuran from the *Sample solution*
r_S = peak response of tetrahydrofuran from the *Standard solution*
C_S = concentration of tetrahydrofuran in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Tetrahydrofuran: NMT 0.4%

- **PROCEDURE 2: LIMIT OF EARLY ELUTING IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Diluent A: Methanol, water, and hydrochloric acid (20:5:0.01)
Diluent B: Methanol and water (3:2)
Standard solution A: 0.5 µg/mL of USP Ziprasidone Related Compound A RS and 0.8 µg/mL of USP Ziprasidone Related Compound B RS in *Diluent A*
Standard solution B: 0.4 µg/mL of USP Ziprasidone Related Compound B RS in *Diluent A*
System suitability solution: 0.24 mg/mL of USP Ziprasidone Hydrochloride RS in *Standard solution A*
Sample solution A: 0.4 mg/mL of Ziprasidone Hydrochloride in *Diluent A*
Sample solution B: 0.2 mg/mL of Ziprasidone Hydrochloride in *Diluent B*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the ziprasidone related compound B and ziprasidone peaks
Relative standard deviation: NMT 10% for the ziprasidone related compound B peak
Analysis: [NOTE—Run the *Sample solution* NLT 7 times the ziprasidone retention time in *Sample solution A*.]
Samples: *Standard solution A* and *Sample solution A*
Calculate the percentages of ziprasidone related compounds A and B relative to the content of ziprasidone free base in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the ziprasidone related compound from the *Sample solution A*
r_S = peak response of the ziprasidone related compound from the *Standard solution A*

In-Process Revision

C_S = concentration of the ziprasidone related compound in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in *Sample solution A* (mg/mL)
M_{r1} = molecular weight of ziprasidone free base $C_{21}H_{21}ClN_4OS$, 412.94
M_{r2} = molecular weight of Ziprasidone Hydrochloride $C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$, 467.41

Samples: *Standard solution B* and *Sample solution B*

Calculate the percentage of ziprasidone open ring relative to the content of ziprasidone free base in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone open ring from *Sample solution A*
r_S = peak response of ziprasidone related compound B from *Standard solution B*
C_S = concentration of ziprasidone related compound B in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in *Sample solution B* (mg/mL)
M_{r1} = molecular weight of ziprasidone free base $C_{21}H_{21}ClN_4OS$, 412.94
M_{r2} = molecular weight of Ziprasidone Hydrochloride $C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$, 467.41

Samples: *Diluent A*, *Diluent B*, *Standard solution B*, and *Sample solution A*

Calculate the percentage of unspecified impurity relative to the content of ziprasidone free base in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified impurity from *Sample solution A*
r_S = peak response of ziprasidone related compound B from *Standard solution B*
C_S = concentration of ziprasidone related compound B in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in the *Sample solution A* (mg/mL)
M_{r1} = molecular weight of ziprasidone free base $C_{21}H_{21}ClN_4OS$, 412.94
M_{r2} = molecular weight of Ziprasidone Hydrochloride $C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$, 467.41

[NOTE—Disregard the peaks in the injections of *Diluent A* and *Diluent B*.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time (RRT)	Limit (%)
Ziprasidone related compound A	0.4	0.1
Ziprasidone related compound B	0.8	0.2
Ziprasidone open ring[a]	0.9	0.2
Ziprasidone	1.0	—
Any individual unspecified impurity	—	0.1

[a] 2-(2-Amino-5-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)-4-chlorophenyl)acetic acid.

- **PROCEDURE 3: LIMIT OF LATE ELUTING IMPURITIES**

Buffer: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile, methanol, and *Buffer* (11:1:8)
Diluent: Methanol, water, and hydrochloric acid (20:5:0.01)
Standard solution: 0.8 µg/mL each of USP Ziprasidone Related Compound C RS and USP Ziprasidone Related Compound D RS in *Diluent*
System suitability solution: 0.24 mg/mL of USP Ziprasidone Hydrochloride RS in *Standard solution*
Sample solution: 0.45 mg/mL of Ziprasidone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm
Refrigerated autosampler temperature: 5°
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 6.0 between the ziprasidone and ziprasidone related compound C peaks
Relative standard deviation: NMT 10% for the ziprasidone related compound C peak
Analysis [NOTE—Run the *Sample solution* NLT 12 times the ziprasidone retention time in the *Sample solution*.]
Samples: *Standard solution* and *Sample solution*

Calculate the percentages of ziprasidone related compounds C and D relative to the content of ziprasidone free base in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the ziprasidone related compound from the *Sample solution*
r_S = peak response of the ziprasidone related compound from the *Standard solution*
C_S = concentration of the ziprasidone related compound in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of Ziprasidone Hydrochloride free base $C_{21}H_{21}ClN_4OS$, 412.94
M_{r2} = molecular weight of Ziprasidone Hydrochloride $C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$, 467.41

Samples: *Standard solution, Sample solution,* and *Diluent*

Calculate the percentage of unspecified impurity relative to the content of ziprasidone free base in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of ziprasidone related compound D from the *Standard solution*
C_S = concentration of ziprasidone related compound D in the *Standard solution* (mg/mL)
C_U = nominal concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of Ziprasidone Hydrochloride free base $C_{21}H_{21}ClN_4OS$, 412.94
M_{r2} = molecular weight of Ziprasidone Hydrochloride $C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$, 467.41

[NOTE—Disregard the peaks in the injection of *Diluent*.]

Acceptance criteria

Individual impurities: See *Impurity Table 2*.
Total impurities: NMT 0.5%. [NOTE—The total impurities are the sum of related compounds A, B, C, D, ziprasidone open ring and all unspecified impurities found in *Procedure 2: Limit of Early Eluting Impurities* and *Procedure 3: Limit of Late Eluting Impurities*.]

In-Process Revision

Impurity Table 2

Name	Relative Retention Time (RRT)	Limit (%)
Ziprasidone	1.0	—
Ziprasidone related compound C	2.0	0.2
Ziprasidone related compound D	3.0	0.2
Any individual unspecified impurity	—	0.1

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** Between 3.7% and 5.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light–resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Ziprasidone Hydrochloride RS
  USP Ziprasidone Related Compound A RS
  USP Ziprasidone Related Compound B RS
  USP Ziprasidone Related Compound C RS
  USP Ziprasidone Related Compound D RS■1S *(USP33)*

BRIEFING

Zolpidem Tartrate Extended-Release Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analysis performed with an Inertsil ODS-2 5-µm C18, 4.6-mm × 15-cm brand of L1 column. The typical retention time for zolpidem is about 10 min.

(MD-PP: R. Ravichandran. BPC: M. Marques.) RTS—C47819

Add the following:

■Zolpidem Tartrate Extended-Release Tablets

DEFINITION
Zolpidem Tartrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$).

IDENTIFICATION
- **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
  Sample: 25 µg/mL of zolpidem tartrate in 0.01 M HCl from a suitable quantity of powder obtained by grinding one Tablet
- **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  Solution A: 3.3 mL of phosphoric acid in 1 L of water. Adjust the pH to 5.5 with triethylamine.
  Mobile phase: Acetonitrile, methanol, *Solution A* (4:5:11)
  Standard stock solution: 0.5 mg/mL of USP Zolpidem Tartrate RS in a mixture of alcohol and 0.01 M hydrochloric acid (4:1)
  Standard solution: 0.1 mg/mL of USP Zolpidem Tartrate RS in *Mobile phase* from *Standard stock solution*
  Sample stock solution: Finely powder NLT 8 Tablets. Transfer the powder quantitatively to a suitable volumetric flask to obtain 0.5 mg/mL of zolpidem tartrate. Add 70% of the flask volume of a mixture of alcohol and 0.01 M hydrochloric acid (5:2), and stir on a magnetic stirrer for 1 h. Dilute with alcohol to volume. Allow solid particles to settle, and pass the supernatant through a suitable filter (Whatman 40 or equivalent).
  Sample solution: 0.1 mg/mL of zolpidem tartrate from filtered *Sample stock solution* and *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection size: 15 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 3.0 for zolpidem
  Relative standard deviation: NMT 2.0 for zolpidem
  Analysis
  Sample: *Sample solution* and *Standard solution*
  Calculate the percentage label claim of $C_{42}H_{48}N_6O_8$ in the portion of Tablets taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of the *Sample solution* (mg/mL)
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨**711**⟩
  Medium: 0.01 N hydrochloric acid; 500 mL
  Apparatus 1: 100 rpm
  Times: 30, 90, and 240 min
  Standard solution: Solution of USP Zolpidem Tartrate RS in *Medium* containing L/500 mg/mL, where L is the Tablet label claim in mg
  Sample solution: Pass a portion of the solution under test through a suitable filter.
  Detector: UV 295 nm
  Blank: *Medium*
  Calculate the quantity dissolved as a percentage of the labeled amount of $C_{42}H_{48}N_6O_8$ in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

  A_U = absorbance from the *Sample solution*
  A_S = absorbance from the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  V = 500 mL
  L = label claim/Tablet (mg)
  Tolerances: The percentage of the labeled amount of $C_{42}H_{48}N_6O_8$ dissolved in the times specified conform to *Acceptance Table 1*.

Acceptance Table 1

Time (min)	Amount Dissolved (%)
30	50–70
90	70–85
240	NLT 90

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Solution A, Mobile phase and **Standard stock solution:** Proceed as directed under *Assay*.

System suitability solution: Dissolve a suitable amount of USP Zolpidem Related Compound A RS in *Standard stock solution* to obtain a solution containing 1 µg/mL of zolpidem related compound A. Dilute 1 mL of the resulting solution to 5 mL with *Mobile phase*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Prepare as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between zolpidem related compound A and zolpidem, *System suitability solution*

Tailing factor: NMT 3.0 for the zolpidem peak

Relative standard deviation: NMT 2.0% for the zolpidem peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_T = sum of the peak responses for all the peaks from the *Sample solution*

F = relative response factor of the corresponding impurity obtained from the *Impurity Table*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolpidem acid[1]	0.3	0.7	0.20
Zolpidem related compound A[2]	0.9	1.0	0.20
Zolpidem	1.0	—	—
Any unspecified degradation product	—	1.0	0.20

[1] 2-(6-Methyl-2-*p*-tolylimidazo[1,2-a]pyridin-3-yl)acetic acid.

[2] *N*,*N*-Dimethyl-2-(7-methyl-2-*p*-tolylimidazo[1,2-a]pyridin-3-yl)acetamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Zolpidem Tartrate RS

USP Zolpidem Related Compound A RS■1S *(USP33)*

DIETARY SUPPLEMENTS—MONOGRAPHS

BRIEFING

Vitamin A Oral Liquid Preparation. Because there is no existing *USP* monograph for this article, the following new monograph is being proposed. The development of this monograph has been requested by the United Nations International Children's Emergency Fund (UNICEF), the United States Agency for International Development (USAID), Micronutrient Initiative, and the World Health Organization (WHO) in support of one of their children's health initiatives. Vitamin A Oral Liquid Preparation packaged in soft gelatin capsules is not intended for ingestion as is, but rather acts as a single-unit container and delivery device for the Oral Liquid. Strength is described in terms of Vitamin A/dosage unit. The *Assay* is conducted using the same chromatographic procedure in the USP General Chapter *Vitamin A Assay* ⟨571⟩, which clearly separates the different *cis* isomers of vitamin A and the different ester forms. Typical retention times obtained using a Zorbax-NH2 brand column of L8 packing are 7.5 min for 13-*cis* retinyl palmitate, 14.5 min for all-*trans* retinyl palmitate, 14.5 min for 13-*cis* retinyl acetate, 17.2 min for 9-*cis* retinyl acetate, and 20.0 min for all-*trans* retinyl acetate. The average of the contents expelled from the Oral Liquid Preparations packaged in capsules is used in the calculation to reflect the deliverable dose.

(DSN: C. Phinney.) RTS—C70858

Add the following:

■Vitamin A Oral Liquid Preparation

DEFINITION

Vitamin A Oral Liquid Preparation is an emulsion, suspension, or solution that contains retinyl acetate or retinyl palmitate in an amount equivalent to NLT 95.0% and NMT 120.0% of the labeled amount of vitamin A in each dosage unit.

IDENTIFICATION

[NOTE—Use low-actinic glassware.]

• **A. PROCEDURE**

Analysis: Prepare a solution in methylene chloride containing approximately 6 µg of all-*trans* retinol in 1 mL. Add 10 mL of antimony trichloride TS.

Acceptance criteria: A transient blue color appears at once.

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Standard solution: Dissolve the contents of 1 ampule of USP Vitamin A RS in methylene chloride to obtain 25.0 mL.

Sample solution: Dissolve a quantity of Oral Liquid Preparation equivalent to about 4.5 mg of all-*trans* retinol in methylene chloride to obtain 10 mL of solution.

Application volume: 10 µL as an 8-mm band

Developing solvent system: A mixture of cyclohexane and ether (4:1)

Spray reagent: 0.2 g/mL phosphomolybdic acid in alcohol. Filter and use only the clear filtrate.

Analysis: Apply at the starting point of the chromatogram and 10 µL of the *Sample solution*, as an 8-mm band, and

In-Process Revision

proceed as directed for *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*. Allow the solvent front to move a distance of 10 cm, remove the plate, and air-dry. Spray with phosphomolybdic acid TS.

Acceptance criteria: The blue-green spot formed is indicative of the presence of retinol. The approximate R_F values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol form, 0.45 for the acetate, and 0.7 for the palmitate.

ASSAY

• **VITAMIN A**

[NOTE—Use low-actinic glassware.]

Standard solution: Dissolve a quantity of USP Vitamin A RS in *n*-hexane, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 15 µg of retinyl acetate/mL.

Sample solution

For oral liquid preparations in oil vehicles packaged in single-unit containers: Empty the contents of NLT 30 single-unit containers following the directions for use as stated in the labeling. Record the mass of the content delivered from each single-unit container, and calculate the average. Mix the contents to obtain a homogeneous sample. Transfer an amount of the composite to a suitable volumetric flask; dissolve it with hexane, and dilute with hexane quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg all-*trans* retinol/mL, based on the labeled content.

For oral liquid preparations in oil vehicles packaged in multiple-unit containers: Dissolve an accurately measured volume of Oral Liquid Preparation in a suitable volume of hexane and dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent to about 13 µg all-*trans* retinol/mL, based on the labeled content.

For oral liquid preparations in aqueous vehicles: Transfer a mass, or an accurately measured volume of Oral Liquid Preparation, into a separatory funnel, and extract quantitatively with hexane or other suitable solvent. Dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg all-*trans* retinol/mL, based on the labeled content.

System suitability solution: Dissolve a quantity of retinyl palmitate and USP Vitamin A RS in *n*-hexane to obtain a solution containing about 7.5 µg/mL of each.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; packing L8

Flow rate: 1 mL/min

Injection size: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between retinyl acetate and retinyl palmitate

Relative standard deviation NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of all-*trans* retinol in each individual container:

$$\text{Result} = (V/D) \times (C/W) \times (r_U/r_S) \times U \times (100/L)$$

V = volume of the *Sample solution* (mL)
D = dilution factor (dilution volume/aliquot volume)
C = concentration of all-*trans* retinol in the *Standard solution* (mg/mL)
W = mass or volume of the Oral Liquid Preparation composite taken (mg or mL)
r_U = peak response from the corresponding all-*trans* retinyl ester in the *Sample solution*
r_S = peak response from the all-*trans* retinyl acetate in the *Standard solution*
U = for multiple-unit containers: labeled volume of each dosage unit (mL); or for single-unit containers: average mass (mg) of the contents delivered from each individual container following the directions for use as stated in the labeling
L = labeled content of all-*trans* retinol in each dosage unit (mg)

Acceptance criteria: 95.0%–120.0% of the labeled amount of all-*trans* retinol in each dosage unit

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩

For oral liquid preparations packaged in muiltiple-unit containers: Meets the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For oral liquid preparations packaged in single-unit containers: Empty the single-unit containers following the directions for use as stated in the labeling. The contents so expelled from the single-unit containers meet the requirements. [NOTE—Capsules intended for use as single-unit containers are not rinsed after expulsion of the contents.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. It may be packaged in single-unit containers. [NOTE—Capsules may be suitable as single-unit containers provided they are packaged in critical secondary containers as described in *Good Packaging Practices* ⟨1177⟩.]

• **LABELING:** The label states that the product is Vitamin A Oral Liquid Preparation. Label the Oral Liquid Preparation to indicate the ester form in which the vitamin is present, and to indicate the content of vitamin A in terms of the equivalent amount of all-*trans* retinol in mg/dosage unit. The content of vitamin A may be stated also in USP Units/dosage unit, on the basis that 1 USP Vitamin A Unit equals the biological activity of 0.3 µg of all-*trans* retinol. Capsules used as single-unit containers may be exempted from the requirements of individual labeling, provided they are packaged in an appropriately labeled secondary container, including directions for use and delivery of the Oral Liquid Preparation. Label the Oral Liquid Preparations packaged in multiple-unit containers to indicate the volume of each dosage unit.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Vitamin A RS■1S *(USP33)*

BRIEFING

Zinc Gluconate Tablets. Because there is no existing *USP* monograph for this article, a new monograph is being proposed. The development of this monograph has been requested by the United Nations International Children's Emergency Fund (UNICEF), the United States Agency for International Development (USAID), and the World Health Organization (WHO), in support of one of their Children's Health Initiatives. A disintegration test with a short test time is proposed for Tablets to be dissolved or dispersed in a small volume, typically a teaspoon of diluent before oral administration to infants, similar to the existing *USP* monograph for Zinc Sulfate Tablets. For other types of Tablets, dissolution requirements are proposed. Because Zinc Gluconate Tablets are also used as a dietary supplement in the United States, this monograph is proposed as part of the Dietary Supplements section of the *USP*.

(DSN: C. Phinney.) RTS—C70857

Add the following:

■Zinc Gluconate Tablets

DEFINITION
Zinc Gluconate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of Zn, in the form of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).

IDENTIFICATION
- **A: THIN-LAYER CHROMATOGRAPHY**
  Sample solution: Transfer a weighed quantity of powdered Tablets to a suitable flask, and add the necessary volume of water to obtain a solution containing about 10 mg/mL of zinc gluconate. Shake and sonicate, heating in a water bath at 60°, if necessary, and filter.
  Standard solution: USP Potassium Gluconate RS in water; 10 mg/mL
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography)*
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 5 µL
  Developing solvent system: Alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10)
  Spray reagent: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  When the solvent front has moved about three-fourths the length of the plate, remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, spray with *Spray reagent*, and heat the plate at 110° for about 10 min.
  Acceptance criteria: The principal spot obtained from the *Sample solution* corresponds in color, size, and R_F value to that obtained from the *Standard solution*.
- **B: PROCEDURE**
  Sodium hydroxide solution: 42 mg/mL of sodium hydroxide
  Ammonium chloride solution: 107 mg/mL of ammonium chloride
  Glycerin solution: A mixture of glycerin and water (85:15)
  Sodium sulfide solution: Dissolve 12 g of sodium sulfide with heating in a 45-mL mixture of *Glycerin solution* and water (29:10), allow to cool, and dilute with the same mixture of solvents to 100 mL. The solution should be colorless.
  Sample solution: Shake and sonicate the amount of powdered Tablets with the necessary volume of water to obtain a solution containing 100 mg/mL of zinc gluconate. Heat in a water bath at 60°, if necessary, and filter.
  Analysis: To 5 mL of the *Sample solution* add 0.2 mL of *Sodium hydroxide solution*. Add an additional 2 mL of *Sodium hydroxide solution*. Add 10 mL of *Ammonium chloride solution*. Add 0.1 mL of *Sodium sulfide solution*.
  Acceptance criteria: A white precipitate is formed after the first addition of the *Sodium hydroxide solution*. The precipitate dissolves after the second addition of the *Sodium hydroxide solution*. The solution remains clear after addition of the *Ammonium chloride solution*, and a white precipitate forms after addition of the *Sodium sulfide solution*.

STRENGTH
- **PROCEDURE**
  Sample solution: Weigh and finely powder NLT 20 Tablets. Weigh a portion of the powder, equivalent to about 80 mg of zinc, transfer to a suitable crucible, and ignite, gently at first, until free from carbon. Cool the crucible, add 25 mL of water and 5 mL of hydrochloric acid, and stir. Heat on a steam bath for 5 min, and filter, rinsing the filter with several portions of water. Dilute the combined filtrate and washes with water to about 100 mL.
  Analysis: Add ammonia–ammonium chloride buffer TS until the solution is neutral to litmus. Add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.27 mg of Zn or 22.78 mg of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).
  Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨**701**⟩ (For Tablets intended to be mixed with water prior to intake as oral liquids)
  Time: NMT 60 s
- **DISINGETRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS** ⟨**2040**⟩ (For Tablets not to be mixed with water prior to ingestion)
  Medium: Hydrochloric acid 0.01 N; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Analysis: Determine the amount of $C_{12}H_{22}O_{14}Zn$ dissolved, employing atomic absorption spectrophotometry at the resonance emission line for zinc, at 213.8 nm, on filtered portions of the solution under test, suitably diluted with water, in comparison with a *Standard solution* having a known concentration of zinc in the same *Medium.*
  Tolerances: NLT 75% of the labeled amount of $C_{12}H_{22}O_{14}Zn$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **LABELING:** Label the Tablets in terms of elemental zinc, and also in terms of zinc gluconate ($C_{12}H_{22}O_{14}Zn$). The labeling indicates whether the Tablets are intended to be mixed with water before intake.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Potassium Gluconate RS■1S *(USP33)*

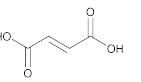
MONOGRAPHS (NF)

BRIEFING

Fumaric Acid, *NF* 27 page 1238. It is proposed to replace the existing non-specific *Identification* test in this monograph with a spectrophotometric *Identification* test employing *Infrared Absorption* ⟨197A⟩.

(EM1: R. Lafaver.) RTS—C72964

Fumaric Acid



$C_4H_4O_4$ 116.07
2-Butenedioic acid, *E-*;
Fumaric acid [110-17-8].

DEFINITION
Fumaric Acid contains NLT 99.5% and NMT 100.5% of $C_4H_4O_4$, calculated on the anhydrous basis.

In-Process Revision

IDENTIFICATION

Change to read:

- **~~PROCEDURE~~**
  ~~Sample solution:~~ ~~0.4 mg/mL~~
  ~~Analysis:~~ ~~To 25 mL of *Sample solution* add 1 mL of a solution prepared by mixing 20 mL of copper sulfate solution (1 in 5) and 8 mL of pyridine.~~
  ~~Acceptance criteria:~~ ~~A precipitate is formed in the blue solution within 1 min.~~
- **■INFRARED ABSORPTION ⟨197A⟩■1S** *(NF28)*

ASSAY

- **PROCEDURE**
  Sample solution: Dissolve 1 g of Fumaric Acid in 50 mL of methanol, and warm gently on a steam bath to effect solution. Cool, and add phenolphthalein TS.
  Analysis: Titrate the *Sample solution* with 0.5 N sodium hydroxide VS to the first appearance of a pink color that persists for NLT 30 s. Perform a blank titration (see *Titrimetry* ⟨541⟩). Each mL of 0.5 N sodium hydroxide is equivalent to 29.02 mg of $C_4H_4O_4$.
  Acceptance criteria: 99.5%–100.5%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

Organic Impurities

- **LIMIT OF MALEIC ACID**
  Mobile phase: 0.005 N sulfuric acid
  Standard solution: 0.001 mg/mL of USP Maleic Acid RS in *Mobile phase*
  Sample solution: 1 mg/mL of Fumaric Acid in *Mobile phase*
  System suitability solution: 10 µg/mL of USP Fumaric Acid RS and 5 µg/mL of USP Maleic Acid RS in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 22-cm; packing L17
  Flow rate: 0.3 mL/min
  Injection size: 5 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times of maleic acid and fumaric acid are about 0.5 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.5 of the maleic acid and fumaric acid peaks
  Relative standard deviation: NMT 2.0% of the maleic acid peak
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Calculate the percentage of maleic acid in the total weight of Fumaric Acid taken:

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

  r_U = response of the maleic acid peaks from the *Sample solution*
  r_S = response of the maleic acid peaks from the *Standard solution*
  C_S = concentration of USP Maleic Acid RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* **⟨921⟩:** 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Fumaric Acid RS
  USP Maleic Acid RS

BRIEFING

Propylene Glycol Dilaurate, *NF 27* page 1326. On the basis of comments and data received, it is proposed to make the following changes:

1. Add the chemical structure and formula, molecular weight, and CAS number to the monograph.
2. Revise the alternate chemical names given for Propylene Glycol Dilaurate to indicate the correct chemical composition.
3. Add the application amount of the *Sample solution* and add Propylene Glycol Dilaurate to the *Sample solution* under *Identification* test *A*.
4. Add the *Standard solution* under *Identification* test *A*.
5. Revise the nomenclature of the sample under *Analysis* in the *Assay* to reflect Propylene Glycol Dilaurate.

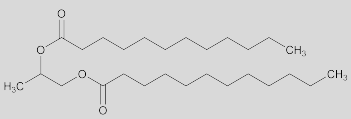
Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver. NOM: A. Wilk.) RTS—C70579

Propylene Glycol Dilaurate

Change to read:

■



$C_{27}H_{52}O_4$■1S *(NF28)*

~~Dodecanoic acid, monoester with 1,2-propanediol;~~ ~~■~~■1S *(NF28)*

Lauric acid, ~~monoester~~ ■diester■1S *(NF28)* with propane-1,2-diol;

■Propane-1,2-diyl didodecanoate 440.70
[22788-19-8].■1S *(NF28)*

DEFINITION

Propylene Glycol Dilaurate is a mixture of the propylene glycol mono- and diesters of lauric acid. It contains NLT 70.0% of diesters and NMT 30.0% of monoesters.

IDENTIFICATION

Change to read:

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  ■Standard solution: 50 mg/mL of USP Propylene Glycol Dilaurate RS in methylene chloride■1S *(NF28)*
  Sample solution: 50 mg/mL ■of Propylene Glycol Dilaurate■1S *(NF28)* in methylene chloride

In-Process Revision

Developing solvent system: Hexane and ether (3:7)
Spray reagent: 0.1 mg/mL of rhodamine 6G in alcohol
Analysis: ■Apply 200 µg of the *Standard solution* and *Sample solution.*■1S (*NF28*) Develop the chromatogram over a path of 15 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent*, and locate the spots on the plate by examination under UV light at a wavelength of 365 nm.
Acceptance criteria: The R_F values of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

- **B. FATS AND FIXED OILS,** *Fatty Acid Composition* ⟨**401**⟩**:** Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**
Mobile phase: Tetrahydrofuran
Sample solution: 200 mg of Propylene Glycol Dilaurate in 5 mL of tetrahydrofuran
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 7-mm × 60-cm; 5-µm packing L21 (100Å)
[NOTE—Two 7-mm × 30-cm L21 columns may be used in place of one 60-cm column, provided *System suitability requirements* are met.]
Temperature
Column: 40°
Detector: 40°
Flow rate: 1 mL/min
Injection size: 40 µL
System suitability
Sample: *Sample solution*
Suitability requirements
Elution: Diesters, monoesters, and propylene glycol
Relative standard deviation: NMT 1.0% is determined from the monoester peak.
Analysis
Sample: *Sample solution*
Calculate the percentage of monoesters or diesters in the Propylene Glycol ~~Monolaurate~~ ■Dilaurate■1S (*NF28*) taken:

$$\text{Result} = (r_U/r_T) \times (100 - D)$$

r_U = peak response for monoesters or diesters
r_T = sum of the responses of the monoester and diester peaks
D = sum of the percentage content of propylene glycol and the percentage content of free fatty acids

Calculate the percentage content of free fatty acids taken:

$$\text{Result} = 200(A/561.1)$$

A = acid value
Acceptance criteria: NLT 70.0% of diesters and NMT 30.0% of monoesters

IMPURITIES

Organic Impurities

- **PROCEDURE: LIMIT OF PROPYLENE GLYCOL**
Mobile phase: Proceed as directed in the *Assay*.
Standard stock solution: 4 mg/mL of USP Propylene Glycol RS in tetrahydrofuran
Standard solutions: Into four 15-mL flasks, introduce respectively 0.25, 0.5, 1.0, and 2.5 mL of *Standard stock solution*, and dilute with tetrahydrofuran to 5.0 mL. In a fifth 15-mL flask, introduce 5.0 mL of *Standard stock solution*.
Sample solution: Use the *Sample solution* from the *Assay*.
Chromatographic system: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solutions* and *Sample solution*
Prepare a standard curve of peak area versus concentration, in mg/mL, of propylene glycol in the *Standard solutions*. Obtain the concentration, C, in mg/mL, of propylene glycol in the *Sample solution* from the standard curve.
Acceptance criteria: NMT 2.0% of propylene glycol is found.

SPECIFIC TESTS

- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 0.1%
- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 4
- **FATS AND FIXED OILS,** *Fatty Acid Composition* ⟨**401**⟩**:** Propylene Glycol Dilaurate exhibits the following composition profile of fatty acids, determined as directed in the chapter.

Fatty Acids	Carbon-Chain Length	Percentage (%)
Caprylic acid	C8	NMT 0.5
Capric acid	C10	NMT 2.0
Lauric acid	C12	NLT 95.0
Myristic acid	C14	NMT 3.0
Palmitic acid	C16	NMT 1.0

- **FATS AND FIXED OILS,** *Iodine Value* ⟨**401**⟩**:** NMT 1
- **FATS AND FIXED OILS,** *Saponification Value* ⟨**401**⟩**:** 230–250
- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** NMT 1.0%, using a mixture of methanol and methylene chloride (1:1) in place of methanol in the titration vessel

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from moisture. No storage requirements are specified.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Propylene Glycol RS
USP Propylene Glycol Dilaurate RS

In-Process Revision

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

⟨**1**⟩ **Injections,** *USP 32* page 31 and page 1020 of *PF* 34(4) [July–Aug. 2008]. As a result of comments received and discussions by the Parenteral Products—Industrial Expert Committee, additions to the *Foreign and Particulate Matter* section have been proposed, specifying requirements for types of injection products in relation to the *USP* general test chapter *Particulate Matter in Injections* ⟨788⟩.

(PPI: D. Hunt) RTS—C70804

Change to read:

INGREDIENTS

Vehicles and Added Substances

Aqueous Vehicles—The vehicles for aqueous Injections meet the requirements of the *Pyrogen Test* ⟨151⟩ or the *Bacterial Endotoxins Test* ⟨85⟩, whichever is specified. *Water for Injection* generally is used as the vehicle, unless otherwise specified in the individual monograph. Sodium chloride may be added in amounts sufficient to render the resulting solution isotonic; and *Sodium Chloride Injection,* or *Ringer's Injection,* may be used in whole or in part instead of *Water for Injection,* unless otherwise specified in the individual monograph. For conditions applying to other adjuvants, see *Added Substances* in this chapter.

Other Vehicles—Fixed oils used as vehicles for nonaqueous Injections are of vegetable origin, are odorless or nearly so, and have no odor suggesting rancidity. They meet the requirements of the test for *Solid paraffin* under *Mineral Oil,* the cooling bath being maintained at 10°, have a *Saponification Value* between 185 and 200 (see *Fats and Fixed Oils* ⟨401⟩), have an *Iodine Value* between 79 and 141 (see *Fats and Fixed Oils* ⟨401⟩), and meet the requirements of the following tests.

~~*Unsaponifiable Matter*—Reflux on a steam bath 10 mL of the oil with 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of alcohol, with occasional shaking until the mixture becomes clear. Transfer the solution to a shallow dish, evaporate the alcohol on a steam bath, and mix the residue with 100 mL of water: a clear solution results.~~

~~*Free Fatty Acids*—The free fatty acids in 10 g of oil require for neutralization not more than 2.0 mL of 0.020 N sodium hydroxide (see *Fats and Fixed Oils* ⟨401⟩).~~

■*Unsaponifiable Matter* (see *Fats and Fixed Oils* ⟨401⟩): not more than 1.5%.

Acid Value (see *Fats and Fixed Oils* ⟨401⟩): not more than 0.2.

Peroxide Value (see *Fats and Fixed Oils* ⟨401⟩): not more than 5.0.

Water, Method I ⟨921⟩: not more than 0.1%, using 50 mL of chloroform instead of 35 to 40 mL of methanol as the solvent.

Limit of Copper, Iron, Lead, and Nickel—Proceed as directed in the section *Trace Metals* under *Fats and Fixed Oils* ⟨401⟩. Not more than 1 ppm of copper is found; not more than 1 ppm of iron is found; not more than 1 ppm of lead is found; and not more than 1 ppm of nickel is found.■*2S (USP32)*

Synthetic mono- or diglycerides of fatty acids may be used as vehicles, provided they are liquid, remain clear when cooled to 10°, and have an *Iodine Value* of not more than 140 (see *Fats and Fixed Oils* ⟨401⟩).

These and other nonaqueous vehicles may be used, provided they are safe in the volume of Injection administered, and also provided they do not interfere with the therapeutic efficacy of the preparation or with its response to prescribed assays and tests.

Added Substances—Suitable substances may be added to preparations intended for injection to increase stability or usefulness, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No coloring agent may be added, solely for the purpose of coloring the finished preparation, to a solution intended for parenteral administration (see also *Added Substances* under *General Notices* and *Antimicrobial Effectiveness Testing* ⟨51⟩).

Observe special care in the choice and use of added substances in preparations for injection that are administered in a volume exceeding 5 mL. The following maximum limits prevail unless otherwise directed: for agents containing mercury and the cationic, surface-active compounds, 0.01%; for chlorobutanol, cresol, phenol, and similar types of substances, 0.5%; and for sulfur dioxide, or an equivalent amount of the sulfite, bisulfite, or metabisulfite of potassium or sodium, 0.2%.

A suitable substance or mixture of substances to prevent the growth of microorganisms must be added to preparations intended for injection that are packaged in multiple-dose containers, regardless of the method of sterilization employed, unless one of the following conditions prevails: (1) there are different directions in the individual monograph; (2) the substance contains a radionuclide with a physical half-life of less than 24 hours; and (3) the active ingredients are themselves antimicrobial. Such substances are used in concentrations that will prevent the growth of or kill micro-organisms in the preparations for injection. Such substances also meet the requirements of *Antimicrobial Effectiveness Testing* ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩. Sterilization processes are employed even though such substances are used (see also *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩). The air

In-Process Revision

in the container may be evacuated or be displaced by a chemically inert gas. Where specified in a monograph, information regarding sensitivity of the article to oxygen is to be provided in the labeling.

Change to read:

FOREIGN AND PARTICULATE MATTER

All articles intended for parenteral administration shall be prepared in a manner designed to exclude particulate matter as defined in *Particulate Matter in Injections* ⟨788⟩ and other foreign matter. Each final container of all parenteral preparations shall be inspected to the extent possible for the presence of observable foreign and particulate matter (hereafter termed ''visible particulates'') in its contents. The inspection process shall be designed and qualified to ensure that every lot of all parenteral preparations is essentially free from visible particulates. Qualification of the inspection process shall be performed with reference to particulates in the visible range of a type that might emanate from the manufacturing or filling process. Every container whose contents shows evidence of visible particulates shall be rejected. The inspection for visible particulates may take place when inspecting for other critical defects, such as cracked or defective containers or seals, or when characterizing the appearance of a lyophilized product.

Where the nature of the contents or the container-closure system permits only limited capability for the inspection of the total contents, the 100% inspection of a lot shall be supplemented with the inspection of constituted (e.g., dried) or withdrawn (e.g., dark amber container) contents of a sample of containers from the lot.

All large-volume Injections for single-dose infusion and small-volume Injections are subject to the light obscuration or microscopic procedures and limits for subvisible particulate matter set forth in *Particulate Matter in Injections* ⟨788⟩, unless otherwise specified in the individual monograph. An article packaged as both a large-volume and a small-volume Injection meets the requirements set forth for small-volume Injections where the container is labeled as containing 100 mL or less, if the individual monograph states a test for *Particulate Matter in Injections* ⟨788⟩; it meets the requirements set forth for large-volume Injections for single-dose infusion where the container is labeled as containing more than 100 mL. ~~Injections administered exclusively by the intramuscular or subcutaneous route or packaged and labeled for use as irrigating solutions are exempt from requirements for *Particulate Matter in Injections* ⟨788⟩.~~

■Solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenteral products for which the labeling specifies the use of a final filter prior to administration are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.■1S (*USP33*)

BRIEFING

⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests. Two new *USP* general chapters on Topical and Transdermal Drug Products address the quality and performance aspects of topical dermal pharmaceutical dosage forms. The general chapters are *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, which covers the basic quality control tests for these dosage forms, and *Topical and Transdermal Drug Products—Product Performance Tests* ⟨725⟩, which covers the apparatus and procedures used to evaluate the in vitro drug release. Comments and suggestions regarding these two general chapters should be sent to Margareth Marques at MRM@usp.org no later than July 31, 2009. USP is planning a workshop on these general chapters and the comments and suggestions received.

(PDF: M. Marques.) RTS—C71036

Add the following:

■⟨3⟩ TOPICAL AND TRANSDERMAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

i. **Introduction**

ii. **Glossary of Terms**

iii. **Product Quality Tests for All Topically Applied Drug Products**

  a. **Product Quality Tests for Topical Drug Products Intended for Local Action**

  b. **Product Quality Tests for Transdermal Drug Products**

iv. **Product Performance Test for Topical Drug Products**

v. **In Vitro Drug Release from Semisolid Dosage Forms**

  a. **Theory**

  b. **Application of In Vitro Drug Release**

I. INTRODUCTION

Drug products topically administered via the skin fall into two general categories: those applied to achieve local action and those to achieve systemic effects. Local ac-

In-Process Revision

tion can occur at or on the surface of the skin (the stratum corneum) and also in the epidermis and/or dermis. Locally acting drug products include creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions. Creams, ointments, and gels are generally referred to as semisolid dosage forms. Drug products applied to the skin to achieve systemic effects are referred to as self-adhering transdermal patches or transdermal drug delivery systems (TDS).

Quality tests with procedures and acceptable criteria for both types of topically administered drug products can be divided into those that assess general quality attributes and those that assess performance. The former include identification, assay (strength), content uniformity, pH, microbial limits, and minimum fill. The latter assess drug release from the finished dosage form. For locally acting topical drug products, a product performance test exists only for semisolid formulations. TDS are physical devices that are applied to the skin and vary in their composition and method of fabrication. They release their active ingredients by different mechanisms. Several product performance tests are available to assess in vitro drug release from TDS. Performance tests considered for topically applied products may also be applicable to drug products of similar composition when administered by other routes of administration, e.g., ophthalmic drug products.

II. GLOSSARY OF TERMS

Definitions of topical drug products, brief information about their manufacture, and a glossary of dosage form names can be found in the general information chapter *Pharmaceutical Dosage Forms* ⟨1151⟩.

Absorption Bases—This class of bases may be divided into two groups: bases that permit the incorporation of aqueous solutions with the formation of a water-in-oil emulsion (e.g., *Hydrophilic Petrolatum* and *Lanolin*, both *USP*), and water-in-oil emulsions that permit the incorporation of additional quantities of aqueous solutions (e.g., *Lanolin*, *USP*). Absorption bases also are useful as emollients.

Choice of Base—The choice of an ointment base depends on many factors, such as the action desired, the nature of the medicament to be incorporated and its bioavailability and stability, and the requisite shelf life of the finished product. In some cases, it is necessary to use a base that is less than ideal in order to achieve the stability required. Drugs that hydrolyze rapidly, for example, are more stable in hydrocarbon bases than in bases that contain water, even though they may be more effective in the latter.

Collodion—Collodion (pyroxylin solution; see *USP* monograph *Collodion*) is a solution of nitrocellulose in ether and acetone, sometimes with the addition of alcohol. As the volatile solvents evaporate, a dry celluloid-like film is left on the skin. Because the medicinal use of a collodion depends on the formation of a protective film, the film should be durable, tenacious in adherence, flexible, and occlusive.

Creams—Creams are semisolid dosage forms that contain one or more drug substances dissolved or dispersed in a suitable base. This term traditionally has been applied to semisolids that possess a relatively soft, spreadable consistency formulated as either water-in-oil or oil-in-water emulsions. However, more recently the term has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water washable and more cosmetically and aesthetically acceptable.

Emulsions—Emulsions are viscid multiphase systems in which one or more liquids are dispersed throughout another immiscible liquid in the form of small droplets. When oil is the dispersed phase and an aqueous solution is the continuous phase, the system is designated an oil-in-water emulsion. Conversely, when water or an aque-

ous solution is the dispersed phase and oil or oleaginous material is the continuous phase, the system is designated a water-in-oil emulsion. Emulsions are stabilized by emulsifying agents that prevent coalescence, the merging of small droplets into larger droplets and, ultimately, into a single separated phase. Emulsifying agents (surfactants) act by concentrating at the interface between the immiscible liquids, thereby providing a physical barrier that reduces the tendency for coalescence. Surfactants also reduce the interfacial tension between the phases, facilitating the formation of small droplets upon mixing. The term emulsion is not used if a more specific term is applicable, e.g., cream or ointment.

Foams—Foams are emulsified systems packaged in pressurized containers or special dispensing devices that contain dispersed gas bubbles, usually in a liquid continuous phase, that when dispensed have a fluffy, semisolid consistency.

Gels—Gels (sometimes called jellies) are semisolid systems that consist of either suspensions composed of small inorganic particles or large organic molecules interpenetrated by a liquid. When the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system (e.g., *Aluminum Hydroxide Gel*, *USP*). In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes referred to as a magma (e.g., *Bentonite Magma*, *NF*). Both gels and magmas may be thixotropic, forming semisolids after standing and becoming liquid when agitated. They should be shaken before use to ensure homogeneity and should be labeled to that effect (see *Topical Suspensions*, below). Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid with no apparent boundary between the dispersed macromolecule and liquid.

Hydrocarbon Bases—Hydrocarbon bases, known also as oleaginous ointment bases, are represented by *White Petrolatum* and *White Ointment* (both *USP*). Only small amounts of an aqueous component can be incorporated into these bases. Hydrocarbon bases keep medicaments in prolonged contact with the skin and act as occlusive dressings. These bases are used chiefly for their emollient effects and are difficult to wash off. They do not ''dry out'' or change noticeably on aging.

Lotions—Although the term lotion may be applied to a solution, lotions usually are fluid, somewhat viscid emulsion dosage forms for external application to the skin. Lotions share many characteristics with creams. See *Creams, Topical Solutions,* and *Topical Suspensions*, herein.

Ointments—Ointments are semisolids intended for external application to the skin or mucous membranes. They usually contain less than 20% water and volatiles and more than 50% hydrocarbons, waxes, or polyols as the vehicle. Ointment bases recognized for use as vehicles fall into four general classes: hydrocarbon bases, absorption bases, water-removable bases, and water-soluble bases. Each therapeutic ointment possesses as its base one of these four general classes.

Ophthalmic Ointments—Ophthalmic ointments are semisolids for application to the eye. Special precautions must be taken in the preparation of ophthalmic ointments. They are manufactured from sterilized ingredients under rigidly aseptic conditions, must meet the requirements under *Sterility Tests* ⟨71⟩, and must be free of large particles. The medicinal agent is added to the ointment base either as a solution or as a micronized powder.

Pastes—Pastes are semisolid dosage forms that contain a high percentage (often $\geq$ 50%) of finely dispersed solids with a stiff consistency and intended for topical application. One class is made from a single-phase aqueous gel (e.g., *Carboxymethylcellulose Sodium Paste*, *USP*). The other class, the fatty pastes (e.g., *Zinc Oxide Paste*, *USP*),

consists of thick, stiff ointments that do not ordinarily flow at body temperature and therefore serve as protective coatings over the areas to which they are applied.

Powders—Powders are solids or mixture of solids in a dry, finely divided state for external (or internal) use.

Sprays—Sprays are products formed by the generation of droplets of solution containing dissolved drug for application to the skin or mucous membranes. The droplets may be formed in a variety of ways but generally result when a liquid is forced through a specially designed nozzle assembly. One example of a spray dosage form is a metered-dose topical transdermal spray that delivers a precisely controlled quantity of solution or suspension on each activation.

Transdermal Delivery Systems (TDS)—TDS are self-contained, discrete dosage forms that, when applied to intact skin, are designed to deliver the drug(s) through the skin to the systemic circulation. Systems typically comprise an outer covering (barrier), a drug reservoir that may have a drug release-controlling membrane, a contact adhesive applied to some or all parts of the system and the system/skin interface, and a protective liner that is removed before the patient applies the system. The dose of these systems is defined in terms of the release rate of the drug(s) from the system and surface area of the patch and is expressed as mass per unit time for a given surface area. With these drug products, the skin typically is the rate-controlling membrane for the drug input into the body. The total duration of drug release from the system and system surface area also may be stated.

TDS work by diffusion: the drug diffuses from the drug reservoir, directly or through the rate-controlling membrane and/or contact adhesive if present, and then through the skin into the general circulation. Typically, modified-release systems are designed to provide drug delivery at a constant rate so that a true steady-state blood concentration is achieved and maintained until the system is removed. Following removal of the system, blood concentration declines at a rate consistent with the pharmacokinetics of the drug.

Topical Aerosols—Topical aerosols are products that are packaged under pressure. The active ingredients are released in the form of fine liquid droplets or fine powder particles upon activation of an appropriate valve system. A special form is a metered-dose aerosol that delivers an exact volume (dose) per each actuation.

Topical Solutions—Topical solutions are liquid preparations that usually are aqueous but often contain other solvents such as alcohol and polyols that contain one or more dissolved chemical substances intended for topical application to the skin or, as in the case of *Lidocaine Oral Topical Solution*, *USP*, to the oral mucosal surface.

Topical Suspensions—Topical suspensions are liquid preparations that contain solid particles dispersed in a liquid vehicle intended for application to the skin. Some suspensions labeled as lotions fall into this category.

Water-removable Bases—Water-removable bases are oil-in-water emulsions (e.g., *Hydrophilic Ointment*, *USP*) and are more correctly called creams (see *Creams*, above). They also are described as ''water-washable'' because they may be readily washed from the skin or clothing with water, an attribute that makes them more acceptable for cosmetic purposes. Some medicaments may be more effective in these bases than in hydrocarbon bases. Other advantages of the water-removable bases are that they can be diluted with water and that they favor the absorption of serous discharges in dermatological conditions.

Water-soluble Bases—This group of so-called ''greaseless ointment bases'' comprises water-soluble constituents. *Polyethylene Glycol Ointment*, *NF*, is the only pharmacopeial preparation in this group. Bases of this type offer many of the advantages of the water-removable bases and, in addition, contain no water-insoluble

substances such as petrolatum, anhydrous lanolin, or waxes. They are more correctly called gels (see *Gels*, above).

III. PRODUCT QUALITY TESTS FOR ALL TOPICALLY APPLIED DRUG PRODUCTS

Universal tests are listed below and should be applied to all topically applied drug products.

Description—A qualitative description of the dosage form should be provided. The acceptance criteria should include the final acceptable appearance. If color changes during storage, a quantitative procedure may be appropriate. It specifies the content or the label claim of the article.

Identification—Identification tests are discussed in *Procedures* under *Tests and Assays* in the *General Notices and Requirements*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structure that are likely to be present. Identity tests should be specific for the drug substances. The most conclusive test for identity is the infrared absorption spectrum (see *Spectrophotometry and Light-Scattering* ⟨851⟩ and *Spectrophotometric Identification Tests* ⟨197⟩). If no suitable infrared spectrum can be obtained, other analytical techniques can be used. Near infrared (NIR) or Raman spectrophotometric methods also could be acceptable for the sole identification of the drug product formulation (see *Near-infrared Spectrophotometry* ⟨1119⟩ and *Raman Spectroscopy* ⟨1120⟩). Identification solely by a single chromatographic retention time is not regarded as specific. However, the use of two chromatographic procedures for which the separation is based on different principles or a combination of tests in a single procedure can be acceptable. See *Chromatography* ⟨621⟩ and *Thin-layer Chromatographic Identification Test* ⟨201⟩.

Assay—A specific and stability-indicating test should be used to determine the strength (content) of the drug product. See *Antibiotics—Microbial Assays* ⟨81⟩, *Chromatography* ⟨621⟩, or *Assay for Steroids* ⟨351⟩. In cases when the use of a nonspecific assay is justified (e.g., *Titrimetry* ⟨541⟩), other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay.

Impurities—Process impurities, synthetic by-products, and other inorganic and organic impurities may be present in the drug substance and excipients used in the manufacture of the drug product. These impurities are controlled by the drug substance and excipients monographs. Organic impurities arising from the degradation of the drug substance and those arising during the manufacturing process of the drug product should be monitored.

In addition to the universal tests listed above, the following specific tests may be considered on a case-by-case basis.

Physicochemical Properties—These are properties such as *pH* ⟨791⟩, *Viscosity* ⟨911⟩, and *Specific Gravity* ⟨841⟩.

Uniformity of Dosage Units—This test is applicable for TDS and for dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form. The test can be performed by either content uniformity or weight variation (see *Uniformity of Dosage Units* ⟨905⟩).

Water Content—A test for water content should be included when appropriate (see *Water Determination* ⟨921⟩).

Microbial Limits—The type of microbial test(s) and acceptance criteria should be based on the nature of the drug substance, method of manufacture, and the intended use of the drug product. See *Microbiological Ex-*

amination of Nonsterile Products: Microbial Enumeration Tests ⟨61⟩ and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩).

Antimicrobial Preservative Content—Acceptance criteria for preservative content in multidose products should be established. They should be based on the levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* ⟨51⟩).

Antioxidant Preservative Content—If antioxidant preservatives are present in the drug product, tests of their content normally should be determined.

Sterility—Depending on the use of the dosage form (e.g., ophthalmic preparations), sterility of the product should be demonstrated as appropriate (see *Sterility Tests* ⟨71⟩).

III. a. PRODUCT QUALITY TESTS FOR TOPICAL DRUG PRODUCTS INTENDED FOR LOCAL ACTION

Additional tests for locally acting topical dosage forms are provided in this section. Some of these may also be used for some nitroglycerin semisolids.

Viscosity—Rheological properties such as viscosity of semisolid dosage forms can influence their drug delivery. Viscosity may directly influence the diffusion rate of a drug at the microstructural level. Yet semisolid drug products with comparatively high viscosity still can exhibit high diffusion rates when compared to semisolid products of comparatively lower viscosity. These observations emphasize the importance of rheologic properties of semisolid dosage forms, specifically viscosity, on drug product performance.

Depending on its viscosity, the rheological behavior of a semisolid drug product may affect its application to treatment site(s) and consistency of treatment and thus the delivered dose. Therefore, maintaining reproducibility of a product's flow behavior at the time of release is an important product manufacturing control that manufacturers should use to maintain and demonstrate batch-to-batch consistency. Most semisolid dosage forms, when sheared, exhibit non-Newtonian behavior. Structures formed within semisolid drug products during manufacturing can show a wide range of behaviors, including shear thinning viscosity, thixotropy, and structural damage that may be irreversible or only partially reversible. In addition, the viscosity of a semisolid dosage form is highly influenced by factors such as the inherent physical structure of the product, product sampling technique, sample temperature for viscosity testing, container size and shape, and specific methodology employed in the measurement of viscosity.

A variety of methods can be used to characterize the consistency of semisolid dosage forms, including penetrometry, viscometry, and rheometry. With all methods significant attention is warranted to the shear history of the sample. For semisolids, viscometer geometries typically fall into the following categories: concentric cylinders, cone-plates, and spindles. Concentric cylinders and spindles typically are used for more fluid, flowable semisolid dosage forms. Cone-plate geometries are more typically used when the sample size is small or the test samples are more viscous and less flowable.

When contemplating what viscosity parameter(s) to test, one must consider the properties of the semisolid drug product both ''at rest'' (in its container) and as it is sheared during application. The rheological properties of the drug product at rest can influence the product's shelf life, and its properties under extensive shear can influence its spreadability and, therefore, its application rate that will affect the safety and efficacy of the drug product. Further, although it is necessary to precisely control the temperature of the test sample during the viscosity measurement, one should link the specific choice

of the temperature to the intended use of the drug product (e.g., skin temperature for external application effects).

Because semisolid dosage forms frequently display non-Newtonian flow properties, formulators should give close attention to the shear history of the sample being tested, such as the shear applied during the filling operation, shear applied dispensing the product from its container, and shear when introducing the sample into the viscometer. The point of reemphasizing this aspect is that considerable variability and many failures to meet specifications can be directly attributed to a lack of attention to this detail rather than a change of viscosity (or flow properties) of the drug product.

Tube (Content) Uniformity—Tube uniformity is the degree of uniformity of the amount of active drug substance among containers, i.e., tubes containing multiple doses of the semisolid topical product. The uniformity of dosage is demonstrated by assay of top, middle, and bottom samples (typically 0.25–1.0 g) obtained from a tube cut open to withdraw respective samples for drug assay.

Various topical semisolid products may show some physical separation at accelerated storage temperatures because emulsions, creams, and topical lotions are prone to mild separation due to the nature of the vehicle.

The following procedure should be followed for testing tube uniformity of semisolid topical dosage forms:

1. Carefully remove or cut off the bottom tube seal and make a vertical cut up the face of the tube. Then carefully cut the tube around the upper rim and pry open the two ''flaps'' to expose the semisolid.
2. At the batch release and/or designated stability time point remove and test 0.25- to 1.0-g samples from the top, middle, and bottom of a tube. If assay values for those tests are within 90.0%–110.0% of the labeled amount of active drug, and the relative standard deviation (RSD) is not more than 6%, then the results are acceptable.
3. If at least one value of the testing described above is outside 90.0%–110.0% of the labeled amount of drug and none is outside 85.0%–115.0% and/or the RSD is more than 6%, then test an additional three randomly sampled tubes using top, middle, and bottom samples as described. Not more than 3 of the 12 determinations should be outside the range of 90%–110.0% of the labeled amount of drug, none should be outside 85.0%–115.0%, and the RSD should not be not more than 7%.
4. For very small tubes (e.g., 5 g or less), test only top and bottom samples, and all values should be within the range of 90.0%–110.0% of the labeled amount of drug.

pH—When applicable, semisolid drug products should be tested for pH at the time of batch release and designated stability test time points for batch-to-batch monitoring. Because most semisolid dosage forms contain very limited quantities of water or aqueous phase, pH measurements may be warranted only as a quality control measure, as appropriate.

Particle Size—Particle size of the active drug substance in semisolid dosage forms is determined and controlled at the formulation development stage. When applicable, semisolid drug products should be tested for any change in the particle size or habit of the active drug substance at the time of batch release and designated stability test time points (for batch-to-batch monitoring) that could compromise the integrity and/or performance of the drug product, as appropriate.

Ophthalmic Dosage Forms—Ophthalmic dosage forms must meet the requirements of *Sterility Tests* ⟨71⟩. If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described under *Sterility Tests* ⟨71⟩, along with aseptic manufacture, may be employed. Ophthalmic ointments must contain a suitable substance or mixture of sub-

stances to prevent growth of, or to destroy, microorganisms accidentally introduced when the container is opened during use, unless otherwise directed in the individual monograph or unless the formula itself is bacteriostatic (see *Added Substances* under *Ophthalmic Ointments* ⟨771⟩). The finished ointment must be free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* in *Ophthalmic Ointments* ⟨771⟩. The immediate containers for ophthalmic ointments shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic ointments be sealed and tamper-proof so that sterility is assured at time of first use.

III. b. PRODUCT QUALITY TESTS FOR TRANSDERMAL DRUG PRODUCTS

The product quality tests for TDS drug products include assay, content uniformity, homogeneity, and adhesive.

Uniformity of Dosage Units—This test is applicable for TDS and for dosage forms that are packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form. It can be done by either content uniformity or weight variation (see *Uniformity of Dosage Units* ⟨905⟩).

Assay of excipient(s) critical to the performance of the product should be considered; e.g., residual solvent content can affect certain patches.

Adhesive Test—Three types of adhesive tests generally are performed to ensure the performance of the TDS dosage forms. These are the peel adhesion test, tack test, and shear strength test. The peel adhesion test measures the force required to peel away a transdermal patch attached to a stainless steel test panel substrate at panel angles of 90° or 180° following a dwell time of 1 minute and peel rate of 300 mm/minute.

The tack test is used to measure the tack adhesive properties of TDS dosage forms. With this test a probe touches the adhesive surface with light pressure, and the force required to break the adhesion after a brief period of contact is measured.

The shear strength or creep compliance test is a measure of the cohesive strength of TDS dosage forms. Two types of shear testing are performed: dynamic and static. During dynamic testing the TDS is pulled from the test panel at a constant rate. With the static test the TDS is subjected to a shearing force by means of a suspended weight.

Leak Test—A test that is discriminating and capable of detecting sudden drug release, such as leakage, from the TDS should be performed. Although form, fill, and seal TDS are more likely to display leak problems, all TDS should be checked for sudden drug release (dose dumping) during release testing of the dosage form.

IV. PRODUCT PERFORMANCE TEST FOR TOPICAL DRUG PRODUCTS

A performance test for topical drug products must have the ability to measure drug release from the finished dosage form. It must be reproducible and reliable, and although it is not a measure of bioavailability, the performance test must be capable of detecting changes in drug release characteristics from the finished product. The latter have the potential to alter the biological performance of the drug in the dosage form. Those changes may be related to active or inactive/inert ingredients in the formulation, physical or chemical attributes of the finished formulation, manufacturing variables, shipping and storage effects, aging effects, and other formulation factors critical to the quality characteristics of the finished drug product. Product performance tests can serve many useful purposes in product development and in post-approval drug product monitoring. They provide assurance of equivalent performance for products that

have undergone postapproval raw material changes, relocation or change in manufacturing site, and other changes as detailed in the FDA's Guidance for Industry SUPAC-SS: *Nonsterile Semisolid Dosage Forms; Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation* (May 1997) (available at www.fda.gov/cder/guidance/1447fnl.pdf). In this general chapter, a USP performance test for semi-solid dosage forms to support batch release is considered. Details of the procedure are provided in the general chapter *Topical and Transdermal Drug Products—Product Performance Tests* ⟨*725*⟩ (proposed).

V. IN VITRO DRUG RELEASE FROM SEMISOLID DOSAGE FORMS

V. a. THEORY

The vertical diffusion cell (VDC) system is a simple, reliable, and reproducible means of measuring drug release from semisolid dosage forms. A thick layer (200–400 mg) of the test semisolid is placed in contact with a reservoir. Diffusive communication between the delivery system and the reservoir takes place through an inert, highly permeable support membrane. The membrane keeps the product and the receptor medium separate and distinct. Membranes are chosen to offer the least possible diffusional resistance and not to be rate controlling. Samples are withdrawn from the reservoir at various times. In most cases, a 5- to 6-hour time period is all that is needed to characterize drug release from a semisolid, and when this is the case samples usually are withdrawn hourly.

After a short lag period, release of drug from the semisolid dosage form in the VDC system is kinetically describable by diffusion of a chemical out of a semi-infinite medium into a sink. The momentary release rate tracks the depth of penetration of the forming gradient within the semisolid. Beginning at the moment when the receding boundary layer's diffusional resistance assumes dominance of the kinetics of release, the amount of the drug released, *M*, becomes proportional to $\sqrt{t}$ (where $\sqrt{t}$ = time) for solution, suspension, or emulsion semisolid systems alike. The momentary rate of release, *dM/dt*, becomes proportional to $1/\sqrt{t}$, which reflects the slowing of drug release with the passage of time. The reservoir is kept large so that drug release is into a medium that remains highly dilute over the entire course of the experiment relative to the concentration of drug dissolved in the semisolid. In this circumstance drug release is said to take place into a diffusional sink.

When a drug is totally in solution within the dosage form, the amount of drug released as a function of time can be described by:

$$M = 2 \cdot Co\sqrt{D \cdot t} / \pi$$

where:

M = amount of drug released into the sink per cm^2

Co = drug concentration in releasing matrix

D = drug diffusion coefficient through the matrix.

A plot of *M* vs $\sqrt{t}$ will be linear with a slope of

$$2 \cdot Co\sqrt{D} / \pi$$

Equation 2 describes drug release when the drug is in the form of a suspension within the dosage form:

$$M = \sqrt{2 \cdot Dm \cdot Cs(Q - Cs/2)t}$$

where:

Dm = drug diffusion coefficient in the semisolid matrix

Cs = drug solubility in the releasing matrix

In-Process Revision

Q = total amount of the drug in solution and suspended in the matrix.

When $Q >> Cs$, Equation 2 simplifies to Equation 3.

$$M = \sqrt{2 \cdot Q \cdot Dm \cdot Cs \cdot t}$$

A plot of M vs $\sqrt{t}$ will be linear with a slope of $\sqrt{2QDmCs}$.

Coarse particles may dissolve so slowly that the moving boundary layer recedes to some extent behind the particles. That situation introduces noticeable curvature in the *t* plot because of a particle size effect. During release rate experiments, every attempt should be made to keep the composition of the formulation intact during the releasing period.

V. b. APPLICATION OF DRUG RELEASE

Drug release results can be utilized for purposes such as ensuring product sameness after SUPAC-SS-related changes or successive batch release comparisons. This is illustrated by the following example in which the initial drug batch is referred to as Reference Batch (R) and the changed or subsequent batch is referred to as Test Batch (T). The individual amount released from R is plotted vs time, and the resulting slope is determined. These are the reference slopes (RS). The process is repeated to determine the test slopes (TS).

The T/R ratios are calculated for each Test to Reference Slope. This is facilitated if one creates a table where the TS are listed down the left side of the table and the RS are listed across the top of the table. The T/R ratios are then calculated and entered in the body of the table.

After the T/R ratios have been calculated they are ordered from lowest to highest. The 8th and 29th T/R ratios are extracted and converted to percent (multiply by 100). To pass the first stage these ratios must fall within the range of 75%–133.33%.

If the results do not meet this criterion, the SUPAC-SS Guidance requires that four more tests of six cells each should be run, resulting in 12 additional slopes per product tested. The T/R ratios are calculated for all 18 slopes per product tested. All 324 individual T/R ratios are calculated and ordered lowest to highest. The 110th and the 215th ratios are evaluated against the specification of 75%–133.33%.

Third stage testing is not suggested.

	RS1	RS2	RS3	RS4	RS5	RS6
TS1	TS1/RS1	TS1/RS2	TS1/RS3	TS1/RS4	TS1/RS5	TS1/RS6
TS2	TS2/RS1	TS2/RS2	TS2/RS3	TS2/RS4	TS2/RS5	TS2/RS6
TS3	TS3/RS1	TS3/RS2	TS3/RS3	TS3/RS4	TS3/RS5	TS3/RS6
TS4	TS4/RS1	TS4/RS2	TS4/RS3	TS4/RS4	TS4/RS5	TS4/RS6
TS5	TS5/RS1	TS5/RS2	TS5/RS3	TS5/RS4	TS5/RS5	TS5/RS6
TS6	TS6/RS1	TS6/RS2	TS6/RS3	TS6/RS4	TS6/RS5	TS6/RS6

■1S (*USP33*)

BRIEFING

〈**11**〉 **USP Reference Standards,** *USP 32* page 35 and page 2022 of *PF* 29(6) [Nov.–Dec. 2003], page 1674 of *PF* 30(5) [Sept.–Oct. 2004], page 507 of *PF* 31(2) [Mar.–Apr. 2005], page 1154 of *PF* 31(4) [July–Aug. 2005], page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 1161 of *PF* 32(4) [July–Aug. 2006], page 95 of *PF* 33(1) [Jan.–Feb. 2007], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 332 of *PF* 34(2) [Mar–Apr. 2008], page 680 of *PF* 34(3) [May–June 2008], page 1021 of *PF* 34(4) [July–Aug. 2008], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], page 144 of *PF* 35(1) [Jan.–Feb. 2009], and page 330 of *PF* 35(2) [Mar–Apr. 2009].

(HDQ) RTS—C42543; C43499; C44129; C44603; C44606; C46368; C49314; C49319; C52047; C57642; C57642; C57726; C62481; C57472; C66966; C68834; C72721

Add the following:

■**USP Articaine RS.**■1S (*USP33*)

Add the following:

■**USP Articaine Hydrochloride RS.**■1S (*USP33*)

Add the following:

■**USP Articaine Related Compound A RS [**methyl 4-methyl-3-[2-(propylamino) acetamido] thiophene-2-carboxylate**]** ($C_{12}H_{18}N_2O_3S$ ✧270.35).■1S (*USP33*)

Add the following:

■**USP Articaine Related Compound E RS [**methyl 3-[2-(isopropylamino) propanamido]-4-methylthiophene-2-carboxylate**]** ($C_{13}H_{20}N_2O_3S$ ✧284.37).■1S (*USP33*)

Change to read:

USP Capecitabine Related Compound A RS

■**[**5′-deoxy-5-fluorocytidine**]** ($C_9H_{12}FN_3O_4$ ✧ 245.21).■1S (*USP33*)

Change to read:

USP Capecitabine Related Compound B RS

■**[**5′-deoxy-5-fluorouridine**]** ($C_9H_{11}FN_2O_5$ ✧ 246.19).■1S (*USP33*)

Change to read:

USP Capecitabine Related Compound C RS

■**[**2′,3′-*O*-carbonyl-5′-deoxy-5-fluoro-*N*-4-(pentyloxycarbonyl)cytidine**]** ($C_{16}H_{20}FN_3O_7$ ✧385.34).■1S (*USP33*)

Add the following:

■**USP Carmustine RS.**■1S (*USP33*)

Add the following:

■**USP Carmustine Related Compound A RS [**1,3-bis(2-chloroethyl) urea**]** ($C_5H_{10}Cl_2N_2O$ ✧ 185.05).■1S (*USP33*)

Add the following:

■**USP Levothyroxine for Peak Identification RS**—Mixture containing liothyronine, T3-acetic acid, and T4-acetic acid in a matrix of levothyroxine sodium.■1S (*USP33*)

Add the following:

■**USP Misoprostol RS.**■1S (*USP33*)

Add the following:

■**USP Morphine Related Compound A RS [**7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol, *N*-oxide**]** ($C_{17}H_{19}NO_4$ ✧301.34).■1S (*USP33*)

Add the following:

■**USP Olopatadine Hydrochloride RS.**■1S (*USP33*)

Add the following:

■**USP Olopatadine Hydrochloride Related Compound B RS [**(*Z*)-3-{2-(carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}-*N,N*-dimethylpropan-1-amine oxide**]** ($C_{21}H_{23}NO_4$ ✧353.41).■1S (*USP33*)

Add the following:

■**USP Olopatadine Hydrochloride Related Compound C RS [**11-oxo-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl acetic acid**]** ($C_{16}H_{12}O_4$ ✧268.26).■1S (*USP33*)

In-Process Revision

Add the following:

▪**USP Pentamidine Isethionate RS** [$C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$].▪1S (*USP33*)

Add the following:

▪**USP Primidone Related Compound A RS** [2-ethyl-2-phenylmalonamide] ($C_{11}H_{14}N_2O_2$ ✧206.24).▪1S (*USP33*)

Add the following:

▪**USP Primidone Related Compound C RS** [2-phenylbutyramide] ($C_{10}H_{13}NO$ ✧163.22).▪1S (*USP33*)

Add the following:

▪**USP Propafenone Related Compound B RS** [(*RS*,*E*)-1-[2-[2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one] ($C_{21}H_{25}NO_3$ ✧ 339.43).▪1S (*USP33*)

Add the following:

▪**USP Telmisartan RS**.▪1S (*USP33*)

Add the following:

▪**USP Telmisartan Related Compound A RS** [1,7′-dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole] ($C_{19}H_{20}N_4$ ✧304.39).▪1S (*USP33*)

Add the following:

▪**USP Telmisartan Related Compound B RS** [4′-[(1,7′-dimethyl-2′-propyl-1*H*,1′*H*-2,5′-bibenzo[*d*]imidazol-1′-yl)methyl]biphenyl-2-carboxylic acid] ($C_{33}H_{30}N_4O_2$ ✧514.62).▪1S (*USP33*)

Delete the following:

~~▪**USP Δ⁸-Tetrahydrocannabinol RS**.~~▪1S (*USP33*)

Add the following:

▪**USP Ticlopidine Hydrochloride RS**.▪1S (*USP33*)

Add the following:

▪**USP Ticlopidine Related Compound A RS** [4-oxo-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine].▪1S (*USP33*)

Add the following:

▪**USP Ticlopidine Related Compound B RS** [5-(2-chlorobenzyl)- 4-oxo-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine].▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Hydrochloride RS**.▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Hydrochloride Related Compound C RS** [2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate] ($C_{14}H_{22}N_6O_4$ ✧338.36).▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Related Compound D RS** [2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate] ($C_{15}H_{24}N_6O_4$ ✧352.39).▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Related Compound E RS** [2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-benzyloxy)carbonyl]-L-valinate] ($C_{20}H_{24}N_6O_6$ ✧ 444.44).▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Related Compound F RS** [2-hydroxyethyl -L-valinate] ($C_7H_{15}NO_3$ ✧161.20.▪1S (*USP33*)

Add the following:

▪**USP Valacyclovir Related Compound G RS** [*N*,*N*-dimethylpyridin-4-amine] ($C_7H_{10}N_2$ ✧122.17).▪1S (*USP33*)

Add the following:

▪**USP Ziprasidone Hydrochloride RS**.▪1S (*USP33*)

Add the following:

▪**USP Ziprasidone Related Compound A RS** [3-(piperazin-1-yl)benzo[*d*]isothiazole monohydrochloride] ($C_{11}H_{13}N_3S \cdot HCl$ ✧255.77).▪1S (*USP33*)

Add the following:

▪USP Ziprasidone Related Compound B RS [5-(2-(4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl)ethyl)-6-chloroindoline-2,3-dione**]** ($C_{21}H_{19}ClN_4O_2S$ ✧426.92).▪1S (*USP33*)

Add the following:

▪USP Ziprasidone Related Compound C RS [5,5′-bis(2-(4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl)ethyl)-6,6′-dichloro-3-hydroxy-3,3′-biindoline-2,2′-dione**]** ($C_{42}H_{40}Cl_2N_8O_3S_2$ ✧839.85).▪1S (*USP33*)

Add the following:

▪USP Ziprasidone Related Compound D RS [3-(benzo[*d*]isothiazol-3-yl)-5-(2-(4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl)ethyl)-6-chloroindolin-2-one**]** ($C_{28}H_{24}ClN_5OS_2$ ✧546.11).▪1S (*USP33*)

OTHER TESTS AND ASSAYS

BRIEFING

〈**381**〉 **Elastomeric Closures for Injections ,** *USP 32* page 145. On the basis of comments received and discussion by the Parenteral Products—Industrial Expert Committee, it is proposed to make editorial changes to clarify the *Introduction* section.

(PPI: D. Hunt) RTS—C70911

Change to read:

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in the general test chapter *Injections* 〈1〉 are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

•This chapter applies to closures used for long-term storage of preparations defined in the general test chapter *Injections* 〈1〉. Such closures are typically used as part of a vial, bottle, or pre-fill syringe package system.• (RB 1-May-2009)

This chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer; however, it does apply to closures treated with silicone (e.g., Dimethicone, *NF*). When performing the tests in this chapter, it is not required that closures be treated with silicone, although there is no restriction prohibiting the use of siliconized closures.

•This chapter also applies to closures coated with other lubricious materials (e.g., materials chemically or mechanically bonded to the closure) that are not intended to, and in fact do not provide, a barrier to the base elastomer. When performing the tests, closures with lubricious nonbarrier coatings are to be tested in their coated state.

The following comments relate solely to closures laminated or coated with materials intended to provide, or in fact function as, a barrier to the base elastomer (e.g., PTFE or lacquer coatings). It is not permissible to use a barrier material in an attempt to change a closure that does not meet compendial requirements to one that does conform. Therefore, all *Physicochemical Tests* apply to the base formula of such closures, as well as to the coated or laminated closure. To obtain *Physicochemical Tests* results, the tests are to be performed on uncoated or nonlaminated closures of the same elastomeric compound, as well as to the laminated or coated closure. The *Functionality Tests* apply to and are to be performed using the laminated or coated elastomeric closure. *Biological Tests* apply to the lamination or coating material, as well as to the base formula. *Biological Tests* may be performed on the laminated or coated closure, or they may be performed on the laminate/coating material and the uncoated or nonlaminated closures of the same elastomeric compound. In the latter case, the results are to be reported separately. The base formula used for physicochemical or biological tests intended to support the compendial compliance of a barrier-coated closure should be similar to the corresponding coated closure in configuration and size.

For all *Elastomeric Closures for Injection* 〈381〉 tests performed on any closure type, it is important to document the closure being tested, including a full description of the elastomer, and any lubrication, coating, laminations, or treatments applied.• (RB 1-May-2009)

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are those used for aqueous preparations. Type II closures are typically intended for nonaqueous preparations and are those which, having properties optimized for special uses, may not meet all requirements listed for Type I closures because of physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements, but still meets the Type II requirements for the test(s), the closure is assigned a final classification of Type II.

▪All elastomeric closures suitable for use with injectable preparations must comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.▪1S (*USP33*)

~~This chapter is intended as an initial screen to identify elastomeric closures that might be appropriate for use with injectable preparations on the basis of their biological compatibility~~

▪It is appropriate to use this chapter when identifying elastomeric closures that might be acceptable for use with injectable preparations on the basis of their biological reactivity,▪1S (*USP33*)
their aqueous extract physicochemical properties, and their functionality. ~~All elastomeric closures suitable for use with injectable preparations comply with either Type I or Type II test~~

In-Process Revision

~~limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.~~

■1S (*USP33*)
The following closure evaluation requirements are beyond the scope of this chapter:
— The establishment of closure identification tests and specifications
— The verification of closure–product physicochemical compatibility
— The identification and safety determination of closure leachables found in the packaged product
— The verification of packaged product closure functionality under actual storage and use conditions

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, compatibility testing must be repeated, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and sterilized prior to use in packaging injectable products.

Physical Tests and Determinations

BRIEFING

⟨725⟩ Topical and Transdermal Drug Products—Product Performance Tests. Two new *USP* general chapters on Topical and Transdermal Drug Products address the quality and performance aspects of topical dermal pharmaceutical dosage forms. The general chapters are *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩, which covers the basic quality control tests for these dosage forms and *Topical and Transdermal Drug Products—Product Performance Tests* ⟨725⟩, which covers the apparatus and procedures to be used to evaluate the in vitro drug release. The HPLC procedure for the hydrocortisone analysis was validated using a Symmetry C18 brand of packing L1. Comments and suggestions regarding these two general chapters should be sent to Margareth Marques at MRM@usp.org no later than July 31, 2009. USP is planning a workshop on these general chapters and the comments and suggestions received.

(BPC: M. Marques.) RTS—C71037

Add the following:

■⟨725⟩ TOPICAL AND TRANSDERMAL DRUG PRODUCTS—PRODUCT PERFORMANCE TESTS

i. **Introduction**
ii. **Performance Test for Topical Drug Products—In Vitro Drug Release Using the Vertical Diffusion Cell**
  a. **Apparatus**
  b. **Calculation of Release Rate (Flux) and Amount of Drug Released**
  c. **Performance Verification Test Method for USP Hydrocortisone Cream Reference Standard Product**
iii. **Product Test for Transdermal Drug Products**
  a. **Apparatus 5—Paddle over Disk Method**
  b. **Apparatus 6—Rotating Cylinder Method**
  c. **Apparatus 7—Reciprocating Holder Method**
iv. **Product Quality Tests**

I. INTRODUCTION

A performance test for topical drug products must be able to measure drug release from the finished dosage form. It must be reproducible and reliable, and although it is not a measure of bioavailability, the performance test must be capable of detecting changes in the finished product's drug release characteristics that have the potential to alter the biological performance of the drug in the dosage form. This product performance test is provided to determine compliance with drug release requirements where specified in individual monographs.

II. PERFORMANCE TEST FOR TOPICAL (SEMISOLID) DRUG PRODUCTS—IN VITRO DRUG RELEASE USING THE VERTICAL DIFFUSION CELL

The vertical diffusion cell (VDC) system is a simple, reliable, and reproducible means of measuring drug release from semisolid (cream, ointment, and gel) dosage forms. Typically, 200–400 mg of a cream, ointment, or gel is spread evenly over a suitable synthetic, inert support membrane. The membrane, with its application side up, is placed in a VDC (typically a 15-mm diameter orifice), e.g., a Franz cell. Diffusive communication between the delivery system and the reservoir takes place through an inert, highly permeable support membrane. The membrane keeps the product and the receptor medium separate and distinct. Membranes are chosen to offer the least possible diffusional resistance and not to be rate controlling. The release rate experiment is carried out at $32 \pm 1^{\circ}$, except in the case of vaginal creams when the temperature should be $37 \pm 1^{\circ}$. Sampling generally is performed during 4–5 h, and the volume withdrawn is replaced with fresh receptor medium. To achieve sink condition, the receptor medium must have a high capacity to dissolve or carry away the drug, and the receptor media should not exceed 10% of the concentration of the standard at the end of the test. The test is done with groups of six cells. Results from 12 cells—two runs of 6 cells—are used to document the release rate.

II. a. Apparatus

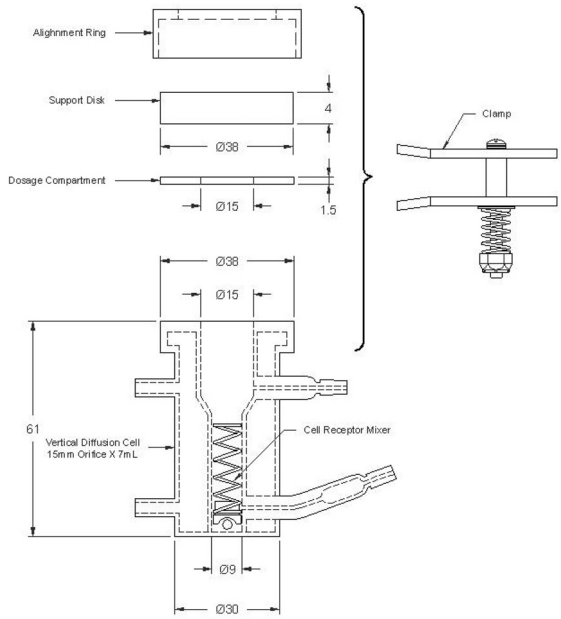


Fig. 1. Vertical Diffusion Cell.
(All measurements are expressed in mm unless noted otherwise.)

The VDC assembly consists of two chambers (a donor chamber and a receptor chamber), separated by a donor compartment and held together by a clamp (see *Figure 1*). This type of cell is commonly used for testing the in vitro release rate of topical drug products such as creams, gels, and ointments. Alternative diffusion cells that conform to the same general design and size can be used.

The VDC body normally is made from borosilicate glass, although different materials may be used to manufacture the body and other parts of the VDC assembly. None of the materials should react with or absorb the test product or samples.

In the donor compartment, the semisolid dosage form sample sits on a synthetic membrane within the cavity of the dosage compartment that is covered with a glass disk.

The diameters of the orifices of the donor chamber and the dosage compartment, which defines the dosage delivery area for the test, should be sized within ±5% of the specified diameter. The receptor chamber orifice should never be smaller than the orifice of the donor chamber and should be fabricated to the same size as the donor chamber orifice. The design of the VDC should facilitate proper alignment of the dosage compartment and receptor orifices.

The thickness of the dosage compartment normally is 1.5 mm. This thickness should be sized within ± 10% of the specified thickness.

The cell body should be manufactured consistently, with uniform height and geometry. Cells should appear the same, and their internal receptor volumes should fall within ±5% of their specified volume.

Volume—Before conducting testing, determine the true volume of each receptor chamber in the VDC. The volume of each VDC should be determined with the internal stirring device in place.

Temperature—The temperature of the receptor media during the test should remain within ±1.0° of the target temperature (typically 32°).

Speed—The rotational speed tolerance is ±10% from the target speed (normally 600–800 rpm). The speed selected should ensure adequate mixing of the receptor media during the test.

Sampling Time—Samples should be taken at the specified times within a tolerance of ±2% or ±2 minutes, whichever is greatest.

Qualification—The qualification of the apparatus is demonstrated by verifying the test temperature and speed requirements are met, along with a performance verification test (PVT). The PVT is passed if two tests of six cells comply with FDA's requirements of a 90% confidence interval (see FDA *Guidance for Industry: Nonsterile Semisolid Dosage Forms; Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation,* May 1997, available at www.fda.gov/cder.guidance/1447fnl.pdf). The PVT is performed by one analyst testing the specified reference standard in duplicate. The first test with six cells is performed and is defined as the reference. The second test of six cells is defined as the test. The PVT is passed if the second test passes the 90% confidence interval with reference to the first reference test.

Procedure—Unless otherwise specified in the individual monograph, degas the medium using an appropriate technique. With the stirring device in place, fill the VDC with the specified media and allow time for it to come to a temperature of 32°. If necessary, saturate the membrane in the specified media (generally receptor media) for 30 minutes. Place the membrane on the dosage compartment and invert. Apply the material that will be tested into the cavity of the dosage compartment, and spread the material out to fill the entire cavity of the dosage compartment.

Assemble each of the prepared dosage compartments to each VDC with the membrane down and in contact with the receptor media. During this application it is important to ensure that there are no bubbles under the membrane. When all dosage compartments and the remaining components are in place, turn on the stirring device, which constitutes time zero.

Follow the specified sampling procedure, and collect an aliquot from each VDC for analysis. Ensure that during the sampling process bubbles are not introduced into the cell. Exercise care during sampling and replenishment of the medium in order not to introduce bubbles.

With some cells it is acceptable to have up to three bubbles under the membrane if the bubbles are less than 1 mm in diameter. With some cells, bubbles may be removed from the receptor chamber during the test by tipping the cell as long as this process is required only one time per position.

II. b. Calculation of Rate (Flux) and Amount of Drug Released

Creams and ointments are considered extended-release preparations. Their drug release largely depends on the formulation and method of preparation. The release rate of a given drug product from different manufacturers is likely to be different. It is assumed that the drug release of the product is linked to the clinical batch.

Unless otherwise specified in the individual monograph, the release requirements are met if the following have been achieved.

The amount released (μg/cm²) at a given time (t_1, t_2, etc.) is calculated for each sample as follows:

$$\text{Amount Released } t_1 = A_U / A_S \cdot C_S \cdot 1{,}000 \cdot V_C / A_O$$

$$\text{Amount Released } t_2 = A_U / A_S \cdot C_S \cdot 1{,}000 \cdot V_C / A_O + (AR_{t1} \cdot V_S / V_C)$$

where

A_U = area of the current sample

$$\text{Amount Released } t_n = \frac{A_U}{A_S} \cdot C_S \cdot 1000 \cdot \frac{V_C}{A_O} + \sum_{i=1}^{n-1} \left(AR_{t_{n-1}} \cdot \frac{V_S}{V_C} \right)$$

A_S = average area of the standard

C_S = concentration of the standard (mg/mL)

V_C = volume of the diffusion cell (mL)

A_O = area of the orifice (cm²)

AR = amount released (μg/cm²)

V_S = volume of the sample aliquot (mL).

For each cell the individual amount released is plotted vs time, and the slope of the resulting line (rate of drug release, flux) is determined. The average of 6 + 6 slopes represents the drug release of the dosage form and serves as the standard for the drug product.

II. c. Performance Verification Test of VDC Systems using the USP Hydrocortisone Cream Reference Standard

Materials and Equipment—USP Hydrocortisone Cream RS; 25-mm, 0.45-μm hydrophilic polysulfone membrane filters (*1*); vacuum filtration apparatus consisting of a filter holder with a medium or fine-porosity sintered glass holder base, funnel with a 250-mL capacity, and magnetic stirrer; small and smooth jeweler's forceps; depression porcelain color plate; diffusion cell system with six diffusion cells and temperature control circulator; and sampling syringe or device and collection vials.

Procedure

Receptor Medium—Mix 60 mL of Alcohol with 140 mL of water to prepare a 30% alcoholic media. Degas the media by filtering through a 47-mm, 0.45-μm membrane by vacuum filtration. Assemble the filtration apparatus placing a magnetic stir bar (approximately 1 in. × 0.25 in.) in the receiving flask. Place the apparatus on a magnetic stirring plate, and spin the bar at a moderate rate. Apply vacuum and pass the media through the filter while stirring. After all media have passed through the filter, continue stirring while maintaining a vacuum for 2 minutes. Applying vacuum and stirring beyond 2 minutes may change the composition of the water-alcohol media. Care should be taken to ensure that the period of time that the media is under vacuum after the filtration is complete is limited to 2 minutes.

Immediately transfer the degassed receptor medium to a suitable receptor medium bottle and stopper. Place the receptor medium bottle in the jacketed beaker and allow the media to equilibrate for 30 minutes before use.

Preparation of Apparatus

Set the circulating bath to a temperature (typically 32.5°) that will maintain the temperature in the diffusion cells at 32° during the test. Place the appropriate magnetic stirrer in each diffusion cell. Allow the system to equilibrate for at least 30 minutes before beginning the test.

Membrane Preparation

Thirty minutes before use, prepare at least seven membranes. Using a Pasteur pipette, apply receptor medium to the surface of each membrane until covered. Allow the membranes to equilibrate for 30 minutes to saturate the membranes.

Sample Preparation

Carefully lift one membrane at its very edge with jeweler's forceps. Place the membrane on a paper tissue and blot any extreme excess solution (a slight excess solution is desired). Carefully place the membrane in the center of the dosage compartment. Place the dosage compartment, with the membrane centered on the underside, onto a tissue and press down on the compartment. Apply an appropriate amount of USP Hydrocortisone Cream RS (between 200 and 400 mg) on top of the membrane and inside of the dosage compartment cavity. Use a spatula to carefully smooth the material over the membrane, filling the entire cavity of the dosage compartment. Wipe any excess material from the surface of the dosage compartment. Repeat for a total of six sample preparations.

Performing the Test

Fill the diffusion cells with receptor media, and allow time to equilibrate to 32°. Ensure that the stirrers are not rotating and that there is a positive meniscus covering the complete top of each diffusion cell. Place the glass disk on top of the dosage compartment against the sample. Place the dosage compartment/glass disk assembly on the top of the diffusion cell, avoiding bubbles. Inspect under the membrane for bubbles. Assemble the cell. Repeat for each cell. Begin the test according to the following test parameters: temperature: 32°; stir speed: 600–800 rpm; total test time: 6 hours; sampling times: 1, 2, 3, 4, and 6 hours.

Sampling Procedure

At each of the stated sampling times, collect a sample from each cell as follows: stop the stirrer 30 seconds before sampling. Repeat sampling procedure for each cell in order from 1 to 6. Replace the medium. After the sixth cell has been sampled, resume the stirrer rotation.

High-Performance Liquid Chromatography (HPLC) Hydrocortisone Analysis

USP Hydrocortisone RS; acetonitrile; water; alcohol, 95%; 47-mm, 0.45-μm polysulfone membrane filters; 50 mm × 3.9 mm, 5-μm packing L1.

Procedure

Mobile Phase Preparation

Prepare and degas a sufficient volume of mobile phase to complete the analysis of the samples collected. For each 1 L of mobile phase mix 200 mL of acetonitrile with

800 mL of water. If necessary, adjust the mobile phase composition to achieve an approximate retention time of 7 minutes for the hydrocortisone peak.

Standard Preparation

Prepare a stock standard solution at a concentration of approximately 0.20 mg/mL of USP Hydrocortisone RS in alcohol. For example, a solution of 20 mg of hydrocortisone in 100 mL of alcohol is suggested. Prepare a working standard solution by making a 5-fold dilution of the stock standard in a solution of 30 : 70 alcohol:water mixture. For example, dilute 2 mL to 10 mL.

Chromatographic Conditions

Wavelength—242 nm; flow rate: 1 mL/min; injection volume: 10 μL; run time: 10 minutes; column: 5 cm × 3.9 mm, 5-μm packing L1; mobile phase: 20 : 80 acetonitrile:water. Begin the analysis by making five replicate injections of the working hydrocortisone standard solution for system suitability.

System Suitability Requirements—Relative standard deviation: < 2%; tailing factor: NMT 1.5. Make single injections of each of the samples obtained during the in vitro release testing. Bracket injections of samples with single standard injections after the analysis of the 2-, 4-, and 6-hour samples. Calculate the results as specified.

III. PERFORMANCE TESTS FOR TRANSDERMAL DRUG PRODUCTS

As with topical drug products, a performance test for transdermal drug products also must have the ability to measure drug release from the finished dosage form, must be reproducible and reliable, and must be capable of detecting changes in the finished product's drug release characteristics that have the potential to alter the desired pharmacologic effect(s) of the active ingredient. Such changes could be related to active or inactive/inert ingredients in the formulation or physical dosage form, physical or chemical attributes of the finished preparation, manufacturing variables, shipping and storage, age, and other characteristics that are critical to quality.

When based on sound scientific principles, product performance tests can be used for pre- and postmanufacturing purposes such as during the product research and development phase, basic quality control, demonstration of product similarity, or demonstration of compliance with FDA Guidelines (e.g., approval and postapproval changes in the dosage form).

In vitro drug release testing of transdermal patches can be carried out using USP Apparatus 5, Apparatus 6, or Apparatus 7. In general, researchers have found that Apparatus 5, the modified paddle method, is simpler and is applicable to most types, sizes, and shapes of transdermal delivery systems. At this time, no PVT Reference Standard exists for Apparatus 5, 6, or 7.

III. a. Apparatus 5 (Paddle Over Disk Method)

Apparatus—Use the paddle and vessel assembly from *Apparatus 2* as described in *Dissolution* ⟨711⟩, with the addition of a stainless steel disk assembly (*2*) designed for holding the transdermal system at the bottom of the vessel. Other appropriate devices may be used, provided they do not absorb, react with, or interfere with the specimen being tested (*3*). The temperature should be maintained at 32 ± 0.5°. During the test maintain a distance of 25 ± 2 mm between the paddle blade and the surface of the disk assembly. The vessel may be covered during the test to minimize evaporation. The disk assembly for holding the transdermal system is designed to minimize any dead volume between the disk assembly and the bottom of the vessel. The disk assembly holds the

system flat and is positioned so that the release surface is parallel with the bottom of the paddle blade (see *Figure 2*).

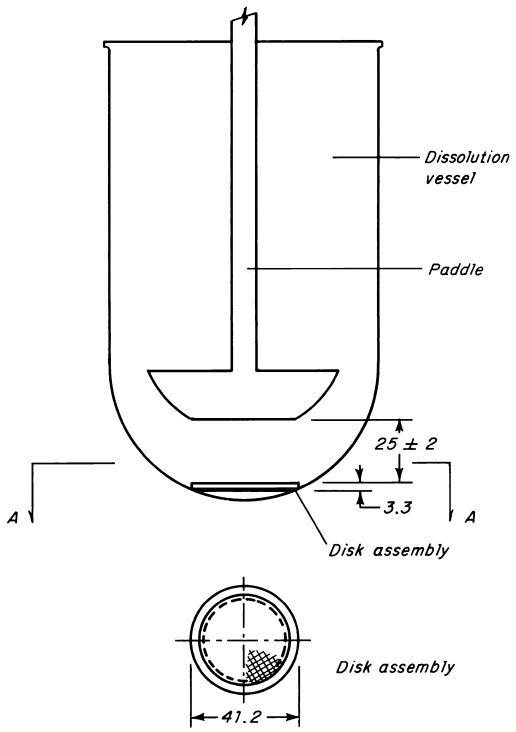


Fig. 2. Paddle over Disk.
(All measurements are expressed in mm unless noted otherwise.)

Performance Verification Test and Dissolution Medium—Proceed as directed for *Apparatus 2* in *Dissolution* ⟨711⟩.

Procedure—Place the stated volume of the *Dissolution Medium* in the vessel, assemble the apparatus without the disk assembly, and equilibrate the medium to 32 ± 0.5°. Apply the transdermal system to the disk assembly, ensuring that the release surface of the system is as flat as possible. The system may be attached to the disk by applying a suitable adhesive to the disk assembly. Dry for 1 minute. Press the system, release surface side up, onto the adhesive-coated side of the disk assembly. If a membrane[3] is used to support the system, it should be applied in such a way that no air bubbles occur between the membrane and the release surface. Place the disk assembly flat at the bottom of the vessel with the release surface facing up and parallel to the edge of the paddle blade and surface of the *Dissolution Medium*. The bottom edge of the paddle should be 25 ± 2 mm from the surface of the disk assembly. Immediately start operation of the apparatus at the rate specified in the monograph. At each sampling time interval, withdraw a specimen from a zone midway between the surface of the *Dissolution Medium* and the top of the blade, not less than 1 cm from the vessel wall. Perform the analysis on each sampled aliquot as directed in the individual monograph, correcting for any volume losses, as necessary. Repeat the test with additional transdermal systems.

Sampling Time—The test time points, generally three, are expressed in hours. Specimens should be withdrawn within a tolerance of ±15 minutes or ±2% of the stated time; select the tolerance that results in the narrowest time interval.

In Vitro Release Criteria—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Acceptance Table 1

Level	Number Tested	Criteria
L_1	6	No individual value lies outside the stated range.
L_2	6	The average value of the 12 units ($L_1 + L_2$) lies within the stated range. No individual value is outside the stated range by more than 10% of the average of the stated range.
L_3	12	The average value of the 24 units ($L_1 + L_2 + L_3$) lies within the stated range. Not more than 2 of the 24 units are outside the stated range by more than 10% of the average of the stated range, and none of the units is outside the stated range by more than 20% of the average of the stated range.

III. b. Apparatus *6* (Rotating Cylinder Method)

Apparatus—Use the vessel assembly from *Apparatus 1* as described in *Dissolution* ⟨711⟩, but replace the basket and shaft with a stainless steel cylinder stirring element and maintain the temperature at 32 ± 0.5° during the test. The shaft and cylinder components of the stirring element are fabricated from stainless steel to the specifications shown in *Figure 3*. The dosage unit is placed on the cylinder at the beginning of each test. The distance between the inside bottom of the vessel and the cylinder is maintained at 25 ± 2 mm during the test.

Dissolution Medium—Use the medium specified in the individual monograph (see *Dissolution* ⟨711⟩).

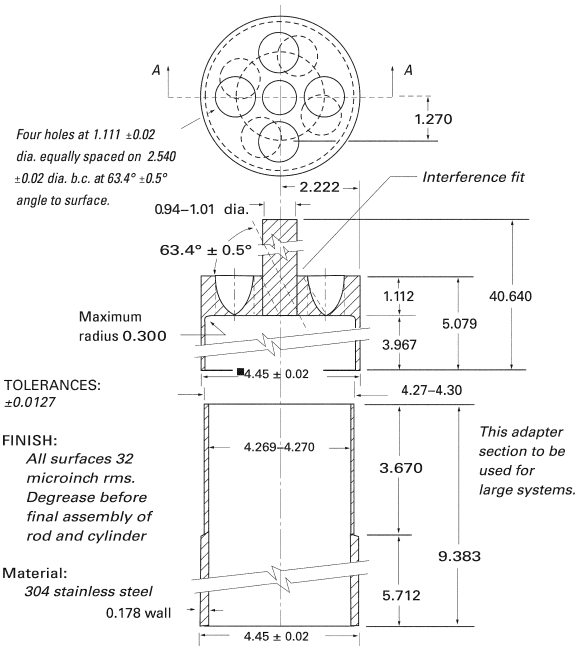


Fig. 3. Cylinder Stirring Element (*5*).
(All measurements are expressed in cm unless noted otherwise.)

Procedure—Place the stated volume of the *Dissolution Medium* in the vessel of the apparatus specified in the individual monograph, assemble the apparatus, and equilibrate the *Dissolution Medium* to 32 ± 0.5°. Unless otherwise directed in the individual monograph, prepare the test system before the test as follows: remove

In-Process Revision

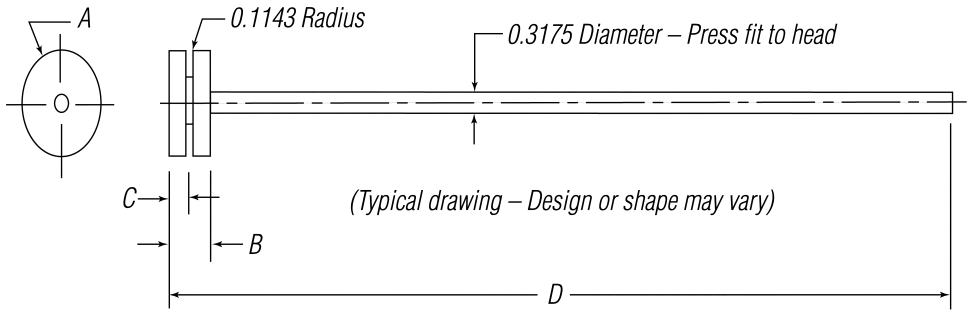
the protective liner from the system, and place the adhesive side on a piece of Cuprophan (*4*) membrane that is not less than 1 cm larger on all sides than the system. Place the system, Cuprophan-covered side down, on a clean surface, and apply a suitable adhesive (*6*) to the exposed Cuprophan borders. If necessary, apply additional adhesive to the back of the system. Dry for 1 minute. Carefully apply the adhesive-coated side of the system to the exterior of the cylinder so that the long axis of the system fits around the circumference of the cylinder. Press the Cuprophan covering to remove trapped air bubbles. Place the cylinder in the apparatus, and immediately rotate at the rate specified in the individual monograph. Within the time interval specified, or at each of the times stated, withdraw a quantity of *Dissolution Medium* for analysis from a zone midway between the surface of the *Dissolution Medium* and the top of the rotating cylinder, not less than 1 cm from the vessel wall. Perform the analysis as directed in the individual monograph, correcting for any volume losses as necessary. Repeat the test with additional transdermal drug delivery systems.

Sampling Time—Proceed as directed for *Apparatus 5 (Paddle Over Disk Method)*.

In Vitro Release Criteria—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

III. c. Apparatus 7 (Reciprocating Holder Method)

Apparatus—The assembly consists of a set of volumetrically calibrated or tared solution containers made of glass or other suitable inert material, a motor and drive assembly to reciprocate the system vertically and to index the system horizontally to a different row of vessels automatically if desired, and a set of suitable sample holders (see *Figure 4* (*7*) and *Figures 5a* and *5b*). The solution containers are partially immersed in a suitable water bath of any convenient size that permits maintaining the temperature, *T*, inside the containers at $32 \pm 0.5^\circ$ or within the allowable range, as specified in the individual monograph, during the test. No part of the assembly, including the environment in which the assembly is placed, should contribute motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating sample holder. An apparatus that permits observation of the system and holder during the test is preferable. Use the size container and sample holder specified in the individual monograph.



Dimensions are in centimeters

	HEAD				ROD		O-RING
System[a]	*A (Diameter)*	*B*	*C*	Material[b]	*D*	Material[c]	*(not shown)*
$1.6cm^2$	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-113-V884-75
$2.5cm^2$	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-016-V884-75
$5cm^2$	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2-022-V884-75
$7cm^2$	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2-124-V884-75
$10cm^2$	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2-225-V884-75

[a] Typical system sizes.
[b] SS/VT=Either stainless steel or virgin Teflon.
[c] SS/P=Either stainless steel or Plexiglas.

Fig. 4. Reciprocating Disk Sample Holder (*7*).

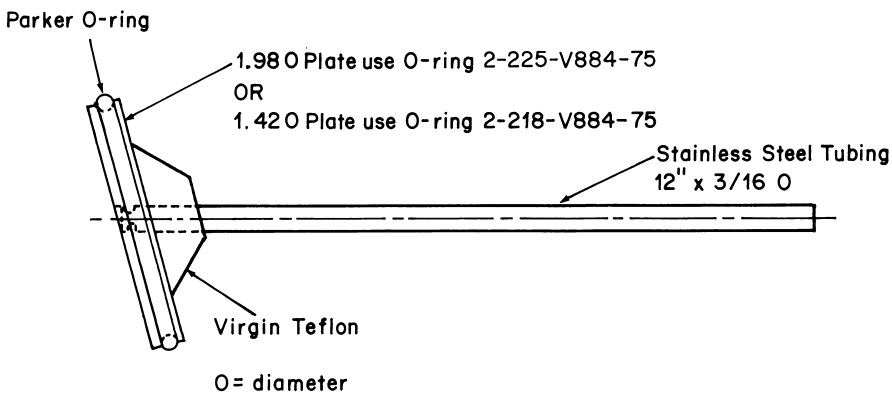


Fig. 5a. Transdermal System Holder—Angled Disk.

In-Process Revision

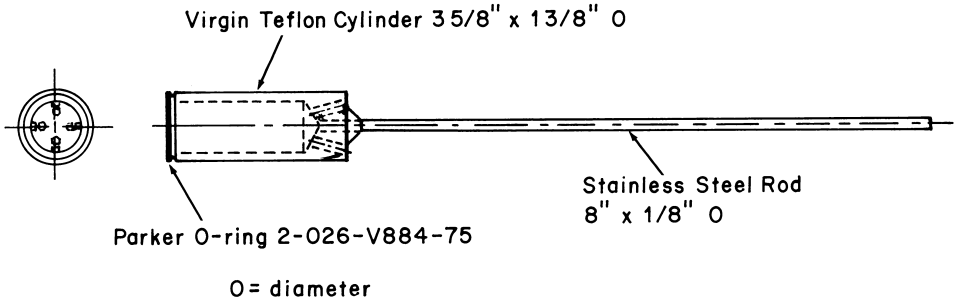


Fig. 5b. Transdermal System Holder—Cylinder

Preparation A—Attach the system to be tested to a suitable sample holder with 2-cyano acrylate glue.

Preparation B—Press the system onto a dry, unused piece of Cuprophan (*4*), nylon netting, or equivalent with the adhesive side against the selected substrate, taking care to eliminate air bubbles between the substrate and the release surface. Attach the system to a suitably sized sample holder with a suitable O-ring so that the back of the system is adjacent to and centered on the bottom of the disk-shaped sample holder or centered around the circumference of the cylindrical-shaped sample holder. Trim the excess substrate with a sharp blade.

Preparation C—Attach the system to a suitable holder as described in the individual monograph.

Dissolution Medium—Use the *Dissolution Medium* specified in the individual monograph (see *Dissolution* ⟨711⟩).

Procedure—Suspend each sample holder from a vertically reciprocating shaker so that each system is continuously immersed in an accurately measured volume of *Dissolution Medium* within a calibrated container pre-equilibrated to temperature, *T*. Reciprocate at a frequency of about 30 cycles/minute with an amplitude of about 2 cm, or as specified in the individual monograph, for the specified time in the medium specified for each time point. Remove the solution containers from the bath, cool to room temperature, and add sufficient solution (water in most cases) to correct for evaporative losses. Perform the analysis as directed in the individual monograph. Repeat the test with additional drug delivery systems as required in the individual monograph.

Sampling Time—Proceed as directed for *Apparatus 5 (Paddle Over Disk Method)*.

In Vitro Release Criteria—Dug release should be measured at least at three time points: the first time point around 1 hour, second around 50% of total drug release, and third around 85% drug release. Unless otherwise specified in the individual monograph, the requirements are met if the quantities of the active ingredients released from the system conform to *Acceptance Table 1* in *Drug Release* ⟨724⟩ for transdermal drug delivery systems, or as specified in the individual monograph. Continue testing through the three levels unless the results conform at either L_1 or L_2.

IV. PRODUCT QUALITY TESTS

General product quality tests that are part of the compendial monograph such as identification, assay, content uniformity (uniformity of dosage units), impurities, pH, water content, and microbial limits; specific tests such as viscosity, tube (content) uniformity, and particle size for topical drug products; and adhesive and leak tests for transdermal drug products should be performed. For details of drug product quality tests for topical and transdermal dosage forms, see the general chapter *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩.

REFERENCES

1. Polysulfone membrane filter—possible supplier www.pall.com, marketed as Tuffryn filters.
2. Disk assembly (stainless support disk) may be obtained from www.millipore.com.
3. A suitable device is the watchglass-patch-polytef mesh sandwich assembly available as the Transdermal Sandwich from www.hansonresearch.com.
4. Use Cuprophan, Type 150 pm, 11 ± 0.5-μm thick, an inert, porous cellulosic material, that is available from www.medicell.co.uk or www.varianinc.com.
5. The cylinder stirring element is available from www.varianinc.com.
6. Use Dow Corning, MD7-4502 Silicone Adhesive 65% in ethyl acetate, or the equivalent.
7. The reciprocating disk sample holder may be purchased from www.varianinc.com.■1S (*USP33*)

BRIEFING

⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions, *USP 32* page 283, and page 341 of *PF* 34(2) [Mar.–Apr. 2008]. On the basis of comments received and discussions by the Parenteral Products—Industrial Expert Committee, the following changes to the section *Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method* are being proposed:

1. The requirement for degassing water by sonication is removed from the *Water* section.
2. The *System Suitability* section is revised to clarify its intent, as well to as to make it consistent with other USP particle-counting chapters.
3. The *Standard Preparation* section is revised to clarify the replicate requirements, the definition of acceptable error, and the frequency of testing.
4. The 25-μm standard sample testing requirement is being deleted from the *Standard Preparation* section.

(PPI: D. Hunt) RTS—C66264

Change to read:

METHOD II—MEASUREMENT OF LARGE GLOBULE CONTENT BY LIGHT OBSCURATION OR EXTINCTION METHOD

For determination of the extent of the large-diameter droplet tail (>5 μm) of lipid injectable emulsions, a light obscuration (LO) or light extinction (LE) method that employs a single-particle (globule) optical sizing (SPOS) technique is used. During application of the LE/SPOS technique, passage of a droplet through a thin optical sensing zone results in blockage of a portion of the incident light beam, causing a momentary decrease in the light intensity reaching the "extinction" detector. The magnitude of this decrease in the signal is ideally proportional to the cross-sectional area of the droplet (assumed smaller than the sensing zone thickness), i.e., to the square of the droplet diameter. During optimization of the LE/SPOS instrument for a given emulsion sample, a series of dilutions should be tested to achieve ~~an acceptably low coefficient of variation (i.e., <10%)~~

■consistency■1S (*USP33*)
between samples. The goal is to identify a standard range of dilutions that yield consistent data and are most applicable to the formulation tested. Ideally, when comparing different emulsions, the same approximate number of globules are sized each time, and once a standard is achieved, it should be incorporated into the routine sampling plan for validation testing. As long as the fat globule concentration is below the "coincidence limit" of the sensor (determined by the flow cell and optical design), only one globule at most will pass through the sensing zone at any given time, allowing it to be counted and accurately sized (with less than 1% coincidence events). Both the coincidence limit and the optimal flow rate must be known for the LE/SPOS sensor used. Furthermore, it is prudent to perform the large-diameter measurements at a reduced emulsion concentration such that the measurable droplet concentration ~~(i.e, >1.8 μm)~~

■at threshold of detection (e.g., >1.8 μm) to an upper limit (e.g., 50 μm)■1S (*USP33*)
is only approximately one-third of the nominal coincidence limit for the sensor used. The resulting single pulse heights are converted to droplet diameters using a standard calibration curve previously constructed from NIST-traceable monosized polystyrene microspheres of known diameters. For additional guidance in the use of the light obscuration methodology, see the general chapter *Particulate Matter in Injections* ⟨788⟩.

Apparatus—A suitable light obscuration instrument with or without the capability of automatic sample dilution and controlled by a personal computer (PC) is used for the measurement. The number- and volume-weighted particle size distribution data are reported, provided it is clearly stated which values are given and that the necessary parameter values required for all necessary calculations are also given.

Water—Pass distilled water through a filter having a 0.2-μm porosity~~, and degas by sonication,~~

■■1S (*USP33*)
or use *Sterile Water for Injection* stored in a glass container.

Standard Preparation—To a pre-established volume of *Water* add an appropriate amount of concentrated suspension, containing NIST-traceable polystyrene latex standard particles

In-Process Revision

or other suitable microspheres. Gently mix the fluids to achieve a homogeneous suspension. If the light obscuration instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe or Teflon sample line. Further dilution of the sample then occurs automatically to optimize the particle concentration for analysis. Alternatively, the sample would require greater manual dilution with water (typically by at least a factor of 10 over the first dilution). The resulting diluted sample is then instilled in an appropriate, clean container, such as a sterile Type I glass container, before being passed through the sensor. In either case the final particle concentration is caused to lie below the coincidence limit of the sensor. The sizing and counting accuracy of the light obscuration instrument should be obtained using ~~three different size standards of approximately 5 μm, 10 μm, and 25 μm (triplicate analyses per size). The corresponding results for the mean diameter should coincide with the expected values, within 10% acceptable error. In addition, the number of particle counts obtained per unit volume of diluted sample suspension should also agree, within 10% acceptable error, with the concentration values certified in the documentation provided with each NIST traceable size standard.~~

■two different size standards of approximately 5 μm and 10 μm (triplicate analyses per size). For the standards after system calibration, set the instrument threshold of detection at 1.8 μm, extended to an upper limit of 50 μm. The corresponding results for the mean diameter should coincide with the expected values, within 10% of the relative standard deviation and 90%–110% size accuracy. In addition, the number of particle counts obtained per mL should also agree within ± 10% with the concentration values certified in the documentation provided with each NIST-traceable size standard.■1S (*USP33*)

Test Preparation—To a pre-established volume of *Water* add an appropriate volume of sample from the lipid injectable emulsion

■(triplicate analyses per sample).■1S (*USP33*)
Gently mix the fluids to achieve a homogeneous suspension. The diluted emulsion will be slightly turbid in appearance. If the light obscuration instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe or nonreactive* Teflon sample line. Further dilution then occurs automatically to optimize the droplet/globule concentration for analysis. Alternatively, the sample would require greater manual dilution with water (typically by at least a factor of 10 over the first dilution). The resulting diluted sample is then instilled in an appropriate, clean container such as a sterile Type I glass container. In either case the final droplet/globule concentration is caused to lie below the coincidence limit of the sensor.

System Suitability— ~~Using the *Standard Preparation*, measure the number-weighted particle diameter and the corresponding standard deviation. The system is suitable once the sample has equilibrated and the results have stabilized and triplicate mean number-weighted particle diameter measurements are obtained within 10% of each other. The measured coefficient of variation (CV) for the number-weighted particle size distribution should not deviate by more than 25% from the CV value stipulated for the NIST traceable standard. The latter value is usually very small, assuming nearly uniform size standard particles. Therefore, in practice the measured CV value is usually considerably larger than this ideal value, being dictated instead by the resolution of the LE/SPOS sensor. The resolution of the sensor should be sufficiently good that the measured CV value does not exceed 15% of the mean diameter of the NIST traceable standard. A larger CV value indicates that the latex microspheres are not suitable as a standard because they either inherently lack uniformity or have become agglomerated to an unacceptable extent. In this case, another standard latex suspension must be selected and tested.~~

■Perform prior to the test procedure, using the *Standard Preparation* of a 5- and 10-μm NIST traceable particle. Measure in triplicate the number-weighted particle diameter and the counts/mL of the standard. The system is suitable when the triplicate mean number-weighted particle diameter measurements are within 10% of the target value, both in terms of repeatability (CV) and closeness to the certified size on the label of the NIST traceable standard.■1S (*USP33*)

Procedure and Interpretation—If the light obscuration instrument is equipped with an automatic dilution system, use a disposable syringe or Teflon sample line to load the *Standard Preparation* or *Test Preparation.* If no automatic dilution system is used, transfer the sample to an appropriate large-volume, clean container such as a sterile Type I glass vessel containing an appropriate volume of *Water.* Allow the sample and *Water* to mix thoroughly to achieve a homogeneous suspension. Set the instrument threshold of detection at 1.8 μm, extended to an upper limit of 50 μm, and ~~employ measurement times of 120, 180, and 240 seconds for each run of each replicate of the sample (*n* = 3 runs per sample).~~

■vary the concentration and/or data collection times such that there is at least a factor of two in the difference of the total number of globules that measure > 5 μm between at least two sample runs. In any case, the number of globules that measure > 5 μm should be large enough so that it represents an adequate number of globules that are statistically representative of the large-diameter tail population of the native emulsion.■1S (*USP33*)
~~As long as the three~~

~~■■1S (*USP32*)~~

~~measurements of the volume-weighted percentage of fat greater than 5 μm (PFAT5) for each sample fall within 10% of each other (irrespective of run-time), the results for the *Test Preparation* are acceptable. Values exceeding this reproducibility tolerance suggest either that the sample is unstable or that the dilution has not been optimized.~~

■■1S (*USP33*)

* Polyvinyl chloride (PVC) with diethylhexylphthalate (DEHP) has been shown to induce breakdown of lipid injectable emulsions (Drug Product Problem Reporting System. USP File Access No. 11173, May 15, 1991).

The volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 μm (PFAT5) for a given lipid injectable emulsion ~~must be less than~~

~~■are not to exceed■1S (USP32)~~

■must not exceed■1S (*USP33*) 0.05%.

BRIEFING

⟨**788**⟩ **Particulate Matter in Injections,** *USP 32* page 310. As a result of comments received and discussions by the Parenteral Products—Industrial Expert Committee, the following additions are being proposed:

1. Solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter In Injections* ⟨788⟩.
2. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter In Injections* ⟨788⟩.
3. Parenteral products for which the labeling specifies use of a final filter prior to administration are exempt from the requirements of *Particulate Matter In Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.
4. Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of *Particulate Matter In Injections* ⟨788⟩.

(PPI: D. Hunt.) RTS—C63370

Change to read:

This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia.* These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter. Portions of the present general chapter text that are national USP text, and therefore not part of the harmonized text, are marked with symbols (◆◆) to specify this fact.

Particulate matter in injections and parenteral infusions consists of mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

■◆As stated in *Injections* ⟨1⟩, solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenteral products for which the labeling specifies use of a final filter prior to administration are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.◆■1S (*USP33*)

For the determination of particulate matter, two procedures, *Method 1 (Light Obscuration Particle Count Test)* and *Method 2 (Microscopic Particle Count Test),* are specified hereinafter. When examining injections and parenteral infusions for subvisible particles, *Method 1* is preferably applied. However, it may be necessary to test some preparations by the *Light Obscuration Particle Count Test* followed by the *Microscopic Particle Count Test* to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for subvisible particles by one or both of these methods. When *Method 1* is not applicable, e.g., in the case of preparations having reduced clarity or increased viscosity, the test should be carried out according to *Method 2*. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units.

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1024** ⟩ **Bovine Serum.** Because of extensive changes in the previously proposed chapter (see page 776 of *PF* 34(3) [May–June 2008]), that proposal is canceled, and a new draft is proposed for this general information chapter.

(BB CGT: F. Atouf.) RTS—C69217

In-Process Revision

Add the following:

■⟨1024⟩ BOVINE SERUM

INTRODUCTION

Bovine serum is the liquid fraction of clotted blood, obtained from an ox (*Bos taurus,* among others), that has been depleted of cells, fibrin, and clotting factors. Since the advent of modern cell culture, manufacturers of biological products have used bovine serum extensively as a cell culture growth supplement. Its rich nutritional composition of proteins, growth factors, hormones, amino acids, vitamins, sugars, lipids, trace elements, and other components supports a broad range of cell culture applications in research and commercial manufacture of vaccines, natural source and recombinant biologics (hereafter biologics), engineered tissues, and other emerging cell-based therapeutic products intended for human or veterinary use. The predominant type of serum used in research applications is Fetal Bovine Serum (FBS). Calf serum (from newborn and older animals) is used much less frequently, but because of its lower cost it may be widely used in commercial manufacturing.

As is the case with other animal-derived products, bovine serum carries a potential risk of introducing extraneous agents into cell culture. Serum manufacturers and regulators must adopt rigorous sourcing and testing procedures and strict processing and production guidelines to ensure the quality of bovine serum.

The objective of increasing the quality and safety of biologics produced with bovine serum, coupled with attempts to mitigate regulatory burden, have caused developers to investigate alternatives to serum supplementation, resulting in application-specific serum-free medium formulations. Although it is recognized that bovine serum should be avoided when there is an option to use serum-free medium, there are cases where this is technically impossible or impractical.

This chapter describes issues related to sourcing, production, and characterization of bovine serum to ensure its safe use. A list of relevant regulatory and guidance documents is presented in *Appendix 1.* Serum manufacturers and serum end users (manufacturers of biological products) should consider and apply as needed the controls and procedures outlined in this chapter to ensure the safe use of bovine serum components in research and pharmaceutical manufacturing.

Types of Bovine Serum

- FBS is obtained from the fetuses of healthy, prepartum bovine dams that had been deemed fit for human consumption through ante- and postmortem inspection by licensed veterinarians. It is collected in government-inspected and -registered slaughterhouses.
- Newborn calf serum (also known as newborn bovine serum) is obtained in government-inspected and -registered slaughterhouses from animals aged less than 20 days.
- Calf serum is obtained in government-inspected and -registered slaughterhouses from animals aged between 20 days and 12 months.
- Donor bovine serum (also known as donor calf serum) is obtained by the repeated bleeding of donor animals from controlled government-inspected and -registered donor herds. The animals are 12–36 months old.
- Adult bovine serum is obtained in government inspected and -registered slaughterhouses from cattle older than 12 months that are declared fit for human consumption.

BOVINE SERUM: HISTORY AND TYPES OF USE

History of Bovine Serum Use

Animal serum and other complex biological materials have been employed in the cultivation of mammalian cells for approximately 100 years. Several factors led to the wide adoption of bovine serum as a standard tissue culture supplement. In comparison to serum from other animal species (horse, goat), bovine serum is easily sourced, and thereby more affordable. Many investigators choose to use fetal serum in their experimental systems because of concerns associated with antibodies present in newborn and adult serum that could cross-react with cells in culture and cause cell lysis through complement-mediated pathways. To eliminate that concern, heat was introduced to inactivate complement that was potentially present in the serum. Studies of FBS undertaken in the 1950s on the cultivation of low-density human cells to elucidate mechanisms of cell growth found that (1) the albumin component may serve as a carrier of essential small molecules; (2) fetuin, a glycoprotein present at high levels in the alpha globulin fraction, facilitates cell attachment and stretching; and (3) fetuin markedly inhibits trypsin, and this antiproteolytic activity may play a role in the ability of fetuin to stimulate cell growth.

In the 1960s and 1970s, serum supplementation of tissue culture media became the norm, thus facilitating biomedical research as well as the first large-scale vaccine manufacturing processes. Serum supplementation reduced the requirement for optimizing medium formulations for different cell types. FBS was shown to provide a variety of polypeptide growth factors. Albumin promoted cell growth presumably because of its abilities to function as a carrier protein for small molecules or lipids, to bind metal ions, to serve as a pH buffer, and to protect cells against shear. Similar functions were found for other serum components such as transferrin, hormones, and other serum-derived attachment factors such as fibronectin, vitronectin, and laminin.

Uses of Bovine Serum

Serum is a complex mixture of macromolecules that is required for cell growth and virus production, and its use as a raw material presents a number of challenges. These include its batch-to-batch composition and the risk of contamination by adventitious agents. The development of serum-free media has replaced serum in some new biotechnology manufacturing applications, but many cell lines used in manufacturing have not been adapted to these serum-free media. Regulatory constraints and scientific challenges generally make it impractical to alter existing manufacturing processes in which serum is used as a raw material.

FBS sometimes is required in cell and tissue bioprocessing, which often involves the cultivation of cells from tissue explants and biopsies. Some bioprocesses may also require the maintenance of specific cellular characteristics during cultivation. FBS often appears to facilitate such procedures and may affect the biological behavior of fastidious cell types. FBS has been shown to affect the transcription of developmentally important genes, apoptosis, and apoptosis-related gene expression, and to provide neuroprotective and antioxidative factors, all of which may be beneficial to the survival and development of cells in culture. Therefore, FBS will continue to play an important role as a cell culture supplement for production of cell- and tissue-based therapies.

In most viral vaccine manufacturing processes the media used for cell culture expansion and virus infection/production are supplemented with different types of serum at different concentrations. In these processes, bovine serum helps generate a mass of cells in an optimal physiological state for efficient viral replication.

BOVINE SERUM HARVESTING AND PRODUCTION

Blood Collection

For all types of bovine sera, blood should be collected in government-inspected and -registered premises (slaughterhouses, abattoirs, and donor farms). Blood should be collected by trained operators following the written procedures approved by the serum manufacturer and using either single-use disposable collection devices or reusable collection equipment for which cleaning procedures have been validated.

DONOR BOVINE SERUM

For each lot of serum from donor animals, serum manufacturers should ensure traceability to the donor herd of origin via production records and animal health and origin certificates. Donor animals are subjected to regular veterinary inspections and are bled multiple times following established procedures. Animals introduced into the herd should be traceable by source, breeding, and rearing history. Collectors should introduce new animals into the herd following specified and approved procedures that include prepurchase animal inspection and testing, proper transportation, a quarantine period, veterinary examination and testing during the quarantine period, and animal release criteria from quarantine to serum production. The collectors should not vaccinate donor animals for bovine viral diarrhea (BVD). Collectors should test animals for any agent and antibody from which the herd is claimed to be free.

NEWBORN CALF SERUM, CALF SERUM, AND ADULT BOVINE SERUM

Certificates of animal health and origin and/or serum production records should ensure that serum manufacturers can trace bovine serum derived from slaughtered animals back to the abattoir. Serum manufacturers should require abattoirs to maintain documentation of the origin of animals for slaughter. Blood should be collected from animals that have been slaughtered, for human consumption, in abattoirs inspected by the competent authority of the country of origin. Inspectors should routinely inspect animals both antemortem and postmortem to check for the clinical appearance of infections and parasitic diseases and other animal health-related problems or conditions. The animals must be free of clinical evidence of infectious diseases at the time of slaughter. Blood collection procedures must be in place to prevent cross-contamination with other tissues and body fluids and the surrounding environment. The standard procedure of slaughter consists of an approved method of animal stunning followed by exsanguination.

FETAL BOVINE SERUM

FBS product specifications and test procedures are presented in the proposed general chapter *Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩. Serum manufacturers should collect fetal bovine blood from bovine fetuses whose dams have been slaughtered. The dams must have been deemed fit for human consumption and must have been slaughtered in abattoirs that were inspected by the competent authority of the country of origin. Inspectors should examine all animals both antemortem and postmortem to check for the clinical appearance of infections and parasitic diseases and other animal health-related problems or conditions. The animals must be free of clinical evidence of infectious diseases at the time of slaughter. The uterus is removed and transported to a dedicated space for fetal bovine blood harvest, where blood collection personnel evaluate the fetus for signs of fetal death, including bloating, skin discoloration, odor, deformation, and hair sloughing. Collectors also should check the amniotic fluid for

color, quantity, and clarity. Serum manufacturers should collect blood from acceptable fetuses by cardiac puncture into a closed collection system using an aseptic technique. Manufacturers should have in place procedures that will prevent cross-contamination with other fetal tissues and bodily fluids and the surrounding environment.

Serum Harvesting and Processing

Trained personnel should perform serum separation (harvesting) and further processing activities following written and approved procedures. Serum is first separated and pooled, followed by filtration and filling into sterile containers. If the serum is subjected to one or more virus inactivation treatments in the production process, serum manufacturers should validate the virus inactivation processes against a range of relevant viruses. It is recommended that bovine viral diarrhea virus (BVDV) be included in any virus validation study because it is ubiquitous.

SERUM SEPARATION AND HARVESTING

Bovine blood should be processed and serum separated (harvested) in such a way as to minimize bacterial and mycoplasmal contamination, which in turn minimizes endotoxin levels in serum product. Gentle, quick blood processing helps to minimize hemolysis, further enhancing the quality of the serum product. After collection, blood is first allowed to clot for a specified period of time and under controlled conditions, then centrifuged in a refrigerated centrifuge. Serum is then removed from the clot, typically by centrifugation; pooled and mixed in a pooling vessel; transferred to labeled containers; and frozen, unless it is filter-sterilized immediately. Serum manufacturers should describe each process step and carry out serum processing activities, including sample collection and in-process quality control testing, following the manufacturer's approved procedures.

POOLING BEFORE FILTRATION

Because limited amounts of blood can be collected from individual animals, serum manufacturers pool the raw serum from many animals in order to create commercial-sized lots. Serum is pooled, after raw serum thawing and before filtration, in a pooling vessel and mixed at a controlled mixing rate and temperature. Pools or lots of donor bovine serum may consist of many separate collections from individual members of the herd. Lots of FBS may consist of pooled serum from thousands of animals. Serum manufacturers should describe each prefiltration pooling process step and should carry out serum thawing, prefiltration pooling, and mixing activities following the manufacturer's approved procedures.

FILTRATION

Pooled serum is mixed and sterile-filtered through filters of pore size 0.2 μm or smaller, depending on the intended application. Filtration processes should be validated. Triple filtration using filters of pore size 0.1 μm has been shown to result in a high degree of mycoplasma removal. Although filtration may remove some large viruses and viral aggregates from the serum, generally viruses cannot be completely eliminated in this manner. Furthermore, the filters are not known to eliminate the causative agent of bovine spongiform encephalopathy (BSE). Following filtration, serum manufacturers fill filtered serum into sterile containers by aseptic processing in a suitably controlled environment. Serum manufacturers should describe each filtration process step and should perform serum filtration, filling, and sample collection activities following the manufacturer's approved procedures.

IRRADIATION

Serum treatment by gamma irradiation is very common and one of the most effective methods of virus inactivation. The most frequently used minimum dose is 25 kilograys (kGy). Some countries specify higher dose requirements (>30 kGy) for imported serum. Gamma irradiation may inactivate viruses, mycoplasma, and bacteria, but serum end users should ensure that the gamma irradiation process does not negatively affect their specific applications. Irradiation may have adverse effects on serum quality, and these adverse effects tend to increase with higher doses.

Validation of gamma irradiation has two aspects: (1) dose delivery in a defined load configuration and (2) inactivation capacity. Critical irradiation process parameters include product (serum) temperature, packaging size and configuration, dosimeter distribution, and defined minimum/maximum dose received. Dosimeters should be used to monitor the established high-dose and low-dose positions in each irradiation run. If the serum manufacturer makes inactivation claims, these should be supported by the manufacturer's own well-designed viral inactivation studies.

ULTRAVIOLET (UV) TREATMENT

Serum manufacturers may use UV treatment to inactivate viruses, mycoplasma, and bacteria, but manufacturers must validate the process to demonstrate its efficacy. In addition, manufacturers must be aware that UV treatment may have an adverse effect on serum quality and accordingly should consider the effects of UV treatment for each application, as should serum end users.

HEAT INACTIVATION

Heat inactivation involves elevating the temperature of the serum to $>56^{\circ}$ for at least 30 minutes to inactivate complement. Heat inactivation may also inactivate viruses, mycoplasma, and bacteria; but it may have an adverse affect on serum quality, and manufacturers must validate the procedure's suitability for specific applications. Heat inactivation provides significantly less assurance of virus inactivation than does irradiation.

VIRAL CLEARANCE STUDIES

Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin ⟨1050⟩ and other regulatory documents give guidance about conducting viral clearance studies that help validate removal/inactivation processes. Serum manufacturers should also perform formal spiking studies with relevant and representative (model) viruses, and should test and compare inactivated spiked serum samples, negative controls, and positive controls.

CHARCOAL STRIPPING

Some serum manufacturers use charcoal/dextran treatment to reduce the levels of hormones in serum.

DIALYSIS

Some manufacturers use dialysis or dialfiltration to remove low molecular weight components from serum.

CLEANING AND STERILITY OF EQUIPMENT

Stainless steel systems and tubing used in the manufacture of bovine serum must be cleaned and sterilized to prevent cross-contamination and growth of adventitious agents. Serum manufacturers must validate their clean-

ing processes for removing and inactivating agents of concern. Thereafter, manufacturers should implement process controls that routinely verify cleaning cycles. Steam sterilization-in-place is a common and effective sterilization technique. Serum manufacturers that use this technology must validate steam cycles to demonstrate their uniformity and ability to destroy heat-resistant bacterial spores. Alternatively, manufacturers can use sterile disposable systems that do not require cleaning validation.

Quality Control

TRACEABILITY

Abattoir Collection

Materials collected in the U.S. should originate from U.S. Department of Agriculture (USDA)-registered facilities. Serum manufacturers should maintain documentation that traces a given serum sub-lot to the abattoir where it was collected. Slaughterhouses maintain records of animal source. General industry practice is to keep this information as part of the Device Master Record. General record-keeping requirements at USDA-licensed abattoir facilities are outlined in 9 Code of Federal Regulations (CFR) 320.

Materials collected from countries approved by the USDA for importation of bovine products into the U.S. should meet the requirements of the competent authority of the country of origin. In addition, serum manufacturers should keep USDA-required safety testing records of imported materials (if applicable) as part of their Device History Record.

Serum manufacturers should consult 9 CFR 309 and 9 CFR 310 about requirements for inspection of animals for various diseases pre- and postslaughter. These requirements are recommended for materials collected outside the U.S.

Donor Herd Collection

Serum manufacturers should maintain traceability to the donor animal farm where blood was collected from donor animals. In most cases, manufacturers individually identify farm animals and keep records for bleed and processing dates, making it possible to trace blood collection to an individual animal. A licensed veterinarian or a designee under the guidance of a veterinarian should inspect animals regularly and should certify that the animals are free of disease and fit for human consumption, consistent with 9 CFR 309.

PRODUCT STORAGE AND STABILITY

Serum should be stored in the frozen state at –10° or below. Serum is frozen as quickly as possible to preserve product quality and is stored under controlled storage conditions. Serum manufacturers should establish serum product stability in support of a proposed expiration date. Typical expiration dating for bovine serum is 5 years from the date of filtration and filling. Use of any type of bovine serum beyond the stated expiration date depends on the application, and the serum user must establish the product's continued suitability for use.

Labeling

Finished product labels must contain the following information: product description, lot number, storage conditions, name and address of manufacturer, and a statement indicating the intended use. Materials intended for research purposes are exempt from labeling regulations (21 CFR 801). Typically, serum manufacturers supply a lot-specific Certificate of Analysis (COA) that is classified as part of the product's labeling. See COA requirements in the following section.

Certification/Documentation

CERTIFICATE OF ANALYSIS

The COA should provide information about a specific lot of serum, including tests performed and test results (according to the serum manufacturer's specifications for release), as well as critical labeling identifiers such as lot number, catalog number, description of type of bovine serum, country of origin, and either or both dates of manufacture and expiration. This document is distinct from the certificate of health issued by the competent authority of the country of origin.

CERTIFICATE OF ORIGIN AND CERTIFICATION OF ANIMAL STATE OF HEALTH

The Certificate of Origin establishes the country in which the bovine blood was collected and veterinary certification of the health of the animals pre- and postcollection (9 CFR 327.4).

IMPORT/EXPORT DOCUMENTS

Import/export documents contain formal certification of animal disease status of the country of origin and negotiated/agreed certification statements. These vary from country to country. Each country defines import/export requirements in order to control introduction of exotic animal diseases and their economic impact as well as product safety assessments (risk vs. research, diagnostic, and/or therapeutic benefits).

PRODUCTION REPORTS

Production reports typically are batch records that document the raw materials in identifiable and traceable ways, production methods (centrifugation or filtration) used in manufacturing, equipment and facility cleaning, quality control testing, and personnel performing required activities. Raw material with Certificates of Origin or serum production records facilitates traceability to the source of the blood that was used to create the serum. When serum is used as a raw material for further manufacturing, process documentation also helps demonstrate controlled manufacture of the bovine serum.

BSE RISK ASSESSMENT

Despite the low risk potential of transmissible spongiform encephalopathies (TSEs) in bovine serum, various U.S. and international regulatory agencies have developed guidance to help manage and further reduce the potential risks of transmission. In the absence of appropriate test methods of detecting the infectious agent in fluids such as blood, the consensus recommendation from various regulatory agencies is to adopt good risk assessment strategies. This section of the chapter provides some background information on the disease and current methods of detection; it also highlights risk assessment and risk reduction strategies to potentially prevent transmission of the disease through the use of serum in the manufacturing of medicinal products.

Description of the Disease

TSEs are transmissible animal and human diseases that are characterized by degeneration of the brain, associated with severe neurological signs and symptoms. Since the outbreaks of TSE in cattle, termed BSE, which were transmitted to other species, public health officials have been concerned about the risk of TSE infection, including the possibility of TSE transmission by the use of therapeutic products manufactured using bovine serum. In cattle infected with BSE, lower titers have been found in the cerebrospinal fluid, lung, lymph tissue, spleen, kidney, liver, and ileum. Studies have shown that transfusion of blood from sheep infected with either BSE or scrapie but without evident disease can infect naive sheep. Although the

risk of cross-contamination is always present, to date no studies have shown that blood can transmit disease from cattle with BSE. Embryos from BSE-affected cattle have not transmitted diseases to mice. Calves born of dams that received embryos from BSE-affected cattle have survived for up to 7 years, and examination of the brains of both the unaffected dams and their offspring revealed no spongiform encephalopathy.

Detection Strategies

No currently available procedures have been validated as being sufficiently sensitive for routine antemortem screening of asymptomatic animals, although analytical methods are under development for detection and quantitation from low-infectivity materials such as blood. The classic diagnostic test for TSEs is postmortem histological examination of brain tissue to confirm characteristic vacuolar degeneration. Other testing options include immunohistochemical tests that can confirm the presence of PrPSc, the abnormal disease-specific conformation of prion-related protein (PrP), in the vacuolated regions of the brain; and immunochemical tests such as Western blots and enzyme-linked immunosorbent assays that can detect PrPSc in tissues with high or moderately high titers. These tests typically take less time to perform than histological examination (6–8 hours vs. weeks, respectively) and can be partially or fully automated. Although most of these are postmortem tests, studies have demonstrated the feasibility of premortem testing of lymphoid tissue samples from the tonsils or from the third eyelid of infected animals. Immunochemical tests require extensive sample collection and preparation and can be cost prohibitive for routine testing and monitoring the disease state of large herds. Diagnostic strategies must consider the sensitivity of testing in certain tissues as well as the test's ability to detect infectivity in animals before the development of clinical signs of disease. Negative results do not ensure the absence of infectivity. Detection of infectivity is possible if suspect tissue is inoculated into experimental animals intracranially where the causative agent can amplify. This approach for detection of low infectivity can take months to years to yield a positive result.

Risk Assessment and Risk Reduction Strategies

Serum manufacturers should employ risk reduction strategies to eliminate the danger of cross-contamination of fetal blood with other tissues, including appropriate sourcing of animal-derived articles and using practices that have been shown to eliminate or minimize the risk of transmitting TSE, via either foods or health care products. Serum end users should perform a risk assessment of their sourcing strategy that takes into account the amount of bovine serum used in their application and should conduct supplier audits to ensure traceability of sourcing, handling, and appropriate quality control systems.

SOURCE AND AGE OF ANIMALS

Serum manufacturers should monitor the traceability of each lot of serum to ensure the qualification of bovine serum, as described previously in the two sections *Serum Harvesting and Processing* and *Quality Control*. In addition to traceability, careful selection of source materials is the most important criterion for the safety of medicinal products. Certification of the origin must be available from the supplier, and manufacturers should keep this information on file. The U.S. Food and Drug Administration (FDA) recommendations prohibit the use in FDA-regulated products (except gelatin) of any bovine-derived materials that originate from countries that report indigenous cases of BSE. The current proposed rule qualifies FBS as an unlikely source of BSE infectious material, because current evidence suggests that cow-to-calf transmission of BSE is unlikely. The proposed rule also states that prohib-

ited cattle materials do not include materials sourced from fetal calves of cows that were inspected and passed, as long as the materials were obtained by procedures that can prevent contamination with specified risk materials. For veterinary biologics, current regulations enforced by the USDA's Center for Veterinary Biologics (CVB) indicate that ingredients of animal origin should be sourced from countries with no or low BSE risk, as defined by the U.S. National Center for Import and Export and 9 CFR 94.18.

The most satisfactory sources of materials are from countries with the following:

- No reported cases of indigenous BSE
- Compulsory notification of positive tests
- Compulsory clinical and laboratory verification of suspected cases
- Prohibition of the use in ruminant feed of meat and bone meal containing any ruminant protein
- No importation of cattle from countries where a high incidence of BSE has occurred
- No importation of progeny of affected females

BSE infectivity may increase with animal age. Although bovine serum is considered a low-risk material for TSE transmission, some end users consider it prudent to source serum from dams below a set maximum age. If manufacturers cannot determine the date of the dam's birth, they should consider both the implementation date of the feed ban in the country of origin and the incubation period of BSE in order to determine the safety of the source. A ruminant feed ban was imposed in the United Kingdom in July of 1988. These considerations are lot specific, so audits of the raw material supplier should include a review of records.

PRODUCTION PROCESS

End user manufacturing systems should be in place for monitoring the production process and for batch delineation (definition of batch, separation of batches, and cleaning between batches). Of primary importance is control of the potential for cross-contamination with possible infectious material. Because of the documented resistance of TSE agents to most inactivation procedures, controlled sourcing is the most important criterion in achieving acceptable product safety.

Whenever possible, manufacturers should identify steps that theoretically or demonstratively remove or inactivate agents during the manufacture of the material. Manufacturers should continue their investigations into removal and inactivation methods to identify steps/processes that will help ensure the removal or inactivation of TSE agents. Manufacturers should design production processes using available methods that have the greatest likelihood of inactivating or removing TSE agents. For example, prolonged exposure of tissues to high moist heat and high pH inactivates the BSE agent. Such treatments, however, are inappropriate for the extraction of many other types of bovine-derived articles, such as serum, because these treatments lead to the destruction of the material. Conventional chemical and biochemical extraction and isolation procedures may be sufficient to remove the infectious agent. Similar techniques may be effective for other bovine-derived articles. Further research will help to develop an understanding of the most appropriate methodology for validation studies. Issues to consider during validation of a process for removal of TSE agents include the following:

- The nature of the spiked material and its relevance to the natural situation
- Design of the study (including scale-down approaches)

- Method of detecting the agent (in vitro or in vivo assay) after spiking and after the treatment
- Characterization and standardization of reference materials for spiking
- Data treatment and analysis (see *Design and Analysis of Biological Assays* ⟨111⟩)

Because no studies have successfully validated analytical methods for the detection of small amounts of the TSE agent, TSE clearance validation studies typically employ the intracranial injection of in-process material into rodents for amplification and detection of potential residual infectivity.

TESTING AND CONTROL OF ADVENTITIOUS AGENTS

Introduction

Rigorous testing procedures, strict processing and production guidelines, and appropriate risk assessments help ensure the safety of the different types of bovine serum. This section discusses specific tests that can detect and control adventitious agents.

Adventitious Agents Testing

The adventitious agents testing required for the evaluation of master seeds, master cells, and bulk and final products is described in 9 CFR 113.53 and by directives from the European Agency for the Evaluation of Medicinal Products (EMEA) (EMEA/CVMP/743/00 and EMEA/CPMP/BWP/1793/02). The testing methods outlined in these documents can detect a wide range of bovine microbial agents in serum products. These testing methods meet the requirements for most of the world's regulatory agencies. Serum manufacturers should test a representative sample of each batch of serum to determine the presence of adventitious agents. Testing is performed after filtration but before any further processing that is intended to inactivate or remove viruses.

Filtration with multiple 100-nm (0.1-μm) pore size filters, gamma irradiation (> 25 kGy while frozen), and chemical treatment (e.g., with betapropiolactone) are accepted methods of removing or inactivating viruses and mycoplasmas, and serum manufacturers routinely use these tools in both production and testing facilities. These treatments do not remove antibodies that may interfere with some applications. Additionally, the treatments do not ensure complete viral removal or inactivation, but can significantly reduce the risk of viral activity. The testing series to screen bovine serum for the absence of adventitious agents typically includes the following:

- Bacterial and fungal sterility testing as described in 9 CFR 113.26
- Mycoplasma testing as described in 9 CFR 113.28
- Viral testing as described in 9 CFR 113.53

The procedures described in *Sterility Tests* ⟨71⟩ confirm the absence of bacterial and fungal infection. For viruses, only cultivation using suitable substrate cells can indicate viral infectivity and replication. Those who use serum for research or production should test the serum for the absence of adventitious agents in a manner that is consistent with the product's intended application, bearing in mind that testing indicates only presence or absence of adventitious agents within the limits of the test procedures used.

Mycoplasma Testing

Mycoplasma contamination in tissue culture can arise from many animal origin sources, including serum, but more commonly it results from cross-contamination of infected cultures. Mycoplasmas are particularly insidious contaminants in cell culture because they

- cannot be visualized by light microscopy, even at high density ($>10^7$ colony-forming units/mL);
- cause no observable change in turbidity or pH of the culture fluid;
- cannot routinely be removed by single sterilizing filters, although removal can be obtained through a triple series of 0.1-μm filters;
- are unaffected by traditional antibiotics used in cell culture; and
- exert an extremely wide variety of adverse effects in tissue culture.

Classical mycoplasma detection is accomplished by direct sample inoculation in broth and/or agar and cultivation for a period of 28 days, with periodic observation and subculturing. This method is typically supplemented by fluorescent deoxyribonucleic acid (DNA) detection assay. Not all mycoplasma species can be cultivated, which explains the utility of supplemental fluorescent DNA detection assay. The sensitive and highly reliable direct cultivation test requires anaerobic and aerobic incubations for 3 weeks, which is a serious limitation in certain applications where quick results are required. The fluorescent DNA assay also is reliable but can yield equivocal results because it requires skilled staff to analyze a sample. It requires approximately 1 hour to complete.

In addition to the methods described previously, more recent detection procedures include luminescent and polymerase chain reaction (PCR) assay procedures. *Nucleic Acid-Based Techniques—Amplification* ⟨1127⟩ describes the general principles of PCR assays. The sensitive 20-minute luminescent assay measures a specific enzyme activity of mollicutes that converts adenosine diphosphate to adenosine triphosphate via a luciferase/luciferin reaction. Results are unequivocal and semiquantitative. PCR methods are quick and sensitive and display with good reliability, but occasional false positive results are a source of concern with commercial testing service labs. PCR may detect mycoplasmal DNA fragments that are non-infectious.

Viral Testing

The virus testing procedures for serum products are outlined in 9 CFR 113.52 and 9 CFR 113.53. In addition, there are other documents that may include equivalent or relevant testing such the EMEA/CVMP/743/00-Rev.2 from the Committee for Veterinary Medicinal Products (CVMP) *Revised Guideline on Requirements and Controls Applied to Bovine Serum Used in the Production of Immunological Veterinary Medicinal Products* and EMEA/CPMP/BWP/1793/02 from the Committee for Proprietary Medicinal Products (CPMP) *Note for Guidance on the Use of Bovine Serum in the Manufacture of Human Biological Medicinal Products*. Serum manufacturers should perform virus testing in compliance with this regulation, using at least two different and sensitive detector cell lines, one of which should be of bovine origin. The tests include cultivation of detector cells in cell culture media supplemented with 15% test serum for at least 21 days. Cells are subcultured at least twice during this period, usually 7 and 14 days post inoculation. At the conclusion of the last subculture (after a total of at least 21 days of incubation), cells are examined for general signs of virus amplification. The following end points are used for general virus detection: microscopic cell examination for cytopathogenic agents such as infectious bovine rhinotracheitis virus, cell staining and microscopic examination for inclusion bodies, and hemadsorbtion test to detect hemadsorbing agents such as PI-3. In addition to this series of testing and at the conclusion of the last subculture (after a total of at least 21 days of incubation), cells are stained with specific fluorescent antibody against the following specific viral agents:

- BVDV

- Bovine parvovirus
- Bovine adenovirus
- Bluetongue virus
- Bovine respiratory syncytial virus
- Reovirus
- Rabies virus

In addition to the viruses listed above, other viruses can be causative agents of disease and may require testing in various bovine serum applications. The serum end user is responsible for determining whether full 9 CFR testing is sufficient, and if other specific viral agents should be tested for. Examples of specific viruses not covered by the current virus testing guide may include akabane, bovine herpesvirus 1 (BHV-1), Parainvluenza-3 virus (PI-3), bovine leukemia, bovine rotavirus, bovine circovirus, bovine polyomavirus, coronavirus, torovirus, bovine enterovirus, bovine astrovirus, foot-and-mouth disease virus (FMDV), and rinderpest. *Appendix 2* provides a general description of some of these viruses as well as the ones for which testing is required. A serum end user's thorough risk analysis should determine the scope of testing and serum treatment options.

Risk Assessment and Detection Strategies

Serum manufacturers and serum end users should carry out a comprehensive, science-based risk assessment (e.g. Failure Modes and Effects Analysis) in order to better understand the safety profile of the serum product. The following risk assessment elements can be taken into consideration, but other elements can be included as appropriate: country of origin, region of the country, animal disease status of the country/region of origin, animal age, blood collection process, animal stunning method and exsanguination method, serum manufacturing process, type of production quality system, production in-process controls, final product testing, virus inactivation, equipment segregation, equipment cleaning procedure, personnel training, serum use/application, pharmaceutical product type, and intended use.

The species barrier provides a degree of protection against infection by some animal etiologic agents. This barrier is not an alternative to proactively ensuring that pharmaceutical products are manufactured only from raw materials of animal origin that have undetectable levels of adventitious agents. Inoculation of viable organisms into a nonhost species carries a risk that the organisms could cross the species barrier. An appropriate test regimen of serum material should therefore include examination for potential contaminants associated with the species of origin and the species of intent. Serum treatments to inactivate viral agents are a factor in establishing the appropriate test regimen for a particular material. Lowest risk of contamination is associated with biological materials that are terminally sterilized.

Zero risk is neither possible nor reasonable. The serum manufacturers should fully describe specific testing regimens in the product specifications, and these will vary depending on the type and source of the serum. Therefore, the guidelines for screening described in this chapter are examples only, and screening for all viruses listed may not be required for a particular material. Some manufacturers may perform certain tests on the finished product or on in-process materials rather than on individual component(s). Manufacturers must also evaluate the dilution effect in relation to the limit of detection of the test procedure. Interference with growth or neutralization of viral activity by serum may be an indication of a specific antibody or certain nonspecific factors in serum masking the viral agent. It is recommended that serum manufacturers consider this possibility when determining an adequate level of treatment in their viral inactivation studies or in virus testing applications.

Serum manufacturers should confirm that the species of origin is bovine to ensure that no other nonbovine agents may be present. Manufacturers should perform extraneous virus testing in appropriate cell cultures (see *Virology Test Methods* ⟨1237⟩ for appropriate cell line choices dependent on assay and targeted agent). If necessary, seroconversion studies should be conducted in susceptible animal species using a host species immune antibody response as the method of detection. Studies should use this procedure following an inactivation step to detect whether the virus was present before the virus inactivation process.

Serum manufacturing processes should be conducted in a consistent manner, following the established manufacturing procedures, with adequate quality systems built into the production process. Furthermore, equipment segregation (by species of origin), equipment and facility cleaning procedures, and personnel training are important elements in the risk assessment of the process.

Safety Considerations

End users of donor bovine serum may require serum that does not have detectable antibodies against BVDV or other specific agents so that the users can propagate cell cultures used in vaccine production, diagnostic testing, and test kit preparation, especially for the maintenance of master seed and master cell stocks. More than 40 cell types are available for the production of veterinary biologicals, but fewer than 10 media types are available for their propagation. Some researchers have proposed serum-free media as an alternative in propagating certain cells and viruses; but this means adapting culture procedures, which may alter the cells and change production results. If new or different sera are imported into the U.S., serum end users will require confirmation of source, species, and documentation of the origin of the sera in countries that are free of FMD and rinderpest.

CHARACTERIZATION OF BOVINE SERUM

Introduction

In the absence of end product-specific requirements, each lot of FBS should be tested to confirm that the serum meets the requirements of the proposed general chapter *Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩. For all other types of bovine sera, this section describes several key procedures for characterization. These procedures are not mandatory but are guidelines that manufacturers may consider for their individual applications. The table in the *Hemoglobin* section shows samples of specifications for the different types of bovine sera.

Species Identification

Both inter- and intraspecies identification assays should be performed on bovine sera to confirm species identity and the integrity of the serum products, and to ensure that nonbovine agents are not present. The most commonly used assay for the identification of bovine species identity is based on the electrophoretic profile of specific serum proteins. With electrophoresis, the serum proteins usually separate into as many as six fractions: albumin, alpha 1, alpha 2, beta 1, beta 2, and gamma globulins.

Other procedures used for bovine speciation include radial immunodiffusion (RID) and the double diffusion Ouchterlony method. These procedures allow either qualitative or quantitative measurements of the immunoglobulin G levels in serum. The RID method is based on the diffusion of an antigen from a circular well into a homogeneous gel that contains specific antiserum for each particular antigen. A circle of precipitated antigen

and antibody forms and continues to grow until it reaches equilibrium. The diameters of the rings are a function of antigen concentration. The Ouchterlony method is a double gel diffusion test wherein antigen and antibody diffuse toward each other in a semisolid medium to a point in the medium where optimum concentration of each is reached, forming a precipitate. The Ouchterlony plates contain cylindrical wells—a central 8-mm diameter antigen well, surrounded by six 3-mm antisera wells—which make possible the simultaneous monitoring of multiple antigen–antibody systems and the identification of particular antigens in a preparation. The proposed general chapter *Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩ describes the accepted procedure.

Hemoglobin

Hemoglobin is a multi-subunit protein that forms an unstable reversible bond with oxygen in the red blood cells. The oxygen-loaded form is called oxyhemoglobin and is bright red. The oxygen-unloaded form is called deoxyhemoglobin and is purple-blue. Oxyhemoglobin is the predominant form in red blood cells.

Low hemoglobin content in sera is widely accepted as a good general indication of rapid and careful processing of blood that will be used for serum. Red blood cells are fragile and rupture easily, releasing hemoglobin into the serum. Rough handling of the harvested blood, poor temperature control, or delayed processing elevates hemoglobin content in serum. Acceptable levels of hemoglobin may vary with intended application. The hemoglobin levels are measured using spectrophotometric procedures (see *Spectrophotometry and Light-Scattering* ⟨851⟩) as described in the proposed general chapter *Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩.

Chemical Profile

The testing of components such as cholesterol, alpha globulin, beta globulin, gamma globulin, albumin, creatinine, bilirubin, glucose, alanine aminotransferase, aspartate aminotransferase, phosphorus, potassium, calcium, and sodium usually is not considered a criterion for bovine serum lot release. Some manufacturers do not perform the tests on a routine basis but only as auxiliary tests. In some instances hospital clinical laboratories may run the tests. The levels of these chemicals in serum are important to end users and may also be used to assess lot-to-lot variability.

	FBS	Newborn Calf Serum	Calf Serum	Donor Bovine Serum	Adult Bovine Serum
Sterility test	No growth detected	No growth detected	No growth detected	No growth detected	No growth detected
Mycoplasma	Not detected	Not detected	Not detected	Not detected	Not detected
Virus testing	Not detected	Not detected	Not detected	Not detected	Not detected
Hemoglobin (mg/dL)	<30	<30	<30	<30	<30
Total protein (g/dL)	3.0–4.5	3.5–6.0	5.0–8.0	5.0–8.0	6.0–10.0
pH	7.00–8.00	7.00–8.00	7.00–8.00	7.00–8.00	7.00–8.00
Osmolality (mOsmol/Kg)	280–360	240–340	240–340	240–340	240–340

Endotoxin Levels

Although high endotoxin levels are not suitable for applications involving injectables, acceptable levels in bovine sera vary depending on the intended application. Some manufacturers may overlook the importance of low endotoxin levels in bovine sera used in cell culture applications. Endotoxin influences more than 30 biological activities. Some of these are macrophage activation, mitogenic stimulation, and induction of interferon and colony-stimulating factor (for some applications, these may be positive activities). Endotoxin can also lead to cytotoxicity by initiating complement activation. The most commonly used methods for endotoxin detection are the semiquantitative gel clot Limulus amebocyte lysate procedure and the quantitative kinetic chromogenic method described in *Bacterial Endotoxins Test* ⟨85⟩. For both the gel clot and the kinetic chromogenic assays, valid endotoxin assays require appropriate treatment by heat or dilution in order to avoid adverse effects of interfering substances in serum. Researchers should include a positive product control in each assay to confirm that any interference has been overcome by the heat or dilution treatment.

Osmolality

The osmolality test is designed to evaluate the electrolyte concentration in bovine serum. Chemicals that affect serum osmolality include sodium, chloride, bicarbonate, potassium, proteins, and glucose. Serum manufacturers should measure the osmolality of each serum batch to verify compliance with product specifications, using equipment calibrated with standards that are traceable to the National Institute of Standards and Technology. *Osmolality and Osmolarity* ⟨785⟩ describes how osmolality is determined by freezing-point depression of the bovine serum solution. Scientists use at least two standards to calibrate the instrument. The osmolality of each sample is calculated and related to the serum water content and is expressed as mOsmol/kg H_2O.

Total Protein Level

The total protein level in serum is measured to verify animal age and compliance with product specifications. *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩ describes two procedures, the Biuret and Bradford methods, for determining protein concentration. The acceptable level of protein in serum should be assessed by the end user based on the intended application.

Cell Growth Properties

Each lot of serum should be tested for its ability to support in vitro growth of specific cell lines. Bovine sera are highly variable, and different lots may yield different results. Because of this variability, end users should characterize and standardize the cell lines that they will use for this type of testing. End users should design cell growth procedures that will help them check the efficacy of bovine serum in promoting cell growth. Serum manufacturers will benefit from monitoring growth promotion over several generations of subcultures to detect any evidence of cytotoxicity or changes in cell morphology. Different serum manufacturers use different cell types, and the growth studies and cell lines used by serum manufacturers also may differ from those applied by serum end users. When serum manufacturers evaluate the growth properties of a specific cell line in response to a specific lot of serum, they should take into account plating efficiency and/or growth promotion or some other functionality tests that qualify the serum lot for its intended use.

Plating efficiency at low cell density is a preferred method for analyzing the proliferative capacity and survival of single cells under optimal growth conditions. This procedure can reveal differences in the growth rate within the population and is capable of distinguishing between changes in growth rate (colony size) and cell survival (colony number). The growth kinetic is another important aspect in the design of cell-based experiments. Determining the growth curve of each cell line helps define optimal culture conditions, because variation in serum and other growth additives may influence growth parameters, which may affect the experimental outcome.

In the absence of specific tests designed for their particular products, serum users can refer to the functionality tests described in the proposed general chapter *Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩ to determine whether a lot of serum is suitable for their application. This chapter provides guidance about how to perform growth promotion and plating efficiency tests.

In Vitro Cytotoxicity

Serum manufacturers should use an appropriate cell line for testing each lot of serum, and should perform growth studies through several subcultured generations to ensure that the serum has no cytotoxic effect on the cells. The choice of cell line depends on the intended use of serum. The cell growth and cytotoxicity assays should be performed on the final batch of serum after any viral inactivation step or any further processing.

CONCLUSION

Bovine serum is likely to remain an important component in the manufacture of many biologics, particularly those relying on cell culture. As with similar materials, bovine serum displays inherently variable quality. As a result, serum end users must establish suitable tests, procedures, and acceptance criteria for introduction of materials into a particular application process that uses serum. This may mean screening multiple lots of bovine serum to determine which lots meet the specification (see the section *Characterization of Bovine Serum*).

Manufacturers of therapeutic products using bovine serum are responsible for ensuring and documenting its quality and its impact on the quality, safety, and efficacy of the final product. In addition, it is important to ensure that each lot of serum performs in an equivalent manner during manufacturing. Serum can also interfere with final product purification; therefore it is important to understand the effect of bovine serum on the manufacturing process in order to understand the effect that various processes might have on the final product. Finally, risks can also be mitigated through the design of processes to include steps to adequately remove the bovine material through dilution, separation, or inactivation as well as the development of analytical assays to assess the bovine-derived residual content during processes and in the final therapeutic product. A number of sensitive assays can provide a quantitative means of detecting bovine material at picogram levels.

APPENDIX 1

Bovine sera and serum-related products used in the manufacture of biological products are regulated in the context of *Requirements for Ingredients of Animal Origin Used for the Production of Biologics*, 9 CFR 113.53. Currently, individual serum manufacturers perform detection studies to identify contaminating viruses. Because of the potential international market for serum, serum manufacturers need to be mindful of other regulatory requirements. Manufacturers can use the documents listed here as guidance for screening bovine sera for contamination by adventitious agents. Because of the risk carried by animal-derived serum products, serum manufacturers and end users should ensure that the country of origin of

the material complies with applicable regulatory requirements. Although no cell performance assays currently demonstrate lack of BSE in serum, serum manufacturers must comply with the regulatory requirements of countries where the serum is marketed to ensure a minimal risk of infection with BSE/TSE.

Beyond relevant *USP* chapters referenced in this chapter, the following list of documents includes regulatory documents as well as best practices in product and process development, manufacturing, quality control, and quality assurance.

CFR

- 9 CFR 94.18 (CVB, 2001)
- 9 CFR 113.46
- 9 CFR 113.47
- 9 CFR 113.52
- 9 CFR 113.53
- 9 CFR 113.55
- 9 CFR 320
- 9 CFR 327.4
- 21 CFR 211 Subpart E
- 21 CFR 801.1
- 21 CFR 809.10

FDA

- FDA. Center for Biologics Evaluation and Research (CBER). 2000. Letter to manufacturers of biological products. http://www.fda.gov/Cber/ltr/bse041900.pdf
- Use of materials derived from cattle in medicinal products intended for use in humans and drugs intended for use in ruminants (Proposed Rule). *Federal Register*. 2007; *72*(8): 1582–1619. Available at http://www.fda.gov/cber/rules/catruminant.pdf.

International Regulations and Guidance Documents

- CPMP/Biotechnology Working Party/EMEA (CPMP/BWP/EMEA). 1996. *Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses*. Available at http://www.emea.europa.eu/pdfs/human/bwp/026895en.pdf.
- CPMP/BWP/EMEA. 2003. *Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products*. Available at http://www.emea.europa.eu/pdfs/human/bwp/179302en.pdf.
- EMEA/CVMP/743/00-Rev.2 from the Committee for Veterinary Medicinal Products (CVMP). *Revised guideline on requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products.* Available at http://www.emea.europa.eu/pdfs/vet/iwp/074300en.pdf.
- EMEA/CPMP/BWP/1793/02, from the CPMP. *Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products*. Available at http://www.emea.europa.eu/pdfs/human/bwp/179302en.pdf.
- CPMP/CVMP. *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*. Available at http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf.
- World Health Organization (WHO), Office International des Epizooties. *Terrestrial animal health code 2007.* Available at http://www.oie.int/eng/normes/mcode/code2007/anc-en_summry.htm.
- WHO. 2006. *WHO guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies*. http://www.who.int/bloodproducts/cs/TSEPUBLISHEDREPORT.pdf.

APPENDIX 2

Following is a general description of viruses that manufacturers can consider when testing bovine serum for the absence of adventitious agents. The list is intended

only to provide general information. The list of required testing is described in this chapter in the section *Viral Testing*.

Akabane—An insect-transmitted virus that causes congenital abnormalities of the central nervous system in ruminants. Disease due to *Akabane* virus has been recognized in Australia, Israel, Japan, and Korea. Antibodies to it have been found in a number of countries in Southeast Asia, the Middle East, and Africa. The disease affects fetuses of cattle, sheep, and goats. Asymptomatic infection has been demonstrated serologically in horses, buffalo, and deer (but not in humans or pigs) in endemic areas.

Bluetongue—An infectious, noncontagious arthropod-borne viral disease primarily of domestic and wild ruminants. Infection with bluetongue virus is common worldwide but is usually subclinical or mild. *Bluetongue* virus is the type-species of the genus *Orbivirus* in the family Reoviridae. Worldwide, 24 serotypes have been identified, although not all serotypes exist in any one geographic area: e.g., only 5 serotypes (2, 10, 11, 13, and 17) have been reported in the U.S. Distribution throughout the world parallels the spatial and temporal distribution of vector species of *Culicoides* biting midges, which are the only significant natural transmitters of the virus.

Bovine adenovirus—Associated with a wide spectrum of diseases. *Bovine adenovirus* type 3 is the serotype most often associated with bovine respiratory disease. *Bovine adenoviruses* are DNA viruses that have been separated into two genera, the *Mastadenovirus,* or *mammalian adenoviruses,* and the *Aviadenovirus,* or *avian adenoviruses.* Within the genus *Mastadenovirus* are numerous species specific serotypes, nine of which have been identified in cattle. *Epitheliotrophic adenoviruses* have also been isolated from ruminants, and usually are clinically unapparent. Clinical disease is dictated by various factors, including the strain of virus, concurrent infection, stress, environmental conditions, and management practices.

Bovine herpesvirus 1 (BHV-1)—Associated with several diseases in cattle, including infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, balanoposthitis, conjunctivitis, abortion, encephalomyelitis, and mastitis. BHV-1 infections are widespread in the cattle population. In feedlot cattle the respiratory form is most common.

Bovine leukemia—An exogenous C-type oncovirus in the family Retroviridae. Bovine leukemia is a viral disease of adult cattle characterized by neoplasia of lymphocytes and lymph nodes. Infection occurs by iatrogenic transfer of infected lymphocytes and is followed by a permanent antibody response. The prevalence of infection in a herd may be high, but only a few animals develop fatal lymphosarcoma. Infection is spread by contact with contaminated blood from an infected animal.

Bovine reovirus—Double-stranded ribonucleic acid (RNA) (dsRNA) viruses with nonenveloped spherical virions 60–80 nm in diameter. They cause bovine respiratory diseases.

Bovine respiratory syncytial virus (BRSV)—An RNA virus classified as a pneumovirus in the Paramyxovirus family. This virus was named for its characteristic cytopathic effect—the formation of syncytial cells. In addition to cattle, sheep and goats can also be infected by respiratory syncytial viruses. Human respiratory syncytial virus (HRSV) is an important respiratory pathogen in infants and young children. HRSV has antigenic subtypes, and preliminary evidence suggests the existence of antigenic subtypes of BRSV. BRSV is distributed worldwide, and the virus is indigenous in the cattle population. BRSV infections associated with respiratory disease occur predominantly in young beef and dairy cattle.

Bovine rotavirus—A dsRNA spherical virion 60–80 nm in diameter without an envelope. It is the most common viral cause of diarrhea in calves and lambs.

Bovine viral diarrhea virus (BVDV)—An RNA virus classified as a *Pestivirus* in the family Flaviviridae. BVDV can cross the placenta and appears to be capable of inducing immunosuppression, which allows the development of secondary bacterial pneumonia. BVDV has been reported to be the virus most frequently associated with multiple viral infections of the respiratory tract of calves.

Foot-and-mouth disease (FMD)—A highly infectious viral disease of cattle, pigs, sheep, goats, buffalo, and artiodactyl wildlife species. In a susceptible population, morbidity approaches 100%. The disease is rarely fatal except in young animals. FMD is caused by an *Aphthovirus* of the family Picornaviridae. Seven immunologically distinct serotypes are known, and within each serotype exist a large number of strains that exhibit a spectrum of antigenic characteristics.

Parainfluenza-3 virus (PI-3)—An RNA virus classified in the Paramyxovirus family. Although PI-3 is capable of causing disease, the virus usually is associated with mild to subclinical infection. The most important role of PI-3 is to serve as an initiator that can lead to the development of secondary bacterial pneumonia. Infections caused by PI-3 are common in cattle.

Parvovirus—A relatively heat-stabile single-stranded DNA virus approximately 20 nm in diameter that has been recovered from cattle but under natural conditions is not known to cause disease.

Rabies—An acute viral encephalomyelitis that principally affects carnivores and bats, although it can affect any mammal. Rabies is caused by *Lyssaviruses* in the Rhabdovirus family. Although they are usually confined to one major reservoir species in a given geographic area, spillover to other species is common.

Rinderpest—A *Morbillivirus,* closely related to the viruses that cause canine distemper and measles. Strains may vary markedly in host range and virulence. Sera from recovered or vaccinated cattle cross-react with all strains in neutralization tests, but minor antigenic differences have been demonstrated. The virus is fragile and becomes rapidly inactivated by heat and light but remains viable for long periods in chilled or frozen tissues. Rinderpest is endemic in many countries in Asia and Africa. Historically, the virus has been widely distributed throughout Europe and Africa but to date has not established itself in North America, Central America, the Caribbean Islands, South America, Australia, or New Zealand. Rinderpest is included in the WHO's Office International des Epizooties list of communicable diseases that have the potential for very serious and rapid spread, irrespective of national borders; which are of serious socioeconomic or public health consequence; and which are of major importance in the international trade of livestock and livestock products.■1S (*USP33*)

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

BRIEFING

***t*-Butylthiol.** It is proposed to add this new reagent used in the *Content of Alliin* test in the monograph for *Garlic*.

(HDQ: M. Marques.) RTS—C73260

Add the following:

■***t*-Butylthiol** (*tert-Butylthiol; tert-Butyl Mercaptan; 2-Methyl-2-propanethiol; TBM*), $(CH_3)_3CSH$—**90.19** [*75-66-1*]—Use a suitable grade with a content of NLT 98.0%.

[NOTE— A suitable grade is available as catalog number 20230 at www.sigma-aldrich.com.]■1S *(USP33)*

BRIEFING

Cobalt Nitrate, *USP 32* page 816. It is proposed to correct the CAS number for this reagent.

(HDQ: M. Marques.) RTS—C73254

Change to read:

Cobalt Nitrate, $Co(NO_3)_2 \cdot 6H_2O$—**291.03** ~~[*10141-05-6*]~~

■[*10026-22-9*]■1S *(USP33)*
—Use ACS reagent grade.

BRIEFING

Diaveridine, *USP 32* page 818. It is proposed to include information about a possible supplier of this reagent.

(HDQ: M. Marques.) RTS—73261

Diaveridine *(5-([3,4-Dimethoxyphenyl]methyl)-2,4-pyrimidinediamine),* $C_{13}H_{16}N_4O_2$—**260.3** [*5355-16-8*]—Use a suitable grade.

■[NOTE—A suitable grade is available as catalog number 46174 from www.sigma-aldrich.com.]■1S *(USP33)*

BRIEFING

***p*-Naphtholbenzein,** *USP 32* page 834. It is proposed to correct the CAS number for this reagent.

(HDQ: M. Marques.) RTS—C73222

Change to read:

***p*-Naphtholbenzein,** $C_{27}H_{18}O_2$—**374.43** ~~[*6948-88-5*]~~

■[*145-50-6*]■1S *(USP33)*
—Red-brown powder. Use a suitable grade.

BRIEFING

Sodium Biphenyl, *USP 32* page 847 and page 178 of *PF* 35(1) [Jan.–Feb. 2009]. It is proposed to add information about other possible suppliers of this reagent.

(HDQ: M. Marques.) RTS—C73178

Change to read:

Sodium Biphenyl, $C_{12}H_9Na$—**176.19**~~—Supplied as a solution (10 percent to 30 percent, w/w) in a mixture of dimethoxyethane and toluene or xylene. The solution is a viscous, dark green liquid. [NOTE—The solution deteriorates at a rate of about 10% per month. Use only freshly prepared solution.]~~

▲Available as a solution in 2-ethoxyethyl ether,▲*USP33*

■or in 1,2-dimethoxyethane (diethylene glycol diether).■1S *(USP33)*

Activity—Place 20 mL of dry toluene in a titration flask equipped with a magnetic stirring bar and a stopper having a hole through which the delivery tip of a weight buret may be inserted. Add a quantity of sodium biphenyl sufficient to produce a blue color in the mixture, and titrate with amyl alcohol, contained in a weight buret, to the disappearance of the blue color. (Disregard the amounts of sodium biphenyl and amyl alcohol used in this adjustment.) Weigh accurately the weight buret containing the amyl alcohol. Transfer the contents of a vial of well-mixed test specimen to the titration flask, and titrate quickly with the amyl alcohol to the

disappearance of the blue color. Weigh the buret to determine the weight of amyl alcohol consumed, and calculate the activity, in mEq per vial, by the formula:

$$11.25W$$

in which *W* is the weight of amyl alcohol consumed. Not less than 10% activity is found.

Iodine content—Add 10 mL to 5 mL of toluene contained in a 125-mL separator fitted with a suitable inert plastic stopcock, and shake vigorously for 2 minutes. Extract gently with three 10-mL portions of dilute phosphoric acid (1 in 3), combining the lower phases in a 125-mL iodine flask. Add sodium hypochlorite TS, dropwise, to the combined extracts until the solution turns brown, then add 0.5 mL in excess. Shake intermittently for 3 minutes, add 5 mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 minute, accurately timed. Add 1 g of potassium iodide, shake for 30 seconds, add 3 mL of starch TS, and titrate with 0.1 N sodium thiosulfate VS: not more than 0.1 mL of 0.1 N sodium thiosulfate is consumed.

▲[NOTE—A suitable grade is available as catalog number 277134 from www.sigma-aldrich.com▲*USP33*

■or as catalog number 54101 from www.gfschemicals.com.]■1S *(USP33)*

BRIEFING

Stannous Chloride, *USP 32* page 851. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques.) RTS—C73278

Change to read:

Stannous Chloride, $SnCl_2 \cdot 2H_2O$—**225.65** ~~[7772-99-8]~~

■[*10025-69-1*]■1S *(USP33)*
—Use ACS reagent grade.

BRIEFING

Sulfuric Acid, Nitrogen Free. It is proposed to add this new reagent used in the test for *Limit of Nitrate* in the monograph for *Calcium Acetate*.

(HDQ: M. Marques.) RTS—C73250

Add the following:

■**Sulfuric Acid, Nitrogen Free,** H_2SO_4—**98.08**

[*7664-93-9*]—Use a suitable grade.

[NOTE—A suitable grade is available as Sulfuric Acid, Ultrex II, catalog number 6902-05 from www.mallbaker.com.]■1S *(USP33)*

Test Solutions

BRIEFING

Dibasic Sodium Phosphate TS, *USP 32* page 869. It is proposed to make some editorial changes in the entry for this *Test Solution*.

(HDQ: M. Marques.) RTS—C73221

Change to read:

Dibasic Sodium Phosphate TS—Dissolve 12 g of ~~clear crystals of~~

■■1S *(USP33)*
dibasic sodium phosphate in water to 100 mL.

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 32* page 881, and page 462 of *PF* 35(2) [Mar.–Apr. 2009].

(HDQ) RTS—C40389; C47819; C57642; C62529; C64170; C70857

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	$T_{▲USP33}$
Add the following:	
■Amlodipine Besylate Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
■Azithromycin Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Cabergoline Tablets	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Calcium Citrate Tablets	$W_{■1S\ (USP32)}$
Add the following:	
■Clonazepam Orally Disintegrating Tablets	W, $LR_{■1S\ (USP32)}$
Change to read:	
Dantrolene Sodium Capsules	T, ~~LR~~ ■ $_{■2S\ (USP32)}$
Add the following:	
■Doxycycline Hyclate Tablets, Delayed-Release	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Fluconazole Tablets	$W_{■2S\ (USP32)}$
Add the following:	
■Granisetron Hydrochloride Tablets	W, $LR_{■2S\ (USP32)}$

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
▲Guggul Tablets	W, $LR_{▲USP33}$
Add the following:	
■Ivermectin and Pyrantel Pamoate Tablets	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Ketoprofen Capsules, Extended-Release	$T_{■2S\ (USP32)}$
Add the following:	
■Lamivudine and Zidovudine Tablets	W, $LR_{■1S\ (USP33)}$
Add the following:	
▲Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	$LR_{▲USP33}$
Add the following:	
▲Loratadine Orally Disintegrating Tablets	$T_{▲USP33}$
Add the following:	
■Losartan Potassium Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Metronidazole Capsules	W, $LR_{■1S\ (USP32)}$
Add the following:	
■Nateglinide Tablets	$T_{■1S\ (USP33)}$
Add the following:	
■Olanzapine Tablets	T, $LR_{■1S\ (USP33)}$
Add the following:	
■Ondansetron Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
■Orbifloxacin Tablets	$T_{■1S\ (USP32)}$
Add the following:	
■Orphenadrine Citrate Tablets, Extended-Release	T, $LR_{■1S\ (USP32)}$
Add the following:	
▲Oseltamivir Phosphate Capsules	$W_{▲USP33}$
Add the following:	
▲Oxcarbazepine Tablets	$W_{▲USP33}$

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Pilocarpine Hydrochloride Tablets	T ■1S (*USP32*)
Add the following:	
■Potassium Citrate Tablets	W■1S (*USP32*)
Add the following:	
■Ribavirin Capsules	W■1S (*USP33*)
Add the following:	
■Risedronate Sodium Tablets	W■2S (*USP32*)
Add the following:	
■Tacrolimus Capsules	T■1S (*USP33*)
Add the following:	
■Tamsulosin Hydrochloride Capsules	T■2S (*USP32*)
Add the following:	
■Telmisartan Tablets	W■1S (*USP33*)
Add the following:	
■Ticlopidine Hydrochloride Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Sulfate Tablets	W■1S (*USP33*)
Add the following:	
■Valganciclovir Tablets	T■2S (*USP32*)
Add the following:	
■Zinc Citrate Tablets	W■1S (*USP32*)
Add the following:	
■Zinc Gluconate Tablets	T, LR■1S (*USP33*)
Add the following:	
▲Zolpidem Tartrate	W▲*USP33*
Add the following:	
■Zolpidem Tartrate Extended-Release Tablets	W■1S (*USP33*)

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 32* page 890, page 266 of *PF* 29(1) [Jan.–Feb. 2003], page 591 of *PF* 31(2) [Mar.–Apr. 2005], page 1053 of *PF* 33(5) [Sept.–Oct. 2007], page 450 of *PF* 34(2) [Mar.–Apr. 2008], page 817 of *PF* 34(3) [May–June 2008], page 1046 of *PF* 34(4) [July–Aug. 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], page 188 of *PF* 35(1) [Jan.–Feb. 2009], and page 464 of *PF* 34(2) [Mar.–Apr. 2009].

(HDQ) RTS—C44129; C44603; C44606; C49314; C49319; C52047; C57726; C62481; C62812; C68834

Add the following:

■**Articaine Hydrochloride:** White or almost white, crystalline powder. Freely soluble in water and in alcohol.■1S (*USP33*)

Add the following:

■**Carmustine:** Light yellow powder. Freely soluble in ether.■1S (*USP33*)

Add the following:

■**Misoprostol:** Clear, colorless or light yellow viscous liquid. Very slightly soluble in water.■1S (*USP33*)

Add the following:

■**Olopatadine Hydrochloride:** White crystalline powder. Very soluble in formic acid; sparingly soluble in water; very slightly soluble in dehydrated alcohol.■1S (*USP33*)

Add the following:

■**Pentamidine Isethionate:** White or almost white powder or colorless crystals, hygroscopic. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in methylene chloride.■1S (*USP33*)

Add the following:

■**Telmisartan:** White or slightly yellowish, crystalline powder. Sparingly soluble in methylene chloride; slightly soluble in methanol; practically insoluble in water. It dissolves in 1 M sodium hydroxide.■1S (*USP33*)

Add the following:

■**Ticlopidine:** White or almost white crystalline powder. Sparingly soluble in water and in alcohol; very slightly soluble in ethyl acetate.■1S (*USP33*)

Change to read:

Tretinoin: Yellow to light-orange, crystalline powder. Slightly soluble in alcohol, ~~and in chloroform.~~ ■in chloroform, and in methanol;■1S (*USP33*) insoluble in water.

Add the following:

■**Valacyclovir Hydrochloride:** White to off-white powder. Soluble in water; insoluble in dichloromethane.■1S (*USP33*)

Add the following:

■**Ziprasidone Hydrochloride:** White to slightly pink powder. Very soluble in methanol; slightly soluble in isopropyl alcohol, and in hot tetrahydrofuran; practically insoluble in water.■1S (*USP33*)

In-Process Revision

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* or an *IRA.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents; Indicators; and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
General Notices (entire *General Notices and Requirements* revised)	34	1	40
USP Monographs			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetaminophen and Tramadol Hydrochloride Tablets (new)	35	1	56
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Sulfate—*USP Reference standards, Assay*	34	2	242
Albuterol Tablets—*Assay*	31	3	726
Alendronate Sodium Tablets—*Dissolution*	35	1	59
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Aluminum Acetate Topical Solution—*Identification*	34	2	242
Aluminum Subacetate Topical Solution—*Identification*	34	2	242
Amantadine Hydrochloride Capsules—*Labeling* (add), *Dissolution*	35	1	61
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	35	1	62
Amodiaquine Hydrochloride—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	2	243
Amodiaquine Hydrochloride Tablets—*USP Reference standards, Identification, Assay*	34	3	558
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140
Ampicillin Sodium—*Dimethylaniline*	35	1	65
Anastrozole (new)	34	2	244
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Aspirin—*Readily carbonizable substances*	34	5	1143
Atenolol Tablets—*Dissolution*	35	1	66
Atorvastatin Calcium (new)	35	1	66
Atovaquone—*Assay*	34	2	247
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam for Injection—*Assay*	34	4	906
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Benzoin—*Botanic characteristics, Identification*	35	1	70
Betamethasone Oral Solution—*Packaging and storage, Thin-layer chromatographic identification test* (delete), *Identification A, B* (add), *Microbial limits* (add), *pH* (add), *Deliverable volume* (add), *Related compounds* (add), *Assay*	34	3	567
Bicalutamide Tablets—*Labeling, Dissolution*	34	5	1147
Bisoctrizole (new)	32	2	309
Bisoprolol Fumarate Tablets—*Dissolution*	34	3	570
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Related compounds*	34	4	907
Bupropion Hydrochloride Extended-Release Tablets—*Dissolution, Related compounds*	35	1	70
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Cabergoline Tablets (new)	34	3	572
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Capecitabine Tablets—*Dissolution*	35	1	72
Carbidopa—*Specific rotation*	35	1	73
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Cefaclor Capsules—*Identification, Related compounds, Assay*	34	2	248
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Chlorhexidine Acetate (new)	34	3	582
Chlorhexidine Gluconate Oral Rinse—*Labeling, USP Reference standards*	34	2	250
Chlorhexidine Gluconate Solution—*USP Reference standards, Limit of p-chloroaniline, Assay*	34	2	250
Chlorhexidine Hydrochloride (new)	34	3	585
Chloroquine Phosphate—*USP Reference standards, Identification, Assay*	34	2	251
Chloroquine Phosphate Tablets—*USP Reference standards, Identification, Assay*	34	3	587
Cilostazol—*Loss on drying*	34	3	589
Cisapride (new)	34	2	253
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Anhydrous Citric Acid *(Harmonization)*—*Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)*—*Sulfate*	31	3	750
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49
Clarithromycin Tablets—*Dissolution*	35	1	73
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Climbazole (new)	33	5	891
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clonazepam Orally Disintegrating Tablets (new)	34	2	254
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Clozapine Tablets—*Uniformity of dosage units* (add)	34	3	589
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclazuril (new)	35	1	73
Diclofenac Sodium Delayed-Release Tablets—*Dissolution*	35	2	271
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Dolasetron Mesylate—*Impurities*	35	2	272
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Doxycycline Hyclate Delayed-Release Tablets (new)	34	3	589
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Enalaprilat Injection (new)	34	3	593
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Epirubicin Hydrochloride (new)	35	2	273
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158
Estradiol Tablets—*USP Reference standards, Chromatographic purity* (add)	34	3	596
Estradiol Vaginal Inserts (new)	34	3	597
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Ethinyl Estradiol Tablets—*Dissolution* (add)	31	4	1067
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate—*Definition, Assay*	35	2	275
Fenoprofen Calcium—*Chromatographic purity*	34	3	601
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931
Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets—*Labeling, USP Reference standards, Identification, Dissolution, Related compounds, Assay*	34	6	1445
Fluconazole Injection (new)	34	2	266
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766
Fluticasone Propionate Cream (new)	34	3	609
Fluticasone Propionate Ointment (new)	34	3	611
Fluvestrant (new)	33	5	99

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Fosinopril Sodium—*Related compounds*	34	3	613
Fosphenytoin Sodium—*Related compounds, Assay*	34	2	270
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Labeling* (add), *Dissolution*	34	6	1452
Glimepiride Tablets—*Dissolution*	33	3	411
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide Tablets—*Dissolution*	33	4	651
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163
Haloperidol Decanoate (new)	34	3	614
Heparin Sodium (entire monograph revised)	35	2	257
Heparin Sodium Injection (entire monograph revised)	35	2	266
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxyzine Pamoate—*Identification, Residue on ignition, Heavy metals, Pamoic acid content* (delete), *Assay*	34	2	271
Hydroxyzine Pamoate Capsules—*Identification, Assay*	34	2	272
Hydroxyzine Pamoate Oral Suspension—*Identification, Assay*	34	2	273
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Irbesartan—*Limit of azide*	34	5	1164
Isotretinoin Capsules—*Labeling* (add), *Chromatographic purity, Assay*	34	4	942
Itraconazole (new)	34	4	947
Ivermectin and Pyrantel Pamoate Tablets (new)	34	2	277
Ketoprofen—*USP Reference standards, Chromatographic purity*	34	3	617
Ketoprofen Extended-Release Capsules (new)	34	4	951
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamivudine and Zidovudine Tablets (new)	35	2	277
Lamotrigine (new)	34	3	617
Levonorgestrel—*USP Reference standards, Chromatographic purity, Assay*	34	3	620
Levorphanol Tartrate—*Assay*	34	2	280
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lipid Injectable Emulsion—*Definition, Limit of free fatty acids*	34	3	621
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium—*Limit of cyclohexane and isopropyl alcohol* (delete)	34	3	626
Losartan Potassium Tablets (new)	34	5	1164
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Mafenide Acetate Cream—*Identification*	34	2	280
Mafenide Acetate for Topical Solution—*Content of acetic acid*	34	3	627
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Meclocycline Sulfosalicylate—*Assay*	34	3	627
Meclocycline Sulfosalicylate Cream—*Assay*	34	3	628
Mefenamic Acid—*Heavy metals*	34	2	281
Megestrol Acetate Oral Suspension—*Dissolution*	35	1	75
Meloxicam—*Impurities, Procedure 1*	35	2	278
Mesna (new)	34	5	1168
Metformin Hydrochloride Extended-Release Tablets—*Dissolution*	35	1	76
Methacholine Chloride—*Identification, Melting range* (delete)	34	3	629
Methotrexate—*USP Reference standards, Chromatographic purity*	34	3	630
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Metronidazole—*Packaging and storage, USP Reference standards, Identification, Melting range* (delete), *Non-basic substances* (delete), *Chromatographic purity* (delete), *Related compounds* (add), *Assay*	34	3	631
Metronidazole Capsules (new)	34	3	633
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Mometasone Furoate Cream—*Packaging and storage, Related compounds* (add), *Assay*	35	1	82
Mometasone Furoate Ointment—*Packaging and storage, Related compounds* (add), *Assay*	35	1	84
Mometasone Furoate Topical Solution—*Packaging and storage, Related compounds* (add), *Assay*	35	1	87
Morantel Tartrate—*pH*	32	6	1735
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Cream—*Related compounds, Assay*	34	2	281
Mupirocin Nasal Ointment (new)	34	4	966
Mycophenolate Mofetil—*Identification, Melting range* (delete), *Related compounds, Assay*	35	1	89
Naltrexone Hydrochloride—*Related compounds*	34	2	283
Naratriptan Hydrochloride Oral Suspension (new)	35	1	90
Nateglinide (new)	34	6	1463
Nateglinide Tablets (new)	35	2	281
Niacinamide—*Readily carbonizable substances*	34	5	1176
Nitrofurantoin—*Packaging and storage*	35	1	92
Nitrofurantoin Capsules—*Packaging and storage*	35	1	92
Nitrofurantoin Oral Suspension—*Packaging and storage*	35	1	92
Nitrofurantoin Tablets—*Packaging and storage*	35	1	92
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethynodrel (delete entire monograph)	35	1	92
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine (new)	34	3	641
Olanzapine Tablets (new)	35	2	282
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Orbifloxacin (new)	34	2	283
Orbifloxacin Tablets (new)	34	2	286
Orlistat Capsules (new)	32	6	1739
Orphenadrine Citrate Extended-Release Tablets (new)	34	3	643
Oseltamivir Phosphate (new)	34	6	1468
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin Injection (new)	35	2	284
Oxaliplatin for Injection (new)	34	6	1473
Oxazepam Capsules—*Dissolution*	35	2	286
Oxcarbazepine (new)	34	5	1177

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Oxcarbazepine Tablets (new)	34	6	1478
Oxybutynin Chloride Tablets—*Dissolution*	35	1	93
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxycodone Hydrochloride Extended-Release Tablets—*Related compounds*	31	4	1104
Oxymetazoline Hydrochloride Nasal Solution—*pH*	33	5	932
Oxytocin—*Definition, USP Reference standards, Identification, Vasopressor activity* (delete), *Acetic acid content* (add)	34	3	647
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Paricalcitol—*Identification, Assay*	33	2	252
Pectin—*Chemical information; Definition; Identification—A, B, C, D* (delete), *Procedure* (add); *Assay—Methoxy Groups* (name change), *Galacturonic Acid, Methoxy Groups* (add); *Impurities—Lead, Procedure 1, Procedure 2* (add), *Procedure 3* (add); *Microbial Enumeration Tests; Packaging and Storage; Labeling; USP Reference Standards* (add)	35	2	287
Penicillamine Capsules—*Dissolution*	31	2	436
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Pergolide Oral Suspension, Veterinary (new)	34	2	289
Permethrin (new)	32	4	1100
Permethrin Cream (new)	34	1	103
Petrolatum (new)—*Harmonization*	28	2	569
White Petrolatum (new)—*Harmonization*	28	2	570
Liquefied Phenol—*Identification* (add), *Other requirements*	35	1	93
Phenylephrine Hydrochloride—*Assay*	34	2	291
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride Tablets (new)	34	2	291
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180
Pravastatin Sodium—*Chromatographic purity, Assay*	34	2	294
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Praziquantel Tablets—*Dissolution*	35	2	291
Proguanil Hydrochloride (new)	34	2	296
Promethazine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	292
Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution (new)	35	2	295
Promethazine and Phenylephrine Hydrochloride Oral Solution (new)	35	2	298
Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	301
Propafenone Hydrochloride—*USP Reference standards, Chromatographic purity* (delete), *Related compounds* (add)	35	1	94

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Pseudoephedrine Hydrochloride—*Definition, USP Reference standards, Ordinary impurities* (delete), *Chromatographic purity* (add), *Assay*	34	2	298
Psyllium Husk—*Impurities—Heavy Metals* (add), *Procedure 3* (add)	35	2	304
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ramipril—*Definition, Assay*	31	3	787
Ranitidine Hydrochloride—*Chromatograpic purity, Assay*	34	2	299
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Repaglinide Tablets—*Loss on Drying* (delete)	35	2	306
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Ritonavir—*Identification*	35	1	95
Rocuronium Bromide (new)	34	3	648
Salmeterol Xinafoate (new)	35	2	307
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sennosides—*Content of Sennosides A and B* (add), *USP Reference Standards* (add)	35	2	308
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Simethicone Emulsion—*Assay*	34	3	652
Simethicone Tablets—*Disintegration*	34	3	652
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Fluoride—*Assay*	34	3	653
Sodium Sulfate—*Assay*	34	5	1192
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Stavudine—*Specific rotation*	34	3	653
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfadoxine and Pyrimethamine Tablets—*Assay*	34	2	301
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfasalazine—*Identification*	34	3	653
Sulfazalazine Tablets—*Identification*	34	3	653
Sulfinpyrazone—*Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	34	6	1483
Tacrolimus (new)	35	2	310
Tacrolimus Capsules (new)	35	2	312
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Tazobactam—*Identification, Specific rotation, Related compounds, Organic volatile impurities* (delete), *Assay*	34	2	302
Terbinafine Hydrochloride—*Melting range*	34	5	1197
Terbinafine Oral Suspension (new)	35	1	96
Terbutaline Oral Suspension (new)	35	1	97
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride—*Chromatographic purity*	34	2	306
Tiagabine Hydrochloride Oral Suspension (new)	35	1	98
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798
Tobramycin Inhalation Solution—*Identification* (add), *Osmolarity, Chromatographic purity, Other requirements* (delete), *Assay*	34	2	307
Topiramate Tablets (new)	34	5	1197
Tramadol Hydrochloride (new)	34	5	1200

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Trandolapril (new)	34	2	310
Tranylcypromine Sulfate (new)	35	2	314
Travoprost (new)	32	4	1115
Travoprost Ophthalmic Solution (new)	32	4	1118
Trenbolone Acetate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Limit of trenbolone acetate 17α-isomer* (delete), *Related compounds* (add), *Assay*	35	1	100
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800
Triamterine Capsules—*USP Reference standards, Related compounds* (add), *Assay*	34	3	654
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tromethamine—*Melting Range or Temperature*	35	2	316
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Valganciclovir Tablets (new)	33	1	89
Valrubicin—*Definition, USP Reference standards, Identification, Loss on drying* (delete), *Water* (add), *Limit of residual solvents* (delete), *Related compounds, Assay*	35	1	103
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486
Vancomycin Hydrochloride—*Labeling* (add), *USP Reference standards, Chromatographic purity, Other requirements* (add)	34	1	111
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Labeling* (add), *Identification* (add), *Water* (add), *pH* (add), *Uniformity of dosage units* (add), *Chromatographic purity, Assay*	34	4	992
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Vincristine Sulfate Injection—*Identification*	35	1	106
Vincristine Sulfate for Injection—*Identification*	35	1	106
Pure Steam (new)	31	2	467
Water for Hemodialysis—*Bacterial endotoxins*	31	2	468
Water for Injection—*Definition, Bacterial Endotoxins Test, Water Conductivity, Sterility Tests* (add), *Packaging and Storage* (add), *Labeling* (add)	35	2	316
Purified Water—*Definition, Packaging and Storage* (add), *Labeling* (add)	35	2	317
Sterile Water for Inhalation—*pH* (delete), *Ammonia* (delete), *Calcium* (delete), *Carbon dioxide* (delete), *Chloride* (delete), *Sulfate* (delete), *Conductivity* (add), *Oxidizable substances*	31	3	802
Sterile Water for Injection—*Oxidizable substances*	31	3	803
Sterile Water for Irrigation—*Oxidizable substances*	31	3	804
Sterile Purified Water—*Oxidizable substances*	31	3	804
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Zidovudine—*Assay*	34	3	656
Zidovudine Capsules—*Related compounds, Assay*	34	3	657
Zidovudine Injection—*Related compounds, Assay*	34	3	658
Zolpidem Tartrate (new)	34	6	1487
Zonisamide (new)	34	6	1489
Dietary Supplements Monographs			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
N-Acetyltyrosine (new)	35	1	107

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Calcium Citrate Tablets (new)	34	2	312
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Glucosamine Hydrochloride—*Assay*	33	4	691
Glucosamine Sulfate Potassium Chloride—*Assay*	33	4	692
Glucosamine Sulfate Sodium Chloride—*Assay*	33	4	692
Glutamic Acid (new)	34	4	997
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004
Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Powdered Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Omega-3 Acid Triglycerides (new)	34	3	662
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Potassium Citrate Tablets (new)	34	2	313
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Citrate (new)	34	2	315
Zinc Citrate Tablets (new)	34	2	316
Zinc and Vitamin C Lozenges (new)	34	2	317
USP General Test Chapters			
⟨1⟩ Injections—*Ingredients*	34	4	1020
⟨11⟩ USP Reference Standards	29	6	2022
	30	5	1674
	31	2	507
	31	4	1154
	31	6	1680
	32	4	1161
	33	1	95
	33	5	981
	34	2	332
	34	3	680
	34	4	1021
	34	5	1230
	34	6	1531
	35	1	144
	35	2	330
⟨41⟩ Weights and Balances—*Introduction, Repeatability, Verification of Accuracy, Calibration Check*	35	2	331
⟨63⟩ Mycoplasma Tests (new)	35	1	146
⟨85⟩ Bacterial Endotoxins Test—*Harmonization*	33	3	539
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨191⟩ Identification Tests—General—*Introduction*	34	2	333
⟨197⟩ Spectrophotometric Identification Tests (entire chapter revised)	35	1	153
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨223⟩ Dimethylaniline—*Chromatographic System, Procedure*	35	1	156
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨271⟩ Readily Carbonizable Substances Test—*Introduction*	33	6	1258
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨401⟩ Fats and Fixed Oils—*Ester Value, Hydroxyl Value, Iodine Value, Peroxide Value, Saponification Value, Polyunsaturated Fatty Acids Determination and Profile* (add), *Trace Metals* (add), *Sterol Composition* (add)	34	3	736
⟨429⟩ Light Diffraction Measure of Particle Size (new)—*Harmonization*	31	4	1234
⟨467⟩ Organic Volatile Impurities—*Identification, Control, and Quantification of Residual Solvents*	34	3	747
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	35	2	334
⟨525⟩ Sulfur Dioxide—*Method IV* (add), *Method V* (add)	35	2	341

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density—*Harmonization*	31	3	909
⟨621⟩ Chromatography—*System Suitability*	34	5	1238
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨661⟩ Containers—Plastics—*Introduction, Polyethylene Containers, Polypropylene Containers*	34	2	335
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533
⟨671⟩ Containers—Performance Testing—*Introduction, Moisture Permeation, Light Transmission Test*	34	2	337
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	34	2	341
⟨731⟩ Loss on Drying—*Introduction*	34	3	760
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨851⟩ Spectrophotometry and Light-Scattering—(entire chapter revised)	35	1	157
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	35	2	346
⟨941⟩ X-Ray Diffraction (new)—*Harmonization*	31	4	1241
General Information Chapters			
⟨1010⟩ Analytical Data—Interpretation and Treatment—*Prerequisite Laboratory Practices and Principles, Measurement Principles and Variation, Comparison of Analytical Methods, Appendixes B, C, D, E, F*	34	3	764
⟨1033⟩ Biological Assay Validation (new)	35	2	349
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients (entire chapter revised)	34	2	343
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1097⟩ Bulk Powder Sampling Procedures (new)	35	2	367
⟨1113⟩ Microbial Identification (new)	35	1	167
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1180⟩ Human Plasma (new)	35	2	388
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients (new)	34	2	375
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation*	30	5	1729
⟨1225⟩ Validation of Compendial Procedures—*Validation*	35	2	444
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1235⟩ Vaccines for Human Use—General Considerations (new)	34	5	1297
⟨1237⟩ Virology Test Methods (new)	34	2	391
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	35	2	448
⟨1788⟩ Particulate Matter Determination in Parenteral and Ophthalmic Products (new)	34	2	421

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Reagents, Indicators, and Solutions			
Reagents, Indicators, and Solutions—*Introduction*	35	1	176
Acetylactone	34	3	808
Alcohol	35	1	177
Alcohol, Denatured (new)	34	3	808
p-Aminophenol	34	2	442
Ammonium Molybdate	35	1	177
Barium Chloride	34	2	442
Beclomethasone (new)	34	3	808
1-Butanesulfonic Acid Sodium Salt (new)	33	4	766
Calcium Chloride	34	3	808
Chloramine T	34	2	442
Chromotropic Acid	35	1	177
Chromotropic Acid Disodium Salt	35	1	177
Diatomaceous Earth (new)	34	3	809
2,7-Dihydroxynaphthalene (new)	34	3	809
N,N-Dimethyldecylamine (new)	34	4	1041
Dimethyltin Dibromide (new)	34	2	442
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Ethylenediamine (new)	34	2	442
Ferric Chloride	34	2	443
Heptyl *p*-Hydroxybenzoate (new)	35	2	460
Hydrogen Peroxide, 30 Percent	34	2	443
Hydrogen Peroxide, 30 Percent, Unstabilized (new)	34	3	809
Hydrogen Peroxide, 50 Percent in Water (new)	34	3	809
Lead Acetate	34	2	443
Maltotriose (new)	34	3	809
7-Methoxycoumarin (new)	34	2	443
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Methyl Red	35	1	177
Morin (new)	34	2	443
1-Octanol (new)	32	6	1804
Pectate Lysate (new)	35	2	460
Phosphatase Enzyme, Alkaline	34	3	809
Phosphorous Acid (new)	35	1	178
Potassium Metabisulfite (new)	35	1	178
Potassium Sodium Tartrate	35	1	178
Silver Nitrate	34	3	810
Sodium Acetate	35	2	461
Sodium Biphenyl	35	1	178
Sodium Cholate Hydrate (new)	34	3	810
Sodium 1-Decanesulfonate	34	5	1319
Sorbitol (new)	34	3	810
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Tetrabutylammonium Hydroxide 30-Hydrate (new)	34	3	810
Tetrabutylammonium Hydroxide, 40 Percent in Water (delete)	34	3	810
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled	34	3	810
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled	34	3	811
Triethylenediamine (new)	34	2	443
Trimethyltin Bromide (new)	34	2	444
Test Solutions			
Acetic Acid, Glacial, TS	35	1	179
Alcoholic TS (new)	34	3	811
Denatured Alcoholic TS (new)	35	1	179
Ammonia TS 2 (new)	34	2	444
Cupric Citrate TS 2, Alkaline	35	1	179
Iodine and Potassium Iodide TS 3 (new)	34	2	444
Lanthanum Nitrate TS (new)	34	2	444
Methyl Red TS 2 (new)	34	2	445
Potasssium Pyroantimonate TS	34	3	812
Volumetric Solutions			
Hydrochloric Acid, Normal (1 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N) in Methanol	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid	35	1	181
Potassium Iodate, Twentieth-Molar (0.05 M)	34	3	813

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Sodium Tetraphenylboron, Fiftieth-Molar (0.02 M)	34	2	447
Chromatographc Reagents			
Chromatographic Reagents—*Title, Packings*	35	1	182
Reference Tables			
Container Specifications for Capsules and Tablets	35	2	462
Description and Solubility	29	1	266
	31	2	591
	31	4	1193
	33	5	1053
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
	35	1	188
	35	2	464
Atomic Weights—*Standard Atomic Weights of the Elements*	35	1	189
Excipients			
USP and *NF* Excipients, Listed by Category	35	2	318
NF General Notices and Requirements—*Title* (delete), ''*Official*'' *and* ''*Official Articles*'' (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
NF Monographs			
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
Alpha-Lactalbumin (new)	34	3	670
Amino Methacrylate Copolymer—*Definition, Packaging and storage, Viscosity, Limit of monomers*	34	2	326
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Harmonization*	33	3	537
Silicified Microcrystalline Cellulose (new)	34	5	1218
Chitosan (new)	35	1	115
Hydrogenated Coconut Oil (new)	34	2	327
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
High Fructose Corn Syrup—*Total solids, Assay*	34	2	329
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone—*Harmonization*	28	4	1257
Cystine (new)	35	1	122
Desoxycholic Acid (new)	34	6	1523
Egg Phospholipids (new)	33	4	703
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity, Coagulum content*	35	1	123

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ethyl Maltol (new)	34	5	1224
Ethylene Glycol and Vinyl Alcohol Graft Copolymer (new)	35	2	324
Ethylparaben—*Harmonization*	34	6	1594
Hydrogenated Palm Oil (new)	34	2	330
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Hydroxypropyl Cellulose—*Identification*	35	1	124
Low-Substituted Hydroxypropyl Cellulose—*Harmonization*	30	1	338
Anhydrous Lactose—*Harmonization*	32	6	1847
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Viscosity, Limit of monomers, Coagulum content*	35	1	124
Methyl Alcohol—*Readily carbonizable substances*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Definition, Packaging and storage, Assay*	31	4	1145
Nitrogen 97 Percent—*Definition, Packaging and storage, Assay*	31	4	1146
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Olive Oil—*CAS number* (add), *Definition, Packaging and storage, Identification* (add), *Fatty acid composition* (add), *Specific gravity* (delete), *Cottonseed oil* (delete), *Peanut oil* (delete), *Sesame oil* (delete), *Teaseed oil* (delete), *Absence of sesame oil* (add), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Specific absorbance* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add)	35	1	126
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete) *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227
Polyethylene Glycol—*Harmonization*	31	3	897
Polyoxyl 15 Hydroxystearate (new)	35	1	128
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polysorbate 80—*Harmonization*	33	5	1075
Polyvinyl Acetate (new)	34	6	1526
Polyvinyl Acetate Dispersion (new)	35	1	134
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Hydrogenated Starch Hydrolysate (new)	35	1	136
Pea Starch (new)	35	1	140
Rice Starch (new)—*Harmonization*	30	2	721
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Sucrose Palmitate (new)	35	2	326
Sucrose Stearate (new)	35	2	328
Tagatose (new)	30	5	1672
Tetrafluoroethane (new)	31	6	1672

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	***PF Volume, Issue, and Page Numbers of Canceled Proposals*** ***Vol.***	***No.***	***Page(s)***
USP Monographs			
Carvedilol Tablets—*Title* (add), Definition (add), *Packaging and storage* (add), *USP Reference standards* (add),*Identification* (add), *Uniformity of dosage units* (add),*Related compounds* (add), *Assay* (add)	33	5	888
Conjugated Estrogens—*Definition*	30	3	840
Desogestrel and Ethinyl Estradiol Tablets—*Related compounds*	30	5	1604
Estradiol Vaginal Inserts—*Dissolution*	31	6	1617
Flavoxate Hydrochloride Tablets—*Dissolution* (add)	33	6	1174
Hydrocodone Bitartrate and Homatropine Methylbromide Tablets—*Dissolution*	30	3	853
Ketoprofen Extended-Release Capsules—*Drug release*	31	5	1378
Leflunomide Tablets—*Dissolution*	31	5	1383
Mirtazapine Orally Disintegrating Tablets—*Water, Method Ia* (add)	33	6	1189
Norethindrone Tablets—*Dissolution* (add)	32	6	1736
Norethindrone Tablets—*Dissolution* (add)	33	6	1193
Promethazine Hydrochloride—*USP Reference standards, Related substances*	32	2	365
Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add), *Assay*	32	2	367
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add)	32	4	1107
Dietary Supplements			
Asian Ginseng Capsules (entire submission)	30	2	571
USP General Test Chapters			
<191> Identification Tests—General—*Acetate, Ammonium*	33	4	719
USP General Information Chapters			
<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire submission)	31	2	524
†<1024> Bovine Serum (entire submission)	34	3	776
NF Monographs			
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
Sucralose—*Related compounds*	33	6	1255

† New cancellation in *PF* 35(3).

STAGE 4 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (***Stages 1*** through ***7***, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF,* this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

MONOGRAPHS (NF)

BRIEFING

Crospovidone. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Crospovidone monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the revised OFFICIAL INQUIRY STAGE 4 document, is based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia. Differences between this OFFICIAL INQUIRY STAGE 4 document and the current USP monograph include the following.

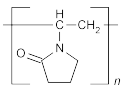
1. *Definition*. Amended to include Type A or Type B, determined by particle size.
2. *Identification*. New test added to distinguish between Type A and Type B using wet sieving.
3. *Residue on Ignition*. New specification proposed (NMT 0.1%).
4. *Peroxides*. New test proposed by EP.
5. *Vinylpyrrolidone*. Replaced titration assay with more sensitive HPLC assay, consistent with other Povidone monographs.
6. *Loss on Drying*. New test to replace *Water Determination*.
7. *Labeling*. Included requirement to label Type A or Type B.

The LC procedure performed for the *Vinylpyrrolidone* test is based on analyses performed by the Nucleosil 120-5 brand of precolumn and Aquasil brand of column containing packing L1.

(EM2: K. Moore.) RTS—C47365

Add the following:

Crospovidone



$(C_6H_9NO)_n$ $(111.1)_n$

1-Ethenyl-2-pyrrolidinone homopolymer;
1-Vinyl-2-pyrrolidinone homopolymer [9003-39-8].

DEFINITION

Crospovidone is the cross-linked homopolymer of 1-ethenylpyrrolidin-2-one. Content: 11.0%–12.8% of nitrogen (N; A_r 14.01) (dried substance). Two types of crospovidone are available, depending on the particle size: Type A and Type B.

IDENTIFICATION

- **A.** Suspend 1 g in 10 mL of water, add 0.1 mL of 0.1 N iodine, and shake for 30 s. Add 1 mL of starch TS, and shake: no blue color develops within 30 s.
- **B.** To 10 mL of water, add 0.1 g and shake. A suspension is formed and no clear solution is obtained within 15 min.
- **C. PROCEDURE**

Analysis: [NOTE—The analytical sieves must be clean and dry. To this purpose the sieves are washed in hot water and allowed to dry overnight in a drying cabinet at 105 °C.] Place 20 g calculated on the dried substance in a 1000-mL conical flask, add 500 mL of water, and shake the suspension for 30 min. Pour the suspension through a 63-µm analytical sieve, previously tared, and rinse the sieve with water until the filtrate is clear. Dry the sieve and sample residue at 105 °C for 5 h in a drying cabinet without circulating air. Cool in a desiccator for 30 min, and weigh. Calculate the sieving residue fraction of sample particles having a diameter of more than 63 µm, in percentage, using the following expression:

$$\text{Result} = (m_1 - m_3 \times 100)/(m_2)$$

m_1 = mass of the sieve and sample residue, after drying for 5 h (g)
m_3 = mass of the sieve (g)
m_2 = initial mass of the sample, calculated on a dried basis (g)

Acceptance criteria: If the sieving residue fraction is more than 15%, the substance is classified as Type A; if the sieving residue fraction is NMT 15%, the substance is classified as Type B.

ASSAY

- **PROCEDURE**

Analysis: Place 0.100 g in a combustion flask and add 5 g of a mixture of 1 g of cupric sulfate, 1 g of titanium dioxide, 33 g of potassium sulfate, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to run down the inside wall of the flask. Gradually heat the flask until the solution has a clear, yellowish-green colour, and the inside wall of the flask is free from carbonized material, and then heat for a further 45 min. After cooling, cautiously add 20 mL of water, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a 40 g/L solution of boric acid, 3 drops of bromocresol green–methyl red solution TS, and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of strong sodium hydroxide solution through a funnel, cautiously rinse the funnel with 10 mL of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to obtain 80–100 mL of distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate the distillate with 0.025 M sulfuric acid until the color of the solution changes from green through pale greyish-blue to pale greyish red-purple. Carry out a blank determination and make any necessary correction. 1 mL of 0.025 M sulfuric acid is equivalent to 0.700 mg of N.
Acceptance criteria: 11.0%–12.8%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%, a 1-g specimen being used

Organic Impurities

- **PROCEDURE 1: PEROXIDES**

Sample suspension A: [NOTE—Use for Type A.] 40 mg/mL in water. To 25 mL of this suspension add 2 mL of titanium trichloride–sulfuric acid TS. Allow to stand for 30 min and filter.
Sample suspension B: [NOTE—Use for Type B.] 16 mg/mL in water. To 25 mL of this suspension add 2 mL of titanium trichloride–sulfuric acid TS. Allow to stand for 30 min and filter.
Compensation liquid A: [NOTE—Use for Type A.] 40 mg/mL in water. Filter, take 25 mL, and add 2 mL of a 13% solution of sulfuric acid.
Compensation liquid B: [NOTE—Use for Type B.] 16 mg/mL in water. Filter, take 25 mL, and add 2 mL of a 13% solution of sulfuric acid.

Analysis: Measure the absorbance of the filtrate at 405 nm against the appropriate compensation liquid.
Acceptance criteria: NMT 0.35. For Type A, this corresponds to NMT 400 ppm expressed as H_2O_2; For Type B, this corresponds to NMT 1000 ppm expressed as H_2O_2.

- **PROCEDURE 2: VINYLPYRROLIDINONE**

Mobile phase: 1:9 Acetonitrile:water
Sample solution: 25 mg/mL suspension in methanol. Shake for 60 min. Leave the bulk to settle, and pass through a 0.2-µm filter.
Reference stock solution A: 5 µg/mL of vinylpyrrolidinone in methanol
Reference stock solution B: 100 µg/mL of vinylpyrrolidinone and 5 mg/mL of vinyl acetate in methanol
Reference solution A: A 1 in 20 solution of *Reference stock solution A* with *Mobile phase*
Reference solution B: A 1 in 100 solution of *Reference stock solution B* with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV
Detection wavelength: 235 nm
Precolumn[1]: L1, 4-mm × 2.5-cm, 5 µm
Column[2]: L1, 4-mm × 25-cm, 5 µm
Temperature: 40°
Flow rate: 1 mL/min
Injection size: 50 µL. After each injection of the *Sample solution*, wash the precolumn by passing the *Mobile phase* backwards, at the same flow rate as applied in the test, for 30 min.
System suitability
Samples: *Reference solution A* and *Reference solution B*
Suitability requirements
Resolution: NLT 2.0 between vinylpyrrolidone and vinyl acetate, *Reference solution B*
Relative standard deviation: NMT 2.0% on 6 injections of *Reference solution A*
Analysis
Sample: *Sample solution*
Acceptance criteria: The area of the peak in the *Sample solution* is NMT the area of the principal peak in the chromatogram obtained with *Reference solution A.* NMT 10 ppm.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 0.500 g at 105° to constant weight.

Acceptance criteria: NMT 5.0% of its weight

- **WATER–SOLUBLE SUBSTANCES**

Analysis: Transfer 25.0 g of Crospovidone to a 400-mL beaker, add 200 mL of water, and stir on a magnetic stirrer, using a 5-cm stirring bar, for 1 h. Transfer to a 250-mL volumetric flask with the aid of 25 mL of water. Add water to volume. Allow the bulk of the solids to settle. Pass 100 mL of the relatively clear supernatant through a membrane filter having a 0.45-µm porosity, protected against clogging by superimposing a membrane filter having a 3-µm porosity. While filtering, stir the solution above the filter manually or with a mechanical stirrer, taking care not to physically damage the membrane filter. Transfer 50.0 mL of the clear filtrate to a tared 100-mL beaker, evaporate to dryness, and dry at 110° for 3 h.
Acceptance criteria: The weight of the residue does not exceed 75 mg (1.5%).

ADDITIONAL REQUIREMENTS

- **LABELING:** The label states the type (Type A or Type B).

[1]Nucleosil 120-5 C18 from Macherey & Nagel is sufficient.
[2]Aquasil C18 from ThermoHypersil is sufficient.

BRIEFING

Hydroxypropyl Cellulose. The United States Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Hydroxypropyl Cellulose monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the revised **OFFICIAL INQUIRY STAGE 4** document, is based in part on comments from the Japanese Pharmacopoeia and the European Pharmacopeia, in response to the **OFFICIAL INQUIRY STAGE 4** draft, prepared by the United States Pharmacopoeia, and published in *PF* 30(1) [Jan.–Feb. 2004]. Differences between this **OFFICIAL INQUIRY STAGE 4** document and the previous draft include a new assay method, where a determination of the molar substitution has been added based on extensive validation work and comparative studies with the existing titration method. This assay applies the general principle for determination of alkoxy groups in substituted celluloses (Zeissel-reaction followed by gas chromatography) which has been presented by the Japanese Pharmaceutical Excipients Council (JPEC) for Hydroxypropylcellulose and Hydroxypropylcellulose, Low Substituted. This method replaces the existing titration method which required the highly toxic reagent chromic acid.

(EM2: K. Moore.) RTS—C51136

Add the following:

Hydroxypropyl Cellulose

Cellulose, 2-hydroxypropyl ether [9004-64-21].

DEFINITION
Hydroxypropyl Cellulose is a partially substituted poly(hydroxypropyl) ether of cellulose. It contains NLT 53.4% and NMT 80.5% of hydroxypropoxy groups, calculated on the dried basis. It may contain suitable anticaking agents.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197**⟩
- **B. PROCEDURE**

Sample solution: 10 mg/mL Hydroxypropyl Cellulose
Analysis: Transfer 10 mL of *Sample solution* to a suitable container. Heat the solution to 45°.
Acceptance criteria: The solution becomes cloudy or a flocculent precipitate is formed, and the turbidity or precipitate disappears on cooling.

- **C. PROCEDURE**

Sample solution: 1 mL of *Sample solution* from *Identification* test *B*
Analysis: Transfer the *Sample solution* to a glass plate, and allow the water to evaporate.
Acceptance criteria: A thin, self-sustaining film is formed.

ASSAY
[NOTE—Determination of hydroxypropoxy groups in Hydroxypropyl Cellulose]

- **PROCEDURE**

Internal standard solution: Methylcyclohexane in *o*-xylene (1 in 50)
Standard solution: Add 60 mg of adipic acid, 2.0 mL of *Internal standard solution*, and 1.0 mL of hydroiodic acid, stopper the vial tightly, and weigh. Add 25 µL of isopropyl iodide for assay, and again weigh. Shake the reaction vial for 30 s, and use the upper layer of the content as the *Standard solution*.

Sample solution: Weigh 30 mg of hydroxypropyl cellulose previously dried, transfer to the reaction vial, add 60 mg of adipic acid, 2.0 mL of *Internal standard solution*, and 1.0 mL of hydroiodic acid, stopper the vial tightly, and weigh. Shake the vial for 30 s, heat at 115° in a heater capable of maintaining the inside temperature within ±1° for 70 min with continuous shaking. Allow the vial to cool, and weigh. If the loss is less than 5 mg, use the upper layer of the mixture as the *Sample solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: Fused silica, 0.53-mm × 30-m; bonded with 3-µm layer of G1. [NOTE—Use a guard column if necessary.]
Temperature: See the Temperature Program Table below.

Temperature Program Table

	Time (min)	Temperature (°)
Detector		280
Column	0	40
	3	40
	9	100
	12	250
	15	250
Injection port		180

Flow rate: Adjust the flow rate so the retention time of the internal standard is about 8 min.
Carrier gas: Helium
Split ratio: 1:50
Injection size: 1 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between isopropyl iodide and methylcyclohexane
Relative standard deviation: NMT 2.0% based on 6 injections

Analysis
Sample: *Sample solution*
[NOTE—Measure all of the peak areas, excluding the solvent peak.]
Calculate the relative response factor (RRF) from the following expression:

$$\text{Result} = (A_1 \times W_1 \times C)/(A_2 \times 100)$$

A_1 = peak area of the internal standard peak from the *Standard solution*
W_1 = weight of isopropyl iodide in the *Standard solution* (mg)
C = percentage content of isopropyl iodide from the certificate of the manufacturer
A_2 = peak area of the isopropyl iodide peak from the *Standard solution*

Calculate the percentage content (w/w) of the hydroxypropoxy group in the portion of Hydroxypropyl Cellulose taken:

$$\text{Result} = (F \times A_3 \times \text{RRF} \times M_1 \times 100)/(A_4 \times W_2 \times M_2)$$

F = correction factor, 1.1 [NOTE—The correction factor is used to correlate the accuracy of the method with historical values for the previous titration assay. This value was determined based on data from the validation robustness studies.]
A_3 = peak area of isopropyl iodide peak from the *Sample solution*
RRF = relative response factor calculated from above
M_1 = molar mass of hydroxypropoxy group, 75.1
A_4 = peak area of the internal standard peak from the *Sample solution*
W_2 = weight of the sample in the *Sample solution* (mg)
M_2 = molar mass of isopropyl iodide, 170.0

Acceptance criteria: 53.4%–80.5%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.8%
- **HEAVY METALS,** *Method III* ⟨**231**⟩**:** NMT 20 ppm

SPECIFIC TESTS
- **pH** ⟨**791**⟩**:** 1% solution
  Acceptance criteria: 5.0–8.0
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry at 105° for 4 h.
  Acceptance criteria: NMT 5.0% of its weight
- **VISCOSITY** ⟨**911**⟩**:** Use an LV-type rotational viscometer with the spindle and speed combination for materials with labeled viscosity of 75 cp or higher.
  Acceptance criteria: 50%–150% of the label claim

ADDITIONAL REQUIREMENTS
- **LABELING:** Label it to indicate the normal viscosity in an aqueous solution of stated concentration and temperature. Suitable anticaking agents should be stated on the label.
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Hydroxypropyl Cellulose RS

BRIEFING

Hydroxypropyl Cellulose, Low Substituted. The United States Pharmacopeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Hydroxypropyl Cellulose, Low Substituted monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the revised **OFFICIAL INQUIRY STAGE 4** document, is based in part on comments from the Japanese Pharmacopoeia and the European Pharmacopoeia, in response to the **OFFICIAL INQUIRY STAGE 4** draft, prepared by the United States Pharmacopeia, and published on page 339 of *PF* 30(1) [Jan.–Feb. 2004]. Differences between this **OFFICIAL INQUIRY STAGE 4** document and the previous draft include a new assay method, in which a determination of the molar substitution has been added, based on extensive validation work and comparative studies with the existing titration method. This assay applies the general principle for determination of alkoxy groups in substituted celluloses (Zeissel-reaction followed by gas chromatography), which has been presented by the Japanese Pharmaceutical Excipients Council (JPEC) for Hydroxypropylcellulose and Hydroxypropylcellulose, Low Substituted. This method replaces the existing gas chromatography method to align with the new method proposed for Hydroxypropyl Cellulose.

(EM2: K. Moore.) RTS—C72689

Add the following:

Hydroxypropyl Cellulose, Low Substituted

Cellulose, 2-hydroxypropyl ether [9004-64-21].

DEFINITION

Hydroxypropyl Cellulose, Low Substituted is a low-substituted poly(hydroxypropyl) ether of cellulose. It contains NLT 5.0% and NMT 16.0% of hydroxypropoxy groups, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197⟩**
- **B. PROCEDURE**

Sample solution: 10 mg/mL Hydroxypropyl Cellulose, Low Substituted. Add 1 g of sodium hydroxide, and shake until it becomes homogeneous.

Analysis: Transfer 5 mL of *Sample solution* to a suitable container, add 10 mL of a mixture of acetone and methanol (4:1), and shake.

Acceptance criteria: A white, flocculent precipitate is formed.

ASSAY

[NOTE—Determination of hydroxypropoxy groups in Hydroxypropyl Cellulose, Low Substituted]

- **PROCEDURE**

Internal standard solution: Methylcyclohexane in *o*-xylene (1 in 50)

Sample solution: Weigh accurately 30 mg of previously dried Hydroxypropyl Cellulose, Low Substituted, transfer to the reaction vial, add 60 mg of adipic acid, 2.0 mL of *Internal standard solution*, and 1.0 mL of hydroiodic acid, stopper the vial tightly, and weigh accurately. Shake the vial for 30 s, and heat at 115° in a heater capable of maintaining the inside temperature within ± 1° for 70 min with continuous shaking. Allow the vial to cool, and weigh accurately. If the loss is less than 5 mg, use the upper layer of the mixture as the *Sample solution*.

Standard solution: Add 60 mg of adipic acid, 2.0 mL of *Internal standard solution*, and 1.0 mL of hydroiodic acid, stopper the vial tightly, and weigh accurately. Add 25 µL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction vial for 30 s, and use the upper layer of the content as the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: Fused silica, 0.53-mm × 30-m; bonded with a 3-µm layer of G1. [NOTE—Use a guard column if necessary.]

Temperature: See the *Temperature Program Table* below.

Temperature Program Table

	Time (min)	Temperature (°)
Detector		280
Column	0	40
	3	40
	9	100
	12	250
	15	250
Injection port		180

Flow rate: Adjust the flow rate so the retention time of the internal standard is about 8 min.

Carrier gas: Helium

Split ratio: 1:50

Injection size: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between isopropyl iodide and methylcyclohexane

Relative standard deviation: NMT 2.0% based on 6 injections

Analysis

Sample: *Sample solution*

[NOTE—Measure all of the peak areas, excluding the solvent peak.]

Calculate the response factor (F) from the following expression:

$$\text{Result} = (A_1 \times W_1 \times C)/(A_2 \times 100)$$

A_1 = peak area of the internal standard peak of the *Standard solution*

W_1 = weight of isopropyl iodide in the *Standard solution* (mg)

C = percentage content of isopropyl iodide from the certificate of the manufacturer

A_2 = peak area of the isopropyl iodide peak of the *Standard solution*

Calculate the percentage content (w/w) of the hydroxypropoxy group in the portion of Hydroxypropyl Cellulose, Low Substituted taken:

$$\text{Result} = (A_3 \times F \times M_1 \times 100)/(A_4 \times W_2 \times M_2)$$

A_3 = peak area of the isopropyl iodide peak of the *Sample solution*

F = response factor calculated above

M_1 = molar mass of hydroxypropoxy group, 75.1

A_4 = peak area of the internal standard peak of the *Sample solution*

W_2 = weight of the sample in the *Sample solution* (mg)

M_2 = molar mass of isopropyl iodide, 170.0

Acceptance criteria: 5.0%–16.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.8%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

SPECIFIC TESTS

- **PH ⟨791⟩:** 1% solution

Acceptance criteria 5.0–7.5

- **LOSS ON DRYING ⟨731⟩:** Dry at 105° for 4 h.

Acceptance criteria: NMT 5.0% of its weight

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Hydroxypropyl Cellulose, Low Substituted RS

GENERAL CHAPTERS

Physical Tests and Determinations

BRIEFING

⟨696⟩ Characterization of Crystallinity Determination by Solution Calorimetry. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for general chapter ⟨696⟩ *Characterization of Crystallinity Determination by Solution Calorimetry,* as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following document, which represents the revised **OFFICIAL INQUIRY STAGE 4** document, is based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia. Readers are urged to review these proposals carefully and to respond to USP no later than July 31, 2009.

(GC: K. Zaidi; K. Moore) RTS—C70392

Add the following:

⟨696⟩ CHARACTERIZATION OF CRYSTALLINITY DETERMINATION BY SOLUTION CALORIMETRY

INTRODUCTION—THE CONCEPT OF CRYSTALLINITY

For the purpose of this chapter, crystalline material, partially crystalline material, and amorphous material are considered as solids.

The perfectly ordered crystal lattice with every molecule in its expected lattice position is an ideal that is seldom, if ever, achieved. The other extreme is the amorphous state, in which a solid contains the maximum possible density of imperfections (defects of various dimensionalities), such that all long-range order is lost while only the short-range order, imposed by its nearest neighbors, remains. Real crystals lie somewhere between these two extremes. A crystal's position on a scale bounded by these two extremes is termed *crystallinity.*

All real crystals, even in the pure state, possess some lattice imperfections or defects, which increase both the energy (enthalpy under conditions of constant atmospheric pressure) and the disorder (expressed as the entropy) of the crystal lattice. A crystal with a relatively small density of imperfections is said to be highly crystalline and to possess a high crystallinity. By contrast, a particle with a relatively high density of imperfections is said to be partially amorphous and to possess a low crystallinity. A totally amorphous particle corresponds to zero crystallinity. Amorphous particles may contain somewhat ordered domains that can act as nuclei for crystallization; such so-called amorphous particles are said to possess a low-level but finite crystallinity.

In reality, a powder probably contains particles with different degrees of crystallinity, just as it may contain particles with different sizes and shapes. The lower the crystallinity of a particle, the greater its enthalpy and entropy. The increase in enthalpy is never totally compensated by the increase in entropy; instead, the Gibbs free energy, which reflects the balance between them, actually increases. Hence, the lower the crystallinity of a particle, and consequently the greater its amorphous character, the greater its apparent intrinsic solubility, dissolution rate, and reactivity, but the lower its stability. Because of the great relevance of these properties, crystallinity is also an important property and requires measurement by a suitable method.

In the following chapter, the crystallinity or the content of amorphous parts of a powder are measured by calorimetric methods such as microcalorimetry or solution calorimetry, although other methods could be used (e.g., see general chapter ⟨941⟩ *Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction*).

Many substances are capable of crystallizing in more than one type of crystal lattice, which is known as polymorphism. If water or a solvent is incorporated in the crystal lattice the crystals are termed hydrates or solvates. Because of the different crystal packaging, and/or molecular conformation and lattice energy, they usually exhibit different physical properties. The control of the crystal form has, therefore, to be taken into account for the characterization of crystallinity.

DETERMINING AMORPHOUS CONTENT BY MICROCALORIMETRY

Most chemical, physical, and biological processes are associated with the exchange of heat. Microcalorimetry is a highly sensitive technique to monitor and quantify both exothermic (heat producing) and endothermic (heat absorbing) changes associated with those processes. The technique allows to determine the rate and extent of chemical reactions, changes of phase, or changes of structure.

Thermal events producing only a fraction of a microwatt can be observed using microcalorimetry. This means that temperature differences less than 10^{-6} K must be detectable. Microcalorimetry typically uses the heat flow (heat leakage) principle, where the heat produced (or absorbed) in a thermally defined vessel flows away (or into) in an effort to re-establish thermal equilibrium with its surroundings. Exceptional thermal stability with its surrounding has to be achieved either by a heat sink or an electronically regulated surrounding.

Heat energy from an active sample in the reaction vessel is channeled typically through Peltier elements; they act as thermoelectric generators using the Seebeck effect. The heat energy is converted into a voltage signal proportional to the heat flow.

Results are typically presented as a measure of the thermal energy produced per unit of time (Watt) as a function of time.

Apparatus

Microcalorimeters are typically designed as twin systems with a measuring vessel and a reference vessel. Vessels are typically made of glass or stainless steel. For certain applications specially designed vessels which allow the addition of a gas, a liquid, or a solid material may be used.

Calibration

The microcalorimeter is calibrated for heat flow (energy per time unit) using either a calibrated external or internal electrical heat source or a suitable standard reaction.

Sensitivity

The sensitivity of the microcalorimetric method can be assessed based on the stability of the instrument baseline noise or by an appropriate standard sample analyzed according to the corresponding method.

Procedure

Weigh in a suitable vessel an appropriate quantity of the substance to be examined. The substance can be a solid, a liquid, or a semi-solid. Close the vessel carefully, to avoid any evaporation of solvents, and place the vessel in the sample holder. If appropriate, allow the vessel to equilibrate at the temperature of the measurement before placing it in the measuring position.

Begin the analysis and record the heat flow, with the time on the abscissa and the heat flow on the ordinate (specify the direction of exothermic or endothermic heat flow).

Applications of Microcalorimetry

Detection and quantification of amorphous content in powders—The ability to detect and to quantify the amount of amorphous material within a highly crystalline substance is of great importance during the development and subsequent manufacture of a preparation. Because the amorphous state is metastable with respect to the crystalline state, recrystallization is likely to occur. The measurement of the heat of recrystallization enables the amorphous content to be determined by the area of the recrystallization peak. By relating the output from the microcalorimeter for a sample to that obtained from an amorphous standard, it is possible to quantify the amorphous content of the sample. The range of amorphous content covered by this method depends on the individual substance to be tested, in favorable cases limits of detection below 1% can be reached.

Recrystallization can be initiated by subjecting the sample to higher relative humidity or an atmosphere containing organic vapor. The sample is typically placed in an ampul which also contains a small test tube containing a saturated salt solution or an organic solvent (pure or saturated with salt) or a solvent mixture.

The heat of recrystallization is typically measured using a fixed sample mass placed in a glass or steel vessel. The test tube containing a saturated salt solution or an organic solvent is chosen large enough to allow a full saturation of the atmosphere above the sample. The mass of the sample and the nature of the vapor atmosphere above the sample is chosen so that recrystallization occurs in such a way that a distinct peak is observed, clearly separated from initial thermal events caused by introduction of the sample.

The conditions under which the transition of the amorphous phase to a thermodynamically more stable crystalline state occurs will have a significant impact on the time of recrystallization. In particular, physical mixtures of purely amorphous and crystalline material will behave differently from a partially crystalline material. These effects have to be considered when developing a method.

A typical response for the recrystallization of a partially amorphous material is shown in *Figure 1*. The first part of the curve represents a clear response caused by different processes taking place simultaneously, such as the absorption of water vapor into the amorphous parts of the powder by the generation of water vapor from the test tube. After this initial response there is a large exothermic response caused by the recrystallization of the amorphous material. Also included, but not seen, is the expulsion of excess water from the recrystallized parts. Thus, the area under this exothermic recrystallization response is proportional to the heat of recrystallization.

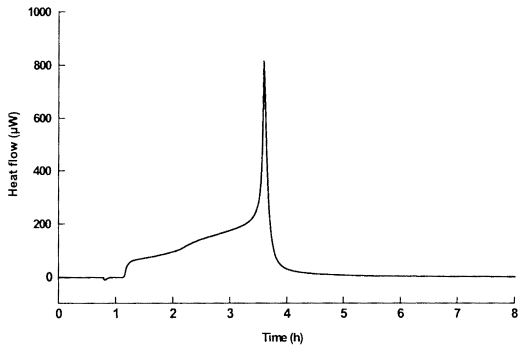


Figure 1. Typical microcalorimetric output of heat flow (in uW) as a function of time (in hours) for partially amorphous lactose at 25 degrees and 57% relative humidity.

DETERMINING CRYSTALLINITY BY SOLUTION CALORIMETRY

Solution calorimetry provides a means of determining enthalpy of solution (i.e., heat of solution under constant atmospheric pressure) of a substance. Enthalpy of solution is defined as the enthalpy of the substance dissolved in the solution to a defined concentration minus the enthalpy of the original substance. The solvent for the dissolution process must be such that the mass of solid

dissolves within a time frame that matches the response time of the calorimeter, as discussed below. The enthalpy of solution is proportional to the amount of solid being dissolved. This amount may be defined as one mole for molar enthalpy or as one gram for specific enthalpy. If the substance is pure and if its molecular mass is known, the molar enthalpy is preferred, otherwise the specific enthalpy must be used. The enthalpy of solution is weakly dependent on both the temperature, which is usually 25.0°, and the final concentration of the dissolved solute.

The crystallinity of the sample under study is given by the enthalpy of solution of the sample ΔH^s_x, minus the enthalpy of solution of the chosen reference standard of the same substance, ΔH^s_R, when determined under the same conditions. Because the reference standard is usually chosen for its perceived high crystallinity, its enthalpy of solution usually is algebraically greater (more endothermic or less exothermic) than that of the sample under study in the same solvent. Consequently, the crystallinity so determined is a negative quantity with the international system units, kJ/mol or J/g (J/kg is avoided because of its unwieldiness and potential for error). The preference for a negative value with respect to a highly crystalline reference standard recognizes the fact that most samples have a lower crystallinity than this reference standard.

A number of substances, including some purified by freeze-drying, may be available in an amorphous form but not in a crystalline form. With such substances, an amorphous form, prepared according to a standard procedure, may be used as the reference standard. The enthalpy of solution may then be algebraically smaller than that of the chosen amorphous reference standard, in which case the crystallinity, as defined above, has a positive value.

The use of a single reference standard for each substance provides a single crystallinity scale, expressed in terms of energy, for each substance and recognizes that each active substance or excipient has unique properties. Furthermore, the crystallinity can be recalculated (if the original reference standard is later replaced by a more crystalline or more amorphous reference standard) because enthalpies of solution are additive quantities according to Hess's Law of constant heat summation.

Because the enthalpy of solution depends not only on the crystallinity of the solid but also on the various other solute–solute interactions and on the solute–solvent and solvent–solvent interactions, a zero value for the enthalpy of solution does not necessarily indicate zero crystallinity of the solid solute, nor does a measurable response necessarily confirm some degree of crystallinity.

It is sometimes preferred to express the crystallinity, P_c, of a substance on a percentage scale. This procedure requires two reference standards, namely a highly crystalline sample assuming 100% crystallinity and having a measured enthalpy of solution of ΔH^s_c, and an essentially amorphous sample assuming 0% crystallinity and having a measured enthalpy of solution of ΔH^s_a. From these values and from the measured enthalpy, ΔH^s_s, of solution of the solid under study, the percentage crystallinity of the solid, P_c, may be calculated as follows:

$$P_c\,(\%) = 100(\Delta H^s_s - \Delta H^s_a)/(\Delta H^s_c - \Delta H^s_a)$$

Clearly, crystallinity expressed on a percentage scale depends on three, not two, measured values and the enthalpies of solution may be replaced by other corresponding physical quantities that depend on crystallinity. The value of the percentage crystallinity of a sample, however, depends not only on the nature and method of preparation of the two reference standards, but also on the choice of the physical quantity that is measured.

The enthalpy of solution is measured either by an isoperibol (constant perimeter, i.e., jacket) solution calorimeter or by an isothermal (constant temperature) solution calorimeter. At least three measurements are made with

each sample until the measured values of the heat of solution do not differ by more than 5%. The mean of these three values is then calculated.

Isoperibol Solution Calorimetry

In the isoperibol solution calorimeter, the heat change during the solution process causes a corresponding change in temperature of the solvent–solute system (i.e., solution). This temperature change is measured by a temperature sensor, which is wired to an electrical circuit that records an electrical signal corresponding to the temperature change. Typically, this temperature change in an electronic form is measured at precisely defined time intervals to produce temperature–time data that are collected, analyzed by a computer, and then plotted. A blank run without addition of the solid solute to the solvent normally shows no discernible change in the slope of the temperature–time plot.

For isoperibol solution calorimeters, response is relatively rapid, but any heat losses to or heat gains from the bath reduce the accuracy and contribute to noise. Therefore, isoperibol solution calorimeters are more advantageous than isothermal solution calorimeters when the solution process is relatively fast. For all measurements of enthalpy of solution using isoperibol solution calorimeters, the choice of solvent and solid is critical. The nature and mass of the solvent and the mass of sample allow the total heat change, corresponding to total dissolution of the solid, to proceed to completion within 10 minutes under vigorous stirring at a constant rotational speed within the range of 400 to 600 revolutions per minute. The rotational speed is checked with a stroboscope.

Isothermal Solution Calorimetry

In the isothermal (constant temperature) solution calorimeter, the heat change during the solution process is compensated for by an equal but opposite energy change, such that the temperature of the solvent–solute system (i.e., solution) remains constant. This equal but opposite energy change is measured and, when its sign is reversed, provides the enthalpy of solution. For isothermal calorimeters, response is relatively slow, but the compensation process eliminates the effects of heat losses to or heat gains from the bath. Therefore, isothermal calorimeters are more advantageous than isoperibol calorimetry when the solution process is relatively slow.

Solution Calorimeter Calibration

To ensure the accuracy of the calorimeter, chemical calibrations must be performed daily. For an endothermic solution process, the calibration of the calorimeter is checked by measuring the heat absorbed during the dissolution of potassium chloride in distilled water at 298.15 K (25.0°). The established enthalpy change in this endothermic process is 235.5 J/g (17.56 kJ/mol). For an exothermic solution process, the calorimeter is checked by measuring the heat evolved during the dissolution of 5 g per L of tromethamine [*tris*(hydroxymethyl) aminomethane, THAM] in a 0.1 mol/L aqueous hydrochloric acid solution at 298.15 K (25.0°). The established heat for the aforementioned process is –246.0 J/g (–29.80 kJ/mol).

The effective heat capacity of the calorimeter cell and its contents is determined for every calorimeter run. This determination is accomplished by electrical heating of the contents of the calorimeter cell. The effective heat capacity is determined according to one of two protocols—either by making one determination after ampul breakage or by making one determination before and a second determination after ampul breakage and then averaging the two results. The accuracy and reliability of the electrical heating are established by the accuracy and reliability of the aforementioned chemical calibrations.

Sample Handling

The stability of solids decreases with decreasing crystallinity. In particular, solids of low crystallinity, especially amorphous solids, tend to sorb water vapor from the atmosphere, leading to crystallization and a corresponding gain in crystallinity. For these reasons, anhydrous samples whose crystallinity is to be determined must be stored at zero humidity in sealed chambers containing a desiccant, preferably containing an indicator of effectiveness. If crystallinity–humidity studies are to be carried out, the sample is stored in a sealed chamber containing a saturated salt solution to provide a defined relative humidity at $25.0 \pm 0.1^{\circ}$.

STAGE 6 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (***Stages 1*** through ***7***, as defined below). This section includes Stage 6 adopted text which is provided for information. USP cannot incorporate public comments at Stage 6 without consulting PDG partners. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

MONOGRAPHS (USP)

BRIEFING

Methylcellulose. The Japanese Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Methylcellulose monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Methylcellulose that was prepared by the Japanese Pharmacopoeia. The Japanese Pharmacopoeia draft was based in part on comments from the European Pharmacopoeia and the United States Pharmacopeia in response to the Provisional Harmonized Text Stage 5A and 5B drafts prepared by the Japanese Pharmacopoeia. Differences between the Japanese Pharmacopoeia Adoption Stage 6 document and the current USP monograph for Methylcellulose include the following.

1. *Definition*. Changed from NLT 27.5% and NMT 31.5% of methoxy to NLT 26.0% and NMT 33.0% of methoxy groups. Drying time changed from 2 h to 1 h.
2. *Identification*. Replaced three existing methods with five new methods.
3. *Assay*. Aligned method with current PDG harmonized monograph for Hypromellose.
4. *Heavy Metals*. Aligned method with current PDG harmonized monograph for Hypromellose.
5. *Loss on Drying*. Changed time from 2 h to 1 h to align with PDG proposal.
6. *pH*. New method proposed by PDG.
7. *Viscosity*. Aligned method with current PDG harmonized monograph for Hypromellose.
8. *Packaging and Storage*. No change.
9. *Labeling*. Added statement to include units of measurement (mPa · s).

(EM2: K. Moore.) RTS—C44010

Add the following:

▲Methylcellulose

Attributes	EP	JP	USP
Definition	+	+	+
Labeling	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Identification C	+	+	+
Identification D	+	+	+
Identification E	+	+	+
Apparent Viscosity, Method 1	+	+	+
Apparent Viscosity, Method 2	+	+	+
pH	+	+	+
Heavy Metals	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+
Assay	+	+	+

Legend: + will adopt and implement; – will not stipulate.
In EP, Viscosity and Assay will be dealt with in the non-mandatory Functionality-Related Characteristics section. The assay limits will not be included in the Definition (EP).
Nonharmonized attributes: Packaging and storage
Specific local attributes: Appearance of solution (EP), Description (JP), Limit of glyoxal (EP).

Cellulose, methyl ether;
Cellulose methyl ether [9004-67-5].

DEFINITION

Methylcellulose is a methyl ether of cellulose. When dried at 105° for 1 h, it contains NLT 26.0% and NMT 33.0% of methoxy (OCH_3) groups.

IDENTIFICATION

- **A. PROCEDURE**
  Analysis: Evenly distribute 1.0 g onto the surface of 100 mL of water in a beaker, tapping the top of the beaker gently if necessary to ensure a uniform layer on the surface, and allow to stand for 1–2 min.
  Acceptance criteria: The powdered material aggregates on the surface.
- **B. PROCEDURE**
  Analysis: Evenly distribute 1.0 g into 100 mL of boiling water, and stir the mixture using a magnetic stirrer with a 25-mm long bar: a slurry is formed and the particles do not dissolve. Allow the slurry to cool to 5° and stir using a magnetic stirrer.
  Acceptance criteria: A clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.
- **C. PROCEDURE**
  Analysis: To 0.1 mL of the sample solution obtained in *Identification* test *B* add 9 mL of diluted sulfuric acid (9 in 10), shake, heat in a water bath for exactly 3 min, immediately cool in an ice bath, add carefully 0.6 mL of ninhydrin TS, shake, and allow to stand at 25°.
  Acceptance criteria: A red color develops immediately, and it does not change to purple within 100 min.
- **D. PROCEDURE**
  Analysis: Add 2–3 mL of the solution obtained in *Identification* test *B* onto a glass slide as a thin film and allow the water to evaporate.
  Acceptance criteria: A coherent, clear film forms on the glass slide.
- **E. PROCEDURE**
  Analysis: Add exactly 50 mL of the sample solution obtained in *Identification* test *A* to exactly 50 mL of water in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate and begin heating at a rate of 2°/min to 5°/min. Determine the temperature at which a turbidity increase begins to occur and designate the temperature as the flocculation temperature.
  Acceptance criteria: The flocculation temperature is higher than 50°.

ASSAY

- **PROCEDURE**
  [**CAUTION—**Perform all steps involving *Hydriodic acid* carefully, in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be exceedingly careful when handling the hot vials, because they are under pressure. In the event of hydriodic exposure, wash with copious amounts of water, and seek medical attention at once.]
  Apparatus
  Reaction vial: A 5-mL pressure-tight serum vial, 20 mm in outside diameter, 50 mm in height, and 20 mm in outside diameter and 13 mm in inside diameter at the mouth,

Stage 6 Harmonization

equipped with a pressure-tight septum having a polytetrafluoroethylene-faced butyl rubber, and air-tight sealing by an aluminum crimp or another sealing system providing a sufficient air-tightness.

Heater: A heating module with a square-shaped aluminum block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vial fits. Capable of mixing the contents of the vial using a magnetic stirrer equipped in the heating module or using a reciprocal shaker which performs a reciprocating motion approximately 100 times/min.

Hydriodic acid: Use a reagent having a specific gravity of at least 1.69, equivalent to 55%–57% HI.

Internal standard solution: 30 mg/mL of *n*-octane in *o*-xylene

Standard solution: Into a suitable serum vial, weigh 60–100 mg of adipic acid, add 2.0 mL of *Hydriodic acid,* then pipet 2.0 mL of *Internal standard solution* into the vial, and close the vial securely with a suitable septum stopper. Weigh the vial and contents, add 45 µL of methyl iodide with a syringe through the septum, weigh again, and calculate the weight of methyl iodide added, by difference. Shake, and allow the layers to separate. Use the upper layer as the *Standard solution*.

Sample solution: Transfer 0.065 g of Methylcellulose to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum closure, add 60–100 mg of adipic acid, and pipet 2.0 mL of *Internal standard solution* into the vial. Cautiously pipet 2.0 mL of *Hydriodic acid* into the mixture, immediately secure the closure, and weigh it. Using a magnetic stirrer equipped in the heating module, or using a reciprocal shaker, mix the contents of the vial continuously for 60 min while heating the block so that the temperature of the contents is maintained at 130 ± 2°. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-min intervals during the initial 30 min of the heating time. Allow the vial to cool, and again weigh. If the weight loss is less than 0.50% of the contents and there is no evidence of a leak, use the upper layer of the mixture as the *Sample solution*.

Chromatographic system

Mode: GC

Detector: Thermal conductivity or hydrogen flame ionization

Column: 1.8 to 3 m × 3 to 4-mm column packed with 10%–20% liquid phase G1, 125–150 µm in diameter on 100- to 120-mesh support S1A

Column temperature: 100°

Carrier gas: Helium for the thermal conductivity detector, and helium or nitrogen for the hydrogen flame ionization detector

Flow rate: Adjust so that the retention time of the internal standard is about 10 min.

Injection size: 1 or 2 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methoxy in the Methylcellulose taken:

$$\text{Result} = 21.864 \times (Q_T/Q_S) \times (W_S/W)$$

21.864 = ratio of the formula weights of methoxy to methyl iodide times 100%

Q_T = ratio of the peak area of methyl iodide to that of internal standard in the *Sample solution*

Q_S = ratio of the peak area of methyl iodide to that of internal standard in the *Standard solution*

W_S = weight of methyl iodide in the *Standard solution* (mg)

W = weight of Methylcellulose calculated on the dried basis taken for the *Assay* (mg)

Acceptance criteria: 26.0%–33.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 1.5%
- **HEAVY METALS,** *Method III* ⟨**231**⟩**:** For the *Standard Preparation*, add the *Standard Lead Solution* prior to digestion. Omit the *Monitor Preparation*.

Acceptance criteria: The color of the test solution is not darker than that of the control solution (NMT 20 ppm).

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 1 h: it loses NMT 5.0% of its weight.
- **pH:** Measure the pH of the solution prepared in the test for *Viscosity* at 20 ± 2°. Read the indicated pH value after the probe has been immersed for 5 ± 0.5 min.

Acceptance criteria: 5.0–8.0

- **VISCOSITY ⟨911⟩**

[NOTE—The density is 1.00 g/mL, so there is no necessity for determining the density at every measurement in the case of having the confirmation data.]

Method 1: This method is applied to samples with a viscosity of less than 600 mPa · s. Weigh a quantity of Methylcellulose, equivalent to 4.000 g, calculated on the dried basis, transfer into a wide mouth bottle, and add hot water to obtain the total weight of the sample and water of 200.0 g. Capping the bottle, stir by mechanical means at 400 ± 50 rpm for 10 or 20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight if necessary to 200.0 g using cold water. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula remove any foam, if present. Perform the test with this solution at 20 ± 0.1° to obtain the kinematic viscosity, ν. Separately, determine the density, ρ, of the solution, and calculate the viscosity, η, as $\eta = \rho\nu$.

Method 2: This method is applied to samples with a viscosity of 600 mPa · s or higher. Weigh a quantity of Methylcellulose, equivalent to 10.00 g, calculated on the dried basis, transfer into a wide mouth bottle, and add hot water to obtain the total weight of the sample and water of 500.0 g. Capping the bottle, stir by mechanical means at 400 ± 50 rpm for 10–20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight if necessary to 500.0 g using cold water. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula remove any foam, if present. Determine the viscosity of this solution at 20 ± 0.1° using a single cylinder type rotational viscometer.

Apparatus: Brookfield type LV model or equivalent. Rotor No., revolution, and calculation multiplier: apply the conditions specified in the following table.

Labeled Viscosity[a] (mPa·s)	Rotor No.	Revolution (rpm)	Calculation Multiplier
600 or more and less than 1400	3	60	20
1400 or more and less than 3500	3	12	100
3500 or more and less than 9500	4	60	100
9500 or more and less than 99,500	4	6	1000
99,500 or more	4	3	2000

[a]The labeled viscosity is based on the manufacturer's specifications.

Operation of apparatus: Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of 2 min between subsequent measurements. Repeat the operation to rotate the spindle specified above twice and average the three readings.

Acceptance criteria: 80.0%–120.0% of that stated on the label for viscosity types less than 600 mPa · s, and 75.0%–140.0% of that stated on the label for viscosity types 600 mPa · s or higher

ADDITIONAL REQUIREMENTS

- **✦PACKAGING AND STORAGE:** Preserve in well-closed containers.✦
- **LABELING:** Label it to indicate its nominal viscosity type [viscosity of a solution (1 in 50)] in milli-Pascal second (mPa · s).▲*USP33*

MONOGRAPHS (NF)

BRIEFING

Benzyl Alcohol. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Benzyl Alcohol monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, includes a minor change to *Procedure: Benzaldehyde and Other Related Substances* (previously labeled *Related compounds*). The procedure has been changed to address the instance in which the substance contains peaks that overlap with peaks due to ethylbenzene or dicyclohexyl. Instruction has been added to correct for this additional peak when calculating the sum, disregard any limit that is 0.01 times the area of ethylbenzene.

(EM1: K. Moore.) RTS—C58790

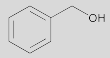
Change to read:

▲Benzyl Alcohol

Attributes	EP	JP	USP
Definition	+	+	+
Refractive Index	+	+	+
Acidity	+	+	+
Benzaldehyde and Other Related Substances	+	+	+
Peroxide Value	+	+	+
Residue on Evaporation	+	+	+
Assay	+	+	+

Legend: + will adopt and implement; – will not stipulate.
Nonharmonized attributes: *Characters, Clarity of Solution, Color of Solution, Labeling,* and *Packaging and Storage*

Reagents and Reference materials: Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.



C_7H_8O 108.1
Phenylmethanol [100-51-6].

DEFINITION

Benzyl Alcohol contains NLT 98.0% and NMT the equivalent of 100.5% of phenylmethanol (C_7H_8O).

IDENTIFICATION

- **INFRARED ABSORPTION ⟨197F⟩:** On undried specimen

ASSAY

- **PROCEDURE**

Phenolphthalein solution: Dissolve 0.1 g of phenolphthalein in 80 mL of ethanol (96%), and dilute with water to 100.0 mL. To test for sensitivity, add 100 mL of carbon dioxide-free water to 0.1 mL of the *Phenolphthalein solution*. The solution is colorless. NMT 0.2 mL of 0.02 M sodium hydroxide is required to change the color to pink.

Sample: 0.900 g

Analysis: To the *Sample*, add 15 mL of a freshly prepared mixture of pyridine and acetic anhydride (7:1), and boil under a reflux condenser on a water bath for 30 min. Cool, and add 25 mL of water. Using 0.25 mL of *Phenolphthalein solution* as the indicator, titrate with 1 M sodium hydroxide VS. Carry out a blank titration. Calculate the percentage content of C_7H_8O from the formula:

$$\text{Result} = 10.81 \times (n_2 - n_1)/m$$

n_2 = amount of 1 M sodium hydroxide used for the sample (mL)
n_1 = amount of 1 M sodium hydroxide used for the blank (mL)
m = amount of sample taken (g)

Acceptance criteria: 98.0%–100.5%

IMPURITIES

Inorganic Impurities

- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5
- **RESIDUE ON EVAPORATION**

Analysis: After ensuring that the Benzyl Alcohol complies with the test for *Fats and Fixed Oils, Peroxide Value*, evaporate 10.0 g to dryness in a tared quartz or porcelain crucible or platinum dish on a hot plate at a temperature not exceeding 200°. Ensure that the Benzyl Alcohol does not boil during evaporation. Dry the residue on the hot plate for 1 h, and allow to cool in a desiccator.

Acceptance criteria: The residue weighs NMT 5 mg, corresponding to NMT 0.05%.

Organic Impurities

- **PROCEDURE: BENZALDEHYDE AND OTHER RELATED SUBSTANCES**

Sample solution: Use the Benzyl Alcohol specimen under examination.

Standard solution A: Dissolve 0.100 g of ethylbenzene in 10.0 mL of the *Sample solution*. Dilute 2.0 mL of this solution to 20.0 mL with the *Sample solution*.

Standard solution B: Dissolve 2.000 g of dicyclohexyl in 10.0 mL of the *Sample solution*. Dilute 2.0 mL of this solution to 20.0 mL with the *Sample solution*.

Reference solution A: (for use in nonparenteral applications) Dissolve 0.750 g of benzaldehyde and 0.500 g of cyclohexylmethanol in the *Sample solution*, and dilute to 25.0 mL with the *Sample solution*. Add 1.0 mL of this solution to a mixture of 2.0 mL of *Standard solution A* and 3.0 mL of *Standard solution B*, and dilute with the *Sample solution* to 20.0 mL.

Reference solution B: (for use in parenteral applications) Dissolve 0.250 g of benzaldehyde and 0.500 g of cyclohexylmethanol in the *Sample solution*, and dilute to 25.0 mL

Stage 6 Harmonization

with the *Sample solution*. Add 1.0 mL of this solution to a mixture of 2.0 mL of *Standard solution A* and 2.0 mL of *Standard solution B*, and dilute with the *Sample solution* to 20.0 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Carrier: Helium, chromatography grade

Carrier linear velocity: 25 cm/s, at 50°

Detector temperature: 310°

Column: 30-m × 0.32-mm, 0.5-µm film thickness, G16.

Column temperature

Time (min)	Temperature (°)
0	50
34	220
69	220

Injection port temperature: 200°

System suitability

Sample: For nonparenteral applications, use *Reference solution A*. For parenteral applications, use *Reference solution B*. [NOTE—The retention time of benzyl alcohol is about 26 min. The relative retention times for ethylbenzene, dicyclohexyl, benzaldehyde, cyclohexylmethanol and benzyl alcohol are about 0.28, 0.59, 0.68, 0.71, 1.0, respectively.]

Injection volume: 0.1 µL without air plug

Suitability requirements

Sensitivity: Adjust the sensitivity of the detector so that the height of the peak due to ethylbenzene is NLT 30% of the full scale of the recorder.

Resolution: NLT 3.0 between the peaks corresponding to benzaldehyde and cyclohexylmethanol

Analysis

Samples: *Sample solution* and *Reference solution A* for non-parenteral applications and *Reference solution B* for parenteral applications

Acceptance criteria: (non-parenteral applications)

If any peaks are present in the chromatogram obtained with the *Sample solution* that have the same retention times as the peaks due to ethyl benzene and dicyclohexyl, subtract the areas of any such peaks from the peak areas at these retention times in the chromatograms of *Reference solution A* or *Reference solution B* (corrected peak areas of ethylbenzene and dicyclohexyl). Any such peaks in the *Sample solution* should be included in the assessments for the total of other peaks.

In the chromatogram obtained with the *Sample solution*, the area of any peak corresponding to benzaldehyde is not greater than the difference between the area of the peak due to benzaldehyde in the chromatogram obtained with *Reference solution A* (0.15%) and the area of the peak due to benzaldehyde in the chromatogram obtained with the *Sample solution*.

In the chromatogram obtained with the *Sample solution*, the area of any peak corresponding to cyclohexylmethanol is not greater than the difference between the area of the peak due to cyclohexylmethanol in the chromatogram obtained with *Reference solution A* (0.10%) and the area of the peak due to cyclohexylmethanol in the chromatogram obtained with the *Sample solution*.

In the chromatogram obtained with the *Sample solution*, the sum of the areas of any peak with a relative retention time less than that of benzyl alcohol and apart from the peaks due to benzaldehyde and cyclohexylmethanol is not greater than four times the area of ethylbenzene in *Reference solution A*, corrected if necessary as described above (0.04%).

In the chromatogram obtained with the *Sample solution*, the sum of the areas of any peak with a relative retention time greater than that of benzyl alcohol is not greater than the area of dicyclohexyl in *Reference solution A*, corrected if necessary as described above (0.3%).

Disregard any peak with an area less than 0.01 times that of the peak due to ethylbenzene in the chromatogram of *Reference solution A*, corrected if necessary as described above.

Acceptance criteria: (parenteral applications)

If any peaks are present in the chromatogram obtained with the *Sample solution* that have the same retention times as the peaks due to ethyl benzene and dicyclohexyl, subtract the areas of any such peaks from the peak areas at these retention times in the chromatograms of *Reference solution A* or *Reference solution B* (corrected peak areas of ethylbenzene and dicyclohexyl). Any such peaks in the *Sample solution* should be included in the assessments for the total of other peaks.

In the chromatogram obtained with the *Sample solution*, the area of any peak corresponding to benzaldehyde is not greater than the difference between the area of the peak due to benzaldehyde in the chromatogram obtained with *Reference solution B* (0.05%) and the area of the peak due to benzaldehyde in the chromatogram obtained with the *Sample solution*.

In the chromatogram obtained with the *Sample solution*, the area of any peak corresponding to cyclohexylmethanol is not greater than the difference between the area of the peak due to cyclohexylmethanol in the chromatogram obtained with *Reference solution B* (0.10%) and the area of the peak due to cyclohexylmethanol in the chromatogram obtained with the *Sample solution*.

In the chromatogram obtained with the *Sample solution*, the sum of the areas of any peak with a relative retention time less than that of benzyl alcohol and apart from the peaks due to benzaldehyde and cyclohexylmethanol is not greater than two times the area of ethylbenzene in *Reference solution B*, corrected if necessary as described above (0.02%).

In the chromatogram obtained with the *Sample solution*, the sum of the areas of any peak with a relative retention time greater than that of benzyl alcohol is not greater than the area of dicyclohexyl in *Reference solution B*, corrected if necessary as described above (0.2%).

Disregard any peak with an area less than 0.01 times that of the peak due to ethylbenzene in the chromatogram of *Reference solution B*, corrected if necessary as described above.

SPECIFIC TESTS

- **ACIDITY**

Phenolphthalein solution: Prepare as directed in the *Assay*.

Analysis: To 10 mL of Benzyl Alcohol add 10 mL of ethanol (96%) and 1 mL of *Phenolphthalein solution*.

Acceptance criteria: NMT 1 mL of 0.1 M sodium hydroxide is required to change the color of the indicator to pink.

- **+CLARITY OF SOLUTION:** [NOTE—The *Sample solution* is to be compared to *Reference suspension 1* in diffused daylight 5 min after preparation of *Reference suspension 1*.]

Hydrazine solution: Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand 4–6 h before use.

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h.

Opalescence standard: [NOTE—This suspension should not be used beyond 24 h after preparation.]

Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Reference suspension 1: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.

Reference suspension 2: Transfer 10.0 mL of the *Opalescence standard* to a second 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Dissolve 2.0 g of Benzyl Alcohol in 60 mL of water.
Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension 1*, *Reference suspension 2*, and water to separate matching test tubes. Compare the *Sample solution*, *Reference suspension 1*, *Reference suspension 2*, and water in diffused daylight, viewing vertically against a black background (see *Spectrophotometry and Light-Scattering* ⟨851⟩, *Visual Comparison*). [NOTE—The diffusion of light must be such that *Reference suspension 1* can readily be distinguished from water, and that *Reference suspension 2* can readily be distinguished from *Reference suspension 1*.]
Acceptance criteria: The *Sample solution* shows the same clarity as that of water, or its opalescence is not more pronounced than that of *Reference suspension 1*.✦

- ✦**COLOR OF SOLUTION**
  Sample solution: Use the *Sample solution* prepared in the test for *Clarity of Solution*.
  Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer a portion of water to a separate matching test tube. Compare the color of the *Sample solution* with that of water in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* ⟨851⟩, *Visual Comparison*).
  Acceptance criteria: The *Sample solution* has the color of water.✦
- **REFRACTIVE INDEX ⟨831⟩:** 1.538 to 1.541 at 20°

ADDITIONAL REQUIREMENTS

- ✦**LABELING:** Where Benzyl Alcohol is intended for use in the manufacture of parenteral applications, it is so labeled.✦
- ✦**PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.✦
- ✦**USP REFERENCE STANDARDS**
  USP Benzyl Alcohol RS✦▲*NF28*

BRIEFING

Corn Starch. The United States Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Corn Starch monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Corn Starch that was revised based on a request from the Japanese Pharmacopoeia. Differences between the **ADOPTION STAGE 6** document and the current USP monograph for Corn Starch include the following: the sentence under *Identification* test *A* "Between crossed nicol prisms" is changed to "Between orthogonally oriented polarizing plates or prisms."

(EM2-05: K. Moore.) RTS—C50840

Change to read:

▲Corn Starch

Attributes	EP	JP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Identification C	+	+	+
pH	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+
Limit of Iron	+	+	+
Limit of Oxidizing Substances	+	+	+
Limit of Sulfur Dioxide	+	+	+

Legend: + will adopt and implement; – will not stipulate
Nonharmonized attributes: Characters, *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests,* and *Tests for Specified Microorganisms*, *Labeling*, and *Packing and Storage* (USP)
Specific local attributes: Foreign matter (EP)
Reagents and reference materials: Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.

DEFINITION
Corn Starch consists of the starch granules separated from the mature grain of corn [*Zea mays* Linnè (Fam. Gramineae)].

IDENTIFICATION

- **A. PROCEDURE:** Examine under a microscope, using NLT 20× magnification and using a mixture of glycerin and water (1:1) as a mounting agent.
  Acceptance criteria: It appears either as angular polyhedral granules of irregular sizes with diameters ranging from 2–23 µm, or as rounded or spheroidal granules of irregular sizes with diameters ranging from 25–35 µm. The central hilum consists of a distinct cavity or two- to five-rayed cleft, and there are no concentric striations. Between orthogonally oriented polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.
- **B. PROCEDURE**
  Sample solution: 20 mg/mL in water
  Analysis: Boil for 1 min, and cool.
  Acceptance criteria: A thin, cloudy mucilage is formed.
- **C. PROCEDURE**
  Sample solution: 1 mL of the mucilage obtained in *Identification* test *B*
  Analysis: Add 0.05 mL of iodine and potassium iodide TS 2 to the *Sample solution*.
  Acceptance criteria: An orange-red to dark blue color is produced, which disappears upon heating.

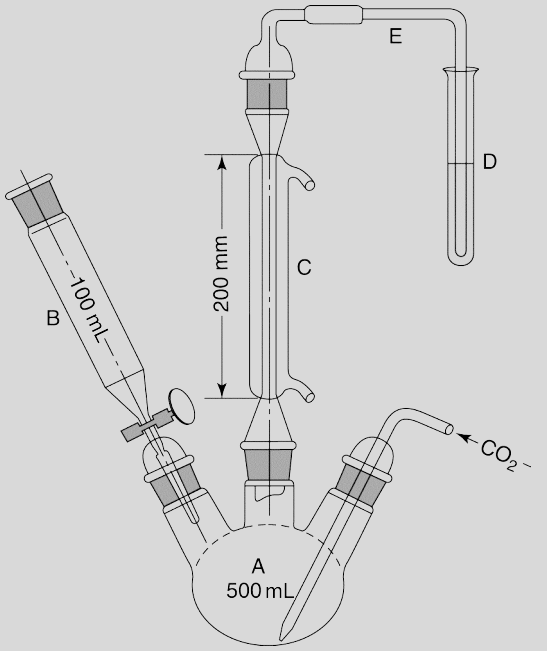
IMPURITIES
Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.6%, determined on a 1.0-g sample
- **LIMIT OF IRON**
  Sample solution: Shake 1.5 g of Corn Starch with 15 mL of 2 N hydrochloric acid, and filter. Transfer 10 mL of the filtrate to a test tube, add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
  Standard iron stock solution A: Equivalent to 10 µg/mL of iron prepared as directed under *Iron* ⟨241⟩

Standard iron stock solution B: 1 µg/mL of iron from *Standard iron stock solution A* in water
[NOTE—Prepare immediately before use.]
Standard iron solution: Transfer 10 mL of *Standard iron stock solution B* to a test tube, and add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
Acceptance criteria: After 5 min, any pink color in the *Sample solution* is not more intense than that in the *Standard iron solution*, corresponding to a limit of 10 ppm of iron.

- **LIMIT OF SULFUR DIOXIDE**

Carbon dioxide: Use carbon dioxide, with a flow regulator that will maintain a flow of 100 ± 10 mL/min.
Bromophenol blue indicator solution: 0.2 mg/mL of bromophenol blue in dilute alcohol. Filter if necessary.
Hydrogen peroxide solution: Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of *Bromophenol blue indicator solution*, and neutralize to a violet-blue endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.
Apparatus: *Figure 1.*



In this test, the sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution where the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali. The apparatus consists essentially of a 500-mL three-neck, round-bottom boiling flask, a separatory funnel having a capacity of 100 mL or greater, a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask, a reflux condenser having a jacket length of 200 mm, and a delivery tube connecting the upper end of the reflux condenser to the bottom of a receiving test tube. Apply a thin film of stopcock grease to the sealing surfaces of all of the joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.
Analysis: Add 150 mL of water to the boiling flask. Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL/min through the *Apparatus*. Start the condenser coolant flow. Add 10 mL of *Hydrogen peroxide solution* to a receiving test tube. After 15 min, without interrupting the flow of carbon dioxide, remove the separatory funnel from the boiling flask, and transfer 25.0 g of Corn Starch into the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against the escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 h. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 min, and allow to cool.
Add 0.1 mL of *Bromophenol blue indicator solution*, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩).
Calculate the content, in ppm, of sulfur dioxide in the *Sample solution* taken:

$$\text{Result} = 1000\,(32.03)\,VN/W$$

32.03 = milliequivalent weight of sulfur dioxide
V = volume of titrant consumed (mL)
N = normality of the titrant
W = weight of the *Sample solution* (g)
Acceptance criteria: NMT 50 ppm

- **LIMIT OF OXIDIZING SUBSTANCES**

Sample solution: Transfer 4.0 g to a glass-stoppered, 125-mL conical flask, and add 50.0 mL of water. Insert the stopper, and swirl for 5 min. Transfer to a glass-stoppered, 50-mL centrifuge tube, and centrifuge to clarify. Transfer 30.0 mL of the clear supernatant to a glass-stoppered, 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5–1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25–30 min in the dark. Add 1 mL of starch TS.
Analysis: Titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch–iodine color. Perform a blank determination, and make any necessary correction. Each mL of 0.002 N sodium thiosulfate is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide.
Acceptance criteria: NMT 1.4 mL of 0.002 N sodium thiosulfate is required (20 ppm, calculated as H_2O_2).

SPECIFIC TESTS

- ✦**MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 1000 cfu/g; the total combined molds and yeasts count does not exceed 100 cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*. Where it is intended for use in preparing Absorbable Dusting Powder, it also meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.✦
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1 g, at 130° for 90 min: it loses NMT 15.0% of its weight.
- **pH** ⟨**791**⟩**:** 4.0–7.0

Sample solution: Prepare a slurry by weighing 5.0 g of Corn Starch, transferring to a suitable nonmetallic container, and adding 25.0 mL of freshly boiled and cooled water.
Analysis: Agitate continuously at a moderate rate for 1 min. Stop the agitation, and allow to stand for 15 min. Determine the pH to the nearest 0.1 unit.

ADDITIONAL REQUIREMENTS

- ✦**PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.✦
- ✦**LABELING:** Where Corn Starch is intended for use in preparing Absorbable Dusting Powder, it is so labeled, and the label states that it must be subjected to further processing during the preparation of Absorbable Dusting Powder.✦▲*NF28*

BRIEFING

Potato Starch. The United States Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Potato Starch monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Potato Starch that was revised based on a request from the Japanese Pharmacopoeia. Differences between the **ADOPTION STAGE 6** document and the current USP monograph for Potato Starch include the following: the sentence under *Identification* test *A* "Between crossed nicol prisms" is changed to "Between orthogonally oriented polarizing plates or prisms."

(EM2: K. Moore.) RTS—C50840

Add the following:

▲Potato Starch

Attributes	EP	JP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Identification C	+	+	+
pH	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+
Limit of Iron	+	+	+
Limit of Oxidizing Substances	+	+	+
Limit of Sulfur Dioxide	+	+	+

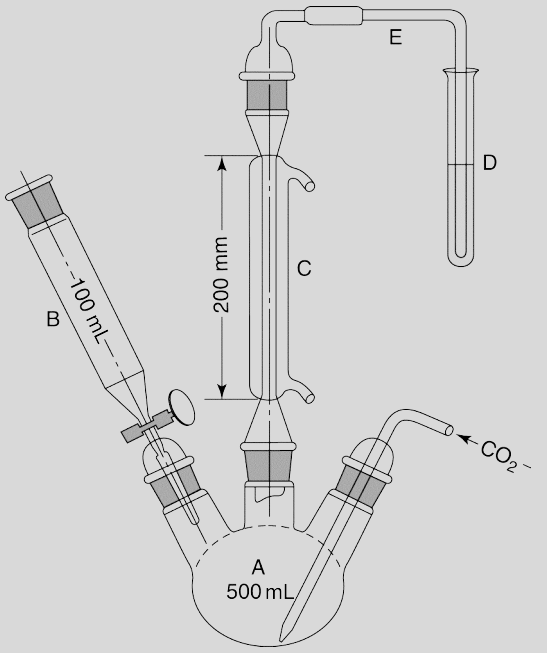
Legend: + will adopt and implement; – will not stipulate
Nonharmonized attributes: Characters, *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* and *Tests for Specified Microorganisms*, *Labeling*, and *Packing and Storage* (USP)
Specific local attributes: Foreign matter (EP)
Reagents and reference materials: Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.

DEFINITION
Potato Starch is obtained from the tuber of *Solanum tuberosum L.*

IDENTIFICATION
- **A. PROCEDURE:** Examine under a microscope using a mixture of equal volumes of glycerol and water.
  Acceptance criteria: It presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30–100 µm in size but occasionally exceeding 100 µm, or rounded, 10–35 µm in size. There are occasional compound granules having two to four components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.
- **B. PROCEDURE**
  Sample solution: 20 mg/mL in water
  Analysis: Boil for 1 min, and cool.
  Acceptance criteria: A thin, cloudy mucilage is formed.
- **C. PROCEDURE**
  Sample solution: 1 mL of the mucilage obtained in *Identification* test *B*
  Analysis: Add 0.05 mL of iodine and potassium iodide TS 2 to the *Sample solution*.
  Acceptance criteria: An orange-red to dark blue color is produced, which disappears upon heating.

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.6%, determined on a 1.0-g sample
- **LIMIT OF IRON**
  Sample solution: Shake 1.5 g of Potato Starch with 15 mL of 2 N hydrochloric acid, and filter. Transfer 10 mL of the filtrate to a test tube, add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
  Standard iron stock solution A: Equivalent of 10 µg/mL of iron prepared as directed under *Iron* ⟨241⟩
  Standard iron stock solution B: 1 µg/mL of iron from *Standard iron stock solution A* in water
  [NOTE—Prepare immediately before use.]
  Standard iron solution: Transfer 10 mL of *Standard iron stock solution B* to a test tube, and add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
  Acceptance criteria: After 5 min, any pink color in the *Sample solution* is not more intense than that in the *Standard iron solution*, corresponding to a limit of 10 ppm of iron.
- **LIMIT OF SULFUR DIOXIDE**
  Carbon dioxide: Use carbon dioxide, with a flow regulator that will maintain a flow of 100 ± 10 mL/min.
  Bromophenol blue indicator solution: 0.2 mg/mL of bromophenol blue in dilute alcohol. Filter if necessary.
  Hydrogen peroxide solution: Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of *Bromophenol blue indicator solution*, and neutralize to a violet-blue endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.

Apparatus: *Figure 1.*



In this test, the sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution where the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali. The apparatus consists essentially of a 500-mL three-neck, round-bottom boiling flask, a separatory funnel having a capacity of 100 mL or greater, a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask, a reflux condenser having a jacket length of 200 mm, and a delivery tube connecting the upper end of the reflux condenser to the bottom of a receiving test tube. Apply a thin film of stopcock grease to the sealing surfaces of all of the joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

Analysis: Add 150 mL of water to the boiling flask. Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL/min through the *Apparatus*. Start the condenser coolant flow. Add 10 mL of *Hydrogen peroxide solution* to a receiving test tube. After 15 min, without interrupting the flow of carbon dioxide, remove the separatory funnel from the boiling flask, and transfer 25.0 g of Potato Starch into the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against the escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 h. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 min, and allow to cool.

Analysis: Add 0.1 mL of *Bromophenol blue indicator solution*, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩).

Calculate the content, in ppm, of sulfur dioxide in the *Sample solution* taken:

$$\text{Result} = 1000\ (32.03)\ VN/W$$

32.03 = milliequivalent weight of sulfur dioxide
V = volume of titrant consumed (mL)
N = normality of the titrant
W = weight of the *Sample solution* (g)

Acceptance criteria: NMT 50 ppm

- **LIMIT OF OXIDIZING SUBSTANCES**

Sample solution: Transfer 4.0 g to a glass-stoppered, 125-mL conical flask, and add 50.0 mL of water. Insert the stopper, and swirl for 5 min. Transfer to a glass-stoppered, 50-mL centrifuge tube, and centrifuge to clarify. Transfer 30.0 mL of the clear supernatant to a glass-stoppered, 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5–1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25–30 min in the dark. Add 1 mL of starch TS.

Analysis: Titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch–iodine color. Perform a blank determination, and make any necessary correction. Each mL of 0.002 N sodium thiosulfate is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide.

Acceptance criteria: NMT 1.4 mL of 0.002 N sodium thiosulfate is required (20 ppm, calculated as H_2O_2).

SPECIFIC TESTS

- **✦MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTIONS: MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 1000 cfu/g; the total combined molds and yeasts count does not exceed 100 cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*.✦
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1 g, at 130° for 90 min: it loses NMT 20.0% of its weight.
- **pH** ⟨**791**⟩**:** 5.0–8.0

Sample solution: Prepare a slurry by weighing 5.0 g of Potato Starch, transferring to a suitable nonmetallic container, and adding 25.0 mL of freshly boiled and cooled water.

Analysis: Agitate continuously at a moderate rate for 1 min. Stop the agitation, and allow to stand for 15 min. Determine the pH to the nearest 0.1 unit.

ADDITIONAL REQUIREMENTS

- **✦PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.✦▲*NF28*

BRIEFING

Rice Starch. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for this monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the ADOPTION STAGE 6 document, is based on the Official Inquiry Stage 4 document, which appeared in PF 30(2). The text presented is similar to the harmonization draft for Corn Starch that appears in this issue of *Pharmacopeial Forum*, with one difference: the pH range, which is 5.0–8.0. Because there is no existing monograph for this excipient, a new monograph based on the ADOPTION STAGE 6 document is proposed.

(EM2: Kevin Moore.) RTS—C44217

Add the following:

▲Rice Starch

Attributes	EP	JP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Identification C	+	+	+
pH	+	+	+
Iron	+	+	+
Oxidizing Substances	+	+	+
Sulfur dioxide	+	+	+
Loss on drying	+	+	+
Sulfated Ash [Residue on Ignition]	+	+	+

Legend: + will adopt and implement; – will not stipulate.
Nonharmonized attributes: Characters, Microbial Enumeration Tests and Tests for Specified Microorganisms, Packaging and Storage
Specific local attributes: Foreign matter (EP)
Reagents and Reference materials: Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.

DEFINITION
Rice Starch is obtained from the caryopsis of *Oryza sativa L.*

IDENTIFICATION
- **A. PROCEDURE**
  Analysis: Examine under a microscope, using NLT 20x magnification and using a mixture of glycerin and water (1:1) as a mounting agent.
  Acceptance criteria: It appears either as angular polyhedral granules of irregular sizes with diameters ranging from 2 µm to 23 µm, or as rounded or spheroidal granules of irregular sizes with diameters ranging from 25 µm to 35 µm. The central hilum consists of a distinct cavity or two- to five-rayed cleft, and there are no concentric striations. Between orthogonally oriented polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.
- **B. PROCEDURE**
  Sample solution: 20 mg/mL in water
  Analysis: Boil for 1 min, and cool.
  Acceptance criteria: A thin, cloudy mucilage is formed.
- **C. PROCEDURE**
  Sample solution: 1 mL of the mucilage obtained in *Identification* test *B*
  Analysis: Add 0.05 mL of iodine and potassium iodide TS 2 to the *Sample solution*.
  Acceptance criteria: An orange-red to dark blue color is produced, which disappears upon heating.

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.6%, determined on a 1.0-g sample
- **LIMIT OF IRON**
  Sample solution: Shake 1.5 g of Rice Starch with 15 mL of 2 N hydrochloric acid, and filter. Transfer 10 mL of the filtrate to a test tube, and add 2 mL of citric acid solution (2 in 10) and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
  Standard iron stock solution A: Equivalent to 10 µg/mL of iron prepared as directed under *Iron* ⟨241⟩
  Standard iron stock solution B: 1 µg/mL of iron from *Standard iron stock solution A* in water
  [NOTE—Prepare immediately before use.]
  Standard iron solution: Transfer 10 mL of *Standard iron stock solution B* to a test tube, and add 2 mL of citric acid solution (2 in 10) and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.
  Acceptance criteria: After 5 min, any pink color in the *Sample solution* is not more intense than that in the *Standard iron solution*, corresponding to a limit of 10 ppm of iron.
- **LIMIT OF SULFUR DIOXIDE**
  Carbon dioxide: Use carbon dioxide, with a flow regulator that will maintain a flow of 100 ± 10 mL/min.
  Bromophenol blue indicator solution: 0.2 mg/mL of bromophenol blue in dilute alcohol. Filter if necessary.
  Hydrogen peroxide solution: Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of *Bromophenol blue indicator solution*, and neutralize to a violet-blue endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.
  Apparatus: *Figure 1.*

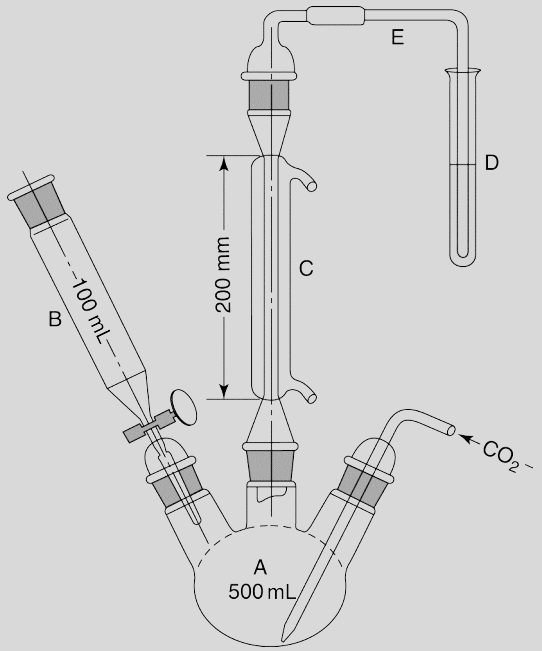


Figure 1

In this test, the sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution where the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali. The apparatus consists essentially of a 500-mL three-neck, round-bottom boiling flask, a separatory funnel having a capacity of 100 mL or greater, a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask, a reflux condenser having a jacket length of 200 mm, and a delivery tube connecting the upper end of the reflux condenser to the bottom of a receiving test tube. Apply a thin film of stopcock grease to the sealing surfaces of all of the joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

Analysis
Add 150 mL of water to the boiling flask. Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL/min through the *Apparatus*. Start the condenser coolant flow. Add 10 mL of *Hydrogen peroxide solution* to a receiving test tube. After 15 min, without interrupting the flow of carbon dioxide, remove the separatory funnel from the boiling flask, and transfer 25.0 g of Rice Starch into the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory

funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against the escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 h. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 min, and allow to cool.

Add 0.1 mL of *Bromophenol blue indicator solution*, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩).

Calculate the content, in ppm, of sulfur dioxide in the sample solution taken:

$$\text{Result} = 1000\ (32.03)\ VN/W$$

32.03 = milliequivalent weight of sulfur dioxide
V = volume of titrant consumed (mL)
N = normality of the titrant
W = weight of the sample solution (g)

Acceptance criteria: NMT 50 ppm

- **LIMIT OF OXIDIZING SUBSTANCES**

Sample solution: Transfer 4.0 g to a glass-stoppered, 125-mL conical flask, and add 50.0 mL of water. Insert the stopper, and swirl for 5 min. Transfer to a glass-stoppered, 50-mL centrifuge tube, and centrifuge to clarify. Transfer 30.0 mL of the clear supernatant to a glass-stoppered, 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5–1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25–30 min in the dark. Add 1 mL of starch TS.

Analysis: Titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch–iodine color. Perform a blank determination, and make any necessary correction. Each mL of 0.002 N sodium thiosulfate is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide.

Acceptance criteria: NMT 1.4 mL of 0.002 N sodium thiosulfate is required (20 ppm, calculated as H_2O_2).

SPECIFIC TESTS

- **✦MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩ :** The total aerobic microbial count does not exceed 1000 cfu/g, the total combined molds and yeasts count does not exceed 100 cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*.✦
- **LOSS ON DRYING ⟨731⟩:** Dry 1 g at 130° for 90 min: it loses NMT 15.0% of its weight.
- **pH ⟨791⟩:** 5.0–8.0

Sample solution: Prepare a slurry by weighing 5.0 g of Rice Starch, transferring to a suitable nonmetallic container, and adding 25.0 mL of freshly boiled and cooled water.

Analysis: Agitate continuously at a moderate rate for 1 min. Stop the agitation, and allow to stand for 15 min. Determine the pH to the nearest 0.1 unit.

ADDITIONAL REQUIREMENTS

- **✦PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.✦▲*NF28*

BRIEFING

Wheat Starch. The Japanese Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Wheat Starch monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Wheat Starch that was revised based on a request from the Japanese Pharmacopoeia. Differences between the **ADOPTION STAGE 6** document and the current USP monograph for Wheat Starch include the following: under *Identification* test *A*, "Between crossed nicol prisms" is changed to "Between orthogonally-oriented polarizing plates or prisms."

(EM2: K. Moore.) RTS—C50840

Add the following:

▲Wheat Starch

Attributes	EP	JP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Identification C	+	+	+
pH	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+
Limit of Iron	+	+	+
Limit of Oxidizing Substances	+	+	+
Limit of Sulfur Dioxide	+	+	+
Total Protein	+	+	+

Legend: + will adopt and implement; - will not stipulate.
Nonharmonized attributes: Characters, *Microbial Enumeration Tests,* and *Tests for Specified Microorganisms*, and *Packing and Storage* (USP)
Specific local attributes: Foreign matter (EP)
Reagents and Reference materials: Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.

DEFINITION

Wheat starch is obtained from the caryopsis of *Triticum aestivum L.* (*T. vulgare* Vill.).

IDENTIFICATION

- **A. PROCEDURE**

Analysis: Examine under a microscope using equal volumes of glycerol and water.

Acceptance criteria: It presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually 10–60 µm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible, and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2–10 µm in diameter. Between orthogonally-oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

- **B. PROCEDURE**

Sample solution: 20 mg/mL in water
Analysis: Boil for 1 min, and cool.
Acceptance criteria: A thin, cloudy mucilage is formed.

- **C. PROCEDURE**

Sample solution: 1 mL of the mucilage obtained in *Identification* test *B*
Analysis: Add 0.05 mL of iodine and potassium iodide TS 2 to the *Sample solution*.

Acceptance criteria: An orange-red to dark blue color is produced, which disappears upon heating.

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.6%, determined on a 1.0-g sample
- **LIMIT OF IRON**

  Sample solution: Shake 1.5 g of Wheat Starch with 15 mL of 2 N hydrochloric acid, and filter. Transfer 10 mL of the filtrate to a test tube, add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.

  Standard iron stock solution A: Equivalent to 10 µg/mL of iron prepared as directed under *Iron* ⟨241⟩

  Standard iron stock solution B: 1 µg/mL of iron from *Standard iron stock solution A* in water
  [NOTE—Prepare immediately before use.]

  Standard iron solution: Transfer 10 mL of *Standard iron stock solution B* to a test tube, and add 2 mL of citric acid solution (2 in 10), and 0.1 mL of thioglycolic acid. Add 10 N ammonium hydroxide until the solution is distinctly alkaline to litmus, and dilute with water to 20 mL.

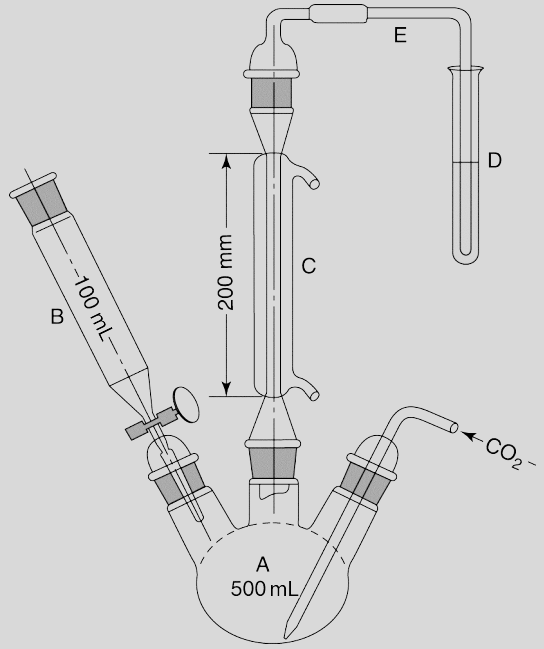
  Acceptance criteria: After 5 min, any pink color in the *Sample solution* is not more intense than that in the *Standard iron solution*, corresponding to a limit of 10 ppm of iron.
- **LIMIT OF SULFUR DIOXIDE**

  Carbon dioxide: Use carbon dioxide, with a flow regulator that will maintain a flow of 100 ± 10 mL/min.

  Bromophenol blue indicator solution: 0.2 mg/mL of bromophenol blue in dilute alcohol. Filter if necessary.

  Hydrogen peroxide solution: Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of *Bromophenol blue indicator solution*, and neutralize to a violet-blue endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.

  Apparatus: *Figure 1.*



  In this test, the sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution where the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali. The apparatus consists essentially of a 500-mL three-neck, round-bottom boiling flask, a separatory funnel having a capacity of 100 mL or greater, a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask, a reflux condenser having a jacket length of 200 mm, and a delivery tube connecting the upper end of the reflux condenser to the bottom of a receiving test tube. Apply a thin film of stopcock grease to the sealing surfaces of all of the joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

  Analysis: Add 150 mL of water to the boiling flask. Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL/min through the *Apparatus*. Start the condenser coolant flow. Add 10 mL of *Hydrogen peroxide solution* to a receiving test tube. After 15 min, without interrupting the flow of carbon dioxide, remove the separatory funnel from the boiling flask, and transfer 25.0 g of Wheat Starch into the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against the escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 h. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 min, and allow to cool. Add 0.1 mL of *Bromophenol blue indicator solution*, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩).

  Calculate the content, in ppm, of sulfur dioxide in the sample solution taken:

$$\text{Result} = 1000\ (32.03)\ VN/W$$

  32.03 = milliequivalent weight of sulfur dioxide
  V = volume of titrant consumed (mL)
  N = normality of the titrant
  W = weight of the sample solution (g)

  Acceptance criteria: NMT 50 ppm
- **LIMIT OF OXIDIZING SUBSTANCES**

  Sample solution: Transfer 4.0 g to a glass-stoppered, 125-mL conical flask, and add 50.0 mL of water. Insert the stopper, and swirl for 5 min. Transfer to a glass-stoppered, 50-mL centrifuge tube, and centrifuge to clarify. Transfer 30.0 mL of the clear supernatant to a glass-stoppered, 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5 g to 1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25–30 min in the dark. Add 1 mL of starch TS.

  Analysis: Titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch–iodine color. Perform a blank determination, and make any necessary correction. Each mL of 0.002 N sodium thiosulfate is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide.

  Acceptance criteria: NMT 1.4 mL of 0.002 N sodium thiosulfate is required (20 ppm, calculated as H_2O_2).

SPECIFIC TESTS

- **•MICROBIAL ENUMERATION TESTS ⟨61⟩ and TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** The total aerobic microbial count does not exceed 1000 cfu/g; the total combined molds and yeasts count does not exceed 100 cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*.•
- **LOSS ON DRYING ⟨731⟩:** Dry 1 g at 130° for 90 min: it loses NMT 15.0% of its weight.
- **pH ⟨791⟩**

  Sample solution: Prepare a slurry by weighing 5.0 g of Wheat Starch, transferring to a suitable nonmetallic container, and adding 25.0 mL of freshly boiled and cooled water.

  Analysis: Agitate continuously at a moderate rate for 1 min. Stop the agitation, and allow to stand for 15 min. Determine the pH to the nearest 0.1 unit.

Acceptance criteria: 4.5–7.0

SPECIFIC TESTS

- **TOTAL PROTEIN**

Analysis: Weigh 6.0 g of sample containing 2 mg of nitrogen, transfer to a combustion flask, add 4 g of a powdered mixture consisting of 100 g of potassium sulfate, 5 g of cupric sulfate, and 2.5 g of selenium, and add three glass beads. Wash any adhering particles from the neck into the flask with 5 mL of sulfuric acid, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulfuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulfuric acid in the neck of the flask; precautions should be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 30 min, unless otherwise prescribed. Cool, dissolve the solid material by cautiously adding to the mixture 25 mL of water, cool again, and place in a steam distillation apparatus. Add 30 mL of sodium hydroxide solution (42 in 100), and distill immediately by passing steam through the mixture. Collect 40 mL of distillate in 20.0 mL of 0.01 N hydrochloric acid and enough water to cover the tip of the condenser. Toward the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with 0.01 N sodium hydroxide, using methyl purple TS as the indicator (n_1 mL of 0.01 N sodium hydroxide). Repeat the test using 50 mg of glucose in place of the substance to be examined (n_2 mL of 0.01 N sodium hydroxide).

$$\text{Content of nitrogen} = [0.01401\ (n_2 - n_1)]/m$$

m = amount of test substance weighed (g)

Acceptance criteria: NMT 0.3% (corresponding to 0.048% N_2, conversion factor: 6.25)

ADDITIONAL REQUIREMENTS

- **✦PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.✦▲*NF28*

GENERAL CHAPTERS

General Tests and Assays

Biological Tests and Assays

BRIEFING

〈 **85**〉 **Bacterial Endotoxins Test,** *USP 32* page 93. The signed-off PDG harmonization Stage 6 document is presented for information purposes only and not for comments.

(MSA 05: R.Tirumalai) RTS—C71157

Change to read:

〈85〉 BACTERIAL ENDOTOXINS TEST

◆Portions of this general chapter have been harmonized with the corresponding texts of the European Pharmacopeia and/or the Japanese Pharmacopeia. Those portions that are not harmonized are marked with symbols (◆ ◆) to specify this fact.◆

This chapter provides a test to detect or quantify bacterial endotoxins that may be present in or on the sample of the article(s) to which the test is applied. It uses Limulus Amebocyte Lysate (LAL) obtained from the aqueous extracts of circulating amebocytes of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*) which has been prepared and characterized for use as an LAL Reagent.◆[1]◆

There are two types of techniques for this test: the gel-clot techniques, which are based on gel formation, and the photometric techniques. The latter include a turbidimetric method, which is based on the development of turbidity after cleavage of an endogenous substrate, and a chromogenic method, which is based on the development of color after cleavage of a synthetic peptide-chromogen complex. Proceed by any one of these techniques, unless otherwise indicated in the monograph. In case of dispute, the final decision is based on the gel-clot techniques, unless otherwise indicated in the monograph.

In the gel-clot techniques, the reaction endpoint is determined from dilutions of the material under test in direct comparison with parallel dilutions of a reference endotoxin, and quantities of endotoxin are expressed in USP Endotoxin Units (USP EU). [NOTE—One USP EU is equal to one IU of endotoxin.]

Because LAL Reagents have been formulated to be used also for turbidimetric or colorimetric tests, such tests may be used to comply with the requirements. These tests require the establishment of a standard regression curve; the endotoxin content of the test material is determined by interpolation from the curve. The procedures include incubation for a preselected time of reacting endotoxin and control solutions with LAL Reagent and reading of the spectrophotometric light absorbance at suitable wavelengths. In the endpoint turbidimetric procedure the reading is made immediately at the end of the incubation period. In the endpoint colorimetric procedure the reaction is arrested at the end of the preselected time by the addition of an enzyme reaction-terminating agent prior to the readings. In the turbidimetric and colorimetric kinetic assays the absorbance is measured throughout the reaction period and rate values are determined from those readings.

APPARATUS AND GLASSWARE

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process.◆[2]◆ Commonly used minimum time and temperature settings are 30 minutes at 250°. If employing plastic apparatus, such as microplates and pipet tips for automatic pipetters, use only that which has been shown to be free of detectable endotoxin and not to interfere with the test. [NOTE—In this chapter, the term "tube" includes any other receptacle such as a micro-titer well.]

PREPARATION OF THE STANDARD ENDOTOXIN STOCK SOLUTION AND STANDARD SOLUTIONS

The USP Endotoxin RS has a defined potency of 10,000 USP Endotoxin Units (EU) per vial. Constitute the entire contents of 1 vial of the RSE with 5 mL of LAL Reagent Water◆[3]◆, mix intermittently for 30 minutes, using a vortex mixer, and use this concentrate for making appropriate serial dilutions. Preserve the concentrate in a refrigerator for making subsequent dilutions for not more than 14 days. Mix vigorously, using a vortex mixer, for not less than 3 minutes before use. Mix each dilution for not less than 30 seconds before proceeding to make the next dilution. Do not store dilutions, because of loss of activity by adsorption, in the absence of supporting data to the contrary.

Preparatory Testing

Use an LAL Reagent of confirmed label sensitivity.

The validity of test results for bacterial endotoxins requires an adequate demonstration that specimens of the article or of solutions, washings, or extracts thereof to which the test is to be applied do not of themselves inhibit or enhance the reaction or otherwise interfere with the test. Validation is accomplished by performing the inhibition or enhancement test described under each of the three techniques indicated. Appropriate negative controls are included. Validation must be repeated if the LAL Reagent source or the method of manufacture or formulation of the article is changed.

Preparation of Sample Solutions

Prepare sample solutions by dissolving or diluting drugs or extracting medical devices using LAL Reagent Water. Some substances or preparations may be more appropriately dissolved, diluted, or extracted in other aqueous solutions. If necessary, adjust the pH of the solution (or dilution thereof) to be examined so that the pH of the mixture of the LAL Reagent and sam-

◆[1] LAL Reagent reacts with some β glucans in addition to endotoxins. Some preparations that are treated will not react with β glucans and must be used for samples that contain glucans.◆

◆[2] For a validity test of the procedure for inactivating endotoxins, see Dry-Heat Sterilization under Sterilization and Sterility Assurance of Compendial Articles 1211. Use an LAL Reagent having a sensitivity of not less than 0.15 Endotoxin Unit per mL.◆

◆[3] Sterile Water for Injection or other water that shows no reaction with the specific LAL Reagent with which it is to be used, at the limit of sensitivity of such reagent.◆

ple falls within the pH range specified by the LAL Reagent manufacturer. This usually applies to a product with a pH in the range of 6.0 to 8.0. The pH may be adjusted using an acid, base, or suitable buffer as recommended by the LAL Reagent manufacturer. Acids and bases may be prepared from concentrates or solids with LAL Reagent Water in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

DETERMINATION OF MAXIMUM VALID DILUTION (MVD)

The Maximum Valid Dilution is the maximum allowable dilution of a specimen at which the endotoxin limit can be determined. It applies to injections or to solutions for parenteral administration in the form constituted or diluted for administration, or, where applicable, to the amount of drug by weight if the volume of the dosage form for administration could be varied. The general equation to determine MVD is:

$$MVD = (\text{Endotoxin limit} \times \text{Concentration of sample solution}) / (\lambda)$$

where the concentration of sample solution and λ are as defined below. Where the endotoxin limit concentration is specified in the individual monograph in terms of volume (in EU per mL), divide the limit by λ, which is the labeled sensitivity (in EU per mL) of the LAL Reagent, to obtain the MVD factor. Where the endotoxin limit concentration is specified in the individual monograph in terms of weight or Units of active drug (in EU per mg or in EU per Unit), multiply the limit by the concentration (in mg per mL or in Units per mL) of the drug in the solution tested or of the drug constituted according to the label instructions, whichever is applicable, and divide the product of the multiplication by λ, to obtain the MVD factor. The MVD factor so obtained is the limit dilution factor for the preparation for the test to be valid.

ESTABLISHMENT OF ENDOTOXIN LIMITS

The endotoxin limit for parenteral drugs, defined on the basis of dose, is equal to K/M,◆[4]◆ where K is the threshold human pyrogenic dose of endotoxin per kg of body weight, and M is equal to the maximum recommended human dose of product per kg of body weight in a single hour period.

The endotoxin limit for parenteral drugs is specified in individual monographs in units such as EU/mL, EU/mg, or EU/Unit of biological activity.

GEL-CLOT TECHNIQUES

The gel-clot techniques detect or quantify endotoxins based on clotting of the LAL Reagent in the presence of endotoxin. The concentration of endotoxin required to cause the lysate to clot under standard conditions is the labeled sensitivity of the LAL Reagent. To ensure both the precision and validity of the test, tests for confirming the labeled LAL Reagent sensitivity and for interfering factors are described under *Preparatory Testing for the Gel-Clot Techniques*.

Preparatory Testing for the Gel-Clot Techniques

Test for Confirmation of Labeled LAL Reagent Sensitivity— Confirm the labeled sensitivity using at least 1 vial of the LAL Reagent lot. Prepare a series of two-fold dilutions of the USP Endotoxin RS in LAL Reagent Water to give concentrations of 2λ, λ, 0.5λ, and 0.25λ, where λ is as defined above. Perform the test on the four standard concentrations in quadruplicate and include negative controls. The test for confirmation of lysate sensitivity is to be carried out when a new batch of LAL Reagent is used or when there is any change in the experimental conditions that may affect the outcome of the test.

Mix a volume of the LAL Reagent with an equal volume (such as 0.1 mL aliquots) of one of the *Standard Endotoxin Solutions* in each test tube. When single test vials or ampuls containing lyophilized LAL Reagent are used, add solutions directly to the vial or ampul. Incubate the reaction mixture for a constant period according to directions of the LAL reagent manufacturer (usually at 37 ± 1° for 60 ± 2 minutes), avoiding vibration. To test the integrity of the gel, take each tube in turn directly from the incubator and invert it through about 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed. The test is not valid unless the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The endpoint is the last positive test in the series of decreasing concentrations of endotoxin. Calculate the mean value of the logarithms of the endpoint concentration and then the antilogarithm of the mean value using the following equation:

$$\text{Geometric Mean Endpoint Concentration} = \text{antilog}\,(\Sigma e/f)$$

where Σe is the sum of the log endpoint concentrations of the dilution series used, and f is the number of replicate test tubes. The geometric mean endpoint concentration is the measured sensitivity of the LAL Reagent (in EU/mL). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate.

Interfering Factors Test for the Gel-Clot Techniques— Prepare solutions A, B, C, and D as shown in *Table 1*, and perform the inhibition/enhancement test on the *Sample Solutions* at a dilution less than the MVD, not containing any detectable endotoxins, following the procedure in the *Test for Confirmation of Labeled LAL Reagent Sensitivity* above. The geometric mean endpoint concentrations of Solutions B and C are determined using the equation in that test.

◆[4] K is 5 USP EU/kg for any route of administration other than intrathecal (for which K is 0.2 USP EU/kg body weight). For radiopharmaceutical products not administered intrathecally the endotoxin limit is calculated as 175/V, where V is the maximum recommended dose in mL. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula 14/V. For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is K/M, where K = 5 EU/kg and M is the (maximum dose/m2/hour × 1.80 m2)/70 Kg.◆

Table 1. Preparation of Solutions for the Inhibition/Enhancement Test for Gel-Clot Techniques

Solution	Endotoxin Concentration/Solution to which Endotoxin is Added	Diluent	Dilution Factor	Initial Endotoxin Concentration	Number of Replicates
A[a]	none/sample solution	—	—	—	4
B[b]	2λ/sample solution	sample solution	1	2λ	4
			2	1λ	4
			4	0.5λ	4
			8	0.25λ	4
C[c]	2λ/LAL Reagent Water	LAL Reagent Water	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D[d]	none/LAL Reagent Water	—	—	—	2

[a] Solution A: a *Sample Solution* of the preparation under test that is free of detectable endotoxins.
[b] Solution B: test for interference.
[c] Solution C: control for labeled LAL Reagent sensitivity.
[d] Solution D: negative control of LAL Reagent Water.

This test must be repeated when any condition that is likely to influence the test results changes. The test is not valid unless Solutions A and D show no reaction and the result of Solution C confirms the labeled sensitivity.

If the sensitivity of the lysate determined in the presence of the sample solution under test of Solution B is not less than 0.5λ and not greater than 2λ, the sample solution does not contain factors that interfere under the experimental conditions used. Otherwise, the sample solution to be examined interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined and this may contribute to the elimination of interference.

Interference may be overcome by suitable treatment, such as filtration, neutralization, dialysis, or heating. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described below using the preparation to be examined to which USP Endotoxin RS has been added and which has been subjected to the selected treatment.

Gel-Clot Limit Test

This test is used when a monograph contains a requirement for endotoxin limits.

Procedure— Prepare Solutions A, B, C, and D as shown in *Table 2*, and perform the test on these solutions following the procedure in the *Test for Confirmation of Labeled LAL Reagent Sensitivity* under *Preparatory Testing for the Gel-Clot Techniques*.

Table 2. Preparation of Solutions for the Gel-Clot Limit Test

Solution[a]	Endotoxin Concentration/Solution to which Endotoxin is Added	Number of Replicates
A	none/diluted *Sample Solution*	2
B	2λ/diluted *Sample Solution*	2

Table 2. Preparation of Solutions for the Gel-Clot Limit Test *(Continued)*

Solution[a]	Endotoxin Concentration/Solution to which Endotoxin is Added	Number of Replicates
C	2λ/LAL Reagent Water	2
D	none/LAL Reagent Water	2

[a] Prepare Solution A and positive product control Solution B using a dilution not greater than the MVD and treatments as directed in the *Interfering Factors Test for the Gel-Clot Techniques* under *Preparatory Testing for the Gel-Clot Techniques*. Positive control Solutions B and C contain the standard endotoxin preparation at a concentration corresponding to twice the labeled LAL Reagent sensitivity. The negative control Solution D is LAL Reagent Water.

Interpretation— The test is not valid unless both replicates of positive control Solutions B and C are positive and those of negative control Solution D are negative. The preparation under test complies with the test when a negative result is found for both tubes containing Solution A. The preparation under test does not comply with the test when a positive result is found for both tubes containing Solution A.

Repeat the test when a positive result is found for 1 tube containing Solution A and a negative result for the other one. The preparation under test complies with the test when a negative result is found for both tubes containing Solution A in the repeat result. If the test is positive for the preparation under test at a dilution less than the MVD, the test may be repeated at a dilution not greater than the MVD.

Gel-Clot Assay

This assay quantifies bacterial endotoxins in sample solutions by titration to an endpoint.

Procedure— Prepare Solutions A, B, C, and D as shown in *Table 3*, and test these solutions by following the procedure in the Test for Confirmation of Labeled LAL Reagent Sensitivity under *Preparatory Testing for the Gel-Clot Techniques*.

Table 3. Preparation of Solutions for the Gel-Clot Assay

Solution	Endotoxin Concentration/ Solution to which Endotoxin is Added	Diluent	Dilution Factor	Initial Endotoxin Concentration	Number of Replicates
A[a]	none/sample solution	sample solution	1	—	2
			2	—	2
			4	—	2
			8	—	2
B[b]	2λ/sample solution	—	1	2λ	2

Table 3. Preparation of Solutions for the Gel-Clot Assay *(Continued)*

Solution	Endotoxin Concentration/ Solution to which Endotoxin is Added	Diluent	Dilution Factor	Initial Endotoxin Concentration	Number of Replicates
C[c]	2λ/LAL Reagent Water		1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D[d]	none/LAL Reagent Water	—	—	—	2

[a] Solution A: a sample solution under test at the dilution, not to exceed the MVD, with which the *Interfering Factors Test for the Gel-Clot Techniques* was completed. Subsequent dilution of the sample solution must not exceed the MVD. Use LAL Reagent Water to make dilution series of four tubes containing the sample solution under test at concentrations of *Interfering Factors Test for the Gel-Clot Techniques* was completed. Other dilutions 1, , , and $\frac{1}{8}$ relative to the dilution with which the may be used as appropriate.

[b] Solution B: Solution A containing standard endotoxin at a concentration of 2λ (positive product control).

[c] Solution C: two series of 4 tubes of LAL Reagent Water containing the standardendotoxin at a concentration of 2λ, λ, 0.5λ, and 0.25λ, respectively.

[d] Solution D: LAL Reagent Water (negative control).

Calculation and Interpretation The test is not valid unless the following conditions are met: (1) both replicates of negative control Solution D are negative; (2) both replicates of positive product control Solution B are positive; and (3) the geometric mean endpoint concentration of Solution C is in the range of 0.5λ to 2λ.

To determine the endotoxin concentration of Solution A, calculate the endpoint concentration for each replicate series of dilutions by multiplying each endpoint dilution factor by λ. The endotoxin concentration in the sample is the geometric mean endpoint concentration of the replicates (see the formula given *Test for Confirmation of Labeled LAL Reagent Sensitivity* in the under *Preparatory Testing for the Gel-Clot Techniques*). If the test is conducted with a diluted *Sample Solution*, calculate the concentration of endotoxin in the original *Sample Solution* by multiplying by the dilution factor. If none of the dilutions of the *Sample Solution* is positive in a valid assay, report the endotoxin concentration as less than λ (if the diluted sample was tested, less than λ times the lowest dilution factor of the sample.) If all dilutions are positive, the endotoxin concentration is reported as equal to or greater than the greatest dilution factor multiplied by λ (e.g., initial dilution factor times 8 times λ in *Table 3*).

The articlemeets the requirements of the test if the concentration of endotoxin is less than that specified in the individual monograph.

PHOTOMETRIC TECHNIQUES

The turbidimetric method measures increases in turbidity. Depending on the test principle used, this technique is classified as either endpoint turbidimetric or kinetic turbidimetric. The endpoint turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation period. The kinetic turbidimetric technique is a method to measure either the onset time needed to reach a predetermined absorbance of the reaction mixture or the rate of turbidity development.

The chromogenic method measures the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with the LAL Reagent. Depending on the test principle employed, this technique is classified as either endpoint chromogenic or kinetic chromogenic. The endpoint chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period. The kinetic chromogenic technique is a method to measure either the onset time needed to reach a predetermined absorbance of the reaction mixture or the rate of color development

All photometric tests are carried out at the incubation temperature recommended by the LAL Reagent manufacturer, which is usually 37 ± 1°.

Preparatory Testing for the Photometric Techniques

To ensure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to verify that the criteria for the standard curve are valid and that the sample solution does not inhibit or enhance the reaction. Revalidation for the test method is required when conditions that are likely to influence the test result change.

Verification of Criteria for the Standard Curve Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve. Perform the test using at least three replicates of each standard endotoxin concentration according to the manufacturer's instructions for the LAL Reagent (with regard to volume ratios, incubation time, temperature, pH, etc.). If the desired range in the kinetic methods is greater than two logs, additional standards should be included to bracket each log increase within the range of the standard curve. The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980 for the range of endotoxin concentrations indicated by the manufacturer of the LAL Reagent.

Interfering Factors Test for the Photometric Techniques Select an endotoxin concentration at or near the middle of the endotoxin standard curve. Prepare Solutions A, B, C, and D as shown in *Table 4*. Perform the test on Solutions A, B, C, and D at least in duplicate following the instructions for the LAL Reagent used (with regard to volume of sample and LAL Reagent, volume ratio of sample to LAL Reagent, incubation time, etc.)

Table 4. Preparation of Solutions for the Inhibition/Enhancement Test for Photometric Techniques

Solution	Endotoxin Concentration	Solution to which Endotoxin is Added	Number of Replicates
A[a]	none	sample solution	not less than 2
B[b]	middle concentration of the standard curve	sample solution	not less than 2

Table 4. Preparation of Solutions for the Inhibition/Enhancement Test for Photometric Techniques *(Continued)*

Solution	Endotoxin Concentration	Solution to which Endotoxin is Added	Number of Replicates
C[c]	at least 3 concentrations (lowest concentration is designated λ.)	LAL Reagent Water	each not less than 2
D[d]	none	LAL Reagent Water	not less than 2

[a] Solution A: the sample solution may be diluted not to exceed MVD.
[b] Solution B: the preparation under test at the same dilution as Solution A, containing added endotoxin at a concentration equal to or near the middle of the standard curve.
[c] Solution C: the standard endotoxin at the concentrations used in the validationof the method described in *Verification of Criteria for the Standard Curve* under *Preparatory Testing for the Photometric Techniques (positive control series)*.
[d] Solution D: LAL Reagent Water(negative control).

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution, (if any) from that containing the added endotoxin. In order to be considered free of interfering factors under the conditions of the test, the measured concentration of the endotoxin added to the sample solution must be within 50% to 200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified ranges, the interfering factors must be removed as described in the *Interfering Factors Test for the Gel-Clot Techniques* under *Preparatory Testing for the Gel-Clot Techniques*. Repeating the *Interfering Factors Test for the Gel-Clot Techniques* validates the treatment.

Procedure for the Photometric Techniques

Follow the procedure described in the *Interfering Factors Test for the Photometric Techniques* under *Preparatory Testing for the Photometric Techniques*.

Calculation for the Photometric Techniques

Calculate the endotoxin concentration of each of the replicates of test Solution A using the standard curve generated by positive control series C. The test is not valid unless the following conditions are met: (1) the results of control series C comply with the requirements for validation defined under *Verification of Criteria for the Standard Curve* under *Preparatory Testing for the Photometric Techniques;* (2) the endotoxin recovery, calculated from the concentration found in Solution B after subtracting the endotoxin concentration found in Solution A is within 50 to 200%; and (3) the result of negative control series D does not exceed the limit of the blank value required in the description of the LAL Reagent used.

Interpretation of Results from the Photometric Techniques

In photometric assays, the preparation under test complies with the test if the mean endotoxin concentration of the replicates of Solution A, after correction for dilution and concentration, is less than the endotoxin limit for the product.

▲◆Portions of this general chapter have been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. Those portions that are not harmonized are marked with symbols (◆◆) to specify this fact.◆

The Bacterial Endotoxins Test (BET) is a test to detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*).

There are three techniques for this test: the gel-clot technique, which is based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. Proceed by any of the three techniques for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot technique unless otherwise indicated in the monograph for the product being tested. The test is carried out in a manner that avoids endotoxin contamination.

APPARATUS

Depyrogenate all glassware and other heat-stable materials in a hot air oven using a validated process. ◆[1]◆ A commonly used minimum time and temperature is 30 minutes at 250°. If employing plastic apparatus, such as microplates and pipet tips for automatic pipetters, use apparatus that is shown to be free of detectable endotoxin and does not interfere in the test. [NOTE—In this chapter, the term ''tube'' includes any other receptacle such as a microtiter well.]

◆[1] For a validity test of the procedure for inactivating endotoxins, see *Dry-Heat Sterilization*under *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩. Use *Lysate TS* having a sensitivity of not less than 0.15 Endotoxin Unit per mL.◆

REAGENTS AND TEST SOLUTIONS

Amoebocyte Lysate—A lyophilized product obtained from the lysate of amoebocytes (white blood cells) from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). This reagent refers only to a product manufactured in accordance with the regulations of the competent authority. [NOTE—*Amoebocyte Lysate* reacts to some β-glucans in addition to endotoxins. *Amoebocyte Lysate* preparations that do not react to glucans are available: they are prepared by removing the G factor reacting to glucans from *Amoebocyte Lysate* or by inhibiting the G factor reacting system of *Amoebocyte Lysate* and may be used for endotoxin testing in the presence of glucans.]

Water for Bacterial Endotoxins Test (BET)—Use *Water for Injection* or water produced by other procedures that shows no reaction with the lysate employed, at the detection limit of the reagent.

Lysate TS—Dissolve *Amoebocyte Lysate* in *Water for BET*, or in a buffer recommended by the lysate manufacturer, by gentle stirring. Store the reconstituted lysate, refrigerated or frozen, according to the specifications of the manufacturer.

PREPARATION OF SOLUTIONS

Standard Endotoxin Stock Solution—A *Standard Endotoxin Stock Solution* is prepared from a USP Endotoxin Reference Standard that has been calibrated to the current WHO International Standard for Endotoxin. Follow the specifications in the package leaflet and on the label for preparation and storage of the *Standard Endotoxin Stock Solution*. Endotoxin is expressed in Endotoxin Units (EU). [NOTE—One USP Endotoxin Unit (EU) is equal to one International Unit (IU) of endotoxin.]

Standard Endotoxin Solutions—After mixing the *Standard Endotoxin Stock Solution* vigorously, prepare appropriate serial dilutions of *Standard Endotoxin Solution*, using *Water for BET*. Use dilutions as soon as possible to avoid loss of activity by adsorption.

Sample Solutions—Prepare the *Sample Solutions* by dissolving or diluting drugs, or taking washes from medical devices using *Water for BET*. Some substances or preparations may be more appropriately dissolved, diluted, or extracted in other aqueous solutions. If necessary, adjust the pH of the solution to be examined (or dilution thereof) so that the pH of the mixture of the lysate and *Sample Solution* falls within the pH range specified by the lysate manufacturer, usually 6.0 to 8.0. The pH may be adjusted by use of an acid, base, or suitable buffer as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with *Water for BET* in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

DETERMINATION OF MAXIMUM VALID DILUTION (MVD)

The Maximum Valid Dilution is the maximum allowable dilution of a specimen at which the endotoxin limit can be determined. Determine the MVD from the following equation:

$$\text{MVD} = (\text{Endotoxin Limit} \times \text{Concentration of } \textit{Sample Solution}) / (\lambda)$$

Endotoxin Limit—The endotoxin limit for parenteral drugs, defined on the basis of dose, equals K/M[◆2◆], where K is a threshold pyrogenic dose of endotoxin per kg of body weight , and M is equal to the maximum recommended bolus dose of product per kg of body weight. When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period. The endotoxin limit for parenteral drugs is specified in the individual monograph in units such as EU/mL, EU/mg, EU/Unit of biological activity, etc.

Concentration of Sample Solution—

mg/mL: in the case of endotoxin limit specified by weight (EU/mg);

Units/mL: in the case of endotoxin limit specified by unit of biological activity (EU/Unit);

mL/mL: when the endotoxin limit is specified by volume (EU/mL).

λ: the labeled sensitivity in the *Gel-Clot Technique* (EU/mL) or the lowest concentration used in the standard regression curve for the *Turbidimetric Technique* or *Chromogenic Technique*.

GEL-CLOT TECHNIQUE

The Gel-Clot Technique is for detecting or quantifying endotoxins based on clotting of the lysate reagent in the presence of endotoxin. The minimum concentration of endotoxin required to cause the lysate to clot under standard conditions is the labeled sensitivity of the lysate reagent. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate sensitivity and for interfering factors as described under *Preparatory Testing*.

Preparatory Testing

Test for Confirmation of Labeled Lysate Sensitivity—Confirm in four replicates the labeled sensitivity, λ, expressed in EU/mL of the lysate prior to use in the test. The test for confirmation of lysate sensitivity is to be carried out when a new batch of lysate is used or when there is any change in the test conditions that may affect the outcome of the test. Prepare standard solutions having at least four concentrations equivalent to 2λ, λ, 0.5λ, and 0.25λ by diluting the USP Endotoxin RS with *Water for BET*.

Mix a volume of the *Lysate TS* with an equal volume (such as 0.1-mL aliquots) of one of the *Standard Endotoxin Solutions* in each test tube. When single test vials or ampuls containing lyophilized lysate are used, add solutions directly to the vial or ampul. Incubate the reaction mixture for a constant period according to the directions of the lysate manufacturer (usually at 37 ± 1° for 60 ± 2 minutes), avoiding vibration. To test the integrity of the gel, take each tube in turn directly from the incubator and invert it through about 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed. The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The endpoint is the smallest concentration in the series of decreasing concentrations of standard endotoxin that clots the lysate. Determine the geometric mean endpoint by calculating the mean of the logarithms of the endpoint concentrations of the four replicate series and then taking the antilogarithm of the mean value, as indicated in the following formula:

$$\text{Geometric Mean Endpoint Concentration} = \text{antilog}\left(\frac{\Sigma e}{f}\right)$$

[◆2] *K* is 5 USP-EU/kg for any route of administration other than intrathecal (for which *K* is 0.2 USP-EU/kg body weight). For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as $175/V$, where V is the maximum recommended dose in mL. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula $14/V$. For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is K/M, where K = 5 EU/kg and M is the (maximum dose/m^2/hour × 1.80 m^2)/70 Kg. ◆

where Σe is the sum of the log endpoint concentrations of the dilution series used, and f is the number of replicate test tubes. The geometric mean endpoint concentration is the measured sensitivity of the lysate (in EU/mL). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate.

Test for Interfering Factors—Usually prepare solutions (A–D) as shown in *Table 1*, and perform the inhibition/enhancement test on the *Sample Solutions* at a dilution less than the MVD, not containing any detectable endotoxins, operating as described for *Test for Confirmation of Labeled Lysate Sensitivity.* The geometric mean endpoint concentrations of Solutions B and C are determined using the formula described in the *Test for Confirmation of Labeled Lysate Sensitivity.*

The test is considered valid when all replicates of *Solutions A* and *D* show no reaction and the result of *Solution C* confirms the labeled sensitivity.

If the sensitivity of the lysate determined in the presence of *Solution B* is not less than 0.5λ and not greater than 2λ, the *Sample Solution* does not contain factors that interfere under the experimental conditions used. Otherwise, the *Sample Solution* to be examined interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined and this may contribute to the elimination of interference.

Interference may be overcome by suitable treatment, such as filtration, neutralization, dialysis, or heating. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which USP Endotoxin RS has been added and which has then been submitted to the chosen treatment.

Table 1. Preparation of Solutions for the Inhibition/Enhancement Test for Gel-Clot Techniques

Solution	Endotoxin Concentration/ Solution to which Endotoxin is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
A[a]	none/*Sample Solution*	—	—	—	4
B[b]	2λ/*Sample Solution*	*Sample Solution*	1	2λ	4
			2	1λ	4
			4	0.5λ	4
			8	0.25λ	4
C[c]	2λ/*Water for BET*	*Water for BET*	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D[d]	none/*Water for BET*	—	—	—	2

[a] Solution A: a *Sample Solution* of the preparation under test that is free of detectable endotoxins.
[b] Solution B: test for interference.
[c] Solution C: control for labeled lysate sensitivity.
[d] Solution D: negative control of *Water for BET*

Limit Test

Procedure—Prepare *Solutions A, B, C,* and *D* as shown in *Table 2*, and perform the test on these solutions following the procedure for *Test for Confirmation of Labeled Lysate Sensitivity* under *Preparatory Testing.*

Table 2. Preparation of Solutions for the Gel-Clot Limit Test

Solution*	Endotoxin Concentration/Solution to which Endotoxin is Added	Number of Replicates
A	none/diluted *Sample Solution*	2
B	2λ/diluted *Sample Solution*	2
C	2λ/*Water for BET*	2
D	none/*Water for BET*	2

* Prepare *Solution A* and the positive product control *Solution B* using a dilution not greater than the MVD and treatments as for the *Test for Interfering Factors* under *Preparatory Testing*. The positive control *Solutions B* and *C* contain the *Standard Endotoxin Solution* at a concentration corresponding to twice the labeled lysate sensitivity. The negative control *Solution D* consists of *Water for BET.*

Interpretation—The test is considered valid when both replicates of *Solution B* and *C* are positive and those of *Solution D* are negative. When a negative result is found for both replicates of *Solution A,* the preparation under test complies with the test. When a positive result is found for both replicates of *Solution A,* the preparation under test does not comply with the test.

When a positive result is found for one replicate of *Solution A* and a negative result is found for the other, repeat the test. In the repeat test, the preparation under test complies with the test if a negative result is found for both replicates of *Solution A.* The preparation does not comply with the test if a positive result is found for one or both replicates of *Solution A.* However, if the preparation does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

Quantitative Test

Procedure—The test quantifies bacterial endotoxins in *Sample Solutions* by titration to an endpoint. Prepare *Solutions A, B, C,* and *D* as shown in *Table 3*, and test these solutions by following the procedure in the *Test for Confirmation of Labeled Lysate Sensitivity* under *Preparatory Testing.*

Table 3. Preparation of Solutions for the Gel-Clot Assay

Solution	Endotoxin Concentration/ Solution to which Endotoxin is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
A[a]	none/*Sample Solution*	*Water for BET*	1	—	2
			2	—	2
			4	—	2
			8	—	2
B[b]	2λ/*Sample Solution*	—	1	2λ	2

Table 3. Preparation of Solutions for the Gel-Clot Assay *(Continued)*

Solution	Endotoxin Concentration/ Solution to which Endotoxin is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
C[c]	2λ/*Water for BET*	*Water for BET*	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D[d]	none/*Water for BET*	—	—	—	2

[a] Solution A: *Sample Solution* under test at the dilution, not to exceed the MVD, with which the *Test for Interfering Factors* was completed. Subsequent dilution of the *Sample Solution* must not exceed the MVD. Use *Water for BET* to make a dilution series of four tubes containing the *Sample Solution* under test at concentrations of 1, , , and $\frac{1}{8}$ relative to the concentration used in the *Test for Interfering Factors* . Other dilutions up to the MVD may be used as appropriate.
[b] Solution B: *Solution A* containing Standard endotoxin at a concentration of 2λ (positive product control).
[c] Solution C: Two replicates of four tubes of *Water for BET* containing the standard endotoxin at a concentration of 2λ, λ, 0.5λ, and 0.25λ, respectively.
[d] Solution D: *Water for BET* (negative control).

Calculation and Interpretation—The test is considered valid when the following three conditions are met: (1) Both replicates of negative control *Solution D* are negative; (2) Both replicates of positive product control *Solution B* are positive; and (3) The geometric mean endpoint concentration of *Solution C* is in the range of 0.5λ to 2λ.

To determine the endotoxin concentration of *Solution A,* calculate the endpoint concentration for each replicate by multiplying each endpoint dilution factor by λ. The endotoxin concentration in the *Sample Solution* is the endpoint concentration of the replicates. If the test is conducted with a diluted *Sample Solution,* calculate the concentration of endotoxin in the original *Sample Solution* by multiplying by the dilution factor. If none of the dilutions of the *Sample Solution* is positive in a valid assay, report the endotoxin concentration as less than λ (if the diluted sample was tested, report as less than λ times the lowest dilution factor of the sample.) If all dilutions are positive, the endotoxin concentration is reported as equal to or greater than the greatest dilution factor multiplied by λ (e.g., initial dilution factor times 8 times λ in *Table 3*).

The preparation under test meets the requirements of the test if the concentration of endotoxin in both replicates is less than that specified in the individual monograph.

PHOTOMETRIC QUANTITATIVE TECHNIQUES

Turbidimetric Technique

This technique is a photometric assay measuring increases in reactant turbidity. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-turbidimetric assay or a kinetic-turbidimetric assay. The endpoint-turbidimetric assay is based on the quantitative relationship between the concentration of endotoxins and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation period. The kinetic-turbidimetric assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance or trans-

mission of the reaction mixture, or the rate of turbidity development. The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually $37 \pm 1^{\circ}$).

Chromogenic Technique

This technique is an assay to measure the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with lysate. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-chromogenic assay or a kinetic-chromogenic assay. The endpoint-chromogenic assay is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period. The kinetic-chromogenic assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance of the reaction mixture, or the rate of color development. The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually $37 \pm 1^{\circ}$).

Preparatory Testing

To assure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to verify that the criteria for the standard curve are valid and that the sample solution does not interfere with the test. Validation for the test method is required when conditions that are likely to influence the test result change.

Assurance of Criteria for the Standard Curve— The test must be carried out for each lot of lysate reagent. Using the *Standard Endotoxin Solution,* prepare at least three endotoxin concentrations within the range indicated by the lysate manufacturer to generate the standard curve. Perform the assay using at least three replicates of each standard endotoxin concentration according to the manufacturer's instructions for the lysate (volume ratios, incubation time, temperature, pH etc.). If the desired range is greater than two logs in the kinetic methods, additional standards should be included to bracket each log increase in the range of the standard curve. The absolute value of the correlation coefficient, *r,* must be greater than or equal to 0.980, for the range of endotoxin concentrations set up.

Test for Interfering Factors—Select an endotoxin concentration at or near the middle of the endotoxin standard curve. Prepare *Solutions A, B, C,* and *D* as shown in *Table 4*. Perform the test on *Solutions A, B, C,* and *D* at least in duplicate according to the instructions for the lysate employed, for example, concerning volume of *Sample Solution* and *Lysate TS,* volume ratio of *Sample Solution* to *Lysate TS,* incubation time, etc.

Table 4. Preparation of Solutions for the Inhibition/Enhancement Test for Photometric Techniques

Solution	Endotoxin Concentration	Solution to which Endotoxin is Added	Number of Replicates
A[a]	none	*Sample Solution*	not less than 2
B[b]	middle concentration of the standard curve	*Sample Solution*	not less than 2

Table 4. Preparation of Solutions for the Inhibition/Enhancement Test for Photometric Techniques *(Continued)*

Solution	Endotoxin Concentration	Solution to which Endotoxin is Added	Number of Replicates
C[c]	at least 3 concentrations (lowest concentration is designated λ)	*Water for BET*	each not less than 2
D[d]	none	*Water for BET*	not less than 2

[a] Solution A: The sample solution may be diluted not to exceed MVD.
[b] Solution B: The preparation under test at the same dilution as *Solution A,* containing added endotoxin at a concentration equal to or near the middle of the standard curve.
[c] Solution C: The standard endotoxin at the concentrations used in the validation of the method described for *Assurance of Criteria for the Standard Curve* under *Preparatory Testing* (positive controls).
[d] Solution D: *Water for BET* (negative control).

The test is considered valid when the following conditions are met.

1. The absolute value of the correlation coefficient of the standard curve generated using *Solution C* is greater than or equal to 0.980.

2. The result with *Solution D* does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution, if any (*Solution A,Table 4*), from that containing the added endotoxin (*Solution B,Table 4*). In order to be considered free of factors that interfere with the assay under the conditions of the test, the measured concentration of the endotoxin added to the sample solution must be within 50% to 200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified range, the *Sample Solution* under test is considered to contain interfering factors. Then, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the *Sample Solution* or diluted *Sample Solution* not to exceed the MVD may be eliminated by suitable validated treatment, such as filtration, neutralization, dialysis, or heat treatment.To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

Test Procedure

Follow the procedure described for *Test for Interfering Factors* under *Preparatory Testing.*

Calculation

Calculate the endotoxin concentration of each of the replicates of *Solution A* using the standard curve generated by the positive control *Solution C.* The test is considered valid when the following three requirements are met.

1. The results of the control *Solution C* comply with the requirements for validation defined for *Assurance of Criteria for the Standard Curve* under *Preparatory Testing*.

2. The endotoxin recovery, calculated from the concentration found in *Solution B* after subtracting the concentration of endotoxin found in *Solution A,* is within the range of 50% to 200%.

3. The result of the negative control *Solution D* does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Interpretation

In photometric assays, the preparation under test complies with the test if the mean endotoxin concentration of the replicates of *Solution A,* after correction for dilution and concentration, is less than the endotoxin limit for the product.▲*USP33*

OTHER TESTS AND ASSAYS

BRIEFING

⟨429⟩ Light Diffraction Measurement of Particle Size. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the general chapter *Light Diffraction Measurement of Particle Size*, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following document, which represents the revised **ADOPTION STAGE 6** document, is based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia.

(GC K. Zaidi; K. Moore) RTS—C71319

Add the following:

▲⟨429⟩ LIGHT DIFFRACTION MEASUREMENT OF PARTICLE SIZE

INTRODUCTION

The method is based on the ISO standards 13320-1(1999) and 9276-1(1998).

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia.*

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider angular range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by single particles and scattering by clusters of primary particles, i.e., by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary particles before measurement.

For nonspherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g., sedimentation, sieving).

This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems (e.g., powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids), through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products.

PRINCIPLE

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light

scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for subsequent analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

INSTRUMENT

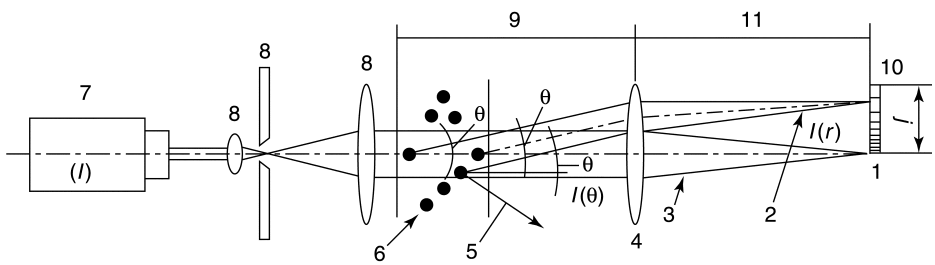
The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity, or direct bright light.

An example of a setup of a laser light diffraction instrument is given in *Figure 1.* Other equipment may be used.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in two positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus in a converging beam. The advantage of the conventional setup is that a reasonable path length for the sample is allowed within the working distance of the lens. The second setup allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.



1. Obscuration detector
2. Scattered beam
3. Direct beam
4. Fourier lens
5. Scattered light not collected by lens (4)
6. Particle ensemble
7. Light source laser
8. Beam processing unit
9. Working distance of lens (4)
10. Multi-element detector
11. Focal distance of lens (4)

Figure 1. Example of a set-up of a laser light diffraction instrument.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

DEVELOPMENT OF THE METHOD

The measurement of particle size by laser diffraction can give reproducible data, even in the submicron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g., dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 μm to 3 mm. Because of recent advances in lens and equipment design, newer instruments are routinely capable of exceeding this range. With the validation report, the user demonstrates the applicability of the method for its intended use.

Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle-size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

Evaluation of the Dispersion Procedure

The sample to be analyzed is inspected, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method, it is highly advisable to check that comminution of the particles does not occur and, conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g., crystallization, milling) of the material has changed, the applicability of the method must be verified (e.g., by microscopic comparison).

Sprays, aerosols, and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes, and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g., agitation, sonication) are often applied for deagglomeration or deaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing is most commonly used. Nonrecirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers that apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol that is blown through

the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free-flowing, coarser particles or granules, the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving, and the mass and percentage of removed material are reported. However, after presieving, note that the sample is no longer representative, unless proven otherwise.

Optimization of the Liquid Dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must

— be transparent at the laser wavelength and practically free from air bubbles or particles;
— have a refractive index that differs from that of the test material;
— be a nonsolvent of the test material (pure liquid or prefiltered, saturated solution);
— not alter the size of the test materials (e.g., by solubility, solubility enhancement, or recrystallization effects);
— favor easy formation and stability of the dispersion;
— be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.); and
— possess a suitable viscosity to facilitate recirculation, stirring, and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH, respectively, can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore, a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

Optimization of the Gas Dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water, and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

Determination of the Concentration Range

In order to produce an acceptable signal-to-noise ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. [NOTE—In different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g., obscuration, optical concentration, proportional number of total mass.]

Determination of the Measuring Time

The time of measurement, the reading time of the detector, and the acquisition frequency is determined experimentally in accordance with the required precision. Generally, the time for measurement permits a large number of detector scans or sweeps at short time intervals.

Selection of an Appropriate Optical Model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly larger amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, because small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about 0.01–0.1 i) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g., shape, surface roughness, and porosity) bear upon the final result.

Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision, and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH is not applicable as it is not possible to discriminate different components into a sample, nor is it possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor signal-to-noise ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly on the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for repeatability such as % RSD $\leq$ 10% [n = 6] for any central value of the distribution (e.g., for x_{50}). Values at the sides of the distribution

(e.g., x_{10} and x_{90}) are oriented towards less stringent acceptance criteria such as % RSD$\leq$ 15% [n = 6]. Below 10 μm, these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

MEASUREMENT

Precautions

The instructions given in the instrument manual are followed:

— never look into the direct path of the laser beam or its reflections;

— earth all instrument components to prevent ignition of solvents or dust explosions;

— check the instrument set-up (e.g., warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight); and

— in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent airflow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

Measurement of the Light Scattering of Dispersed Sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to subtract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated, sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity, and the quantum efficiency. The coordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and hence the particle concentration.

Conversion of Scattering Pattern Into Particle-Size Distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of deviations between measured and calculated scattering patterns (e.g., least squares), some constraints (e.g., non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each make and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

REPORTING RESULTS

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol x is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. Q3(x) denotes the volume fraction undersize at the particle size x. In a graphical representation, x is plotted on the abscissa and the dependent variable Q3 on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as x_{10}, x_{50}, and x_{90}, respectively) are frequently used. x_{50} is also known as the median particle size. It is recognized that the symbol d is also widely used to designate the particle size, thus the symbol x may be replaced by d.

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. Because the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

CONTROL OF THE INSTRUMENT PERFORMANCE

Use the instrument according to the manufacturer's instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument meets the requirements if the mean value of x_{50} from at least three independent measurements does not deviate by more than 3% from the certified range of values of the certified reference material. The mean values for x_{10} and x_{90} must not deviate by more than 5% from the certified range of values. Below 10 μm, these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, nonspherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same nonspherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

Qualification of the System

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software, and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is considered to meet the requirements if the x_{50} value does not deviate by more than 10% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g., x_{10} and x_{90}), then these values must not deviate by more than 15% from the certified range of values. Below 10 μm, these values must be doubled.

NOTE—For calibration of the instrument, stricter requirements are laid down in the paragraph on *Calibration*.▲*USP33*

Physical Tests and Determinations

BRIEFING

⟨616⟩ Bulk Density and Tapped Density of Powders. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Bulk and Tapped Density general chapter, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following general chapter, which represents the **ADOPTION STAGE 6** document, is based on comments from the United States Pharmacopeia and the Japanese Pharmacopoeia. A new section, which includes descriptions for *Measurement in a Vessel* has been added.

(EGC: K. Moore.) RTS—C55768

Add the following:

▲⟨616⟩ BULK DENSITY AND TAPPED DENSITY OF POWDERS

BULK DENSITY

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. ◆The portion that is not harmonized is marked with symbols (◆◆) to specify this fact.◆

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per mL (g/mL) although the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m^3) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm^3). The bulking properties of a powder are dependent upon the preparation, treatment, and storage of the sample, i.e., how it was handled. The particles can be packed to have a range of bulk densities; however, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specifiy how the determination was made. The bulk density of a powder is determined by measuring the volume of a known weight of powder sample, that may have been passed through a screen, into a graduated cylinder (*Method I*), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (*Method II*) or a measuring vessel (*Method III*).

Method I and *Method III* are favored.

Method I—Measurement in a Graduated Cylinder

Procedure—Pass a quantity of material sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated 250-mL cylinder (readable to 2 mL) introduce, without compacting, approximately 100 g of test sample, *M*, weighed with 0.1% accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in g/mL by the formula m/V_0. Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as the test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal

to 60% of the total volume of the cylinder); the weight of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL, a 100-mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Method II—Measurement in a Volumeter

Apparatus—The apparatus (*Figure 1*) consists of a top funnel fitted with a 1.0-mm screen[1]. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup of specified capacity mounted directly below it. The cup may be cylindrical (25.00 $\pm$ 0.05 mL volume with an inside diameter of 30.00 $\pm$ 2.00 mm) or a square (16.39 $\pm$ 0.2 mL volume with inside dimensions of 25.4 $\pm$ 0.076 mm).

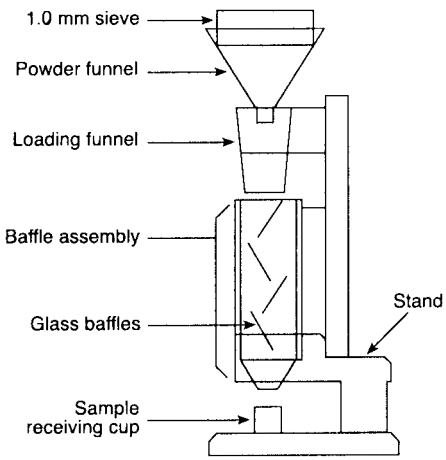


Figure 1.

Procedure—Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm^3 of powder with the square cup and 35 cm^3 of powder with the cylindrical cup. Carefully scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the sides of the cup, and determine the weight, *M,* of the powder to the nearest 0.1%. Calculate the bulk density, in g per mL, by the formula:

$$(M) / (V_0)$$

in which V_0 is the volume, in mL, of the cup. Record the average of three determinations using three different powder samples.

Method III—Measurement in a Vessel

Apparatus—The apparatus consists of a 100-mL cylindrical vessel of stainless steel with dimensions as specified in *Figure 2*.

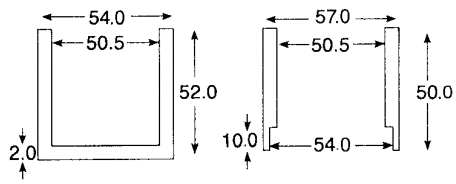


Figure 2.

Procedure—Pass a quantity of powder sufficient to complete the test through a 1.0-mm sieve, if necessary, to break up agglomerates that may have formed during storage, and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for *Method II*. Determine the weight (M_0) of the powder to the nearest 0.1% by subtraction of the previously determined mass of the empty measuring ves-

[1] The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.

sel. Calculate the bulk density (g/mL) by the formula $M_0/100$, and record the average of three determinations using three different powder samples.

TAPPED DENSITY

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. Tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing a powder sample. After observing the initial powder volume or weight, the measuring cylinder or vessel is mechanically tapped, and volume or weight readings are taken until little further volume or weight change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop under its own weight a specified distance by either of three methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

Method I

Apparatus—The apparatus (*Figure 3*) consists of the following:

- A 250-mL graduated cylinder (readable to 2 mL with a mass of 220 $\pm$ 44 g
- A settling apparatus capable of producing, in 1 minute, either nominally 250 $\pm$ 15 taps from a height of 3 $\pm$ 0.2 mm, or nominally 300 $\pm$ 15 taps from a height of 14 $\pm$ 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 $\pm$ 10 g.

Procedure—Proceed as described above for the determination of the bulk volume (V_0). Secure the cylinder in the holder. Carry out 10, 500, and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500}, and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_F in which V_F is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results. If it is not possible to use a 100-g test sample, use a reduced amount and a suitable 100-mL graduated cylinder (readable to 1 mL) weighing 130 $\pm$ 16 g and mounted on a holder weighing 240 $\pm$ 12 g. The modified test conditions are specified in the expression of the results.

Method II

Apparatus and **Procedure—**Proceed as directed under *Method I* except that the mechanical tester provides a fixed drop of 3 $\pm$ 0.2 mm at a nominal rate of 250 taps per minute.

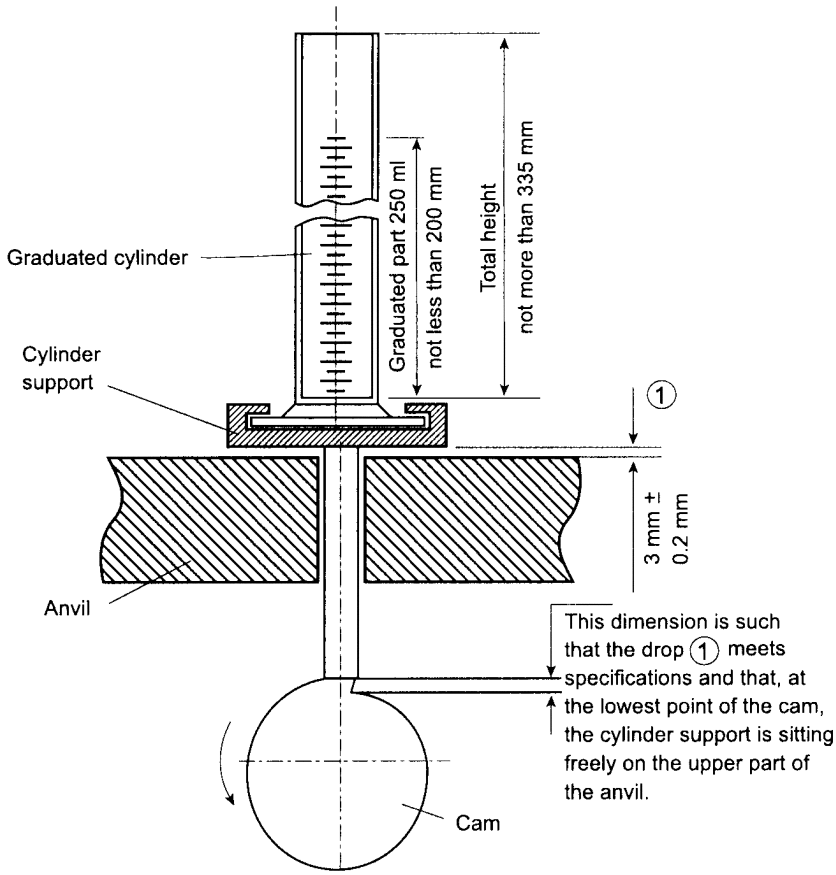


Figure 3.

Method III

Apparatus and **Procedure—**Proceed as directed in *Method III—Measurement in a Vessel* for measuring bulk density using the measuring vessel equipped with the cap shown in *Figure 2*. The measuring vessel with the cap is lifted 50–60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap, and carefully scrape excess powder from the top of the measuring vessel as described in *Method III—Measurement in a Vessel* for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the two masses obtained after 200 and 400 taps exceeds 2%, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2%. Calculate the tapped density (g/mL) using the formula $M_F / 100$ where M_F is the mass of powder in the measuring vessel. Record the average of three determinations using three different powder samples.

MEASURES OF POWDER COMPRESSIBILITY

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the *Compressibility Index* or the *Hausner Ratio* as described below.

The *Compressibility Index* and *Hausner Ratio* are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder's ability to settle, and they permit an assessment

of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the *Compressibility Index* and the *Hausner Ratio.*

Compressibility Index—Calculate by the formula:

$$100(V_0 - V_f)/V_0$$

V_0 = unsettled apparent volume

V_F = final tapped volume

Hausner Ratio—

$$V_0 / V_F$$

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0. ◆[NOTE—If V_{10} is used, it will be clearly stated in the results.]◆▲*USP33*

BRIEFING

〈**711**〉 **Dissolution,** *USP 32* page 268 and page 1243 of *PF* 34(5) [Sept.–Oct. 2008]. The *Apparatus* section of this general test chapter is proposed for revision as a result of comments received on the **ADOPTION STAGE 6** harmonization text. As a result of comments received from the ICH Regulatory Acceptance of Analytical Procedures and Acceptance Criteria evaluation of the dissolution chapters in the *European Pharmacopoeia*, the *Japanese Pharmacopoeia*, and the *United States Pharmacopeia*, the description of the pump used in *Apparatus 4* is revised to include a pump that produces no pulsation.

(BPC: W. Brown) RTS—C69683

Change to read:

APPARATUS

Apparatus 1 (Basket Apparatus)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material[1]; a motor; a metallic drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at 37 ± 0.5° during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. An apparatus that permits observation of the specimen and stirring element during the test is preferable. The vessel is cylindrical, with a hemispherical bottom and ◆with one of the following dimensions and capacities: for a nominal◆ capacity of 1 L, the height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm; ◆for a nominal capacity of 2 L, the height is 280 mm to 300 mm and its inside diameter is 98 mm to 106 mm; and for a nominal capacity of 4 L, the height is 280 mm to 300 mm and its inside diameter is 145 mm to 155 mm◆. Its sides are flanged at the top. A fitted cover may be used to retard evaporation.[2] The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at the specified rate ◆given in the individual monograph,◆ within ± 4%.

Shaft and basket components of the stirring element are fabricated of stainless steel, type 316, or other inert material, to the specifications shown in *Figure 1.* A basket having a gold coating of about 0.0001 inch (2.5 μm) thick may be used. A dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

[1] The materials should not sorb, react, or interfere with the specimen being tested.

[2] If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

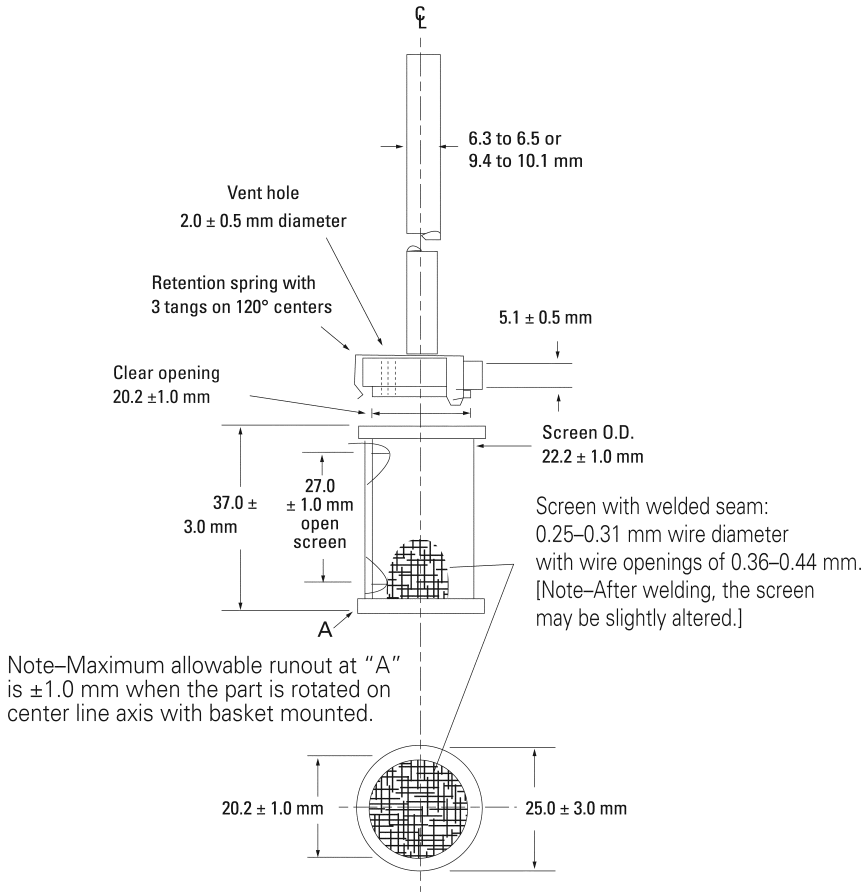


Figure 1. Basket Stirring Element

Apparatus 2 (Paddle Apparatus)

Use the assembly from *Apparatus 1*, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel at any point and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in *Figure 2*. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of nonreactive material, such as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in *Figure 2a.* Other validated sinker devices may be used.

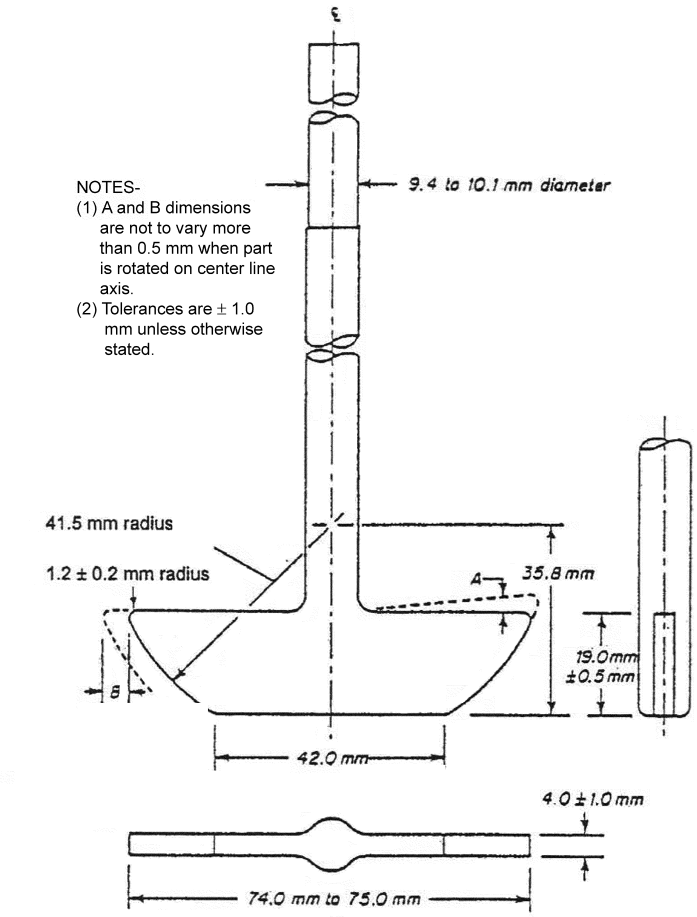


Figure 2. Paddle Stirring Element

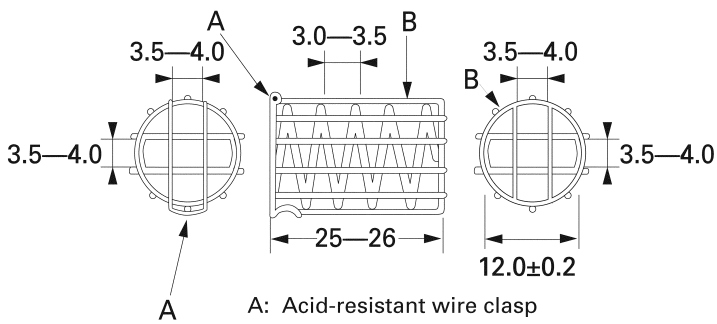


Figure 2a. Alternative sinker. All dimensions are expressed in mm.

Apparatus 3 (Reciprocating Cylinder)

NOT ACCEPTED BY THE JAPANESE PHARMACOPOEIA

The assembly consists of a set of cylindrical, flat-bottomed glass vessels; a set of glass reciprocating cylinders; inert fittings (stainless steel type 316 or other suitable material), and screens that are made of suitable nonsorbing and nonreactive material and that are designed to fit the tops and bottoms of the reciprocating cylinders; and a motor and drive assembly to reciprocate the cylinders vertically inside the vessels and, if desired, index the reciprocating cylinders horizontally to a different row of vessels. The vessels are partially immersed in a suitable water bath of any convenient size that permits holding the temperature at 37 ± 0.5° during the test. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating cylinder. A device is used that allows the reciprocation rate to be selected and maintained at the specified dip rate ◆given in the individual monograph◆ within ± 5%. An apparatus that permits observation of the specimens and reciprocating cylinders is preferable. The vessels are provided with an evaporation cap that remains in place for the duration of the test. The components conform to the dimensions shown in *Figure 3* unless otherwise specified ◆in the individual monograph◆.

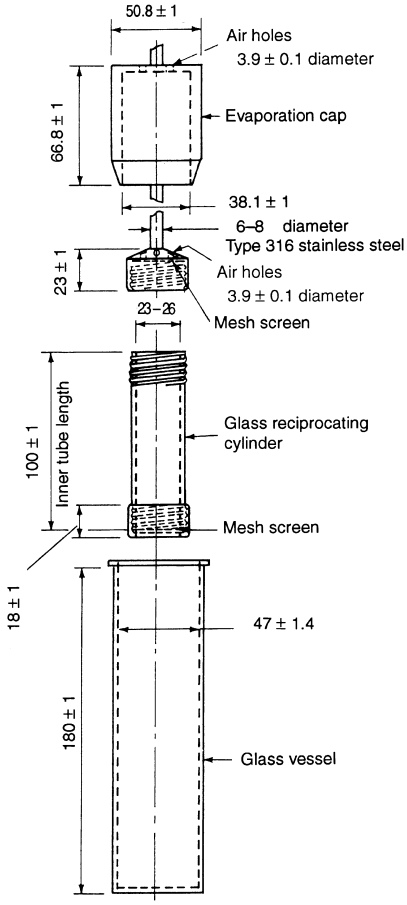


Figure 3. Apparatus 3 (reciprocating cylinder)

Apparatus 4 (Flow-Through Cell)

The assembly consists of a reservoir and a pump for the *Dissolution Medium;* a flow-through cell; and a water bath that maintains the *Dissolution Medium* at 37 ± 0.5°. Use the specified cell size ◆as given in the individual monograph◆.

The pump forces the *Dissolution Medium* upwards through the flow-through cell. The pump has a delivery range between 240 and 960 mL per hour, with standard flow rates of 4, 8, and 16 mL per minute. It must deliver a constant flow (±5% of the nominal flow rate); the flow profile is sinusoidal with a pulsation of 120 ± 10 pulses per minute.

▲A pump without pulsation may also be used. Dissolution test procedures using a flow-through cell must be characterized with respect to rate and any pulsation.▲*USP33*

The flow-through cell (see *Figures 4* and *5*), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph) that prevents escape of undissolved particles from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5 mm positioned at the apex to protect the fluid entry tube; and a tablet holder (see *Figures 4* and *5*) is available for positioning of special dosage forms, for example, inlay tablets. The cell is immersed in a water bath, and the temperature is maintained at 37 ± 0.5°.

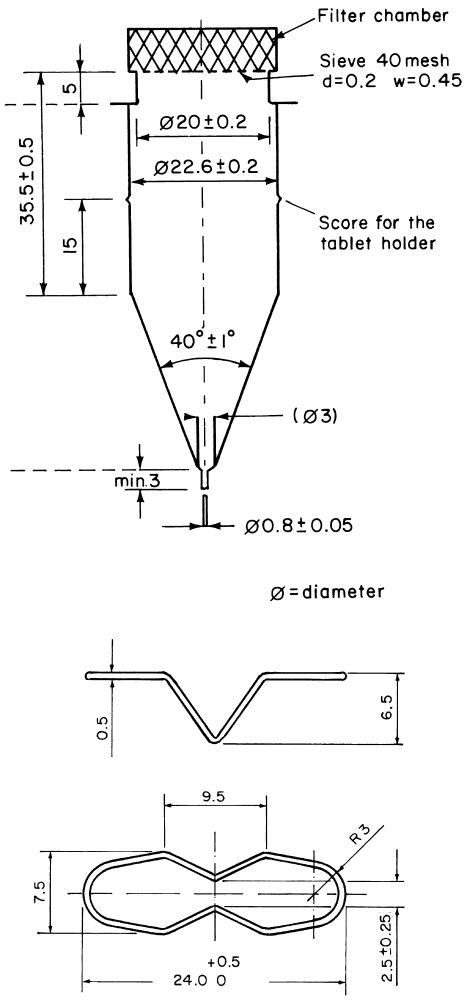


Figure 4. Apparutus 4, large cell for tablets and capsules (top), tablet holder for the large cell (bottom). (All measurements are expressed in mm unless noted otherwise.)

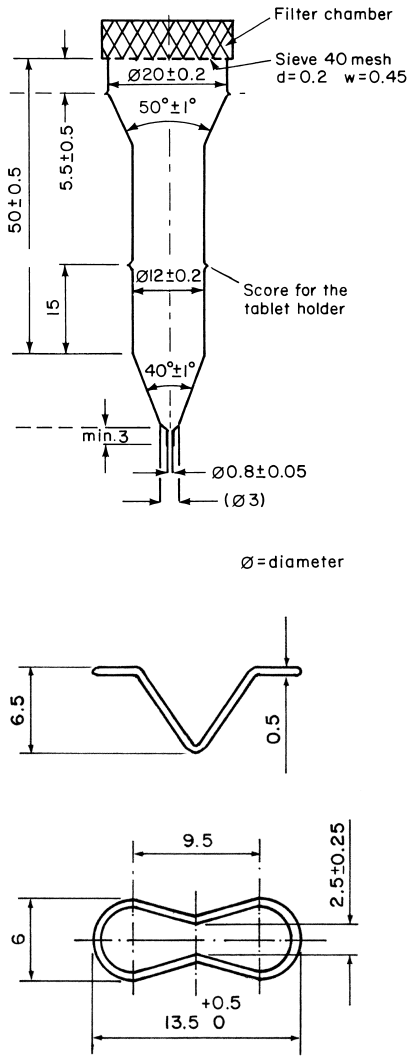


Figure 5. Apparatus 4, small cell for tablets and capsules (top), tablet holder for the small cell (bottom). (All measurements are expressed in mm unless noted otherwise.)

The apparatus uses a clamp mechanism and two O-rings to assemble the cell. The pump is separated from the dissolution unit in order to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytef, with about 1.6-mm inner diameter and chemically inert flanged-end connections.

APPARATUS SUITABILITY

The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and temperature of the *Dissolution Medium,* rotation speed *(Apparatus 1* and *Apparatus 2),* dip rate *(Apparatus 3),* and flow rate of medium *(Apparatus 4).*

Determine the acceptable performance of the dissolution test assembly periodically. ◆The suitability for the individual apparatus is demonstrated by the *Performance Verification Test*.

Performance Verification Test, Apparatus 1 and 2— Individually test 1 tablet of the USP Prednisone Tablets RS and 1 tablet of USP Salicylic Acid Tablets RS, according to the operating conditions specified. The apparatus is suitable if the results obtained are within the acceptable range stated in the certificate for that Reference Standard tablet in the apparatus tested.

Performance Verification Test, Apparatus 3—Individually test 1 tablet of the USP Chlorpheniramine Maleate Extended-Release Tablets RS according to the operating conditions specified. The apparatus is suitable if the results obtained are within the acceptable range stated in the certificate.

Performance Verification Test, Apparatus 4—[To come.]◆

BRIEFING

〈**905**〉 **Uniformity of Dosage Units,** *USP* 32 page 382 and page 1290 of *PF* 34(5) [Sept.–Oct. 2008]. This general test chapter is revised to reduce the quantity of national, *USP*-specific text and more completely represent the harmonized text agreed upon by the Pharmacopeial Discussion Group. The texts of this chapter as presented in the *European Pharmacopoeia*, the *Japanese Pharmacopoeia*, and the *USP* are undergoing evaluation by ICH for Regulatory Approval of Analytical Procedures and Acceptance Criteria. Communications received from the European Pharmacopoeia Commission Secretariat recommended the reduction in *USP*-specific national text to assist the ICH evaluation and approval. USP determined that the changes would not only enhance conformance with the harmonized text but reduce redundant verbiage.

Changes to the *USP* text include the following:

1. Reduction in the number of references to the specific monograph and instead merely citing the specification. In the *USP*, the specification is found in the monograph and thus additional wording to that effect is seen as redundant.
2. Elimination of the evaluation of a special procedure and replacement with harmonized text that refers in more general terms to the possible need to apply a correction factor where differences are observed between the results of the assay and the special procedure for content uniformity.
3. Simplification of the list of dosage forms presented in *Table 1*. This change is also made to the procedures and *Criteria* sections for *Content Uniformity* and *Weight Variation* test sections. The national text procedures and criteria for suppositories, transdermal systems, inhalations packaged in premetered dosage units, and solutions for inhalation packaged in glass or plastic ampuls and intended for use in nebulizers are thereby revised.

(PDF: W. Brown.) RTS—C71696

Change to read:

■This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and the *Japanese Pharmacopoeia*. Portions of the general chapter text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (◆▲) to specify this fact.■2S (*USP32*)

◆[NOTE—In this chapter, *unit* and *dosage unit* are synonymous.]◆

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit. The uniformity of dosage units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for ~~◆topical.◆~~

▲external, cutaneous▲*USP33*
administration.

The term ''uniformity of dosage unit'' is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified ~~◆in the individual monograph.◆~~

▲elsewhere in this pharmacopeia.▲*USP33*

The uniformity of dosage units can be demonstrated by either of two methods, *Content Uniformity* or ◆*Weight*◆*Variation* (see *Table 1*). The test for *Content Uniformity*

▲of preparations presented in dosage units▲*USP33*
is based on the assay of the individual content of drug substance(s) in a number of ~~◆individual◆~~

▲
▲*USP33*
dosage units to determine whether the individual content is within the limits set. The *Content Uniformity* method may be applied in all cases. ~~◆The test for *Content Uniformity* is required for those dosage forms described in *(C1)–(C6)* below:~~

~~(C1) coated tablets, other than film-coated tablets containing 25 mg or more of a drug substance that comprises 25% or more (by weight) of one tablet;~~
~~(C2) transdermal systems;~~
~~(C3) suspensions or emulsions or gels in single-unit containers or in soft capsules that are intended for systemic administration only (not for those drug products that are intended for topical administration);~~
~~(C4) inhalations (other than solutions for inhalation packaged in glass or plastic ampuls and intended for use in nebulizers) packaged in premetered dosage units. For inhalers and premetered dosage units labeled for use with a named inhalation device, also see *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry-Powder Inhalers* 〈601〉;~~
~~(C5) solids (including sterile solids) that are packaged in single-unit containers and that contain active or inactive added substances, except that the test for *Weight Variation* may be applied in the special cases stated in *(W3)* below; and~~
~~(C6) suppositories.◆~~

▲
▲*USP33*

The test for ◆*Weight*◆*Variation* is applicable for the following dosage forms:

(W1) ~~◆solutions for inhalation that are packaged in glass or plastic ampuls and intended for use in nebulizers, and oral◆~~ Solutions ~~◆packaged◆~~

▲enclosed▲*USP33*
in unit-dose containers and into soft capsules;

(W2) solids (including

▲powders, granules, and▲*USP33*
sterile solids) that are packaged in single-unit containers and contain no ~~added substances, whether active or inactive~~

▲active or inactive added substances;▲*USP33*

(W3) solids (including sterile solids) that are packaged in single-unit containers, with or without ~~added substances, whether active or inactive,~~

▲active or inactive added substances,▲*USP33* that have been prepared from true solutions and freeze-dried in the final containers and are labeled to indicate this method of preparation; and

(W4) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting *Content Uniformity* requirements.

The test for *Content Uniformity* is required for all dosage forms not meeting the above conditions for the ◆*Weight Variation* test. ◆Where compliance with the *Content Uniformity* test is required, then, by application of the provision for use of alternative methods provided in the *General Notices* section of this *Pharmacopeia*, it is possible for manufacturers to ensure this compliance by application of the *Weight Variation* test where the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%. This RSD determination may be based on the manufacturer's process validation and product development data.◆ The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in *Table 2*. ◆Where the *Weight Variation* test is used in this way, the product must, if tested, nevertheless comply with the official compendial test for *Content Uniformity.*◆

Change to read:

CONTENT UNIFORMITY

Select not fewer than 30 units, and proceed as follows for the dosage form designated. ◆~~Where the amount of drug substance in a single dosage unit differs from that required in the *Assay*, adjust the degree of dilution of the solutions and/or the volume of aliquots so that the concentration of the drug substances in the final solution is of the same order as that obtained in the *Assay* procedure; or, in the case of a titrimetric assay, use a titrant of a different concentration, if necessary, so that an adequate volume of titrant is required (see *Titrimetry* ⟨541⟩); see also *Procedures* under *Tests and Assays* in the *General Notices and Requirements*. If any such modifications are made in the *Assay* procedure set forth in the individual monograph, make the appropriate corresponding changes in the calculation formula and titration factor.~~

~~Where a special *Procedure for content uniformity* is specified in the test for *Uniformity of dosage units* in the individual monograph, make any necessary correction of the results obtained as follows.~~

~~(1) Prepare a composite specimen of a sufficient number of dosage units to provide the amount of specimen called for in the *Assay* in the individual monograph plus the amount required for the special *Procedure for content uniformity* in the monograph by finely powdering tablets or mixing the contents of capsules or oral solutions, suspensions,~~

Table 1. Application of Content Uniformity (CU) and Weight Variation (WV) Tests for Dosage Forms

Dosage Form	Type	Subtype	Dose & Ratio of Drug Substance	
			≥25 mg &≥25%	<25 mg or<25%
Tablets	Uncoated		WV	CU
	Coated	Film	WV	CU
		Others	CU	CU
Capsules	Hard		WV	CU
	Soft	Suspension, emulsion, or gel	CU	CU
		Solutions	WV	WV
Solids in single-unit containers	Single component		WV	WV
	Multiple components	Solution freeze-dried in final container	WV	WV
		Others	CU	CU
◆~~Suspension, emulsion, or gel for systemic use only, packaged in single-unit containers~~◆			◆~~CU~~◆	◆~~CU~~◆ ▲▲*USP33*
Solutions ◆~~for inhalation packaged in glass or plastic ampuls and intended for use in nebulizers, and oral~~◆ ~~solutions~~ ◆~~packaged~~◆ in unit-dose containers ◆and into soft capsules◆			WV	WV
◆~~Inhalations (other than solutions for inhalation packaged in glass or plastic ampuls and intended for use in nebulizers) packaged in premetered dosage units~~◆			◆~~CU~~◆	◆~~CU~~◆
◆~~Transdermal systems~~◆			◆~~CU~~◆	◆~~CU~~◆
◆~~Suppositories~~◆			◆~~CU~~◆	◆~~CU~~◆ ▲▲*USP33*
Others			CU	CU

~~emulsions, gels, or solids in single-unit containers to obtain a homogeneous mixture. If a homogeneous mixture cannot be obtained in this manner, use suitable solvents or other procedures to prepare a solution containing all of the drug substance, and use appropriate aliquot portions of this solution for the specified procedures.~~

~~(2) Assay separate, accurately measured portions of the composite specimen of capsules or tablets or suspensions or inhalations or solids in single-unit containers, both (a) as directed in the *Assay*, and (b) using the special *Procedure for content uniformity* in the monograph.~~

~~(3) Calculate the weight of drug substance equivalent to 1 average dosage unit, by (a) using the results obtained by the *Assay* procedure, and by (b) using the results obtained by the special procedure.~~

~~(4) Calculate the correction factor, *F*, by the formula:~~

~~$$F = W/P$$~~

~~in which *W* is the weight of drug substance equivalent to 1 average dosage unit obtained by the *Assay* procedure, and *P* is the weight of drug substance equivalent to 1 average dosage unit obtained by the special procedure. If~~

~~$$\frac{100|W - P|}{W}$$~~

~~is greater than 10, the use of a correction factor is not valid.~~

~~(5) The correction factor is to be applied only if *F* is not less than 1.030 nor greater than 1.100, or not less than 0.900 nor greater than 0.970. If *F* is between 0.970 and 1.030, no correction is required.~~

~~(6) If *F* lies between 1.030 and 1.100, or between 0.900 and 0.970, calculate the weight of drug substance in each dosage unit by multiplying each of the weights found using the special procedure by *F*.~~

▲Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.▲*USP33*

~~Uncoated, Coated, or Molded Tablets, Capsules, Oral Solutions in Unit-Dose Containers, Suspensions or Emulsions or Gels in Single-Unit Containers (that are intended for systemic administration only), and Solids (including Sterile Solids) in Single-Unit Containers~~

▲Solid Dosage Forms▲*USP33*
—Assay 10 units individually ~~as directed in the *Assay* in the individual monograph, unless otherwise specified in the *Procedure for content uniformity* in the individual monograph.~~

▲using an appropriate analytical method.▲*USP33* Calculate the acceptance value ~~as directed below~~

▲▲*USP33*

■(see *Table 2*).■2S *(USP32)*

~~For oral solutions in unit-dose containers, and for suspensions, emulsions, or gels in single-unit containers that are intended for systemic administration only,~~

▲Liquid Dosage Forms—▲*USP33*
Carry out the *Assay* on the amount of well-mixed material that ~~drains from an individual container in not more than 5 seconds,~~

■is removed from an individual container in conditions of normal use, and express the results as delivered dose.■2S *(USP32)*
~~or for highly viscous products, conduct the *Assay* on the amount of well-mixed material that is obtained by quantitatively removing the contents from an individual container, and express the results as the delivered dose.~~

▲▲*USP33*

■Calculate the acceptance value (see *Table 2*).■2S *(USP32)*

Calculation of Acceptance Value—Calculate the acceptance value by the formula:

$$\left|M - \overline{X}\right| + ks$$

in which the terms are as defined in *Table 2*.

Table 2

Variable	Definition	Conditions	Value
$\overline{X}$	Mean of individual contents ($\chi_1, \chi_2, \ldots, \chi_n$), expressed as a percentage of the label claim		
$\chi_1, \chi_2, \ldots, \chi_n$	Individual contents of the units tested, expressed as a percentage of the label claim		
n	Sample size (number of units in a sample)		
k	Acceptability constant	If *n* = 10, then *k* =	2.4
		If *n* = 30, then *k* =	2.0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}\left(\chi_i-\overline{X}\right)^2}{n-1}\right]^{\frac{1}{2}}$
RSD	Relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\frac{100s}{\overline{X}}$
M (case 1) to be applied when $T \leq 101.5$	Reference value	If $98.5\% \leq \overline{X} \leq 101.5\%$, then	$M = \overline{X}$ *(AV = ks)*
		If $\overline{X} < 98.5\%$, then	*M* = 98.5% *(AV = 98.5 − $\overline{X}$ + ks)*
		If $\overline{X} > 101.5\%$, then	*M* = 101.5% *(AV = $\overline{X}$ − 101.5 + ks)*
M (case 2) to be applied when $T > 101.5$	Reference value	If $98.5 \leq \overline{X} \leq T$, then	$M = \overline{X}$ *(AV = ks)*
		If $\overline{X} < 98.5\%$, then	*M* = 98.5% *(AV = 98.5 − $\overline{X}$ + ks)*
		If $\overline{X} > T$, then	*M* = *T*% *(AV = $\overline{X}$ − T + ks)*
Acceptance value *(AV)*			general formula: $\left\lvert M-\overline{X}\right\rvert + ks$ (Calculations are specified above for the different cases.)
L1	Maximum allowed acceptance value		*L1* = 15.0 unless otherwise specified~~ in the individual monograph.~~ ▲▲*USP33*

Table 2 *(Continued)*

Variable	Definition	Conditions	Value
L2	Maximum allowed range for deviation of each dosage unit tested from the calculated value of *M*	On the low side, no dosage unit result can be less than [1 – (0.01)(*L2*)]*M,* while on the high side no dosage unit result can be greater than [1 + (0.01)(*L2*)]*M.* (This is based on an *L2* value of 25.0.)	*L2* = 25.0 unless otherwise specified ◆~~in the individual monograph,~~◆ ▲▲*USP33*
T	Target content per dosage unit at the time of manufacture, expressed as a percentage of the label claim. For purposes of this Pharmacopeia, unless otherwise ~~specified in the individual monograph, *T* is the average of the limits specified in the potency definition in the individual monograph.~~■stated in the individual monograph, *T* is 100.0%, and for manufacturing purposes, *T* is the manufacturer's approved target test amount value at the time of manufacture.■2S *(USP32)*		

~~**Suppositories, Transdermal Systems, and Inhalations Packaged in Premetered Dosage Units**~~ ~~[NOTE—Acceptance value calculations are not required for these dosage forms.] Assay 10 units individually as directed in the *Assay* in the individual monograph, unless otherwise specified in the *Procedure for content uniformity*.~~

▲*USP33*

Change to read:

◆WEIGHT◆ VARIATION

~~Select not fewer than 30 dosage units, and proceed as follows for the dosage form designated. The result of the *Assay,* ◆obtained as directed in the individual monograph,◆ is designated as result *A,* expressed as % of label claim (see *Calculation of Acceptance Value*). Assume that the concentration (weight of drug substance per weight of dosage unit) is uniform. [◆NOTE—Specimens other than these test units may be drawn from the same batch for assay determinations.◆]~~

▲Carry out an assay for the drug substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as percent of label claim (see *Calculation of Acceptance Value*). Assume that the concentration (weight of drug substance per weight of dosage unit) is uniform. Select not fewer than 30 dosage units, and proceed as follows for the dosage form designated.▲*USP33*

Uncoated or Film-Coated Tablets—Accurately weigh 10 tablets individually. Calculate the ◆~~drug substance~~◆

▲*USP33*
content, expressed as % of label claim, of each tablet from the ◆weight◆ of the individual tablet and the result of the *Assay.* Calculate the acceptance value.

Hard Capsules—Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by a suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net ◆weight◆ of its contents by subtracting the ◆weight◆ of the shell from the respective gross ◆weight◆. Calculate the drug substance content◆~~, expressed as % of label claim,~~◆

▲*USP33*
of each capsule from the ◆net weight◆ of the individual capsule ◆content◆ and the result of the assay. Calculate the acceptance value.

Soft Capsules—Accurately weigh 10 intact capsules individually to obtain their gross ◆weights◆, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 minutes, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content ◆~~, expressed as % of label claim,~~◆

▲*USP33*
in each capsule from the ◆~~net~~

▲*USP33*
weight◆ of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

~~**Solids ◆(Including Sterile Solids) in Single Unit Containers**◆~~

▲Solid Dosage Forms Other Than Tablets and Capsules▲*USP32*
—Proceed as directed for *Hard Capsules,* treating each unit as described therein. Calculate the acceptance value.

~~◆**Oral Solutions Packaged in Unit Dose Containers**◆~~

▲Liquid Dosage Forms▲*USP33*
—Accurately weigh the amount of liquid that ~~drains in not more than 5 seconds~~

■is removed■2S (*USP32*)
from each of 10 individual containers

■in conditions of normal use.■2S (*USP32*)
If necessary, compute the equivalent volume after determining the density. Calculate the drug substance content ◆~~, expressed as % of label claim, in the liquid drained from each unit from the net weight of the individual container content~~◆

▲in each container from the mass of product removed from the individual containers▲*USP33*
and the result of the assay. Calculate the acceptance value.

Calculation of Acceptance Value—Calculate the acceptance value as shown in *Content Uniformity,* except that the individual contents of the units are replaced with the individual estimated contents defined below.

$\chi_1, \chi_2, \dots, \chi_n$	=	individual estimated contents of the units tested, where $\chi_i = w_i \times A/\overline{W}$
$w_1, w_2, \dots, w_n$	=	individual ◆weights◆ of the units tested ~~for weight variation,~~
		■■2S (*USP32*)
A	=	content of drug substance (% of label claim) ◆~~determined as described in the *Assay;* and~~◆
		▲obtained using an appropriate analytical method▲*USP33*
$\overline{W}$	=	mean of individual ◆weights◆ ($w_1, w_2, \dots, w_n$) ~~of the units used in the Assay.~~
		■■2S (*USP32*)

~~◆**Solutions for Inhalation Packaged in Glass or Plastic Ampuls and Intended for Use in Nebulizers**~~ ~~[NOTE—Acceptance value calculations are not required for these dosage forms.] Accurately weigh 10 containers individually, taking care to preserve the identity of each container. Remove the contents of each container by a suitable means. Accurately weigh the emptied containers individually, and calculate for each container the net weight of its contents by subtracting the weight of the container from the respective gross weight. From the results of the *Assay,* obtained as directed in the individual monograph, calculate the drug substance content, expressed as % of label claim, in each of the containers.~~◆

▲*USP33*

Change to read:

CRITERIA

Apply the following criteria, unless otherwise specified. ◆~~in the individual monograph~~.◆

▲ ▲*USP33*

◆~~Uncoated, Coated, or Molded Tablets, Capsules, Oral Solutions in Unit-Dose Containers, Suspensions or Emulsions or Gels in Single Unit Containers (that are intended for systemic administration only), and Solids (including Sterile Solids) in Single Unit Containers~~.◆

▲Solid and Liquid Dosage Forms▲*USP33*
—The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to *L1*%. If the acceptance value is greater than *L1*%, test the next 20 units, and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to *L1*%, and no individual content of ◆any◆ dosage unit is less than [1 – (0.01)(*L2*)] *M* nor more than [1 + (0.01)(*L2*)] *M* ◆as specified◆ in the *Calculation of Acceptance Value* under *Content Uniformity* or under ◆*Weight◆ Variation.* Unless otherwise specified, ◆~~in the individual monograph,~~◆

▲ ▲*USP33*
L1 is 15.0 and *L2* is 25.0.

◆~~Suppositories~~

~~*Limit A* (if the average of the limits specified in the potency definition in the individual monograph is 100.0 percent or less)—Unless otherwise specified in the individual monograph, the requirements for dosage uniformity are met if the amount of the drug substance in each of the 10 dosage units as determined from the *Content Uniformity* method lies within the range of 85.0% to 115.0% of the label claim, and the RSD is less than or equal to 6.0%.~~

~~If 1 unit is outside the range of 85.0% to 115.0% of label claim, and no unit is outside the range of 75.0% to 125.0% of label claim, or if the RSD is greater than 6.0%, or if both conditions prevail, test 20 additional units. The requirements are met if not more than 1 unit of the 30 is outside the range of 85.0% to 115.0% of label claim, and no unit is outside the range of 75.0% to 125.0% of label claim and the RSD of the 30 dosage units does not exceed 7.8%.~~

~~*Limit B* (if the average of the limits specified in the potency definition in the individual monograph is greater than 100.0 percent)—~~

~~(1) If the average value of the dosage units tested is 100.0 percent or less, the requirements are as in *Limit A*.~~

~~(2) If the average value of the dosage units tested is greater than or equal to the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under *Limit A*, except that the words "label claim" are replaced by the words "label claim multiplied by the average of the limits specified in the potency definition in the monograph divided by 100".~~

~~(3) If the average value of the dosage units tested is between 100 percent and the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under *Limit A*, except that the words "label claim" are replaced by the words "label claim multiplied by the average value of the dosage units tested (expressed as a percent of label claim) divided by 100".~~◆

◆~~Transdermal Systems, and~~■~~2S (USP32)~~ ~~Inhalations Packaged in Premetered Dosage Units,~~■~~and Solutions for Inhalation Packaged in Glass or Plastic Ampuls and Intended for Use in Nebulizers~~■~~2S (USP32)~~

~~*Limit A* (if the average of the limits specified in the potency definition in the individual monograph is 100.0 percent or less)—Unless otherwise specified in the individual monograph, the requirements for dosage uniformity are met if the amount of the drug substance in not fewer than 9 of the 10 dosage units as determined from the *Content Uniformity* method (or, in the case of solutions for inhalation packaged in glass or plastic ampuls and intended for use in nebulizers, from either the *Content Uniformity* or the *Weight Variation* method) lies within the range of 85.0% to 115.0% of label claim, and no unit is outside the range of 75.0% to 125.0% of label claim, and the RSD of the 10 dosage units is less than or equal to 6.0%.~~

~~If 2 or 3 dosage units are outside the range of 85.0% to 115.0% of label claim, but not outside the range of 75.0% to 125.0% of label claim, or if the RSD is greater than 6.0% or if both conditions prevail, test 20 additional units. The requirements are met if not more than 3 units of the 30 are outside the range of 85.0% to 115.0% of label claim and no unit is outside the range of 75.0% to 125.0% of label claim, and the RSD of the 30 dosage units does not exceed 7.8%.~~

~~*Limit B* (if the average of the limits specified in the potency definition in the individual monograph is greater than 100.0 percent)—~~

~~(1) If the average value of the dosage units tested is 100.0 percent or less, the requirements are as in *Limit A*.~~

~~(2) If the average value of the dosage units tested is greater than or equal to the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under *Limit A*, except that the words "label claim" are replaced by the words "label claim multiplied by the average of the limits specified in the potency definition in the monograph divided by 100".~~

~~(3) If the average value of the dosage units tested is between 100 percent and the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under *Limit A*, except that the words "label claim" are replaced by the words "label claim multiplied by the average value of the dosage units tested (expressed as a percent of label claim) divided by 100".~~◆

▲ ▲*USP33*

BRIEFING

⟨**941**⟩ **X-Ray Diffraction.** *USP* 32 page 390. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of compendial standards for this chapter as part of the process of international harmonization of chapters and general analytical methods of the European, Japanese, and United States pharmacopeias. This chapter, which represents the **ADOPTION STAGE 6** draft in the harmonization process, is based on the Official Inquiry Stage 4 document, which appeared in *PF* 31(4) [July–Aug. 2005].

(GC: H. Pappa) RTS—C47061

Add the following:

▲⟨941⟩ CHARACTERIZATION OF CRYSTALLINE AND PARTIALLY CRYSTALLINE SOLIDS BY X-RAY POWDER DIFFRACTION (XRPD)

INTRODUCTION

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially three types of information can be derived from a powder diffraction pattern: the angular position of diffraction lines (depending on geometry and size of the unit cell), the intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample), and diffraction line profiles (depending on instrumental resolution, crystallite size, strain, and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (e.g., identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions[1] can also be made.

The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually nondestructive in nature (to ensure a randomly oriented sample, specimen preparation is usually limited to grinding). XRPD investigations can also be carried out under *in situ* conditions on specimens exposed to non-ambient conditions such as low or high temperature and humidity.

PRINCIPLES

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive when the path difference between two diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see *Figure 1*).

$$2d_{hkl}\sin\theta_{hkl} = n\lambda$$

[1] There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances, such as determination of crystal structures, refinement of crystal structures, determination of the crystallographic purity of crystalline phases, and characterization of crystallographic texture. These applications are not described in this chapter.

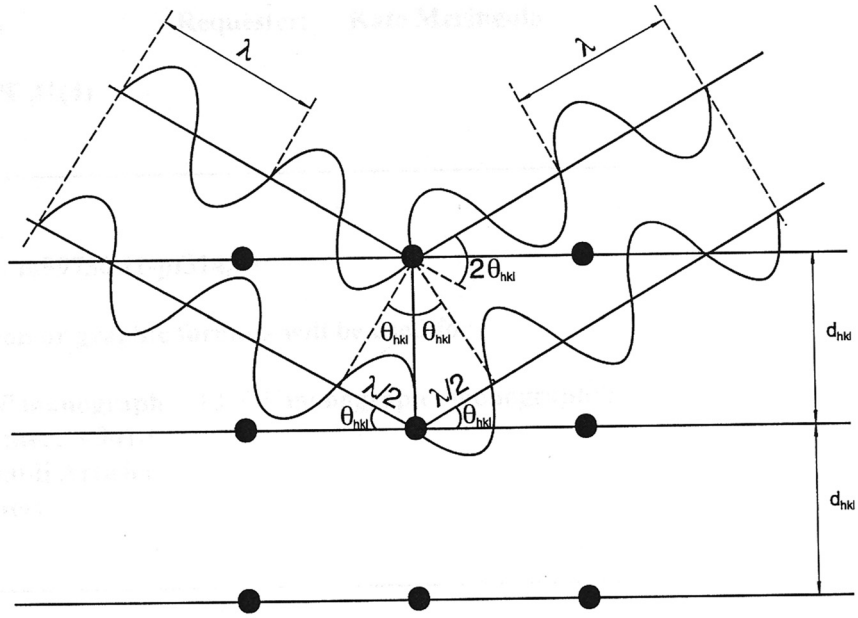


Figure 1. Diffraction of X-rays by a crystal according to Bragg's Law.

The wavelength, λ, of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or d_{hkl} (also called *d*-spacings). θ_{hkl} is the angle between the incident ray and the family of lattice planes, and sin θ_{hkl} is inversely proportional to the distance between successive crystal planes or *d*-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices {*hkl*}. These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings *a*, *b,* and *c,* and the angles between them α, β, and γ.

The interplanar spacing for a specified set of parallel *hkl* planes is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the *d* values for the related families of planes *nh, nk, nl* are diminished by the factor 1/*n* (*n* being an integer: 2, 3, 4, etc.).

Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with it (for a specific λ).

A powder specimen is assumed to be polycrystalline so that at any angle θ_{hkl} there are always crystallites in an orientation allowing diffraction according to Bragg's law.[2] For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as ''lines'', ''reflections'', or ''Bragg reflections'') are characteristic of the crystal lattice (*d*-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles depend on the perfection and extent of the crystal lattice. Under these conditions, the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion, and structural imperfections, as well as from instrument characteristics.

The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity, and Lorentz factor.

[2] An ideal powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.

The main characteristics of diffraction line profiles are 2θ position, peak height, peak area, and shape (characterized by, e.g., peak width, or asymmetry, analytical function, and empirical representation). An example of the type of powder patterns obtained for five different solid phases of a substance are shown in *Figure 2.*

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more or less uniform background, upon which the peaks are superimposed. Besides specimen preparation, other factors contribute to the background—for example, sample holder, diffuse scattering from air and equipment, and other instrumental parameters such as detector noise and general radiation from the X-ray tube. The peak-to-background ratio can be increased by minimizing background and by choosing prolonged exposure times.

INSTRUMENT

Instrument Setup

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras.

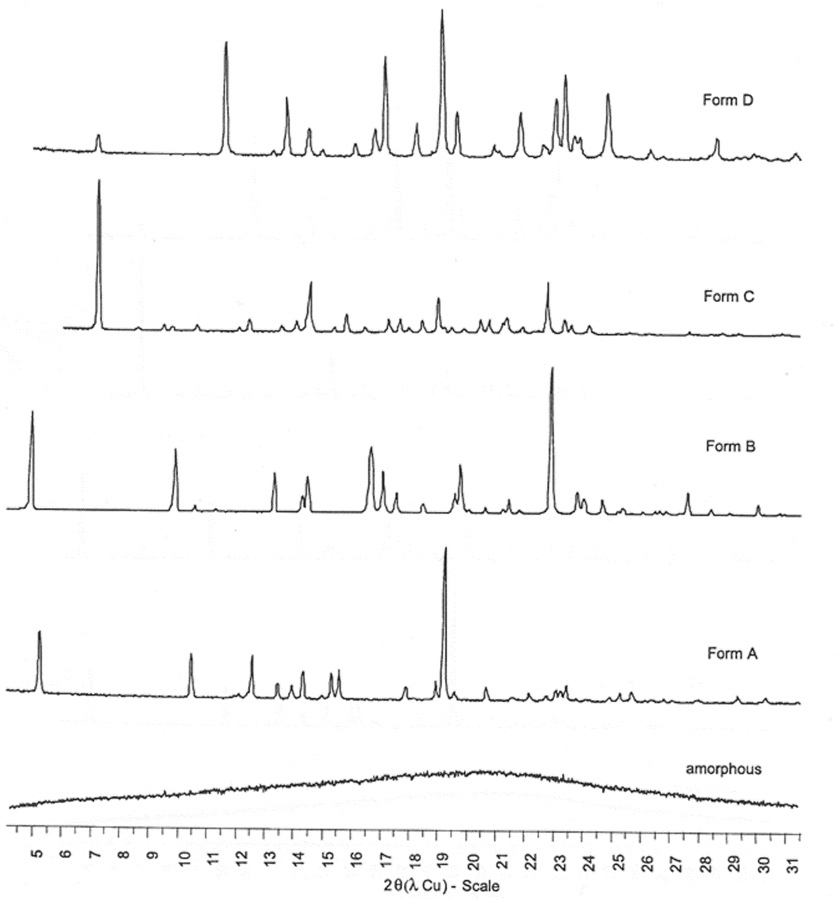
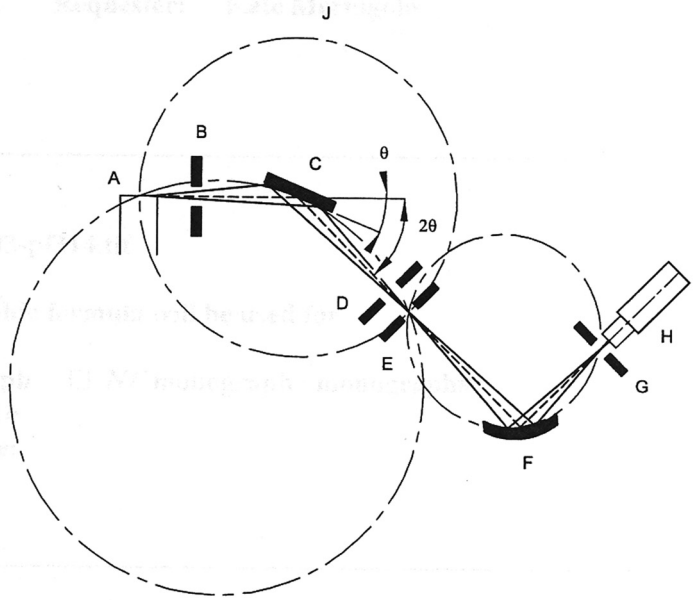


Figure 2. X-ray powder diffraction patterns collected for five different solid phases of a substance (the intensities are normalized).

A powder diffractometer generally comprises five main parts: an X-ray source; the incident beam optics, which may perform monochromatization, filtering, collimation, and/or focusing of the beam; a goniometer; the diffraction beam optics, which may include monochromatization, filtering, collimation, and focusing or parallelizing of beam; and a detector. Data collection and data processing systems are also required and are generally included in current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure powder patterns are powder cameras. Replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing, but parafocusing, such as in Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical $\theta/2\theta$ geometry or a vertical θ/θ geometry. For both geometries, the incident X-ray beam forms an angle θ with the specimen surface plane, and the diffracted X-ray beam forms an angle 2θ with the direction of the incident X-ray beam (an angle θ with the specimen surface plane). The basic geometric arrangement is represented in *Figure 3*. The divergent beam of radiation from the X-ray tube (the so-called primary beam) passes through the parallel plate collimators and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle 2θ converge to a line at the receiving slit. A second set of parallel plate collimators and a scatter slit may be placed either behind or before the receiving slit. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-ray quanta are counted by a radiation detector, usually a scintillation counter, a sealed-gas proportional counter, or a position-sensitive solid-state detector such as an imaging plate or CCD detector. The receiving slit assembly and the detector are coupled together and move tangentially to the focusing circle. For $\theta/2\theta$ scans, the goniometer rotates the specimen around the same axis as that of the detector, but at half the rotational speed, in a $\theta/2\theta$ motion. The surface of the specimen thus remains tangential to the focusing circle. The parallel plate collimator limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.



A. X-ray tube
B. divergence slit
C. sample
D. anti-diffusion slit
E. receiving slit
F. monochromator
G. detector receiving slit
H. detector
J. focusing circle

Figure 3. Geometric arrangement of the Bragg-Brentano parafocusing geometry.

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5–2 mm thickness can also be used for small sample amounts.

X-Ray Radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is converted to heat, which limits the power of the tubes and requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained by using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation and additional characteristic radiation that depends on the type of anode. Only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources used for X-ray diffraction are vacuum tubes using copper, molybdenum, iron, cobalt, or chromium as anodes; copper, molybdenum, or cobalt X-rays are employed most commonly for organic substances (the use of a cobalt anode can especially be preferred to separate distinct X-ray lines). The choice of radiation to be used depends on the absorption characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the K_α radiation from the anode. Consequently, it is advantageous to make the X-ray beam ''monochromatic'' by eliminating all the other components of the emission spectrum. This can be partly achieved using K_β filters—that is, metal filters selected as having an absorption edge between the K_α and K_β wavelengths emitted by the tube. Such a filter is usually inserted between the X-ray tube and the specimen. Another more commonly used way to obtain a monochromatic X-ray beam is via a large monochroma-

tor crystal (usually referred to as a ''monochromator''). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e., K_α and K_β) at different angles so that only one of them may be selected to enter into the detector. It is even possible to separate $K_{\alpha 1}$ and $K_{\alpha 2}$ radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating K_α and K_β wavelengths is by using curved X-ray mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

RADIATION PROTECTION

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions be taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

SPECIMEN PREPARATION AND MOUNTING

The preparation of the powdered material and the mounting of the specimen in a suitable holder are critical steps in many analytical methods, particularly for X-ray powder diffraction analysis, since they can greatly affect the quality of the data to be collected.[3] The main sources of errors due to specimen preparation and mounting are briefly discussed in the following section for instruments in Bragg-Brentano parafocusing geometry.

[3] Similarly, changes in the specimen can occur during data collection in the case of a nonequilibrium specimen (temperature, humidity).

Specimen Preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is particularly evident for needle-like or platelike crystals when size reduction yields finer needles or platelets. Preferred orientation in the specimen influences the intensities of various reflections so that some are more intense and others less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution, and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 μm will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately 0.5 μm) may cause line broadening and significant changes to the sample itself, such as

- specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.),
- reduced degree of crystallinity,
- solid-state transition to another polymorph,
- chemical decomposition,
- introduction of internal stress, and
- solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the nonground specimen with that corresponding to a specimen of smaller particle size (e.g., a milled specimen). If the X-ray powder diffraction pattern obtained is of adequate quality considering its intended use, then grinding may not be required.

It should be noted that if a sample contains more than one phase and if sieving is used to isolate particles to a specific size, the initial composition may be altered.

SPECIMEN MOUNTING

EFFECT OF SPECIMEN DISPLACEMENT

A specimen surface that is offset by D with reference to the diffractometer rotation axis causes systematic errors that are very difficult to avoid entirely; for the reflection mode, this results in absolute $D \cdot \cos\theta$ shifts[4] in 2θ positions (typically of the order of 0.01° in 2θ at low angles

$$[\cos\theta \simeq 1]$$

for a displacement $D = 15$ μm) and asymmetric broadening of the profile toward low 2θ values. Use of an appropriate internal standard allows the detection and correction of this effect simultaneously with that arising from specimen transparency. This effect is by far the largest source of errors in data collected on well-aligned diffractometers.

EFFECT OF SPECIMEN THICKNESS AND TRANSPARENCY

When the XRPD method in reflection mode is applied, it is often preferable to work with specimens of ''infinite thickness''. To minimize the transparency effect, it is advisable to use a nondiffracting substrate (zero background holder)—for example, a plate of single crystalline silicon cut parallel to the 510 lattice planes.[5] One advantage of the transmission mode is that problems with sample height and specimen transparency are less important.

The use of an appropriate internal standard allows the detection and correction of this effect simultaneously with that arising from specimen displacement.

CONTROL OF THE INSTRUMENT PERFORMANCE

The goniometer and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to adequately minimize systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought while performing the alignment procedure. There are many different configurations, and each supplier's equipment requires specific alignment procedures. The overall diffractometer performance must be tested and monitored periodically, using suitable certified reference materials. Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

QUALITATIVE PHASE ANALYSIS (IDENTIFICATION OF PHASES)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its X-ray powder pattern to the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase

[4] Note that a goniometer zero alignment shift would result in a constant shift on all observed 2θ-line positions; in other words, the whole diffraction pattern is, in this case, translated by an offset of Z° in 2θ.

[5] In the case of a thin specimen with low attenuation, accurate measurements of line positions can be made with focusing diffractometer configurations in either transmission or reflection geometry. Accurate measurements of line positions on specimens with low attenuation are preferably made using diffractometers with parallel beam optics. This helps to reduce the effects of specimen thickness.

specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ-diffraction angles or *d*-spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the comparison data can be based on either a more or less extended 2θ range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list of *d*-spacings and normalized intensities, I_{norm}, a so-called (d, I_{norm}) list extracted from the pattern, is the crystallographic fingerprint of the material and can be compared to (d, I_{norm}) lists of single-phase samples compiled in databases.

For most organic crystals, when using Cu K_α radiation, it is appropriate to record the diffraction pattern in a 2θ-range from as near 0° as possible to at least 40°. The agreement in the 2θ-diffraction angles between specimen and reference is within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions, and as such, shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2-θ positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g., inorganic salts), it may be necessary to extend the 2θ region scanned to well beyond 40°. It is generally sufficient to scan past the 10 strongest reflections identified in single-phase X-ray powder diffraction database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

- noncrystallized or amorphous substances,
- the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10% *m/m*),
- pronounced preferred orientation effects,
- the phase has not been filed in the database used,
- the formation of solid solutions,
- the presence of disordered structures that alter the unit cell,
- the specimen comprises too many phases,
- the presence of lattice deformations,
- the structural similarity of different phases.

QUANTITATIVE PHASE ANALYSIS

If the sample under investigation is a mixture of two or more known phases, of which not more than one is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can in many cases be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines,[6] or on the full pattern. These integrated intensities, peak heights, or full-pattern data points are compared to the corresponding values of reference materials. These reference materials must be single phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require, in particular, homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects.

In favorable cases, amounts of crystalline phases as small as 10% may be determined in solid matrices.

[6] If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components are not known, the Pawley method or the partial least-squares (PLS) method can be used.

Polymorphic Samples

For a sample composed of two polymorphic phases *a* and *b*, the following expression may be used to quantify the fraction F_α of phase *a*:

$$F_\alpha = \frac{1}{1+\mathrm{K}(I_b / I_\alpha)}$$

The fraction is derived by measuring the intensity ratio between the two phases, knowing the value of the constant K. K is the ratio of the absolute intensities of the two pure polymorphic phases I_{oa} / I_{ob}. Its value can be determined by measuring standard samples.

Methods Using a Standard

The most commonly used methods for quantitative analysis are

- the external standard method,
- the internal standard method, and
- the spiking method (also often called the standard addition method).

The external standard method is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material can be used that has a crystallite size and X-ray absorption coefficient comparable to those of the components of the sample and with a diffraction pattern that does not overlap at all that of the sample to be analyzed. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the internal standard method, requires precise measurement of diffraction intensities.

In the spiking method (or standard addition method), some of the pure phase *a* is added to the mixture containing the unknown concentration of *a*. Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative *x*-intercept is the concentration of the phase *a* in the original sample.

ESTIMATE OF THE AMORPHOUS AND CRYSTALLINE FRACTIONS

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

- If the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances, as described above. The amorphous fraction is then deduced indirectly by subtraction.

- If the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase (the ''degree of crystallinity'') can be estimated by measuring three areas of the diffractogram:

  A = total area of the peaks arising from diffraction from the crystalline fraction of the sample,

  B = total area below area A,

  C = background area (due to air scattering, fluorescence, equipment, etc).

When these areas have been measured, the degree of crystallinity can be roughly estimated as:

$$\% \text{ crystallinity} = 100A/(A + B - C)$$

It is noteworthy that this method does not yield an absolute degree of crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

SINGLE CRYSTAL STRUCTURE

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low, and the scattering properties are normally very low. For any given crystalline form of a substance, the knowledge of the crystal structure allows for calculating the corresponding XRPD pattern, thereby providing a preferred orientation-free reference XRPD pattern, which may be used for phase identification.▲USP33

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- articles relevant to compendial issues.

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Drug Product Performance and Interchangeability of Multisource Drug Substances and Drug Products

Leon Shargel, PhD, *Applied Biopharmaceutics*[a]

ABSTRACT Multisource drug products may contain drug substances and drug products that meet *USP–NF* monograph standards of strength, quality, purity, and identity. Pharmaceutical equivalence per se does not ensure equivalent drug product performance as demonstrated by bioequivalence, nor does it ensure therapeutic equivalence. This *Stimuli* article examines pharmaceutical equivalence and suggests that multisource drug products that meet *USP* monograph requirements may not be pharmaceutical equivalents and/or may not have the same drug product performance.

INTRODUCTION

Multisource drug products are products marketed by more than one manufacturer that contain the same active pharmaceutical ingredient (API) or drug substance in the same dosage form and are given by the same route of administration. Many of these multisource drug products contain drug substances that meet *USP–NF* monograph standards of strength, quality, purity, and identity. However, drug substances and drug products that solely meet the same *USP–NF* monograph standards should not be considered automatically as interchangeable products. The objective of this *Stimuli* article is to provide an understanding of interchangeability and substitutability of drug substances and drug products that meet *USP–NF* monographs.

Pharmaceutical equivalents are drug products that contain the same active ingredient(s), are of the same dosage form, are administered by the same route, and are identical in strength or concentration. Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient in the same dosage form and to meet the same or compendial or other applicable standards (i.e., strength, quality, purity, and identity), but they may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, and preservatives), expiration time, and, within certain limits, labeling.

Multisource drug products that meet *USP–NF* monographs may not be pharmaceutical equivalents and/or may not have the same drug product performance. Designation of multisource drug products for interchangeability (substitution) generally is a matter of governmental (regulatory) approval. Regulatory approval for interchangeable multisource generic drug products is not identical in all countries. The US Food and Drug Administration (FDA) has very strict rules for the approval and marketing of generic drug products. These regulations must be considered during the development of both branded and generic drug products. Drug product performance also must be considered if any changes occur in the finished dosage form, including scale up, site changes, process changes, or other modifications that could have an effect on the drug product. Only multisource drug products that are pharmaceutical equivalents, bioequivalent, and therapeutic equivalents that have been approved by the appropriate regulatory agency (e.g., FDA), may be marketed as interchangeable. *USP* General Information Chapter *In Vivo Bioequivalence Guidances* ⟨1090⟩ discusses the basis for therapeutic equivalence.

USP–NF contains science-based standards for drugs, biologics, dietary supplements, and excipients used in dosage forms and products. A *USP–NF* monograph for an official substance or preparation includes applicable standards of strength, quality, purity, and identity, including also the article's definition, packaging, storage, and other requirements and specifications. The specification consists of a series of universal (description, identification, impurities, and assay) and specific tests, one or more analytical procedures for each test, and acceptance criteria.

Ingredients are defined as either drug substances or excipients. An excipient is any component, other than the active substance(s), intentionally added to the formulation of a dosage form. Excipients are not necessarily inert and may affect the performance of the finished dosage form (drug product). Quality standards are important attributes that must be built into the drug product. Products that meet *USP–NF* standards are accepted globally as articles with an assurance of high quality.

In addition to meeting *USP–NF* quality standards, multisource drug products must meet certain in vivo and/or in vitro performance standards to be considered therapeutically equivalent and interchangeable (see *USP* General Information Chapter ⟨1090⟩. Drug product performance may be defined as the release of the active pharmaceutical ingredient (API) from the drug product dosage form, leading to systemic availability of the API necessary to achieve a desired therapeutic response.

[a] Correspondence should be addressed to: Darrell R. Abernethy, MD, PhD, Chief Science Officer, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8184; e-mail dra@usp.org.

The number of multisource drug products now available on the open market and on the Internet has greatly increased and has caused some confusion among pharmacy practitioners regarding their quality, performance, and interchangeability. This chapter provides a framework for comparing drug substances and drug products in terms of equivalence and substitutability.

MULTISOURCE DRUG SUBSTANCES

Pharmaceutical Equivalence

Pharmaceutical equivalence of drug substances and drug products is determined by the relevant regulatory agencies who review the manufacturer's submission that contains experimental evidence that the drug substances meet quality standards and perform similarly as measured by bioequivalence or other tests. Pharmaceutical equivalence, by itself, does not ensure bioequivalence or therapeutic equivalence.

Due to patents and different approaches in chemical or biological synthesis, the drug substance may differ in various physicochemical properties. Depending on the synthetic route and method of purification, some drugs can exist in polymorphic forms. A manufacturer may obtain a patent for a specific crystalline form of the drug substance. An alternative manufacturer of this drug substance may use a different synthetic route and method of purification, resulting in a crystalline form that is different from the patented form. Because these two drug substances have different crystalline forms, they are not identical. In another case, the hydrous form of a drug substance is not considered chemically identical to its anhydrous form. In both of these cases, the two drug substances (e.g., crystalline vs noncrystalline or hydrous vs anhydrous) may be considered pharmaceutical equivalents if their in vivo performances are equivalent.

Various factors can affect the performance of a drug substance in the finished drug product. These factors include:

- Physicochemical attributes of the drug substance
- Differences in excipients in the formulation
- Differences in the manufacturing process.

Physicochemical Attributes

As noted, drug substances can differ in polymorphic forms including hydrous and anhydrous forms, various crystalline forms, or an amorphous form. Hydrous, anhydrous, amorphous, and different crystalline forms may dissolve at different rates, and therefore, the rate of absorption from the same dosage form (e.g., oral tablet) may be different. For example, crystalline structures are more rigid and thermodynamically stable compared to amorphous forms that dissolve more quickly. Particle size and the particle size distribution of the drug may also affect the rate of dissolution. Although mean particle size may be similar, a particle size distribution that contains a larger number of very fine (smaller) particles may dissolve more quickly and may be systemically absorbed more quickly than the same drug substance whose particle size distribution contains a greater number of larger particles.

Differences in Excipients in the Formulation

The World Health Organization (WHO) states that ''pharmaceutical equivalence does not necessarily imply bioequivalence and therapeutic equivalence, as differences in the excipients and/or the manufacturing process can lead to differences in product performance'' (*1*). However, amorphous and crystalline drug substances can be bioequivalent when manufactured in the same dosage form (e.g., oral tablet) if a manufacturer uses different excipients and manufacturing processes that produce bioequivalence in vivo.

Pharmaceutical Alternatives

Pharmaceutical alternatives are defined in General Information Chapter ⟨1090⟩. FDA considers pharmaceutical alternatives as drug products that contain the same therapeutic moiety but are different salts, esters, or complexes of that moiety or are different dosage forms or strengths.

WHO considers pharmaceutical alternatives as products that contain the same molar amount of the same active pharmaceutical moiety(s) but differ in dosage form (e.g., tablets vs capsules), and/or chemical form (e.g., different salts or esters). Pharmaceutical alternatives deliver the same active moiety by the same route of administration but are otherwise not pharmaceutically equivalent. They may or may not be bioequivalent or therapeutically equivalent with the comparator product.

Different Salt Forms

Salt forms of drugs such as hydrochloride, phosphate, sulfate, potassium, and sodium, among others, are ionizable substances that have different ionization equilibrium between salt form and non-ionized form. Additionally, different salt forms of the same drug may have different aqueous solubility and may dissolve at different rates. For example, tetracycline phosphate or tetracycline hydrochloride equivalent to 250 mg tetracycline base are considered pharmaceutical alternatives. Sometimes pharmaceutical manufacturers can use different excipients and manufacturing processes to produce pharmaceutical alternatives that may be bioequivalent in vivo and therapeutically equivalent to the reference drug product.

Esters and Prodrugs

Esters of the active pharmaceutical ingredient essentially are prodrugs. Esters of fatty acids such as stearates and palmitates have been used. Generally, the ester prodrug is inactive and must be hydrolyzed to the parent drug to exert its pharmacodynamic activity. Hydrolysis can proceed by enzymatic pathways via esterases in the tissues or plasma or by nonenzymatic means such as acid hydrolysis in the stomach. Esters can be absorbed both as the prodrug and as the parent drug, and the distribution of the intact prodrug may differ from the distribution of

the parent drug. Therefore, prodrugs are considered to be different drugs compared to the parent active drug substance.

EXCIPIENTS

A pharmaceutical dosage form typically consists of both active ingredient(s) and excipients. Excipients, which may have no pharmacodynamic activity, play a critical role in manufacturing, stability, and performance (*2*). Some excipients are manufactured to comply with compendial standards. However, the performance of the finished dosage form depends extensively on the physical and chemical properties of the excipients as well as the physical and chemical properties of the drug substance and the method of manufacture.

INTERCHANGEABILITY OF MULTISOURCE DRUG PRODUCTS

The interchangeability of multisource generic drug products is a major concern for physicians, pharmacists, and others who prescribe, dispense, purchase, or pay/reimburse for drugs. Because the formulation and method of manufacture of the drug product can affect its bioavailability and stability, the multisource generic drug manufacturer must demonstrate that the generic drug product is bioequivalent and therapeutically equivalent to the reference listed drug.

Manufacturing Process

Quality cannot be tested into drug products. Quality should be built in or should be designed and confirmed by testing. With a greater understanding of the drug product and its manufacturing process, regulatory agencies and pharmaceutical manufacturers are developing a systematic approach to achieve quality and drug product performance (*3*).

As discussed in ICH Q8(R1), manufacturing should be based on sound scientific principles. Pharmaceutical development should include, at a minimum, the following elements:

- Defining the target product profile as it relates to quality, safety, and efficacy considering, e.g., the route of administration, dosage form, bioavailability, dosage, and stability
- Identifying critical quality attributes (CQAs) of the drug product so that those product characteristics that have an impact on product quality can be studied and controlled
- Determining the quality attributes of the drug substance and excipients and selecting the type and amount of excipients to deliver drug product of the desired quality
- Selecting an appropriate manufacturing process
- Identifying a control strategy.

An enhanced quality by design (QbD) approach to product development additionally would include the following elements:

- A systematic evaluation, understanding, and refining of the formulation and manufacturing process, including:
  - — Identifying by, e.g., prior knowledge, experimentation, and risk assessment, the material attributes and process parameters that can have an effect on product CQAs
  - — Determining the functional relationships that link material attributes and process parameters to product CQAs
- Using the enhanced process understanding in combination with quality risk management to establish an appropriate control strategy that can include a proposal for design space(s) and/or real-time release.

Scale-up and Postapproval Changes

Scale-up and Postapproval Changes (SUPAC) relate to change(s) in a formulation after market approval (*4*, *5*). These may include a change in (1) the components or composition, (2) the site of manufacture, (3) the scale-up/scale-down of manufacture, and/or (4) the manufacturing (process and equipment) of a modified-release solid oral dosage form during the postapproval period.

Any scale-up and postapproval change can potentially affect the performance of the finished drug product. Moreover, changes in the manufacture of drug substance or excipients have the potential to change the performance of the finished dosage form. FDA SUPAC guidances define (1) levels of change, (2) recommended chemistry, manufacturing, and controls (CMC) tests for each level of change, (3) recommended in vitro dissolution tests and/or in vivo bioequivalence tests for each level of change, and (4) documentation that should support the change. The possibility that a change may affect the safety and performance of the drug product is tabulated by levels of change (*Table 1*).

Table 1: Levels of Change and Their Impact on Drug Product Performance

Change Level	Example	Comment
Level 1	Deletion or partial deletion of an ingredient to affect the color or flavor of the drug product.	Level 1 changes are those that are unlikely to have any detectable impact on formulation quality and performance.
Level 2	Quantitative change in excipients greater than that allowed in a Level 1 change.	Level 2 changes are those that could have a significant impact on formulation quality and performance.
Level 3	Qualitative change in excipients.	Level 3 changes are those that are likely to have a significant impact on formulation quality and performance. A Level 3 change may require in vivo bioequivalence testing.

The manufacturer must provide evidence that the performance of the product is not impaired because of the change.

Generic Substitution and Interchangeability of Multisource Drug Products

Generic substitution is the process of dispensing a different brand or an unbranded therapeutically equivalent drug product in place of the prescribed drug product. The substituted drug product must be approved by the regulatory agency as a therapeutic equivalent. FDA lists therapeutic equivalents in the *Orange Book* (discussed below). In most cases, generic substitution does not require permission from the prescriber if the drug product has been approved by FDA and is listed as a therapeutic equivalent.

Therapeutic Equivalents

Drug products are considered to be *therapeutic equivalents* only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling. FDA classifies as therapeutically equivalent those products that meet the following general criteria:

- they are approved as safe and effective
- they are pharmaceutical equivalents because they
  - contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, and
  - meet compendial or other applicable standards of strength, quality, purity, and identity
- they are bioequivalent because
  - they do not present a known or potential bioequivalence problem, and they meet an acceptable in vitro standard, or
  - if they do present a known or potential problem, they are shown to meet an appropriate bioequivalence standard
- they are adequately labeled
- they are manufactured in compliance with cGMP regulations.

WHO and certain countries may have different definitions of therapeutic equivalence. Drug products that are approved for marketing as therapeutically equivalent can be substituted with the full expectation that the substituted product will produce the same clinical effect and safety profile as the prescribed product.

Orange Book

FDA publishes *Approved Drug Products with Therapeutic Equivalence Evaluations,* also known as the *Orange Book* (www.fda.gov/cder/ob/default.htm; accessed 8 December 2008). The *Orange Book* identifies drug products approved under the 1938 Federal Food, Drug, and Cosmetic Act by FDA on the basis of safety and effectiveness and contains therapeutic equivalence evaluations for approved multisource prescription drug products. It does not include drugs approved under the Public Health Service Act (biologics), pre-1938 drugs, or drugs approved by FDA only on the basis of safety (those covered by the so-called ongoing DESI review). *Orange Book* evaluations serve as public information and advice to health agencies, prescribers, and pharmacists to promote public education in the area of drug product selection and to foster containment of health care costs.

Therapeutic Equivalence Evaluations Codes

The coding system for therapeutic equivalence evaluations in the *Orange Book* (http://www.fda.gov/cder/ob/docs/preface/ecpreface.htm#Therapeutic%20Equivalence%20Evaluations%20Codes; accessed 8 December 2008) is constructed to allow users to determine quickly whether FDA has evaluated a particular approved product as therapeutically equivalent to other pharmaceutically equivalent products (first letter) and to provide additional information about the basis of FDA's evaluations (second letter). With few exceptions, the therapeutic equivalence evaluation date is the same as the approval date.

The two basic categories into which multisource drugs have been placed are indicated by the first letter:

- Drug products that FDA considers to be therapeutically equivalent to other pharmaceutically equivalent products. These are drug products for which there are no known or suspected bioequivalence problems. These are designated AA, AN, AO, AP, or AT, depending on the dosage form
- Actual or potential bioequivalence problems have been resolved with adequate in vivo and/or in vitro evidence supporting bioequivalence. These are designated AB.

FDA at this time considers some drug products not to be therapeutically equivalent to other pharmaceutically equivalent products, i.e., drug products for which actual or potential bioequivalence problems have not been resolved by adequate evidence of bioequivalence. Often the problem is with specific dosage forms rather than with the active ingredients. These are designated BC, BD, BE, BN, BP, BR, BS, BT, BX, or B*. The *Orange Book* should be consulted for a complete listing of therapeutically equivalent drug products.

Reference Listed Drug

The reference listed drug (RLD) is identified by FDA as the drug product on which an applicant relies when seeking approval of an Abbreviated New Drug Application (ANDA). The RLD generally is the brand-name drug that has been approved on the basis of a full New Drug Application (NDA) with substantial evidence of safety and efficacy. FDA designates a single reference listed drug as the standard to which all generic versions must be shown to be bioequivalent. FDA hopes to avoid possible significant variations among generic drugs and their brand-name counterparts. Such variations could result if generic drugs were compared to different RLDs.

Pharmaceutically Equivalent Drug Products

Drug products are considered pharmaceutical equivalents (i.e., are listed in the *Orange Book*) if they contain the same active ingredient(s), are of the same dosage form, are administered by the same route of administration, and are identical in strength or concentration (e.g., chlordiazepoxide hydrochloride, 5-mg capsules). Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient in the same dosage form and to meet the same or compendial or other applicable standards (i.e., strength, quality, purity, and identity), but they may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, and preservatives), expiration time, and, within certain limits, labeling.

Pharmaceutically equivalent or pharmaceutically alternative products may or may not be therapeutically equivalent. Multisource pharmaceutical products that are therapeutically equivalent are interchangeable. An interchangeable pharmaceutical product is one that is therapeutically equivalent to a comparator product and can be interchanged with the comparator in clinical practice.

Pharmaceutical Alternatives

Pharmaceutical alternatives are drug products that contain the same therapeutic moiety but are different salts, esters, or complexes of that moiety or are different dosage forms or strengths (e.g., tetracycline hydrochloride, 250-mg capsules vs tetracycline phosphate complex, 250-mg capsules; quinidine sulfate, 200-mg tablets vs quinidine sulfate, 200-mg capsules). Different dosage forms and strengths within a single manufacturer's product line are thus pharmaceutical alternatives, as are extended-release products when compared with immediate-release or standard-release formulations of the same active ingredient.

Capsules vs Tablets

The bioavailability of the same drug substance from a tablet compared to another tablet may demonstrate bioequivalence. However, FDA currently considers a tablet and capsule containing the same active ingredient in the same dosage strength as pharmaceutical alternatives, and the two dosage forms cannot be interchanged. In contrast, several countries have concluded that bioequivalent capsules and tablets containing the same active ingredient in the same dosage strength are therapeutic equivalents and therefore can be interchanged.

Pharmaceutical Substitution

Pharmaceutical substitution is the process of dispensing a pharmaceutical alternative for the prescribed drug product. This occurs, for example, if ampicillin suspension is dispensed in place of ampicillin capsules or if tetracycline hydrochloride is dispensed in place of tetracycline phosphate. Pharmaceutical substitution generally requires the physician's approval.

Therapeutic Alternatives

Therapeutic alternatives are drug products that contain different active ingredients and are indicated for the same therapeutic or clinical objectives. Active ingredients in therapeutic alternatives are from the same pharmacologic class and are expected to have the same therapeutic effect when administered to patients for identical indications. In such cases, for example, ibuprofen may be given instead of naproxen, and cimetidine may be given instead of ranitidine.

Therapeutic Substitution

Therapeutic substitution is the process of dispensing a therapeutic alternative in place of the prescribed drug product. For example, amoxicillin may be dispensed instead of ampicillin for the treatment of a Staphylococcus infection, or ibuprofen may be dispensed instead of naproxen for the treatment of pain. Therapeutic substitution also can occur when one NDA-approved drug is substituted for the same drug that has been approved by a different NDA, e.g., the substitution of Nicoderm (nicotine transdermal system) for Nicotrol (nicotine transdermal system).

Because these drug products are not therapeutic equivalents as defined above, they may not be marketed or labeled as interchangeable. Generally, the prescriber must be notified before a substitution is performed. However, in certain institutions such as in a hospital or nursing facility, a formulary may list drug products that can be interchanged without the need to contact the physician. In this case, a pharmacy and therapeutics committee has reviewed the products in the formulary and has listed those products that can be substituted.

CONCLUSIONS

USP–NF contains science-based standards for drugs, biologics, dietary supplements, and excipients used in dosage forms and products. A *USP–NF* monograph for an official substance or preparation includes applicable standards of strength, quality, purity, and identity, including the article's definition, and packaging, storage, and other requirements and specifications. Multisource drug products may contain drug substances and drug products that meet *USP–NF* monograph standards of strength, quality, purity, and identity. Pharmaceutical equivalence per se does not ensure equivalent drug product performance as demonstrated by bioequivalence, nor does it ensure therapeutic equivalence. The approval of interchangeability of multisource, generic drug products depends on the review of an appropriate application (e.g., an ANDA) by an appropriate regulatory agency such as FDA.

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Topical and Transdermal Drug Products

The Topical/Transdermal Ad Hoc Advisory Panel for the USP Performance Tests of Topical and Transdermal Dosage Forms: Clarence T. Ueda (Chair), Vinod P. Shah (USP Scientific Liaison), Kris Derdzinski, Gary Ewing, Gordon Flynn, Howard Maibach, Margareth Marques (USP Scientific Liaison),[a] Howard Rytting,[b] Steve Shaw, Kailas Thakker, and Avi Yacobi.

ABSTRACT This *Stimuli* article provides general information about the test methods that should be employed to ensure the quality and performance of topical and transdermal drug products. The term *topical drug products* refers to all formulations applied to the skin except transdermal delivery systems (TDS) or transdermal patches that will be addressed separately.

INTRODUCTION

Drug products topically administered via the skin fall into two general categories, those applied for local action and those for systemic effects. Local actions include those at or on the surface of the skin, those that exert their actions on the stratum corneum, and those that modulate the function of the epidermis and/or the dermis. Common products in the former category include creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions. Creams, ointments, and gels generally are referred to as semisolid dosage forms. The most common drug products applied to the skin for systemic effects are referred to as self-adhering transdermal drug delivery systems (TDS) or transdermal patches.

Two categories of tests, product quality tests and product performance tests, are performed with drug products to provide assurances of batch-to-batch quality, reproducibility, reliability, and performance. Product quality tests are performed to assess attributes such as assay, identification, content uniformity, pH, microbial limits, and minimum fill and are part of the compendial monograph. Product performance tests are conducted to assess drug release from the finished dosage form.

This *Stimuli* article provides general information about the test methods that should be employed to ensure the quality and performance of topical and transdermal drug products. The term *topical drug products* refers to all formulations applied to the skin except transdermal delivery systems (TDS) or transdermal patches that will be addressed separately.

Topical dosage forms include solutions (for which release testing is not indicated), collodion, suspensions, emulsions (e.g., lotions), semisolids (e.g., foams, ointments, pastes, creams, and gels), solids (e.g., powders and aerosols), and sprays. The physical characteristics of these dosage forms vary widely.

Therefore, the in vitro release test for those products also may differ significantly and may require different types of apparatus. At present, a product performance test exists only for semisolid formulations, specifically creams, ointments, and gels. That test employs the vertical diffusion cell (VDC) system. The VDC system is simple to operate and yields reliable and reproducible results when employed by properly trained laboratory personnel.

TDS or transdermal patches are physical devices applied to the skin and vary in their composition and method of fabrication. Therefore, they release their active ingredients by different mechanisms.

GLOSSARY OF TERMS

Definitions of topical drug products, some aspects related to the manufacture of these products, and a glossary of dosage form names commonly used can be found in General Information Chapter *Pharmaceutical Dosage Forms* ⟨1151⟩.

Collodion

Collodion (pyroxylin solution; see *USP* monograph), is a solution of nitrocellulose in ether and acetone, sometimes with the addition of alcohol. As the volatile solvents evaporate, a dry celluloid-like film is left on the skin. Because the medicinal use of a collodion depends on the formation of a protective film, the film should be durable, tenacious in adherence, flexible, and occlusive.

Creams

Creams are semisolid dosage forms that contain one or more drug substances dissolved or dispersed in a suitable base. This term traditionally has been applied to semisolids that possess a relatively soft, spreadable consistency formulated as either water-in-oil or oil-in-water emulsions. However, more recently the term has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water washable and more cosmetically and aesthetically acceptable.

[a] Correspondence should be addressed to: Margareth Marques, PhD, Senior Scientist, Documentary Standards Division, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8106; e-mail mrm@usp.org.

[b] Deceased.

Emulsions

Emulsions are viscid, multiphase systems in which one or more liquids are dispersed throughout another immiscible liquid in the form of small droplets. When oil is the dispersed phase and an aqueous solution is the continuous phase, the system is designated as an oil-in-water emulsion. Conversely, when water or an aqueous solution is the dispersed phase and oil or oleaginous material is the continuous phase, the system is designated as a water-in-oil emulsion. Emulsions are stabilized by emulsifying agents that prevent coalescence, the merging of small droplets into larger droplets, and, ultimately, into a single separated phase. Emulsifying agents (surfactants) act by concentrating at the interface between the immiscible liquids, thereby providing a physical barrier that reduces the tendency for coalescence. Surfactants also reduce the interfacial tension between the phases, facilitating the formation of small droplets upon mixing. The term emulsion is not used if a more specific term is applicable, e.g., cream or ointment.

Foams

Foams are emulsified systems packaged in pressurized containers or special dispensing devices that contain dispersed gas bubbles, usually in a liquid continuous phase, that when dispensed has a fluffy, semisolid consistency.

Gels

Gels (sometimes called Jellies) are semisolid systems consisting of either suspensions composed of small inorganic particles or large organic molecules interpenetrated by a liquid. When the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system (e.g., Aluminum Hydroxide Gel, *USP*). In a two-phase system if the particle size of the dispersed phase is relatively large, the gel mass is sometimes referred to as a magma (e.g., Bentonite Magma, *NF*). Both gels and magmas may be thixotropic, forming semisolids after standing and becoming liquid when agitated. They should be shaken before use to ensure homogeneity and should be labeled to that effect (see Topical Suspensions, below). Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid with no apparent boundary between the dispersed macromolecule and liquid.

Lotions

Although the term lotion may be applied to a solution, lotions usually are fluid, somewhat viscid emulsion dosage forms for external application to the skin. Lotions share many characteristics with creams. See Creams, Topical Solutions, and Topical Suspensions, herein.

Ointments

Ointments are semisolids intended for external application to the skin or mucous membranes. They usually contain less than 20% water and volatiles and more than 50% hydrocarbons, waxes, or polyols as the vehicle. Ointment bases recognized for use as vehicles fall into four general classes: hydrocarbon bases, absorption bases, water-removable bases, and water-soluble bases. Each therapeutic ointment possesses as its base one of these four general classes.

***Hydrocarbon Bases*—**Hydrocarbon bases, known also as ''oleaginous ointment bases,'' are represented by White Petrolatum and White Ointment (both *USP*). Only small amounts of an aqueous component can be incorporated into these bases. Hydrocarbon bases serve to keep medicaments in prolonged contact with the skin and act as occlusive dressings. These bases are used chiefly for their emollient effects and are difficult to wash off. They do not ''dry out'' or change noticeably on aging.

***Absorption Bases*—**This class of bases may be divided into two groups: the first consists of bases that permit the incorporation of aqueous solutions with the formation of a water-in-oil emulsion (e.g., Hydrophilic Petrolatum and Lanolin, both *USP*), and the second group consists of water-in-oil emulsions that permit the incorporation of additional quantities of aqueous solutions (Lanolin, *USP*). Absorption bases also are useful as emollients.

***Water-removable Bases*—**Water-removable bases are oil-in-water emulsions (e.g., Hydrophilic Ointment, *USP*), and are more correctly called ''creams'' (see Creams, above). They also are described as ''water-washable'' because they may be readily washed from the skin or clothing with water, an attribute that makes them more acceptable for cosmetic purposes. Some medicaments may be more effective in these bases than in hydrocarbon bases. Other advantages of the water-removable bases are that they may be diluted with water and that they favor the absorption of serous discharges in dermatological conditions.

***Water-soluble Bases*—**This group of so-called ''greaseless ointment bases'' comprises water-soluble constituents. Polyethylene Glycol Ointment, *NF* is the only pharmacopeial preparation in this group. Bases of this type offer many of the advantages of the water-removable bases and, in addition, contain no water-insoluble substances such as petrolatum, anhydrous lanolin, or waxes. They are more correctly called Gels (see Gels, above).

***Choice of Base*—**The choice of an ointment base depends on many factors, such as the action desired, the nature of the medicament to be incorporated and its bioavailability and stability, and the requisite shelf life of the finished product. In some cases, it is necessary to use a base that is less than ideal in order to achieve the stability required. Drugs that hydrolyze rapidly, for example, are more stable in hydrocarbon bases than in bases that contain water, even though they may be more effective in the latter.

Ophthalmic Ointments

Ophthalmic ointments are semisolids for application to the eye. Special precautions must be taken in the preparation of ophthalmic ointments. They are manufactured from sterilized ingredients under rigidly aseptic conditions, must meet the requirements under *Sterility Tests* ⟨71⟩, and must be free of large particles. The medicinal agent is added to the ointment base either as a solution or as a micronized powder.

Pastes

Pastes are semisolid dosage forms that contain a high percentage (often $\geq$ 50%) of finely dispersed solids with a stiff consistency intended for topical application. One class is made from a single-phase aqueous gel (e.g., Carboxymethylcellulose Sodium Paste, *USP*). The other class, the fatty pastes (e.g., Zinc Oxide Paste, *USP*), consists of thick, stiff ointments that do not ordinarily flow at body temperature and therefore serve as protective coatings over the areas to which they are applied.

Powders

Powders are solids or mixture of solids in a dry, finely divided state for external (or internal) use.

Sprays

Sprays are products formed by the generation of droplets of solution containing dissolved drug for application to the skin or mucous membranes. The droplets may be formed in a variety of ways but generally result from forcing the liquid through a specially designed nozzle assembly. One example of a spray dosage form is a metered-dose topical transdermal spray that delivers a precisely controlled quantity of solution or suspension on each activation.

Topical Aerosols

Topical aerosols are products that are packaged under pressure. The active ingredients are released in the form of fine liquid droplets or fine powder particles upon activation of an appropriate valve system. A special form is a metered-dose aerosol that delivers an exact volume (dose) per each actuation.

Topical Solutions

Topical solutions are liquid preparations, that usually are aqueous but often contain other solvents such as alcohol and polyols that contain one or more dissolved chemical substances intended for topical application to the skin, or, as in the case of Lidocaine Oral Topical Solution *USP*, to the oral mucosal surface.

Topical Suspensions

Topical suspensions are liquid preparations that contain solid particles dispersed in a liquid vehicle intended for application to the skin. Some suspensions labeled as ''Lotions'' fall into this category.

Transdermal Delivery Systems

Transdermal delivery systems (TDS) are self-contained, discrete dosage forms that, when applied to intact skin, are designed to deliver the drug(s) through the skin to the systemic circulation. Systems typically comprise an outer covering (barrier), a drug reservoir that may have a drug release–controlling membrane, a contact adhesive applied to some or all parts of the system and the system/skin interface, and a protective liner that is removed before the patient applies the system. The dose of these systems is defined in terms of the release rate of the drug(s) from the system and surface area of the patch and is expressed as mass per unit time for a given surface area. With these drug products, the skin typically is the rate-controlling membrane for the drug input into the body. The total duration of drug release from the system and system surface area may also be stated.

TDS work by diffusion: The drug diffuses from the drug reservoir, directly or through the rate-controlling membrane and/or contact adhesive if present, and then through the skin into the general circulation. Typically, modified-release systems are designed to provide drug delivery at a constant rate so that a true steady-state blood concentration is achieved and maintained until the system is removed. Following removal of the system, blood concentration declines at a rate consistent with the pharmacokinetics of the drug.

PRODUCT QUALITY TESTS—GENERAL

The International Conference on Harmonization (ICH) Guidance Q6A (available at www.ich.org) recommends specifications (tests, procedures, and acceptance criteria) to ensure that commercialized drug products are safe and effective at release and during shelf life. Tests that are universally applied to ensure safety and efficacy include description, identification, assay, and impurities.

Description—A qualitative description of the dosage form should be provided. The acceptance criteria should include the final acceptable appearance. If color changes during storage, a quantitative procedure may be appropriate. It specifies the content or the label claim of the article.

Identification—Identification tests are discussed in *Procedures* under *Tests and Assays* in the *General Notices and Requirements*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structure that are likely to be present. Identity tests should be specific for the drug substances. The most conclusive test for identity is the infrared absorption spectrum (see *Spectrophotometry and Light-scattering* ⟨851⟩

and *Spectrophotometric Identification Tests* ⟨197⟩). If no suitable infrared spectrum can be obtained, other analytical techniques can be used. Near infrared (NIR) or Raman spectrophotometric methods also could be acceptable as the sole identification method of the drug product formulation (see *Near-infrared Spectrophotometry* ⟨1119⟩ and *Raman Spectroscopy* ⟨1120⟩). Identification solely by a single chromatographic retention time is not regarded as specific. However, the use of two chromatographic procedures for which the separation is based on different principles or a combination of tests in a single procedure can be acceptable. See *Chromatography* ⟨621⟩ and *Thin-layer Chromatographic Identification* ⟨201⟩.

***Assay*—**A specific and stability-indicating test should be used to determine the strength (content) of the drug product. See *Antibiotics—Microbial Assays* ⟨81⟩, ⟨621⟩, or *Assay for Steroids* ⟨351⟩. In cases when the use of nonspecific assay is justified, e.g., *Titrimetry* ⟨541⟩, other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay.

***Impurities*—**Process impurities, synthetic by-products, and other inorganic and organic impurities may be present in the drug substance and excipients used in the manufacture of the drug product. These impurities are controlled by the drug substance and excipients monographs. Organic impurities arising from the degradation of the drug substance and those arising during the manufacturing process of the drug product should be monitored.

In addition to the universal tests listed above, the following tests may be considered on a case-by-case basis:

***Physicochemical Properties*—**These are properties such as *pH* ⟨791⟩, *Viscosity* ⟨911⟩, and *Specific Gravity* ⟨841⟩.

***Uniformity of Dosage Units*—**This test is applicable for TDS and for dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form. The test can be performed by either content uniformity or weight variation (see *Uniformity of Dosage Units* ⟨905⟩).

***Water Content*—**A test for water content should be included when appropriate (see *Water Determination* ⟨921⟩).

***Microbial Limits*—**The type of microbial test(s) and acceptance criteria should be based on the nature of the drug substance, method of manufacture, and the intended use of the drug product. See *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩ and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩.

***Antimicrobial Preservative Content*—**Acceptance criteria for preservative content in multidose products should be established. They should be based on the levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* ⟨51⟩).

***Antioxidant Preservative Content*—**If antioxidant preservatives are present in the drug product, tests of their content normally should be determined.

***Sterility*—**Depending on the use of the dosage form, e.g., ophthalmic preparations, sterility of the product should be demonstrated as appropriate (see *Sterility Tests* ⟨71⟩).

PRODUCT QUALITY TESTS FOR TOPICAL DRUG PRODUCTS

General product quality tests such as identification, assay, content uniformity (uniformity of dosage units), impurities, pH, water content, microbial limits, antimicrobial preservative content, antioxidant preservative content, and sterility should be performed for topical drug products as described above. In addition, specific tests for topical dosage forms, as described below, also should be conducted.

***Viscosity*—**Rheological properties such as viscosity of semisolid dosage forms can influence their drug delivery. Viscosity may directly influence the diffusion rate of drug at the microstructural level. Yet semisolid drug products with relatively high viscosity still can exhibit high diffusion rates when compared to semisolid products of comparatively lower viscosity. These observations emphasize the importance of rheologic properties of semisolid dosage forms, specifically viscosity, on drug product performance.

Depending on its viscosity, the rheological behavior of a semisolid drug product may affect its application to treatment site(s) and consistency of treatment and thus the delivered dose. Therefore, maintaining reproducibility of a product's flow behavior at the time of release is an important product manufacturing control to maintain and demonstrate batch-to-batch consistency. Most semisolid dosage forms, when sheared, exhibit non-Newtonian behavior. Structures formed within semisolid drug products during manufacturing can show a wide range of behaviors, including shear thinning viscosity, thixotropy, and structural damage that may be irreversible or only partially reversible. In addition, the viscosity of a semisolid dosage form is highly influenced by such factors as the inherent physical structure of the product, product sampling technique, sample temperature for viscosity testing, container size and shape, and specific methodology employed in the measurement of viscosity.

A variety of methods can be used to characterize the consistency of semisolid dosage forms, such as penetrometry, viscometry, and rheometry. With all methods significant attention is warranted to the shear history of the sample. For semisolids, viscometer geometries typically fall into the following categories: concentric cylinders, cone-plates, and spindles. Concentric cylinders and spindles typically are used for more fluid, flowable semisolid

dosage forms. Cone-plate geometries are more typically used when the sample size is small or the test samples are more viscous and less flowable.

When contemplating what viscosity parameter(s) to test, one must consider the properties of the semisolid drug product both ''at rest'' (in its container) and as it is sheared during application. The rheological properties of the drug product at rest can influence the product's shelf life, and its properties under extensive shear can influence its spreadability and, therefore, its application rate that will affect the safety and efficacy of the drug product. Further, although it is necessary to precisely control the temperature of the test sample during the viscosity measurement, one should link the specific choice of the temperature to the intended use of the drug product (e.g., skin temperature for external application effects).

Because semisolid dosage forms frequently display non-Newtonian flow properties, formulators should give close attention to the shear history of the sample being tested, such as the shear applied during the filling operation, shear applied dispensing the product from its container, and shear introducing the sample into the viscometer. The point of reemphasizing this aspect is that considerable variability and many failures to meet specifications can be directly attributed to a lack of attention to this detail rather than a change of viscosity (or flow properties) of the drug product.

Tube (Content) Uniformity—Tube uniformity is the degree of uniformity of the amount of active drug substance among containers, i.e., tubes containing multiple doses of the semisolid topical product. The uniformity of dosage is demonstrated by assay of top, middle, and bottom samples (typically 0.25–1.0 g) obtained from a tube cut open to withdraw respective samples for drug assay.

Various topical semisolid products may show some physical separation at accelerated storage temperatures because emulsions, creams, and topical lotions are prone to mild separation due to the nature of the vehicle.

The following procedure should be followed for testing tube uniformity of semisolid topical dosage forms:

1. Carefully remove or cut off the bottom tube seal and make a vertical cut up the face of the tube. Then carefully cut the tube around the upper rim and pry open the two ''flaps'' to expose the semisolid.
2. At the batch release and/or designated stability time point remove and test 0.25- to 1.0-g samples from the top, middle, and bottom of a tube. If assay values for those tests are within 90.0% to 110.0% of the labeled amount of active drug, and the relative standard deviation (RSD) is not more than 6%, then the results are acceptable.
3. If at least 1 value of the testing described above is outside 90.0% to 110.0% of the labeled amount of drug and none is outside 85.0% to 115.0% and/or the RSD is more than 6%, then test an additional 3 randomly sampled tubes using top, middle, and bottom samples as described. Not more than 3 of the 12 determinations should be outside the range of 90% to 110.0% of the labeled amount of drug, none should be outside 85.0% to 115.0%, and the RSD should not be not more than 7%.
4. For very small tubes (e.g., 5 g or less), test only top and bottom samples, and all values should be within the range of 90.0% to 110.0% of the labeled amount of drug.

pH—When applicable, semisolid drug products should be tested for pH at the time of batch release and designated stability test time points for batch-to-batch monitoring. Because most semisolid dosage forms contain very limited quantities of water or aqueous phase, pH measurements may be warranted only as a quality control measure, as appropriate.

Particle Size—Particle size of the active drug substance in semisolid dosage forms is determined and controlled at the formulation development stage. When applicable, semisolid drug products should be tested for any change in the particle size or habit of the active drug substance at the time of batch release and designated stability test time points (for batch-to-batch monitoring) that could compromise the integrity and/or performance of the drug product, as appropriate.

Ophthalmic Dosage Forms—Ophthalmic dosage forms must meet the requirements of *Sterility* ⟨71⟩. If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described under *Sterility* ⟨71⟩, along with aseptic manufacture, may be employed. Ophthalmic ointments must contain a suitable substance or mixture of substances to prevent growth of, or to destroy, microorganisms accidentally introduced when the container is opened during use, unless otherwise directed in the individual monograph or unless the formula itself is bacteriostatic (see *Added Substances* under *Ophthalmic Ointments* ⟨771⟩). The finished ointment must be free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* in ⟨771⟩. The immediate containers for ophthalmic ointments shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic ointments be sealed and tamper-proof so that sterility is assured at time of first use.

PRODUCT QUALITY TESTS FOR TOPICAL DRUG PRODUCTS

General product quality tests such as identification, assay, content uniformity (uniformity of dosage units), impurities, pH, water content, microbial limits, antimicrobial preservative content, antioxidant preservative content, and sterility should be performed for topical drug products. In addition, specific tests for topical dosage forms, such as viscosity, tube (content) uniformity, and particle size also should be conducted. For details, see General Chapter *Product Quality Tests: Topical and Transdermal Drug Products* ⟨3⟩.

PRODUCT PERFORMANCE TEST FOR TOPICAL DRUG PRODUCTS

A performance test for topical drug products must have the ability to measure drug release from the finished dosage form. It must be reproducible and reliable, and although it is not a measure of bioavailability, the performance test must be capable of detecting changes in drug release characteristics from the finished product. The latter have the potential to alter the biological performance of the drug in the dosage form. Those changes may be related to active or inactive/inert ingredients in the formulation, physical or chemical attributes of the finished formulation, manufacturing variables, shipping and storage effects, aging effects, and other formulation factors critical to the quality characteristics of the finished drug product.

Product performance tests can serve many useful purposes in product development and in postapproval drug product monitoring. They provide assurance of equivalent performance for products that have undergone postapproval raw material changes, relocation or change in manufacturing site, and other changes as detailed in the FDA *Guidance for Industry—SUPAC-SS: Nonsterile Semisolid Dosage Forms, Manufacturing Equipment Addendum,* Dec 1998, available at http://www.fda.gov/cder/guidance/1722dft.pdf.

Vertical Diffusion Cell Method

***Theory*—**The vertical diffusion cell (VDC) system is a simple, reliable, and reproducible means of measuring drug release from semisolid dosage forms. A thick layer of the test semisolid is placed in contact with a reservoir. Diffusive communication between the delivery system and the reservoir takes place through an inert, highly permeable support membrane. The membrane keeps the product and the receptor medium separate and distinct. Membranes are chosen to offer the least possible diffusional resistance and not to be rate controlling. Samples are withdrawn from the reservoir at various times. In most cases, a five- to six-hour time period is all that is needed to characterize drug release from a semisolid, and when this is the case samples usually are withdrawn hourly.

After a short lag period, release of drug from the semisolid dosage form in the VDC system is kinetically describable by diffusion of a chemical out of a semi-infinite medium into a sink. The momentary release rate tracks the depth of penetration of the forming gradient within the semisolid. Beginning at the moment when the receding boundary layer's diffusional resistance assumes dominance of the kinetics of release, the amount of the drug released, *M*, becomes proportional to $\sqrt{t}$ (where *t* = time) for solution, suspension, or emulsion semisolid systems alike. The momentary rate of release, *dM/dt*, becomes proportional to $1/\sqrt{t}$, which reflects the slowing of drug release with the passage of time. The reservoir is kept large so that drug release is into a medium that remains highly dilute over the entire course of the experiment relative to the concentration of drug dissolved in the semisolid. In this circumstance, drug release is said to take place into a diffusional sink.

When a drug is totally in solution within the dosage form, the amount of drug released as a function of time can be described by:

$$M = 2 \cdot Co \sqrt{\frac{D \cdot t}{\pi}}$$

where:
M = amount of drug released into the sink per cm^2
Co = drug concentration in releasing matrix
D = drug diffusion coefficient through the matrix.

A plot of *M* vs $\sqrt{t}$ will be linear with a slope of

$$2 \cdot Co \sqrt{\frac{D}{\pi}}$$

The following equation describes drug release when the drug is in the form of a suspension within the dosage form:

$$M = \sqrt{2 \cdot Dm \cdot Cs \left(Q - \frac{Cs}{2} \right) t}$$

where
Cs = drug solubility in the releasing matrix
Dm = drug diffusion coefficient in the semisolid matrix
Q = total amount of the drug in solution and suspended in the matrix.

When *Q*>>*Cs*, the previous equation simplifies to:

$$M = \sqrt{2 \cdot Q \cdot Dm \cdot Cs \cdot t}$$

A plot of *M* vs $\sqrt{t}$ will be linear with a slope of $\sqrt{2QDmCs}$.

Coarse particles may dissolve so slowly that the moving boundary layer recedes to some extent behind the particles. That situation introduces noticeable curvature in the $\sqrt{t}$ plot because of a particle size effect. During release rate experiments, every attempt should be made to keep the composition of the formulation intact over the releasing period.

***In Vitro* Drug Release Using the VDC—**A VDC system is used to determine in vitro release of semisolid (cream, ointment, and gel) preparations. Typically, 200–400 mg of a cream, ointment, or gel is spread evenly over a suitable synthetic inert support membrane. The membrane, with its application side up, is placed in a vertical diffusion cell (typically of 15-mm diameter orifice), e.g., a Franz cell. The release rate experiment is carried out at 32 ± 1 °C, except in the case of vaginal creams for which the temperature should be 37 ± 1 °C. Sampling generally is performed over 4–5 hours, and the volume sampled is replaced with fresh receptor medium. To achieve sink conditions, the receptor medium must have a high capacity to dissolve or carry away the drug, and the receptor media should not exceed 10% of *Cs* (drug solubility in

the releasing matrix) at the end of the test. The test is done with groups of 6 cells. Results from 12 cells, 2 runs of 6 cells, are used to document the release rate.

Application of Drug Release—The drug release results can be utilized for purposes such as ensuring product sameness after scale-up and post-approval related changes or successive batch release comparison.

The VDC assembly consists of two chambers, a donor chamber and a receptor chamber, separated by a donor compartment and held together by a clamp (see *Figure 1*). This type of cell is commonly used for testing the in vitro release rate of topical drug products such as creams, gels, and ointments. Alternative diffusion cells that conform to the same general design and size can be used.

The VDC body normally is made from borosilicate glass, although different materials may be used to manufacture the body and other parts of the VDC assembly. None of the materials should react with or absorb the test product or samples.

In the donor compartment, the semisolid dosage form sample sits on a synthetic membrane within the cavity of the dosage compartment that is covered with a glass disk.

The diameters of the orifices of the donor chamber and the dosage compartment, which defines the dosage delivery area for the test, should be sized within ± 5% of the specified diameter. The receptor chamber orifice should never be smaller than the orifice of the donor chamber and should be fabricated to the same size as the donor chamber orifice. The design of the VDC should facilitate proper alignment of the dosage compartment and receptor orifices.

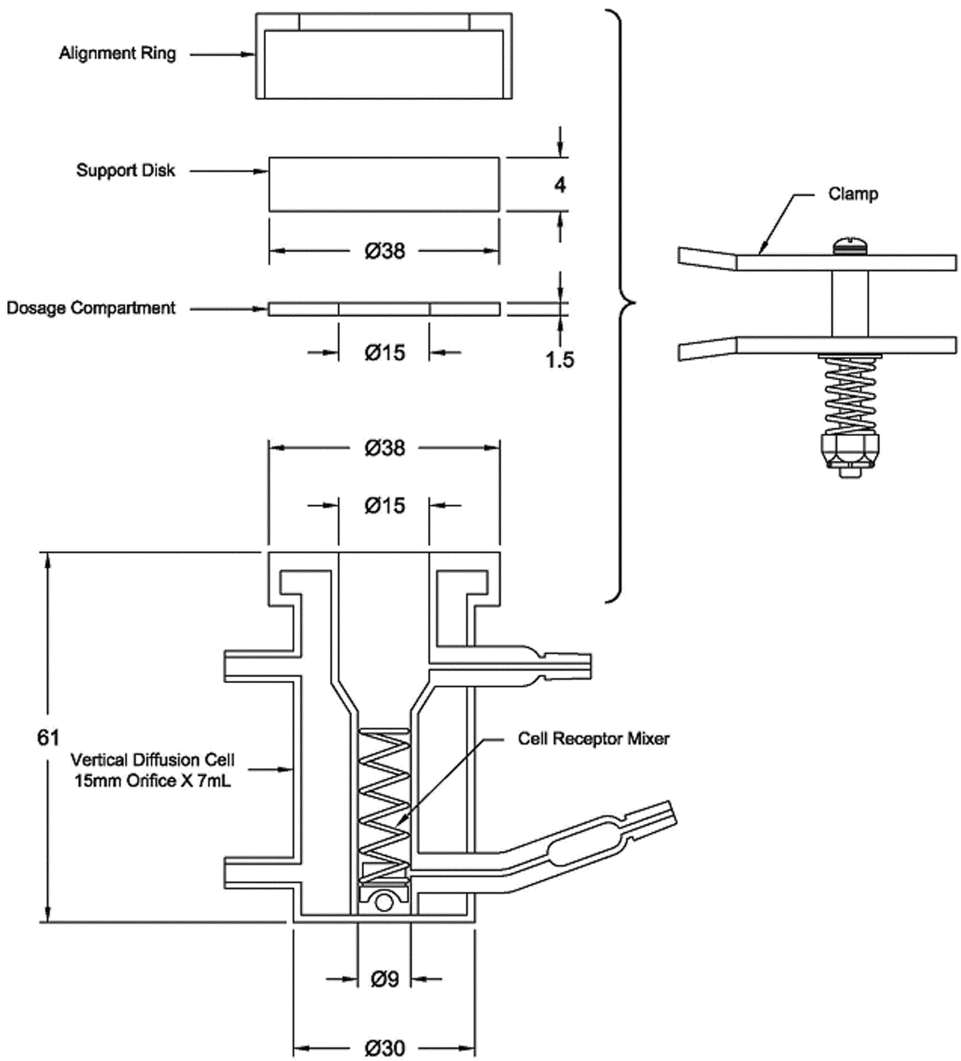


Figure 1. Vertical diffusion cell. (All measurements are expressed in mm unless noted otherwise).

The thickness of the dosage compartment normally is 1.5 mm. This thickness should be sized within ± 10% of the specified thickness.

The cell body should be manufactured consistently, with uniform height and geometry. Cells should appear the same, and their internal receptor volumes should fall within ± 20% of their specified volume.

Volume—Before conducting testing, determine the true volume of each receptor chamber in the VDC. The volume of each VDC should be determined with the internal stirring device in place.

Temperature—The temperature of the receptor media during the test should remain within ± 1.0 °C of the target temperature (typically 32 °C).

Speed—The rotational speed tolerance is ± 10% from the target speed (normally 600–800 rpm). The speed selected should ensure adequate mixing of the receptor media during the test.

Sampling Time—Samples should be taken at the specified times within a tolerance of ± 2% or ± 2 min, whichever is greatest.

Qualification—Unless otherwise specified in the individual monograph, the qualification of the apparatus is demonstrated by verifying the test temperature and speed requirements are met, along with a performance verification test (PVT). The PVT is passed if two tests of 6 cells comply with FDA's *SUPAC-SS* requirements of a 90% confidence interval. The PVT is performed by one analyst testing the specified reference standard in duplicate. The first test with 6 cells is performed and is defined as the reference. The second test of 6 cells is defined as the test. The PVT is passed if the second test passes the 90% confidence interval with reference to the first reference test.

Procedure—Unless otherwise specified in the individual monograph, degas the medium using an appropriate technique. With the stirring device in place, fill the VDC with the specified media and allow time for it to come to a temperature of 32 °C. If necessary, saturate the membrane in the specified media (generally receptor media) for 30 min. Place the membrane on the dosage compartment and invert. Apply the material that will be tested into the cavity of the dosage compartment, and spread the material out to fill the entire cavity of the dosage compartment.

Assemble each of the prepared dosage compartments to each VDC with the membrane down and in contact with the receptor media. During this application it is important to ensure that there are no bubbles under the membrane. When all dosage compartments and the remaining components are in place, turn on the stirring device, which constitutes time zero.

Follow the specified sampling procedure and collect an aliquot from each VDC for analysis. Ensure that during the sampling process bubbles are not introduced into the cell. Exercise care during sampling and replenishment of the medium in order not to introduce bubbles.

With some cells it is acceptable to have up to three bubbles under the membrane if the bubbles are less than 1 mm in diameter. With some cells, bubbles may be removed from the receptor chamber during the test by tipping the cell as long as this process is required only one time per position.

Calculation of Rate (Flux) and Amount of Drug Released

Creams and ointments are considered extended-release preparations. Their drug release largely depends on the formulation and method of preparation. The release rate of a given drug product from different manufacturers is likely to be different. It is assumed that the drug release of the product is linked to the clinical batch.

Unless otherwise specified in the individual monograph, the release requirements are met if the following have been achieved:

The *Amount Released* (μg/cm²) at a given time (t_1, t_2, etc.) is calculated for each sample as follows:

$$\text{Amount Released}_{t_1} = \frac{A_u}{A_s} \bullet C_s \bullet 1{,}000 \bullet \frac{V_c}{A_o}$$

$$\text{Amount Released}_{t_2} = \frac{A_u}{A_s} \bullet C_s \bullet 1{,}000 \bullet \frac{V_c}{A_o} + \left(AR_{t_1} \bullet \frac{V_s}{V_c} \right)$$

$$\text{Amount Released}_{t_n} = \frac{A_u}{A_s} \bullet C_s \bullet 1{,}000 \bullet \frac{V_c}{A_o} + \sum_{i=1}^{n-1} \left(AR_{t_{n-1}} \bullet \frac{V_s}{V_c} \right)$$

where
A_u = Area of the current sample
A_s = Average area of the standard
C_s = Concentration of the standard, mg/mL
V_c = Volume of the diffusion cell, mL
AR = Amount released, μg/cm²
A_o = Area of the orifice, cm²
V_s = Volume of the sample aliquot, mL.

For each cell the individual amount released is plotted vs time, and the slope of the resulting line (rate of drug release, flux) is determined. The average of 6 + 6 slopes represents the drug release of the dosage form and serves as the standard for the drug product.

Application of Drug Release

The drug release results can be utilized for purposes such as ensuring product sameness after *SUPAC-SS*–related changes or successive batch release comparison. This is illustrated by the following example where the initial drug batch is referred to as *Reference Batch* (R) and the changed or subsequent batch is referred to as *Test Batch* (T). The individual amount released from R is plotted vs time, and the resulting slope is determined. These are the reference slopes. The process is repeated to determine the test slopes.

The T/R Ratios are calculated for each Test-to-Reference Slope. This is most easily done by creating a table in which the Test Slopes are listed down the left side of the table and the Reference Slopes are listed across the

top of the table. The T/R ratios are then calculated and entered in the body of the table where the rows and columns intersect (*Table 1*).

After the T/R Ratios have been calculated they are ordered from lowest to highest. The 8th and 29th T/R Ratios are extracted and converted to percent (multiply by 100). To pass the first stage these ratios must fall within the range of 75% to 133.33%.

If the results do not meet this criterion, the FDA *SUPAC-SS* guidance requires that 4 more tests of 6 cells each must be run, resulting in 12 additional slopes per product tested. The T/R ratios are calculated for all 18 slopes per product tested. All 324 individual T/R Ratios are calculated and ordered lowest to highest. The 110th and the 215th ratios are evaluated against the specification of 75% to 133.33%.

No third stage is suggested.

PVT Method for USP Hydrocortisone Cream Reference Standard

Materials and Equipment

USP Hydrocortisone Cream Reference Standard; 25-mm, 0.45-μm hydrophilic polysulfone [NOTE—a suitable filter is Tuffryn available from www.pall.com] membrane filters; vacuum filtration apparatus consisting of a filter holder with a medium or fine-porosity sintered glass holder base, funnel with a 250-mL capacity, and magnetic stirrer; small and smooth jeweler's forceps; depression porcelain color plate; Diffusion Cell System with 6 diffusion cells and temperature control circulator; and sampling syringe or device and collection vials.

Procedure

Receptor Medium Preparation

Mix 60 mL of USP Alcohol with 140 mL of water to prepare a 30% alcoholic media. Degas the media by filtering through a 47-mm, 0.45-μm membrane by vacuum filtration. Assemble the filtration apparatus placing a magnetic stir bar (approximately 1in × 0.25 in) in the receiving flask. Place the apparatus on a magnetic stirring plate, and spin the bar at a moderate rate. Apply vacuum and pass the media through the filter while stirring. After all media have passed through the filter, continue stirring while maintaining a vacuum for 2 min. Applying vacuum and stirring beyond 2 min may change the composition of the water–alcohol media. Care should be taken to ensure that the period of time that the media is under vacuum after the filtration is complete is limited to 2 min.

Immediately transfer the degassed receptor medium to a suitable receptor medium bottle and stopper. Place the receptor medium bottle in the jacketed beaker and allow the media to equilibrate for 30 min before use.

Preparation of Apparatus

Set the circulating bath to a temperature (typically 32.5 °C) that will maintain the temperature in the diffusion cells at 32 °C during the test. Place the appropriate magnetic stirrer in each diffusion cell. Allow the system to equilibrate for at least 30 min before beginning the test.

Sample Preparation

Carefully lift one membrane at its very edge with jeweler's forceps. Place the membrane on a paper tissue, and blot any extreme excess solution (a slight excess solution is desired). Carefully place the membrane in the center of the dosage compartment. Place the dosage compartment, with the membrane centered on the underside, onto a tissue and press down on the compartment. Apply an appropriate amount of Hydrocortisone Cream Reference Standard (between 200 mg and 400 mg) on top of the membrane and inside of the dosage compartment cavity. Use a spatula to carefully smooth the material over the membrane, filling the entire cavity of the dosage compartment. Wipe any excess material from the surface of the dosage compartment. Repeat for a total of 6 sample preparations.

Performing the Test

Fill the diffusion cells with receptor media, and allow time to equilibrate to 32 °C. Ensure that the stirrers are not rotating and that there is a positive meniscus covering the complete top of each diffusion cell. Place the glass disk on top of the dosage compartment against the sample. Place the dosage compartment/glass disk assembly on the top of the diffusion cell, avoiding bubbles. Inspect under the membrane for bubbles. Assemble the cell. Repeat for each cell.

Begin the test according to the following test parameters: temperature: 32 °C; stir speed: 600–800 rpm; total test time: 6 h; sampling times: 1, 2, 3, 4, and 6 h.

Table 1. Calculation of T/R Ratios

	RS1	RS2	RS3	RS4	RS5	RS6
TS1	TS1/RS1	TS1/RS2	TS1/RS3	TS1/RS4	TS1/RS5	TS1/RS6
TS2	TS2/RS1	TS2/RS2	TS2/RS3	TS2/RS4	TS2/RS5	TS2/RS6
TS3	TS3/RS1	TS3/RS2	TS3/RS3	TS3/RS4	TS3/RS5	TS3/RS6
TS4	TS4/RS1	TS4/RS2	TS4/RS3	TS4/RS4	TS4/RS5	TS4/RS6
TS5	TS5/RS1	TS5/RS2	TS5/RS3	TS5/RS4	TS5/RS5	TS5/RS6
TS6	TS6/RS1	TS6/RS2	TS6/RS3	TS6/RS4	TS6/RS5	TS6/RS6

Sampling Procedure

At each of the stated sampling times, collect a sample from each cell as follows:

Stop the stirrer 30 sec before sampling. Repeat sampling procedure for each cell in order from 1 to 6. After the sixth cell has been sampled, resume the stirrer rotation.

High-performance Liquid Chromatography (HPLC) Hydrocortisone Analysis

USP Hydrocortisone Cream Reference Standard; acetonitrile, HPLC grade; water, HPLC grade; USP alcohol, 95%; 47-mm, 0.45-μm hydrophilic polysulfone membrane filters [NOTE—a suitable filter is Tuffryn available from www.pall.com]; HPLC System with UV detector capable of 10-μL injections; 50 mm × 3.9 mm column that contains 5 μm packing L1.

Procedure

Mobile Phase Preparation

Prepare and degas a sufficient volume of mobile phase to complete the analysis of the samples collected. For each 1 L of mobile phase mix 200 mL of acetonitrile with 800 mL of water. If necessary, adjust the mobile phase composition to achieve an approximate retention time of 7 min for the hydrocortisone peak.

Standard Preparation

Obtain a portion of appropriately dried Hydrocortisone Cream Reference Standard. Prepare a stock hydrocortisone standard solution at a concentration of approximately 0.20 mg/mL in USP alcohol. A solution of 20 mg hydrocortisone in 100 mL of USP alcohol is suggested for the stock standard preparation. Prepare a working standard solution by making a 5-fold dilution of the stock standard in a solution of 30:70 USP alcohol/water mixture. For example, dilute 2 mL to 10 mL.

Chromatographic Conditions

Wavelength—242 nm; flow rate: 1 mL/min; injection volume: 10 μL; run time: 10 min; column: 50 mm × 3.9 mm C-18, 5-μm (Symmetry); mobile phase: 20/80 acetonitrile/water. Begin the analysis by making 5 replicate injections of the working hydrocortisone standard solution for system suitability.

System Suitability Requirements—Relative standard deviation: < 2%; tailing factor: NMT 1.5. Make single injections of each of the samples obtained during the in vitro release testing. Bracket injections of samples with single standard injections after the analysis of the 2-h samples, 4-h samples, and 6-h samples. Calculate the results as specified.

Other Test Systems

Other diffusion-type cell devices are available as potential apparatus for drug release testing from topical/dermal drug products. However, only limited data are available for these devices. USP will consider these devices for inclusion in this General Chapter after they are validated and collaborative study data have been evaluated. The devices currently include:

Modified Holding Cell—A sample of semisolid dosage form is placed in an inert holding cell with a suitable membrane separating the dosage form from the receptor medium. The holding cell is positioned at the bottom of a modified, reduced-volume dissolution vessel of the USP Apparatus 2 type and is stirred with a mini paddle.

USP Apparatus 4 (Flow-through Cell) with a Trans-cap Semisolid Cell—A sample of the semisolid dosage form is placed in the trans-cap cell with a suitable membrane separating the dosage form from the receptor medium. The trans-cap cell with membrane facing upward is inserted into the 22.6-mm flow cell of USP Apparatus 4 (Flow-through Cell).

Extraction Cell—A sample of the semisolid dosage form is placed in an inert extraction cell with a suitable membrane separating the dosage form from the receptor medium. The test is performed using the USP Apparatus 2 assembly with the extraction cell positioned at the bottom of the dissolution vessel.

Product Quality Tests for Transdermal Delivery Systems

TDS are physical devices that deliver their active ingredient at a fixed rate over a prolonged period of time, e.g., from days to as long as one month. Their methods of delivery or release mechanisms vary significantly because of differences in their composition and fabrication. TDS systems can be categorized as (1) liquid form, fill, and seal systems, (2) peripheral adhesive systems, or (3) matrix systems. The latter two categories include the subcategories of monolithic, matrix, multi-laminate, and drug-in-adhesive systems. Moreover, recent advances in the design of novel transdermal drug delivery systems has expanded the list of basic TDS categories further, including, systems that employ iontophoresis, heat-assisted drug delivery, or micro-needles.

In all three principal TDS categories, the drug is in solution or suspension. Factors that can influence drug release and, therefore, the performance of TDS dosage forms include changes in formulation composition involving the adhesive, solvents, viscosity-modifying agents, permeation enhancers, and changes in the dosage form's semipermeable film or laminate.

The product quality tests for transdermal drug delivery system include assay, content uniformity, homogeneity, and adhesive test.

Uniformity of dosage units—This test is applicable for transdermal systems and for dosage forms that are packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form. It can be done by either content uniformity or weight variation (see ⟨905⟩).

Assay of excipient(s) critical to the performance of the product should be considered; e.g., residual solvent content can affect certain patches.

Adhesive Test—Three types of adhesive tests generally are performed to ensure the performance of the TDS dosage forms. These are the peel adhesion test, tack test, and shear strength test. The peel adhesion test measures the force required to peel away a transdermal patch attached to a stainless steel test panel substrate at panel angles of 90° or 180° following a dwell time of 1 min and peel rate of 300 mm/min.

The tack test is used to measure the tack adhesive properties of TDS dosage forms. With this test a probe touches the adhesive surface with light pressure, and the force required to break the adhesion after a brief period of contact is measured.

The shear strength or creep compliance test is a measure of the cohesive strength of TDS dosage forms. Two types of shear testing are performed: dynamic and static. During dynamic testing the TDS is pulled from the test panel at a constant rate. With the static test the TDS is subjected to a shearing force by means of a suspended weight.

Leak Test—A test that is discriminating and capable of detecting sudden drug release, such as leakage, from the TDS should be performed. Although form, fill, and seal TDS are more likely to display leak problems, all TDS should be checked for sudden drug release (dose dumping) during release testing of the dosage form.

Product Performance Tests for TDS

As with topical drug products, a performance test for transdermal drug products also must have the ability to measure drug release from the finished dosage form, must be reproducible and reliable, and must be capable of detecting changes in drug release characteristics from the finished product. Again, the latter have the potential to alter the desired pharmacologic effect(s) of the active ingredient. Such changes could be related to active or inactive/inert ingredients in the formulation or physical dosage form, physical or chemical attributes of the finished preparation, manufacturing variables, shipping and storage, age, and other critical-to-quality characteristics of the finished dosage form.

When based on sound scientific principles, product performance tests can be used for pre- and postmanufacturing purposes such as during the product research and development phase, as a basic quality control tool, for demonstrating product similarity, or for demonstrating compliance with FDA guidelines (e.g., approval and postapproval changes in the dosage form).

In vitro drug release methods for transdermal patches include USP Apparatus 5 (Paddle over Disk Method), Apparatus 6 (Rotating Cylinder Method), or Apparatus 7 (Reciprocating Holder Method). In general, it has been found that Apparatus 5, a modified paddle method, is simpler and is applicable for most types, sizes, and shapes of TDS.

Apparatus 5 (Paddle over Disk Method)

Apparatus—Use the paddle and vessel assembly from *Apparatus 2* as described in *Dissolution* ⟨711⟩, with the addition of a stainless steel disk assembly (*1*) designed for holding the transdermal system at the bottom of the vessel. Other appropriate devices may be used, provided they do not absorb, react with, or interfere with the specimen being tested (*2*). The temperature should be maintained at 32 ± 0.5 °C. During the test maintain a distance of 25 ± 2 mm between the paddle blade and the surface of the disk assembly. The vessel may be covered during the test to minimize evaporation. The disk assembly for holding the transdermal system is designed to minimize any dead volume between the disk assembly and the bottom of the vessel. The disk assembly holds the system flat and is positioned so that the release surface is parallel with the bottom of the paddle blade (see *Figure 2*).

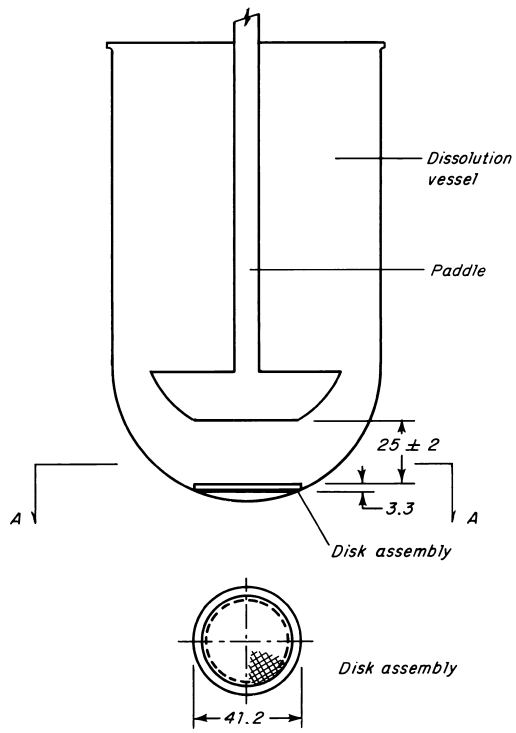


Fig. 2. Paddle over Disk. (All measurements are expressed in mm unless noted otherwise.)

Apparatus Suitability Test and Dissolution Medium—Proceed as directed for *Apparatus 2* in ⟨711⟩.

Procedure—Place the stated volume of the *Dissolution Medium* in the vessel, assemble the apparatus without the disk assembly, and equilibrate the medium to 32 ± 0.5 °C. Apply the transdermal system to the disk assembly, ensuring that the release surface of the system is as flat as possible. The system may be attached to the disk

by applying a suitable adhesive (*3*) to the disk assembly. Dry for 1 min. Press the system, release surface side up, onto the adhesive-coated side of the disk assembly. If a membrane (*4*) is used to support the system, it should be applied in such a way that no air bubbles occur between the membrane and the release surface. Place the disk assembly flat at the bottom of the vessel with the release surface facing up and parallel to the edge of the paddle blade and surface of the *Dissolution Medium*. The bottom edge of the paddle should be 25 ± 2 mm from the surface of the disk assembly. Immediately start operation of the apparatus at the rate specified in the monograph. At each sampling time interval, withdraw a specimen from a zone midway between the surface of the *Dissolution Medium* and the top of the blade, not less than 1 cm from the vessel wall. Perform the analysis on each sampled aliquot as directed in the individual monograph, correcting for any volume losses, as necessary. Repeat the test with additional transdermal systems.

***Sampling Time*—**The test time points, generally three, are expressed in hours. Specimens should be withdrawn within a tolerance of ± 15 min or ± 2% of the stated time; select the tolerance that results in the narrowest time interval.

***In Vitro Release Criteria*—**Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Acceptance Table 1

Level	Number Tested	Criteria
L_1	6	No individual value lies outside the stated range.
L_2	6	The average value of the 12 units ($L_1 + L_2$) lies within the stated range. No individual value is outside the stated range by more than 10% of the average of the stated range.
L_3	12	The average value of the 24 units ($L_1 + L_2 + L_3$) lies within the stated range. Not more than 2 of the 24 units are outside the stated range by more than 10% of the average of the stated range, and none of the units is outside the stated range by more than 20% of the average of the stated range.

Apparatus 6 (Rotating Cylinder Method)

***Apparatus*—**Use the vessel assembly from *Apparatus 1* as described in ⟨711⟩, but replace the basket and shaft with a stainless steel cylinder stirring element and maintain the temperature at 32 ± 0.5 °C during the test. The shaft and cylinder components of the stirring element are fabricated from stainless steel to the specifications shown in *Figure 3*. The dosage unit is placed on the cylinder at the beginning of each test. The distance between the inside bottom of the vessel and the cylinder is maintained at 25 ± 2 mm during the test.

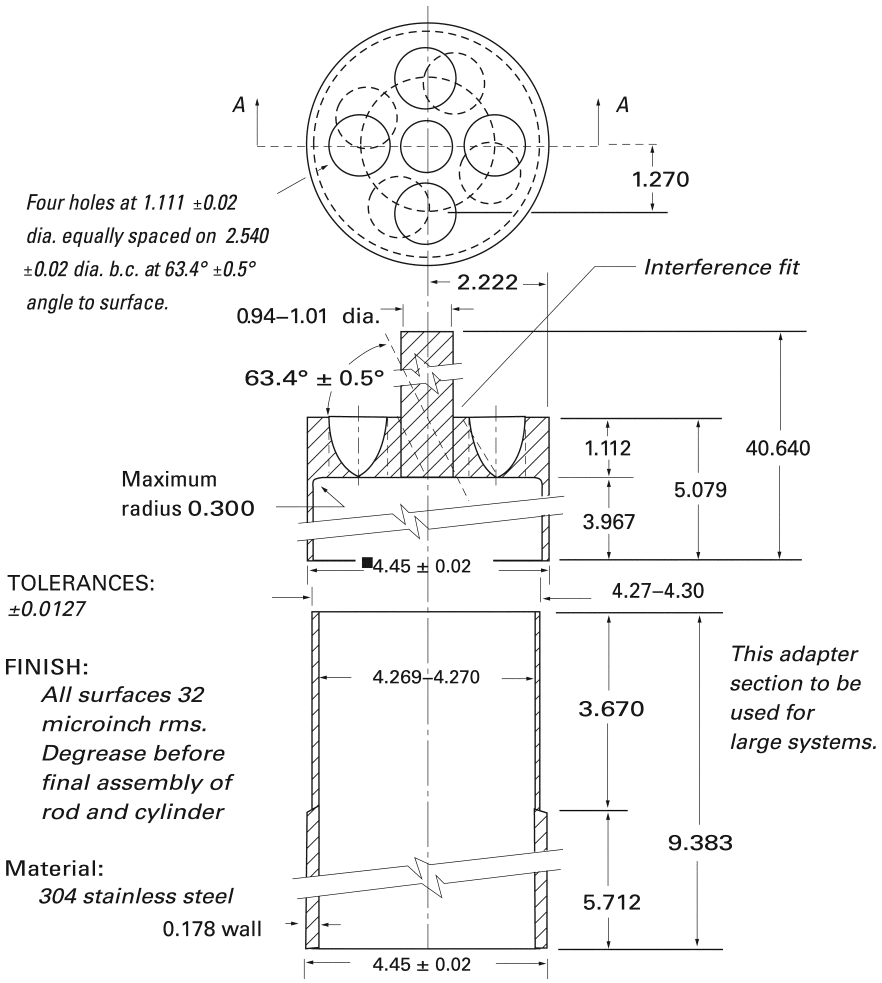


Fig. 3. Cylinder Stirring Element (*5*). (All measurements are expressed in cm unless noted otherwise.)

***Dissolution Medium*—**Use the medium specified in the individual monograph (see ⟨711⟩).

***Procedure*—**Place the stated volume of the *Dissolution Medium* in the vessel of the apparatus specified in the individual monograph, assemble the apparatus, and equilibrate the *Dissolution Medium* to 32 ± 0.5 °C. Unless otherwise directed in the individual monograph, prepare the test system before the test as follows:

Remove the protective liner from the system, and place the adhesive side on a piece of Cuprophan (*4*) that is not less than 1 cm larger on all sides than the system. Place the system, Cuprophan-covered side down, on a clean surface, and apply a suitable adhesive (*3*) to the exposed Cuprophan borders. If necessary, apply additional adhesive to the back of the system. Dry for 1 min. Carefully apply the adhesive-coated side of the system to the exterior of the cylinder so that the long axis of the system fits around the circumference of the cylinder. Press the Cuprophan covering to remove trapped air bubbles. Place the cylinder in the apparatus, and immediately rotate at the rate specified in the individual monograph. Within the time interval specified, or at each of the times stated, withdraw a quantity of *Dissolution Medium* for analysis from a zone midway between the surface of the *Dissolution Medium* and the top of the rotating cylinder, not less than 1 cm from the vessel wall. Perform the analysis as directed in the individual monograph, correcting for any volume losses as necessary. Repeat the test with additional transdermal drug delivery systems.

***Sampling Time*—**Proceed as directed for Apparatus 5.

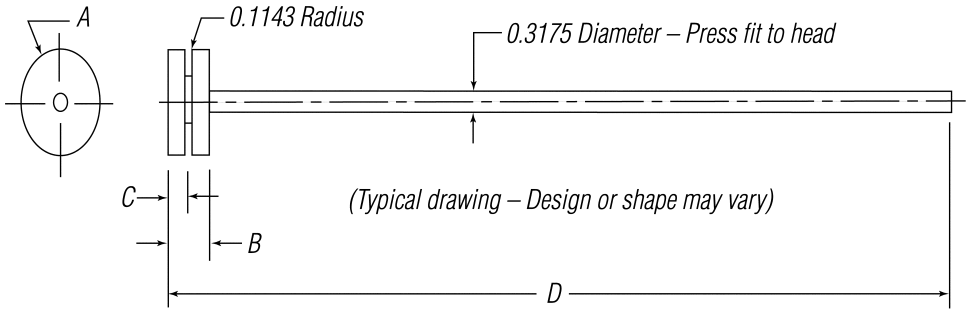
***In Vitro Release Criteria*—**Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Apparatus 7 (Reciprocating Holder Method)

***Apparatus*—**The assembly consists of a set of volumetrically calibrated or tared solution containers made of glass or other suitable inert material (*6*), a motor and drive assembly to reciprocate the system vertically and to index the system horizontally to a different row of vessels auto-

matically if desired, and a set of suitable sample holders [see *Figure 4* (*2*) and *Figures 5a* and *5b*). The solution containers are partially immersed in a suitable water bath of any convenient size that permits maintaining the temperature, *T*, inside the containers at 32 ± 0.5 °C or within the allowable range, as specified in the individual monograph, during the test. No part of the assembly, including the environment in which the assembly is placed, should contribute motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating sample holder.

An apparatus that permits observation of the system and holder during the test is preferable. Use the size container and sample holder specified in the individual monograph.



Dimensions are in centimeters

	HEAD				ROD		O-RING
System[a]	*A (Diameter)*	*B*	*C*	Material[b]	*D*	Material[c]	*(not shown)*
1.6cm^2	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-113-V884-75
2.5cm^2	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-016-V884-75
5cm^2	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2-022-V884-75
7cm^2	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2-124-V884-75
10cm^2	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2-225-V884-75

[a] Typical system sizes.
[b] SS/VT=Either stainless steel or virgin Teflon.
[c] SS/P=Either stainless steel or Plexiglas.

Fig. 4. Reciprocating Disk Sample Holder (*7*).

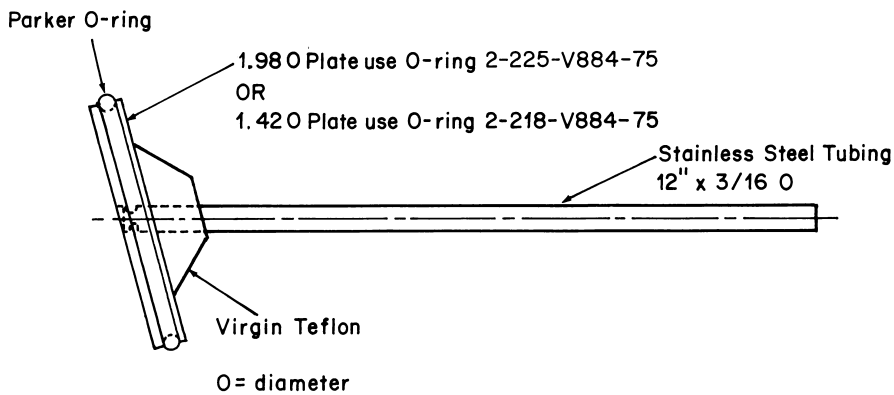


Fig. 5a. Transdermal System Holder—Angled Disk.

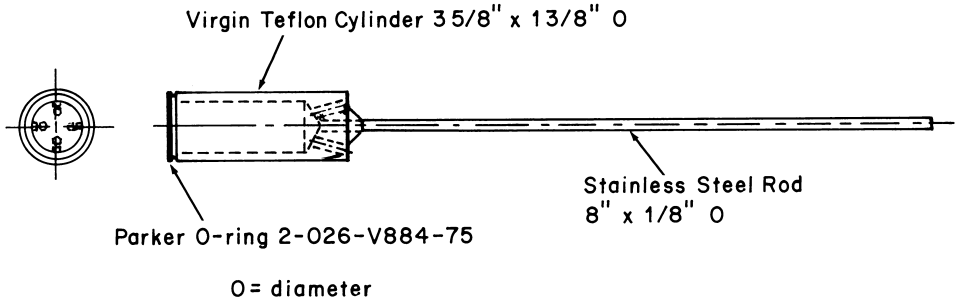


Fig. 5b. Transdermal System Holder—Cylinder.

***Sample Preparation A*—**Attach the system to be tested to a suitable sample holder with 2-cyano acrylate glue.

***Sample Preparation B*—**Press the system onto a dry, unused piece of Cuprophan (*4*), nylon netting, or equivalent with the adhesive side against the selected substrate, taking care to eliminate air bubbles between the substrate and the release surface. Attach the system to a suitably sized sample holder with a suitable O-ring so that the back of the system is adjacent to and centered on the bottom of the disk-shaped sample holder or centered around the circumference of the cylindrical-shaped sample holder. Trim the excess substrate with a sharp blade.

***Sample Preparation C*—**Attach the system to a suitable holder as described in the individual monograph.

***Dissolution Medium*—**Use the *Dissolution Medium* specified in the individual monograph (see ⟨711⟩).

***Procedure*—**Suspend each sample holder from a vertically reciprocating shaker so that each system is continuously immersed in an accurately measured volume of *Dissolution Medium* within a calibrated container preequilibrated to temperature, *T*. Reciprocate at a frequency of about 30 cycles/min with an amplitude of about 2 cm, or as specified in the individual monograph, for the specified time in the medium specified for each time point. Remove the solution containers from the bath, cool to room temperature, and add sufficient solution (water in most cases) to correct for evaporative losses. Perform the analysis as directed in the individual monograph. Repeat the test with additional drug delivery systems as required in the individual monograph.

***Sampling Time*—**Proceed as directed for Apparatus 5.

***In Vitro Release Criteria*—**Drug release should be measured at least at 3 time points, first time point around 1 hour, second around 50% of total drug release, and third around 85% drug release. Unless otherwise specified in the individual monograph, the requirements are met if the quantities of the active ingredients released from the system conform to *Acceptance Table 1* under *Dissolution* ⟨711⟩ for transdermal drug delivery systems, or as specified in the individual monograph. Continue testing through the three levels unless the results conform at either L_1 or L_2.

NOTE

This *Stimuli* article is subdivided into 2 chapters: *Product Quality Tests: Topical and Transdermal Drug Products* ⟨3⟩ and *Product Performance Tests: Topical and Transdermal Drug Products* ⟨725⟩. Both of these appear elsewhere in this issue of *Pharmacopeial Forum*.

ACKNOWLEDGMENT

The authors acknowledge the helpful suggestions of Loice Kikwai, Erika Stippler, and Will Brown of USP and Satish Asotra of Taro Pharmaceuticals.

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1. Disk assembly (stainless support disk) is available from www.millipore.com.
2. A suitable device is the watchglass-patch-polytef mesh sandwich assembly available as the Transdermal Sandwich from www.hansonresearch.com.
3. Use Dow Corning, MD7-4502 Silicone Adhesive 65% in ethyl acetate, or the equivalent.
4. Use Cuprophan, Type 150 pm, 11 ± 0.5-μm thick, an inert, porous cellulosic material, which is available from www.medicell.co.uk or www.varianinc.com.
5. The cylinder stirring element is available from www.varianinc.com.
6. The materials should not sorb, react with, or interfere with the specimen being tested.
7. The reciprocating disk sample holder is available at www.varianinc.com.

Performance-based Monographs

Small Molecules Collaborative Group, R.L. Williams, D.R. Abernethy, W.F. Koch, W.W. Hauck, and T.L. Cecil[a]

ABSTRACT This *Stimuli* article describes a performance-based monograph (PBM) that specifies tests and acceptance criteria for articles in the *US Pharmacopeia* (*USP*) and *National Formulary* (*NF*). For selected tests of the monograph, a certified reference material (CRM) from USP would be provided to assist analysts in developing sound procedures. Availability of a default procedure in association with the PBM is considered, pros and cons of the approach are presented, and comments are solicited.

INTRODUCTION

A *US Pharmacopeia* (*USP*) specification for a drug substance, excipient, or product contains four universal tests (Definition, Identification, Assay, and Impurities) and certain specific tests, with accompanying procedures and acceptance criteria. Each manufacturer develops a unique specification for its ingredients and products. Specifications developed by multiple manufacturers of the same ingredient or product may include tests and acceptance criteria that are similar, but the procedures used in the tests may not be the same. Despite this, USP's intent is to present a monograph establishing a standard specification that encompasses all legally marketed ingredients and products, where appropriate. The procedures presented in *USP* traditionally have been provided by the innovator of a drug substance. As additional manufacturing sources of ingredients and products become available, these original procedures can become too restrictive, causing the *USP–NF* monograph to be out of step with current industry practice. This has led to the development of flexible monographs (*1*) and the need for alternative suppliers of information and reference materials (RMs) to provide evidence that their procedures are adequate for all marketed products (*2*).

In contrast to the current approach wherein all elements of the monograph's specification are explicitly provided, a performance-based monograph (PBM) provides a specification for a drug substance that includes a *Test* and *Acceptance criteria*, but the *Procedure* would define only the criteria needed to show that the procedure used is acceptable. The universal tests and acceptance criteria generally are consistent between monographs [e.g., Assay, 98.0%–102.0%; *Identification* with IR or chromatography; impurities, not more than (NMT) 0.10%; and others] and rarely differ significantly. Therefore, in the case of most drug substances, a default set of tests and acceptance criteria could be used. An example of a performance-based monograph appears in *Appendix 1* in the form of a PBM for Acamprosate Calcium. The general approach fits well within the concepts articulated under the general term *quality by design*, where knowledge and design space requirements conclude with an understanding of the private control space pertinent to a particular ingredient and product (*3, 4*). The control space for the public standard may be viewed as the total array of tests and acceptance criteria of the monograph.

The application of a PBM approach would involve a number of important changes in the way that *USP–NF* monographs and related General Chapters are created, used, and interpreted. The major components of this concept include the PBM itself, to include a definition of an acceptable procedure, primary certified reference materials (CRMs), and, when available, appropriate screening procedures. Initially, only new monographs would be targeted for the PBM approach. In some instances, the performance-based procedure may appear in a General Chapter (*5*).

ACCEPTABLE PROCEDURES

What defines an *acceptable procedure*? There are several means to define an acceptable procedure, including equivalent or better, and others described by Hauck et al. (*6*). In that article, the term *acceptable procedure* is described as a procedure that ''meets a set of minimum performance requirements.'' The term acceptable also means that a procedure is suitable for its intended use. An acceptable procedure also has been defined as a procedure that is validated as described in USP General Chapter *Validation of Compendial Methods* ⟨1225⟩ (*7*). This General Information Chapter provides a set of criteria (reproduced herein as *Table 1*) but does not provide the minimum performance criteria necessary to define an acceptable procedure. Using these criteria as a starting point, one can see that the type of test directly affects the set of performance requirements. In this discussion, we focus on International Conference on Harmonization (ICH) universal tests for ingredients, with the understanding that many of the applications also pertain to drug products.

[a] Correspondence should be addressed to T.L. Cecil, PhD, Vice President, Compendial Affairs, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8234; e-mail tlc@usp.org.

Table 1. Data Elements Required for Validation

Analytical Performance Characteristics	Assay	Impurity Tests		Performance Tests (e.g., Dissolution, Disintegration)	Identification
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	[a]	[a]	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	[a]	Yes
Detection Limit	No	No	Yes	[a]	No
Quantitation Limit	No	Yes	No	[a]	No
Linearity	Yes	Yes	No	[a]	No
Range	Yes	Yes	[a]	[a]	No

[a] May be required depending on the nature of the specific test.

Assay Tests

Table 1 suggests that the critical parameters needed to validate an Assay procedure include precision, accuracy, specificity, linearity, and range. Further review makes it apparent that range is covered by other criteria and thus will not be discussed further. All decisions of procedure and testing include trade-offs between precision, accuracy, costs, time, specificity, and many other parameters. Three criteria that are particularly closely linked are precision, accuracy, and limit of quantitation (LOQ). For an Assay procedure, the LOQ typically is not an issue and therefore is not included in this discussion.

1. Assay—Accuracy and Precision. Trade-offs often are necessary to achieve acceptable accuracy (bias) and precision (standard deviation) in a given procedure. The linkage between the two can be best seen in the Operating Characteristic Curve (OCC) illustrated in *Figure 1*. This curve describes the degree of accuracy and precision needed to ensure that a measurement would have a 95% probability of falling between 98% and 102% if the true value was 100%. Expressed another way, *Figure 2* identifies the boundary conditions for acceptable precision and accuracy values. Any combination of precision and accuracy falling under the shaded portion of the curve would be acceptable for the procedure being evaluated. The accuracy (bias) measurement is determined relative to a USP RM. This bias is estimated as the difference between the mean of three independent determinations of USP RM and the true accepted value. The bias should be evaluated at three points in the range of the procedure. Precision is estimated by testing several separate sample preparations and is calculated as the standard deviation of those measurements. The number of samples to be evaluated should be the number necessary to ensure that the confidence interval for bias and upper confidence bound for the standard deviation fall within the shaded portion of *Figure 2*. For illustration purposes *Figure 2* shows precision and accuracy values on the OCC.

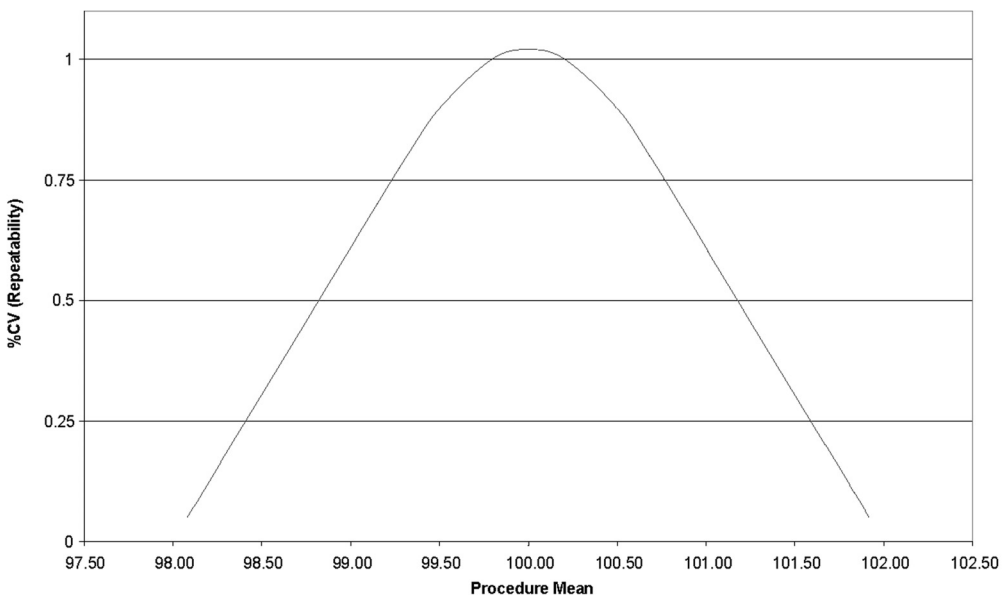

Figure 1: Operational Characteristic Curve for Assay Assuming 98%–102% limits; True Value = 100%; Probability of Passing = 0.95.

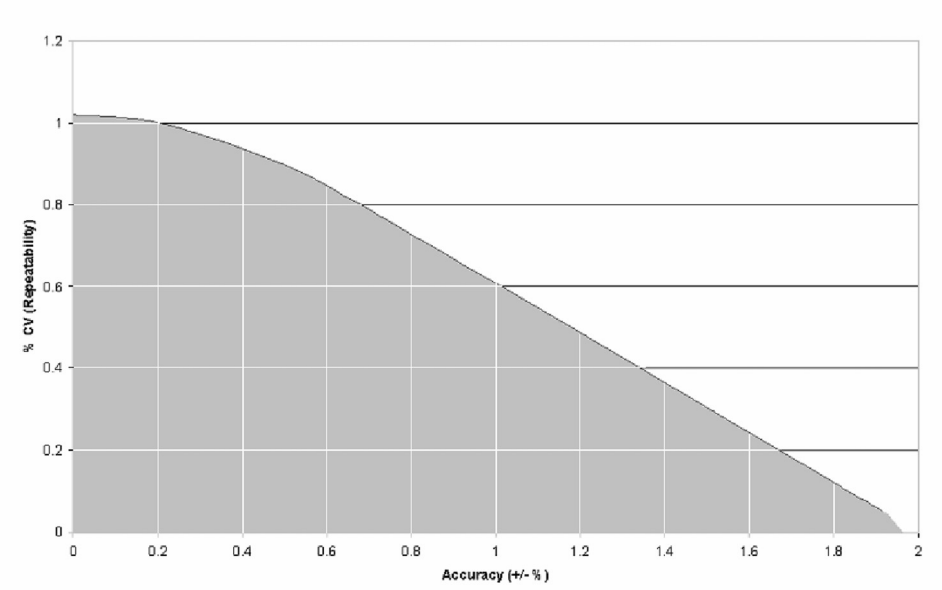

Figure 2: Precision and Accuracy for Assay.

2. Assay—Specificity. The specificity of a chromatographic *Assay* procedure is a measure of the level of interference caused by other peaks in the chromatogram. This is described by the resolution, *R,* between two closely eluting peaks. Generally speaking, an acceptable procedure should have a minimum resolution of 1.25 so that near-baseline resolution is achieved, and the resulting contribution to the error of the main peak would be minimal. The effect on the measurement of a primary peak due to the presence of a secondary peak with a response ratio of 16:1 would be an error of about 0.5% (*8, 9*), recognizing that this level of error is beyond that typically seen in pharmaceutical analytical laboratories. This can best be evaluated by spiking a USP RM with likely impurities at about a 6.25% level. These spiked samples are then compared to the labeled content of the RM using the procedure under evaluation. If the level of error is $< 0.5\%$ then the procedure is acceptable for this performance measure.

3. Assay—Linearity. Linearity is measured as the correlation coefficient (R^2) and slope of a plot of the measured concentration vs actual concentration from 50% to 150% of the expected analytical concentration. If R^2 is greater than 0.99 and the slope is 1.0 ± 0.1, then the procedure is acceptable for this performance measure.

Identification Test

Identification procedures can be broadly categorized as spectral, chromatographic, and classical procedures. These can be further classified as quantitative, semiquantitative, or qualitative in nature. *Table 1* indicates that only specificity is indicated for validation. In a performance-based paradigm, additional information is necessary to provide adequate information about the drug substance.

The criteria that define an adequate identification procedure will vary depending on the type of procedure and the nature of that measurement and are described in *Table 3*. In all cases the specificity is determined by a comparison with USP RM.

Table 2. Precision and Accuracy Characteristics for Small-molecule Assay Procedures

Assay	Measure	Example 1	Example 2	Example 3
Precision	OCC	CV $\leq$ 0.1%	CV $\leq$ 0.5%	CV $\leq$ 1.0%
Accuracy	OCC	$\pm$ 1.84%	$\pm$ 1.18%	$\pm$ 0.20%
Specificity	Contribution to Error	0.5%	0.5%	0.5%
Linearity	R^2	> 0.99	> 0.99	> 0.99

Table 3. Small-molecule Identification Procedures

Identification	Qualitative	Semi-quantitative	Quantitative
Spectral	Provides a Spectrum or Signal That Is Unique to the Moiety of Interest	N/A	See: Impurities Requirements (*Table 4*)
Chromatographic	N/A[a]	See: Impurities Requirements (*Table 4*)	See: Assay Requirements (*Table 2*)
Classical (wet-chemistry)	Provides a Response That Is Selective for the Attribute of Interest	Provides a Response That Is Selective for the Attribute of Interest	N/A

[a] Chromatographic procedures are quantitative but may at times be used for qualitative purposes.

Table 4. Small-molecule Impurities Procedures

Impurities Procedure	Measure	Quantitative	Limit Test (Semi-quantitative)
Precision		[a]	[b]
Accuracy	Difference between Calculated and Known Content	[a]	$\pm 0.01\%$[a]
Specificity	Contribution to Error	NMT 2%	NMT 2%
LOD		N/A	NLT 5:1
LOQ		[a]	N/A
Linearity	R^2	≥ 0.99	N/A

[a] Depends on the uncertainty necessary to meet the acceptance criteria.
[b] May be needed.

Stimuli to the Revision Process

Impurities Test

Impurities tests can be thought of as an *Assay* test for minor components in a mixture of materials. Therefore, parameters to describe an acceptable procedure are very similar to those in *Table 2*. However, as indicated in *Table 1*, impurities have two additional components that should be considered. These are the limits of detection (LOD) and LOQ. An acceptable procedure should be able to quantify impurities accurately and precisely at concentrations that are lower than the acceptance criteria in a monograph or in a drug substance specification. However, the acceptance criteria should be safety based and not capability based. This topic was discussed and a guidance proffered by ICH in the Q3A(R) guideline (*10*). In this guidance, default acceptance criteria for nontoxic impurities was set to 0.10% with the specification that all nontoxic impurities having concentrations of $\leq 0.05\%$ could be disregarded. To achieve this level of quantitation, a procedure must have sufficient accuracy and precision to provide an uncertainty of $<10\%$ of the target level.

1. Quantitative Procedures for Impurities. For quantitative tests, the precision, accuracy, and limit of quantification are linked. LOQ is the lowest concentration at which the analyte can not only be detected reliably but also at which some predefined goals for bias and precision are met (*11*). As stated above, the goals for bias and precision are defined by the requirement to provide a calculated test uncertainty of NMT 10% of the reporting level. The goals would be scaled depending on the uncertainty necessary to report a value consistent with the acceptance criteria (e.g., for NMT 1 ppm, an uncertainty of ≤ 0.1 ppm is necessary).

2. Limit Tests for Impurities. For these procedures the goal is to minimize false negatives by making the acceptance criteria semi-quantitative. Therefore, the precision is not defined, but it may be evaluated. The accuracy is linked to the reporting value for the acceptance criteria.

3. Specificity for Impurities. The evaluation of specificity is based on the ability to quantify all known and unknown impurities in the article. The impurities may be quantified individually or as a group, depending on the procedure. Therefore, specificity is evaluated by comparing the calculated concentration of known impurities spiked into the drug substance matrix relative to the amount added. Rather than an evaluation of all impurities, specificity determinations may be made using only critical impurities or those impurities that occur nearest to the acceptance limit. The authors acknowledge the interrelationship between accuracy and specificity where the latter can be considered in terms of the resolution when a chromatographic procedure is used.

CERTIFIED REFERENCE MATERIALS (CRMS)

Because all measurements are comparisons to a known standard, suitable RMs are required for procedures associated with a PBM in order for the approach to be suc-

cessful. Some RMs are linked to specific SI units (e.g., gram, meter, joule), others to primary standards that are linked or are traced to SI units. The evaluations described in this article all assume the use of an RM with stated content and level of uncertainty of the measurement (e.g., a USP CRM). As compendial procedures become more flexible and undefined, the need for well-characterized RMs traceable, where feasible, to the SI unit for amount of substance (kg) increases. As clearly demonstrated in the previous section, the uncertainty of a measurement has a direct bearing on the acceptability of the procedure and the ability of the procedure to serve its intended purpose. Because all measurements are comparisons, the uncertainty of the RM must be factored into the calculated uncertainty of the procedure. For these reasons, this article postulates that the RM accompanying a PBM should be certified with respect to both quantity (g/g) and uncertainty.

DEFAULT PROCEDURES

In addition to the option of using performance-based standards, some users of the USP's compendia may wish to use a specified default procedure. For these parties a procedure that evaluates identity, strength, quality, and purity via a more inclusive and less case-specific approach is preferred to procedures that are optimized for speed and throughput for a specific process. The more-inclusive, non-optimized procedures are typically developed by a drug manufacturer early in the development cycle and are refined as the process is finalized. These procedures often are used for investigation of forced degradation products, in evaluating potential changes in impurities caused by process changes, and later in assessing generic competition.

To accommodate both those wishing to develop an optimized, and those preferring to use a default procedure, both procedures would be provided for. The primary specification is the PBM discussed above, with specified procedures not provided. Beyond this presentation, a default procedure would be made available. The one or more default procedures would appear in the compendial monograph with a statement to the effect that either the PBM procedure or the default procedure is suitable. In a separate part of the compendia, USP may provide additional, optional, acceptable procedures.

SUMMARY

This article discusses PBMs as a means of allowing flexibility in analysts' choice of acceptable procedures. The general approach has been used in many industries over the years and is part of a larger trend affecting all industries. It also is increasingly used in many regulatory and compendial statements. A sample PBM is included in *Appendix 1*.

The PBM has pros and cons, many of which are interrelated and may be mitigated by the use of CRMs and the presence of alternative screening (default) procedures:

1. Pros
   a. Provides analysts with flexibility in choosing acceptable, equivalent, or better procedures;
   b. Reduces requests to manufacturers who do not wish to share procedures and/or candidate reference materials;
   c. Promotes harmonization;
   d. Aligns with regulatory statements and requirements;
   e. Reduces monograph backlogs and promotes updating of monographs;
   f. Accords well with modern measurement science that allows evaluation and use of different procedures in collaborating studies;
   g. May limit need for pending and non-US monograph approaches;
   h. Would support the application of new technologies;
   i. Would minimize future revisions and changes in the standard.

2. Cons
   a. Compendial test to ensure compliance may be unclear;
   b. Initially may not provide acceptable procedures;
   c. Will take substantial commitment with participating laboratories, including those of USP, to create needed CRMs in the absence of donated procedures.

If the approach articulated in this *Stimuli* article is adopted, following suitable discussion, it would shift monographs in *USP–NF* away from a monograph with all tests, procedures, and acceptance criteria toward a more flexible monograph with allowance for a PBM aproach, optional acceptable procedures coupled with an equally acceptable default procedure. These procedures would necessarily be deemed equivalent or better (*5*). Comments are encouraged and welcomed.

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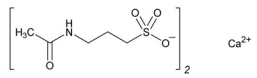
APPENDIX 1: A PERFORMANCE-BASED MONOGRAPH

Acamprosate Calcium



$C_{10}H_{20}CaN_2O_8S_2$ 400.48

1-Propanesulfonic acid, 3-(acetylamino)-, calcium salt (2:1); Calcium 3-(acetylamino)propane-1-sulfonate [77337-76-9]. [77337-73-6] acamprosate.

DEFINITION

Acamprosate Calcium contains NLT 98.0% and NMT 102.0% of $C_{10}H_{20}CaN_2O_8S_2$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B. CALCIUM ⟨191⟩:** Meets the requirements

ASSAY

- **PROCEDURE**

Standard solution: USP Acamprosate Calcium RS in an appropriate diluent [NOTE—Neat determination may be substituted where appropriate.]

Sample solution: Acamprosate Calcium in an appropriate diluent [NOTE—Neat determination may be substituted where appropriate.]

Performance suitability solution: 1 to 1000 dilution of *Standard solution* in an appropriate diluent

System performance

Sample: *Performance suitability solution* and *Standard solution*

Performance requirements

Precision and accuracy

Precision: Standard deviation calculated from NLT 5 determinations of the 5 replicate *Standard solutions*

Accuracy: Difference between the average calculated content of 5 replicate *Standard solutions* and the RS label claim

Suitability requirement: The point described by the precision and accuracy values falls in the shaded portion of *Figure 2*.

Specificity: Contribution to error: NMT 0.5%

Linearity: Plot of calculated content vs measured content

R^2: NLT 0.99

Slope: 1.0 ± 0.1

Acceptance criteria: 98.0%–102.0% of $C_{10}H_{20}CaN_2O_8S_2$

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method I* **⟨231⟩:** 10 ppm

Organic Impurities

- **PROCEDURE**

Standard solution: USP Acamprosate Calcium RS in an appropriate diluent [NOTE—Neat determination may be substituted where appropriate.]

Sample solution: Acamprosate Calcium in an appropriate diluent [NOTE—Neat determination may be substituted where appropriate.]

Performance suitability solution: 1 to 1000 dilution of *Standard solution* in an appropriate diluent

System performance

Sample: *Performance suitability solution* and *Standard solution*

Performance requirements

Precision and accuracy

Precision: Standard deviation calculated from NLT 5 determinations of the 5 replicate *Standard solutions*

Accuracy: Difference between the average calculated content of 5 replicate *Standard solutions* and the RS label claim

Suitability requirement: The point described by the precision and accuracy values falls in the shaded portion of *Figure 2*.

Specificity: Measured values for *Sample solution* spiked with known impurities at reporting level differ from calculated values by NMT 2.0%.

Linearity: Plot of calculated content of each known impurity vs measured content of each known impurity

R^2: NLT 0.99

Slope: 1.0 ± 0.1

Acceptance criteria

Any individual impurity: NMT 0.1%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE** Preserve in well-closed containers, and store at 25°; excursions permitted to 15°–30°.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Acamprosate Calcium RS

Acceptable, Equivalent, or Better: Approaches for Alternatives to Official Compendial Procedures

W.W. Hauck, PhD,[a] A.J. DeStefano, PhD, T.L. Cecil, PhD, D.R. Abernethy, MD, PhD, W.F. Koch, PhD, R.L. Williams, MD

ABSTRACT The *General Notices* in the *US Pharmacopeia* (*USP*) permit analysts to use acceptable (suitable) alternatives to an official procedure. In a revision that will be effective May 1, 2009, the *General Notices* will require that the alternative procedure be demonstrated to give results that are equivalent to or better than those obtained by the official procedure. This *Stimuli* article discusses approaches for determining equivalent or better procedures. The concepts and tests discussed in this paper may become one or more *USP* General Chapters. The preceding *Stimuli* article speaks to approaches that determine an acceptable procedure without requiring a study to document equivalent or better.

INTRODUCTION

In its *General Notices*, the *US Pharmacopeia* (*USP*) states: ''Compliance may be determined also by the use of alternative methods, chosen for advantages in accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction or in other special circumstances. Such alternative or automated procedures or methods shall be validated (*1*).[b] The *General Notices* requirement for validation means the analyst using the alternative procedure has determined the procedure is acceptable (suitable) for its intended use in the specified monograph test. The wording of the *USP General Notices* presumes that the alternative procedure possesses *some* property for which there is an advantage, however defined—otherwise why use it? But the *General Notices* statement does not require comparison to the compendial procedure on advantageous properties. A priori, one would think that the alternative procedure should be not be worse, however defined, than the compendial procedure, but this is not stated in the current *General Notices.* A revision to the *General Notices* (*3*) that will be official May 1, 2009, calls for the alternative procedure to ''give equivalent or better results'' by comparison with the compendial procedure. In addition, elsewhere the *General Notices* state: ''Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with *at least equivalent accuracy*. . .[emphasis added]'' (*1*). General Chapter *Validation of Alternative Microbiological Methods* ⟨1223⟩ states, ''The critical question is whether or not the alternat[iv]e method will yield results *equivalent to, or better than*, the results generated by the conventional method [emphasis added]'' (*1*, p. 681). *USP* does not provide a definition or approaches for alternative procedures, whether they are deemed acceptable, equivalent, or better.

The Food and Drug Administration (FDA) and ICH employ similar language regarding alternative procedures. The Code of Federal Regulations, in the section that covers requirements for Equivalent Methods and Processes for biological products, requires ''that the modification will provide assurances of the safety, purity, potency, and effectiveness of the biological product *equal to or greater than* the assurances provided by the method or process specified in the general standards or additional standards for the biological product [emphasis added]'' (*4*). FDA also uses a similar phrase in its draft guidance, *Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation* (*5*). In discussing alternative analytical procedures this guidance notes, ''A validated alternative analytical procedure should be submitted only if it is shown to perform *equal to or better than* the regulatory analytical procedure [emphasis added]'' (*5*). ICH Q6A, *2.7 Alternative Procedures*, states, ''Alternative procedures are those which may be used to measure an attribute when such procedures control the quality of the drug substance or drug product to an extent that is *comparable or superior* to the official procedure [emphasis added]'' (*6*). In MAPP 5310.7, FDA's Office of New Drug Quality Assessment states the following policy for its reviewers: ''If there is no USP/NF monograph for an excipient, drug substance, or drug product, and the applicant proposes to use an analytical procedure from the BP, EP, or JP in a specification in lieu of the corresponding analytical procedure in the General Chapters of the USP/NF, the BP, EP, or JP procedure is considered an alternative analytical procedure and may be used provided the analytical procedure in the BP, EP, or JP is *equivalent to or better than* the corresponding analytical procedure in the USP/NF [emphasis added].'' ''Equivalent'' is used in this paper rather than the ''equal'' of 21 CFR 610.9(c) in order to avoid the connotation of *equal* as meaning *identical* (*7*).

[a] Correspondence should be addressed to: W.W. Hauck, PhD, Senior Scientific Fellow, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8390; e-mail wh@usp.org.

[b] Using terminology of the International Conference on Harmonization (ICH), the US Pharmacopeial Convention (USPC) uses the term *procedure* to describe the steps to be followed by the analyst in conducting a test. This is in agreement with the *International Vocabulary of Metrology (VIM) (2)*, which describes a measurement procedure as a detailed description in contrast to a measurement method, which is a generic description (such as ''indirect, a direct method, or an instrumental method''). This *Stimuli* article thus will use the terminology *comparison of procedures* rather than *comparison of methods*.

Based on this brief review, the various regulatory and compendial statements appear to lack precision in definition. Following internal discussions, USPC has identified four options (*Table 1*) to allow consideration of alternatives to official procedures, even though the usual wording allows only ''equivalent or better.''

- The first is for *USP* to set minimum performance requirements for alternative procedures. Procedures meeting these requirements are termed *Acceptable Procedures.* For example, for chromatographic procedures, manufacturers could establish minimum requirements for repeatability and peak separation. The concept then would be that any alternative procedure that meets the applicable requirements would be acceptable, i.e., suitable for its intended use. In effect, two procedures that both meet the performance requirements would be considered ''equivalent'' (and hence acceptable) under this option without any direct comparison to the official procedure.
- The remaining options directly compare the alternative procedure to the compendial procedure and require ''method-performance studies,'' (*8*) i.e., procedure comparison studies, thus dealing more explicitly with the requirement to show that the alternative is equivalent to or better than the official procedure. This article proposes three questions that drive the subsequent three options. For the second option, termed *performance equivalence*, the question is: Are the two procedures equivalent or better with respect to validation characteristics? This is similar to Option 1 because it considers validation characteristics but features a direct comparison to the official procedure. For the third and fourth options, the question is: Do the two procedures agree? Agreement can be assessed by considering the numerical result (Option 3) of the procedure or a decision (Option 4) relative to some acceptance criterion that is based on the numerical result. For the third and fourth options, USPC proposes the terms *results equivalence* and *decision equivalence*, respectively, to delineate the two options. Each question leads to different statistical approaches to allow the comparison.

Following the metrological distinctions between *procedure* and *method* in *VIM*, the discussion of this paper applies regardless of whether the comparison is between two procedures that correspond to different methods or between two different procedures for the same method (*2*). The statistical and compendial issues are the same in either case. This paper assumes that the two procedures being compared are measuring the same attribute. Comparison of two bioassays that measure different attributes of an article, for example, is a topic not addressed by this discussion. A similar problem is termed *method transfer* (same procedure but different laboratories). That is not a pharmacopeial issue but is statistically similar to the topic considered here (see *Appendix* for some references).

This paper first addresses each of the four options individually with consideration of advantages and disadvantages and a brief overview of statistical considerations. In the discussion the paper summarizes choices and ways forward. The purpose is to identify issues that should be considered and candidate statistical approaches with the intent of initiating a dialog that could lead to compendial recommendations or requirements in one or more General Chapters. This *Stimuli* article includes an *Appendix* that cites some publications that are relevant to the discussion.

OPTION 1: ACCEPTABLE PROCEDURES

General Comments

The essence of Option 1 is to allow the analyst more than one—perhaps even many—acceptable alternative procedures without a requirement for comparison to another procedure. The comparison, instead, is to a reference material with known properties. This option may be particularly valuable in cases where the current compendial procedure is especially accurate or precise. It would be difficult to demonstrate that the alternative procedure is equivalent to or better than the official procedure in such cases. Implicitly, the judgment of acceptability of a procedure would acknowledge that it is ''equivalent'' in meeting compendial expectations. To this end, USPC has proposed, in a related article in this *Pharmacopeial Forum*, performance-based monographs in which one and perhaps more than one optional acceptable procedure would be deemed acceptable for use in a particular test (*9*). The key characteristic of a performance-based procedure in a monograph is that it would specify performance requirements for a procedure rather than provide a required compendial procedure, although one could be deemed preferred (official) for compliance purposes.

Table 1. Comparison of Options

Option	Name	Demonstrating	Comparison to Official Procedure?	Based on Numerical Results or Conclusion?	Number of Characteristics Considered
1	Acceptable Procedures	Acceptable	No[a]	Results	Many
2	Performance Equivalence	Equivalent or Better	Yes	Results	Many
3	Results Equivalence	Equivalent	Yes	Results	One
4	Decision Equivalence	Equivalent	Yes	Conclusions	One

[a] There need not be an official method.

For Option 1, the criteria for an acceptable procedure could be based on validation characteristics such as specificity, absence of bias, and precision. Criteria for acceptance could be based on the procedure's operating characteristics, specifically on whether articles of known properties pass or fail appropriately when compared to the relevant acceptance criteria. See (*9*) for an example of this approach.

OPTION 2: PERFORMANCE EQUIVALENCE

General Comments

Performance equivalence means demonstrating ''equivalent or better'' results on some subset of validation properties. General Information Chapter ⟨1225⟩ lists many candidate properties. By focusing on a subset, Option 2 is similar to verification in General Information Chapter ⟨1226⟩, where determination of which properties for the procedure's intended use are important. In the context of alternative procedures, the most important generally are specificity and accuracy. Although precision (variability) is important, analysts can sometimes address precision in different ways, such as the choice of the number of replicates (discussed below). Even with focus on a subset of characteristics, a disadvantage of Option 2 is the multiplicity of statistical tests corresponding to the multiple validation properties for a given procedure and the designated attribute of the measurand. One can easily envision an alternative procedure that is better on some properties, equivalent on some, and worse on some. What should be the final decision regarding the alternative? Prioritizing the importance of the validation properties seems to be a key. An additional question is whether the new procedure actually is better than the official procedure on *some* property. An affirmative answer does allow that the alternative procedure may be preferable based on considerations not captured by validation characteristics (e.g., cost or time).

Equivalence Testing

Comparing two procedures to show equivalent or better performance for one or more validation properties leads to a body of statistical work referred to as equivalence testing. The basic idea is to properly align the statistical hypotheses with the intent of the work. If the goal is to positively demonstrate equivalent or better performance, then ''equivalent or better'' needs to be the statistical alternative hypothesis. The statistical null hypothesis is then ''neither equivalent nor better.'' This has not been common practice, although the literature in this field has evolved to equivalence testing. For comparison of means, for example, a common practice has been to declare equivalence if the two means are not found to be statistically different with a standard *t*-test. With a standard *t*-test, the alternative hypothesis is difference, and the null hypothesis is no difference. The papers by Limentani et al. (*10*) and Chambers et al. (*11*) contain good examples of why the equivalence approach is preferred to the still commonly used standard *t*-test.

When comparing two procedures to understand if they are ''equivalent or better,'' the analyst must determine which of three outcomes (questions) are sought in the comparison study: 1) the new procedure may be importantly worse than the current procedure; 2) the new procedure may be importantly better than the current procedure; and 3) there may be no important difference, either better or worse, between the new and current procedures. When outcomes 2 and 3 are found, the results are ''equivalent or better,'' and only the first outcome is excluded. In clinical trials this is referred to as noninferiority. When outcome 3 is found in the comparison results, the procedures are referred to as ''equivalent.'' Thus in a comparison study, the analyst must be clear about the intent of the study in order to derive useful conclusions.

Demonstrating Equivalent or Better—Means

A starting point for comparing two procedures is to compare only means. Ideally, though, this would be a comparison of biases (*accuracy* in ICH and USP terminology). A noninferiority hypothesis would be that the bias of the alternative procedure is equivalent to or better (less) than that of the official procedure. This would require a comparative accuracy (trueness) assessment of the two procedures. If bias cannot be assessed, a related approach would be to suggest that the bias of the official procedure is acceptable. This leads to a two-sided equivalence hypothesis of equivalence of the two procedures' means for the alternative. Specifically, the alternative procedure must be similar on average to the official procedure. Statistical procedures for one- and two-sided equivalence hypotheses for means are well developed (*12, 13*).

The problem with a simple comparison of means is that the difference may vary with the quantity being measured. Analysts may need to compare accuracy separately at each of a range of values rather than perform a single, composite accuracy comparison. Calibration (regression) approaches have been developed and allow for both systematic and proportional differences between the results of the procedures; see the *Appendix* for references. Most of the procedures in the literature determine the regression for the relationship and then, inappropriately, test the null hypotheses of intercept equal to 0 (no systematic difference) and slope equal to 1 (no proportional difference). If both are not rejected, equivalence is claimed. This is the common error of wrong hypotheses for an equivalence problem. Equivalence hypotheses should specify a range for an intercept sufficiently close to 0 and for a slope sufficiently close to 1. These are two-sided equivalence hypotheses.

Demonstrating Equivalent or Better—Variances

Variance comparisons are clearly one-sided (noninferiority); i.e., a procedure that results in an important increase in variance only would not be considered performance equivalence. The general approach is already covered in *USP*'s General Information Chapter *Analytical Data—Interpretation and Treatment* ⟨1010⟩. The larger question relates to which variance(s) to compare. If the context is a single laboratory, reproducibility is not

relevant. For companies with multiple laboratories using a procedure and for compendial statements about procedures that are ''equivalent or better,'' reproducibility is relevant. Where comparisons are confined to a single laboratory, analysts also must bear in mind that repeatability is not a full characterization of the precision of a procedure. The question then becomes, Which intermediate precision components should be assessed?

The other issue with variances is that, in a certain sense, the variance comparison sometimes can be made less important, at least for repeatability. That is, variance may be overcome with increased sample size. If the new procedure appears to be more variable than the official procedure, the sample size can be increased to make the new procedure comparable. With respect to a procedure being developed for use as an alternative procedure, variance can be addressed during validation. The ''sample'' for determining what needs to be increased will depend on the source(s) of variability and the variance of interest. If repeatability is at issue, this may mean simply more samples and/or replicates. If intermediate precision or reproducibility is at issue, improving repeatability is not sufficient and it may not be feasible to increase, e.g., the number of analysts conducting the procedure or the time over which the procedure is conducted in order to reduce the contribution from those components of intermediate precision.

OPTION 3: RESULTS EQUIVALENCE

General Comments

When results equivalence is sought, the hypothesis of the comparison study is that the alternative and official procedures obtain equivalent numerical results. Showing that two procedures obtain similar results could be useful, e.g., for a procedure applied in stability testing when it is desirable to avoid a discontinuity in results with a change of procedure. Option 3 is thus similar to Option 2 in that the focus is on the numerical results obtained by the procedures. Options 2 and 3 differ in the nature of the comparison. In Option 3, there is a direct comparison of results. In Option 2, the comparison is indirect; validation characteristics are evaluated and compared.

Demonstrating Essentially the Same Numerical Result

There are at least two general approaches that could be taken. For the first, a large body of literature examines the general issue of inter-rater reliability. The general idea is to assess the extent to which two raters get the same result when using the same instrument. Many applications of inter-rater reliability involve psychometric studies in which the instrument is a questionnaire, but applications are widespread. For continuous measures, the intraclass correlation coefficient is the most common measure of agreement, and generally accepted terms describe the degree of agreement (*10*). This literature can be adapted to comparison of compendial procedures if one interprets ''rater'' as ''procedure.'' A more recent alternative is to use the concordance correlation coefficient.

Design of experiments for interprocedure agreement should focus on ensuring that the two procedures obtain similar results for each sample tested. If the testing is nondestructive, this is straightforward. For destructive testing, this involves, e.g., preparing replicate aliquots from common preparations.

A difficulty with both intraclass and concordance correlation approaches is the lack of a direct connection to consequences with respect to producer risk (failing to adopt an equivalent alternative procedure) and consumer risk (adopting an inequivalent alternative procedure). With this consideration in mind, the second general approach considered here, the total error approach, was proposed for procedure transfer. The basic idea is to use tolerance intervals to control how often the two procedures give numerical results that differ by an important amount. What constitutes an important amount then depends on the acceptance criteria associated with the procedure.

OPTION 4: DECISION EQUIVALENCE

General Comments

Comments for decision equivalence are similar to those for results equivalence. The difference between Options 3 and 4 is whether agreement occurs with respect to numerical results (Option 3) or a decision relative to some acceptance criteria (Option 4). The distinction leads to different statistical procedures. Thus the key characteristic of Option 4 is that it looks only at the pass/fail results, not the actual numerical results that are the basis of Options 1, 2, and 3.

Demonstrating Essentially the Same Pass/Fail Results

If the only concern is whether or not the two procedures agree on meeting acceptance criteria—rather than the actual numerical value—different statistical procedures apply. A related application arises when a procedure yields only an either/or result (meets/does not meet), as is the case for limit or identity tests. The primary statistical procedure here is the kappa coefficient. Otherwise the issues are the same as for the intraclass and concordance correlations. The kappa coefficient shares the same disadvantage, namely no easy connection to consequences. A possible alternative is to consider the full operating characteristic curves for the two procedures. The operating characteristic curves, by moving the comparison to the probability rather than correlation scale, may make it easier to consider which differences between the procedures are important.

DISCUSSION

This paper has considered four options for what is meant by ''equivalent or better.'' Although there is some similarity across the four, they do differ. An alternative procedure that might be judged equivalent or better by one option need not be judged the same by the other options. One important distinction between Option 2 and Options 3 and 4, is that Options 3 and 4 preclude ''better'' because the alternative procedure must be dif-

ferent to be better. Another distinction is that Option 1, because it does not involve a formal procedure comparison study, yields no guarantee that any two acceptable procedures will agree with respect to either numerical results or decisions relative to some acceptance criterion, though tight criteria about what constitutes ''acceptable'' makes it likely two acceptable procedures will not differ greatly with respect to results.

Discussion regarding alternative procedures tends to return to the decision made based on results from the procedure. The various options differ in how they connect to the accept/reject decision. With Option 4, agreement of procedures with respect to decision is a direct connection. For Options 1, 2, and 3 the connection must be indirect. A general issue, as for any equivalence assessment, is: How close is close enough? A general approach is to link the procedure comparison or the definition of acceptable back to its use in support of accept/reject decisions: What is the impact on consumer and producer risks? The analytic error is just one component of uncertainty. Applied to a product that naturally varies from batch to batch, a good analytic procedure is one whose uncertainty is small relative to the acceptance interval so that the procedure only minimally leads to wrong decisions.

Options based on validation characteristics (Options 1 and 2) share a problem of multiplicity, i.e., many characteristics to be compared. It seems desirable to attempt to reduce the number of comparisons. Partly, this is being selective in the characteristics to compare. Further, one can envision multivariate approaches at least for related characteristics or a combination of some characteristics, e.g., by use of mean squared error rather than bias and repeatability. A further problem is termed *assay sensitivity*. That is, Is the procedure–equivalence study capable of finding an important difference between the two procedures if one exists?

Regulatory and compendial statements have emphasized the importance of ensuring that alternatives to private or public (compendial) procedures be ''equivalent or better.'' As discussed in this article, USPC believes that the requirement or recommendation should be framed somewhat differently. Option 1 simply states that within the acceptance criteria delineated for a test, many procedures using the same or different methods may be acceptable. No further studies are needed, and specifically it isn't necessarily sensible to require additional studies to show equivalence (options 3 and 4) or better (option 2). For USPC this leads to ''performance-based monographs.'' Another *Stimuli* article in this issue of *Pharmacopeial Forum* describes performance-based monographs, some details regarding how *acceptable* might be defined, and USPC's plans for implementation (*9*).

Although USPC is advancing consideration of a performance-based monograph approach (an implementation of Option 1), the choice of option can be viewed as monograph specific. For monographs for which the performance-based option does not apply, one of the other options will be needed. At this writing, USPC is not specifying which option to use for implementing the *General Notices* requirement to demonstrate equivalent or better for a proposed alternative procedure. The choice is to be made and justified by the laboratory. In the future the choice may be specified in the monographs. Should analysts, however, wish to demonstrate ''equivalent or better,'' they must posit the correct hypothesis and conduct the appropriate statistical tests. In so doing, they can readily determine through careful design of experiments whether an alternative procedure is equivalent or better. The general approach relies on good understanding of the control space of the public monograph—the acceptance criteria for the specified tests.

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APPENDIX

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NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–218; Vol. 35 No. 2, pp. 219–498; and Vol. 35 No. 3, pp. 499–784.*]

MONOGRAPHS

Index

Index

CHROMATOGRAPHIC REAGENTS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Reagents Used in *USP-NF* and *Pharmacopeial Forum*
May–June 2009

ANDROGRAPHIS (DSD Mgh #3539)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Luna C18(2)	Identification and Content of	Content of diterpene lactones. 4.6 mm x 25 cm. Manufacturer: Phenomenex

BOSWELLIA SERRATA (DSD Mgh #2775)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Prevail C18	Identification and Content of	Content of keto-derivatives of B-boswellic acids. 4.6 mm x 25 cm. Manufacturer: Grace Alltech

BOSWELLIA SERRATA EXTRACT (DSD Mgh #867)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Prevail C18	Identification and Content of	Content of keto-derivatives of B-boswellic acids. 4.6 mm x 25 cm. Manufacturer: Grace Alltech

FLUCONAZOLE INJECTION (DSD Mgh #33243)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L1	Symmetry C-18	Organic Impurities	Procedure 3. 4.6 mm x 15 cm, 3.5 μm. Manufacturer: Waters Corp.

HEPARIN SODIUM (DSD Mgh #36690)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L##	CarboPac PA20	Limit of	Limit of galactosamine in total hexosamine. Analytical column 3 mm x 15 cm. Guard column 3 mm x 3 cm. Manufacturer: Dionex

MORPHINE SULFATE EXTENDED-RELEASE TABLETS (DSD Mgh #54850)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L1	Keystone ODS-B	Assay, Related Compounds, and Uniformity of Dosage Units	4.6 mm x 15 cm, 5 μm. Manufacturer: Thermo Electron

OIL- AND WATER-SOLUBLE VITAMINS WITH MINERALS ORAL SOLUTION (DSD Mgh #88697)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	L1	Synergi Hydro-RP	Assay	Method 2. 4.6 mm x 75 cm, 4 μm, 80A. Manufacturer: Phenomenex

PECTIN (DSD Mgh #61250)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(2)	G43	Zebron ZB-624	Methanol, Ethanol, and Isopropanol	0.32 mm x 30 m, 1.8 μm. Manufacturer: Phenomenex
35(2)	S3	Porabond Q	Methanol, Ethanol, and Isopropanol	0.32 mm x 25 m, 5 μm. Alternative column. Manufacturer: Varian

PENTAMIDINE ISETHIONATE (DSD Mgh #62125)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L1	Symmetry C-18	Related Compounds	4.6 mm x 25 cm, 5 μm. Manufacturer: Waters Corp.

POWDERED ANDROGRAPHIS (DSD Mgh #3540)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Luna C18(2)	Identification and Content of	Content of diterpene lactones. 4.6 mm x 25 cm. Manufacturer: Phenomenex

POWDERED ANDROGRAPHIS EXTRACT (DSD Mgh #3541)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Luna C18(2)	Content of	Content of diterpene lactones. 4.6 mm x 25 cm. Manufacturer: Phenomenex

VITAMIN A ORAL LIQUID PREPARATION (DSD Mgh #4007)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L8	ZORBAX NH2	Assay	4.6 mm x 15 cm, 5 μm. Manufacturer: Agilent Technologies

ZOLPIDEM TARTRATE EXTENDED-RELEASE TABLETS (DSD Mgh #2061)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L1	Inertsil ODS-2	Related Compounds	4.6 mm x 15 cm, 5 μm. Manufacturer: GL Sciences

Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 4 **JULY–AUG. 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *Pharmacopeial Forum* content to
USP Executive Secretariat
12601 Twinbrook Parkway,
Rockville, MD 20852
execsec@usp.org

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

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

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Our subscribers' records and publication labels are computer-generated. Please send your new address, and your latest label, or an exact copy of it, to:
USPC,
PF Customer Service Dept.,
12601 Twinbrook Parkway,
Rockville, MD 20852.
Fax: (301) 816-8148.

STANDARDS DEVELOPMENT

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

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

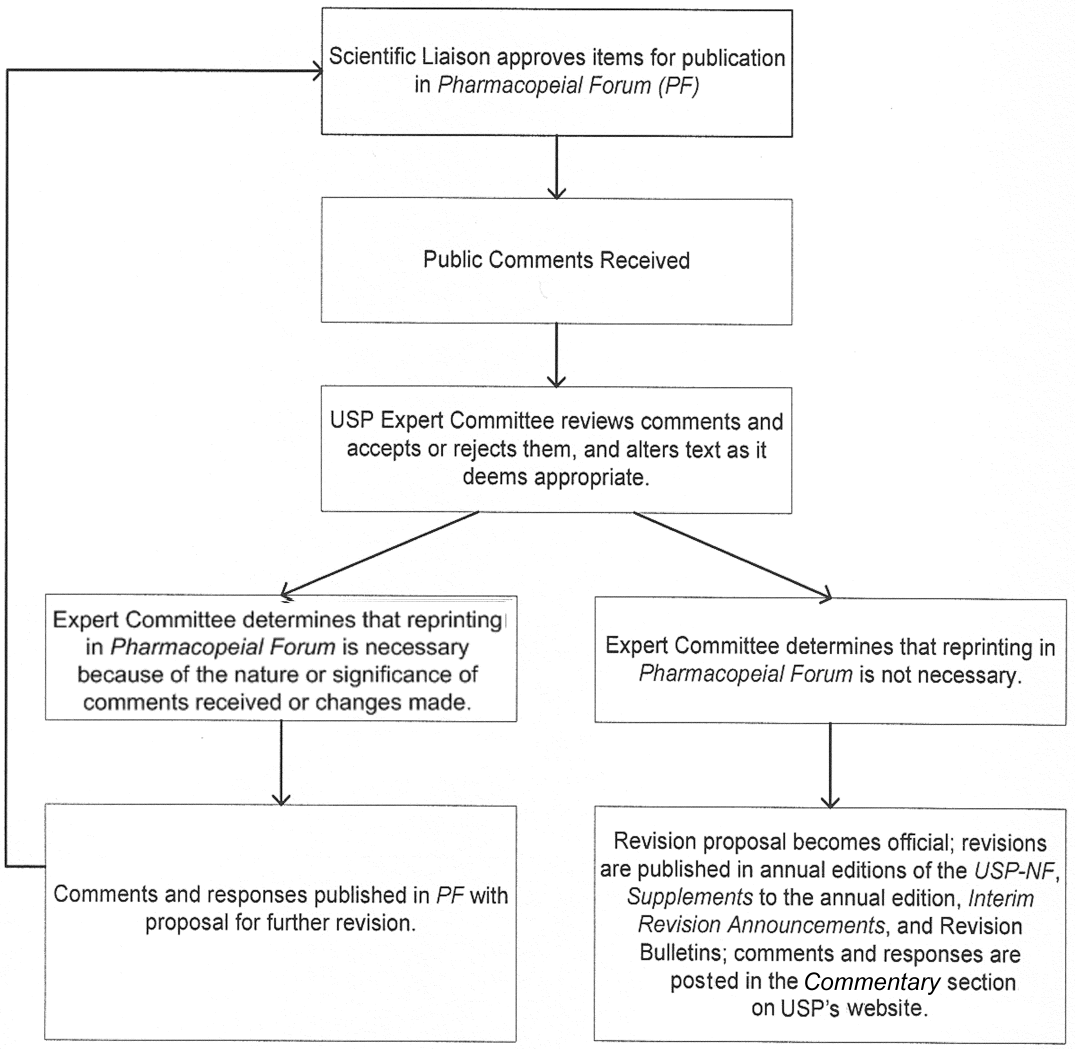
PF includes the following:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's typical Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that reprinting in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Accelerated Revisions**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP website) (e.g., *Interim Revision Announcements*, *Revision Bulletins*, and *Errata*). Accelerated Revisions allow for a revision to become official prior to the next *USP–NF* or *Supplement* and do not always require notice and comment. *Interim Revision Announcements* are first presented for a 60-day public comment period in the *Proposed Interim Revision Announcement* section before becoming official in a later *PF* in the *Interim Revision Announcements* section of the *PF*. Note that *Revision Bulletins* appear only on the USP website.

USP welcomes comments and data on proposed revisions. Comments, along with USP's responses, will be published in the *Commentary* section of the USP website (www.usp.org).

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

''How to Use PF'' describes the various parts of *Pharmacopeial Forum*, lists the *Committee Designations*, and includes the *Staff Directory*.

The contents of the various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
Proposed Interim Revision Announcement Accelerated Revision targeted to become official in an upcoming *PF.*	Proposals for *Interim Revision Announcements (IRAs)* that will be published as official in a future *Pharmacopeial Forum.* BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Scientific Liaison who handled the issue.	Review material and send comments within 60 days of the *PF* publication where the standard was proposed (or per the Briefing). Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing.
Interim Revision Announcement Official Accelerated Revision (on the first day of the second month, unless otherwise indicated).	Standards that have been adopted and will become official on the date that is specified in the section's introductory page or within parentheses following a particular item. New or revised text is marked by the symbols •.	Review material to see whether affected by any of the changes. Note date when standards become official, and ensure compliance.
Errata Accelerated Revision.	Corrections to official standards that will be printed in *USP–NF.*	Review material to see whether affected by any changes.
In-Process Revision Revisions for public review and comment.	Proposals for standards that will be published as official in a future *USP–NF* book or *Supplement*. BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the appropriate USP Scientific Liaison.	Review material and send comments. Comment deadlines are found at the end of the *Policies and Announcements* section. Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839.
Previous PF Proposals	Proposals from previous *PF*s that did not advance to official status in an official USP publication. This section is cumulative.	Review material to track pending proposals.
Canceled Proposals	Items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption in *USP–NF.*	Review material to track canceled proposals.

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
Stage 4 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize	BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. Stage 4 is available for comment.	Review material and provide comments to the appropriate Scientific Liaison cited in the Briefing. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopeia, with a copy to USP, for a given article. The addresses for the European (PhEur) and Japanese (JP) pharmacopoeias are as follows: PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp
Stage 6 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize.	Stage 6 is the final official harmonized standard and is not available for comment. This information is published for informational purposes only. New or revised text to Stage 6 documents is marked with symbols that indicate the publication in which the book or *Supplement* becomes official.	Review material to see whether affected by any changes.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including Scientific Liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Columns Used in USP–NF and Pharmacopeial Forum
Update of chromatographic columns based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* (*Continued*)

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Darrell Abernethy, M.D., Ph.D., Chief Science Officer	dra@usp.org	(301) 816-8184	
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, Senior Scientific Associate	kxb@usp.org	(301) 816-8462	Food Ingredients (FI)
Colleen Brennan, R.Ph., Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	USP–NF Spanish Edition
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D., Scientist	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards (DS-PS)
Anthony DeStefano, Ph.D., Vice President, General Chapters	ajd@usp.org	(301) 230-6303	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	Document Disclosure
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE); Radiopharmaceuticals and Medical Imaging Agents (RMI); Veterinary Drugs (VET)
James Griffiths, Ph.D., Vice President, Food and Dietary Supplement Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	USP Spanish Edition; General Chapters (GC); Pharmaceutical Waters (PW)
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products— Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexy Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B Proteins and Polysaccharides (BB PP)
Randy Kiser, Program Manager	rwk@usp.org	(301) 816-8324	USP Reference Standards Acquisition
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Vice President, RSO	psl@usp.org	(301) 816-8337	Reference Standards Operations
Markus Lipp, Ph.D., Director, Food Ingredients	mxl@usp.org	(301) 816-6366	Food Ingredients (FI)
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Kate Meringolo, Manager, Publications Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Senior Scientific Associate	jm@usp.org	(301) 816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Senior Scientist	ktm@usp.org	(301) 816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Vice President, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements—Non-Botanicals (DSN)
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Senior Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D., Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D., Manager, Pharmacy Compounding EC	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, Director, Excipients	cxs@usp.org	(301) 816-8262	Excipients
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB VV); Proteins and Polysaccharides (BB PP) (small peptides)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Senior Scientist	dwv@usp.org	(301) 998-6828	Monograph Development—Pulmonary and Steroids (MD-PS)
Mary ''Jeanie'' Waddell, Scientist	msw@usp.org	(301) 816-8124	Monograph Development—Pulmonary and Steroids (MD-PS)
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	rstech@usp.org	(301) 816-8129	USP Reference Standards Evaluation
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section provides general information resources for *USP–NF* standards and processes. Information resources include announcements on scientific and policy issues currently under consideration, schedules for USP publications, and schedules for comment periods for proposed standards.

USP ANNOUNCES REVISION TO HAWTHORN MONOGRAPHS. The Dietary Supplements—Information Expert Committee has approved the deletion of the cautionary statement in the *Hawthorn Leaf with Flower* and the *Powdered Hawthorn Leaf with Flower* monographs. These monographs were previously printed as proposed *Interim Revision Announcements* in *Pharmacopeial Forum*, *PF* 34(5) [Sept.–Oct. 2008.] The approved *Interim Revision Announcements*, which appear elsewhere in this issue of *PF* and will become official on August 1, 2009, will supersede the monographs as presented in *USP 32–NF 27* until the revised versions of the monographs are published in *USP 33–NF 28*, which will be released November 1, 2009 and will become official on May 1, 2010.

The revisions to cautionary statements in *Dietary Supplements* are further clarified in the Compendial Notices announcement ''USP Revises Admission Criteria and Safety Classification for Dietary Supplements,'' which is posted on USP's website. The Compendial Notices announcement also describes the cautionary statement revisions that apply to the *Green Tea Extract* monograph.

For further information on the Hawthorn monographs, contact Dandapantula Sarma, Ph.D., at 301-816-8354 or dns@usp.org.

USP ISSUES CALL FOR CANDIDATES FOR 2010–2015 COUNCIL OF EXPERTS, ITS EXPERT COMMITTEES, AND ITS EXPERT PANELS. In accordance with the Bylaws of the USP Convention, USP is issuing a Call for Candidates for the 2010–2015 Council of Experts (COE). The 2010–2015 COE includes Expert Committees in the areas of Nomenclature, Small Molecules, Biologics and Biotechnology, Excipients, General Chapters, Reference Standards, Compounding, Food Ingredients, and Dietary Supplements. In the 2010–2015 cycle, USP is expanding the number of Expert Panels that report to Expert Committees.

These Expert Committees and Panels align with the new USP Strategic Plan, which focuses on expanding and enhancing USP's core compendial and standards-setting activities. The ability to add Expert Panels according to USP's needs introduces flexibility and scalability into USP's activities. USP plans to continue to attract a global base of experts and therefore encourages any qualified individual to apply. Importantly, this approach also enables USP to closely align its documentary and reference standards activities for a more efficient standards-setting process.

Specific Expert Committees and Expert Panels for which USP is seeking candidates are listed at USP's nominations Web site (www.usp.org/goto/nominate). The deadline for applications for the COE (Expert Committee Chairs) is **December 31, 2009**. The deadline for applications for Expert Committee members is **May 15, 2010**. Recruitment for Expert Panel members will begin in July 2010 and will be continuous.

For further information, please contact Nelufar Mohajeri, Director, Volunteer Affairs and Compendial Initiatives (nym@usp.org or nominate@usp.org).

USP POSTS COMMENTARY TO INTERIM REVISION ANNOUNCEMENTS ON THE USP WEB SITE. In order to maintain transparency for revisions made to proposed *Interim Revision Announcements* that become official in the *Pharmacopeial Forum*, USP posts commentary for the proposed *Interim Revision Announcements* on the *Revisions and Commentary* web page on the date that the official standard is released in *Pharmacopeial Forum*. Note that commentary to *In-Process Revisions* is posted on the *Revisions and Commentary* web page under the final book or supplement where the official standard appears.

Commentary is not part of the official text of the monograph and is not intended to be enforceable by regulatory authorities. Rather, it explains the basis of the Expert Committee's response to public comments. If there is a difference between the contents of the *Commentary* section and the official monograph, the text of the official monograph prevails. In case of a dispute or question of interpretation, the language of the official text, alone and independent of the *Commentary* section, shall prevail.

***PHARMACOPEIAL FORUM* PUBLIC REVIEW AND COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section and incorporated in the upcoming *USP–NF* or *Supplement*. They may also be published as *Revision Bulletins* on www.usp.org in the ''New Official Text'' section. The official publication in which an *Interim Revision Announcement* (*IRA*) is incorporated will depend upon publication deadlines. See the table below. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and, therefore, contains all official text up to and including the contents of the latest *Supplement*. The table below outlines the publications and their release and official dates, and the *USP–NF* or *Supplement* that supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
USP 32–NF 27	November 1, 2008	May 1, 2009	*1st Supplement to USP 32–NF 27*
IRA [*PF* 35(1)]	January 1, 2009	February 1, 2009	*2nd Supplement to USP 32–NF 27*
1st Supplement to USP 32–NF 27	February 1, 2009	August 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*

PRIORITY NEW MONOGRAPH ITEMS. USP is seeking monographs for the following drug substances and drug products that are, or soon will be, off patent and thus are of the highest priority. USP also is seeking monographs for the excipients listed below. Monographs are marked ''Received'' upon receipt of a monograph proposal. Received monographs are removed from this list upon publication in *Pharmacopeial Forum* or when posted in the *USP Pending Monographs* section of the USP website (http://www.usp.org/standards/pending/). This list has been updated as of April 15, 2009; monographs received since the last update to the list are noted in bold.

Monograph sponsors should consult USP's Guideline for Submitting Requests for Revision to the *USP–NF* at http://www.usp.org/USPNF/submitMonograph/subGuide.html.

For additional information, contact Karen A. Russo, Ph.D., kar@usp.org.

Small Molecules (Drug Substances)—As of April 15, 2009

1. Allopurinol Sodium	2. Aminopropazine Fumarate	3. Aminopterin Sodium
4. Anagrelide Hydrochloride ***(Received)***	5. Arsenic Trioxide	6. Auranfoin
7. Azelaic Acid ***(Received)***	8. Bentoquatam	9. Benzphetamine Hydrochloride
10. Bivalirudin ***(Received)***	11. Calcipotriene	12. Calcium Trisodium Pentetate
13. Calfactant	14. Candesartan Cilexetil ***(Received)***	15. Ceftibuten
16. Cetrorelix	17. Cevimeline Hydrochloride ***(Received)***	18. Chloroxine
19. Choline Salicylate	20. Cysteamine Bitartrate	21. Dalfopristin

Small Molecules (Drug Substances)—As of April 15, 2009 *(Continued)*

22. Dapirazole Hydrochloride	23. Desirudin	24. Desonide ***(Received)***
25. Dexrazoxane	26. Difenoxin Hydrochloride	27. Entacapone ***(Received)***
28. Epoprostenol Sodium ***(Received)***	29. Erythromycin Phosphate	30. Erythromycin Thiocyanate
31. Esmolol Hydrochloride ***(Received)***	32. Estazolam ***(Received)***	33. Estramustine Phosphate Sodium
34. Ethanolamine Oleate	35. Etomidate ***(Received)***	36. Etoposide Phosphate
37. Exemestane	38. Famciclovir ***(Received)***	39. Felbamate ***(Received)***
40. Fluoromethane F 18	41. Fosfomycin Tromethamine ***(Received)***	42. Gadobenate Dimeglumine
43. Gadopentetic Acid	44. Gallium Nitrate	45. Ganirelix
46. Guanidine Hydrochloride	47. Halobetasol Propionate ***(Received)***	48. Haloperidol Decanoate ***(Received)***
49. Hydrocodone Polistirex	50. Ibandronate Sodium	51. Imipramine Pamoate
52. Imiquimod	53. Isosulfan Blue	54. Latanoprost ***(Received)***
55. Lomustine ***(Received)***	56. Metipranolol Hydrochloride	57. Miglitol
58. Milrinone Lactate	59. Moexipril Hydrochloride	60. Nalbuphine Hydrochloride
61. Nedocromil Sodium	62. **Nicardipine Hydrochloride** ***(Received)***	63. Nilutamide
64. Nisoldipine	65. Olsalazine Sodium ***(Received)***	66. Orlistat ***(Received)***
67. Oxiconazole Nitrate	68. Pemirolast Potassium	69. Pioglitazone Hydrochloride ***(Received)***
70. Piperonyl Butoxide	71. Pirbuterol Acetate ***(Received)***	72. Poractant Alpha
73. Porfimer Sodium	74. Pramiprexole Dihydrochloride ***(Received)***	75. Quetiapine Fumarate ***(Received)***
76. Ranitidine	77. Rivastigmine Tartrate ***(Received)***	78. Ropinirole Hydrochloride ***(Received)***
79. Rose Bengal Disodium	80. Rosiglitazone Maleate	81. Sodium Phenylbutyrate
82. Sodium Phosphates	83. Spectinomycin Sulfate	84. Streptozocin
85. Tenofovir Disoproxil Fumarate ***(Received)***	86. Tiludronate Disodium	87. Tiopronin
88. Trimetrexate Glucuronate	89. Venlafaxine Hydrochloride ***(Received)***	90. Voriconazole ***(Received)***
91. Zaleplon ***(Received)***	92. Zinc Tridosium Pentetate	93. Zoledronic Acid

Small Molecules (Drug Products)—As of April 15, 2009

1. Abacavir Sulfate, Lamivudine, and Zidovudine Tablets	2. Acarbose Tablets	3. Acetaminophen, Butalbital, Caffeine, and Codeine Phosphate Capsules
4. Acetaminophen, Clemastine Fumarate and Pseudoephedrine Hydrochloride Tablets	5. Acetazolamide Extended-Release Capsules	6. Albuterol and Ipratropium Bromide Inhalation Aerosol
7. Albuterol and Ipratropium Bromide Inhalation Solution	8. Albuterol Extended-Release Tablets	9. Albuterol Inhalation Aerosol
10. Albuterol Sulfate Inhalation Solution	11. Albuterol Sulfate Oral Solution	12. Alendronate Sodium Oral Solution
13. Alfuzosin Extended-Release Tablets	14. Allopurinol for Injection	15. Alprazolam Extended-Release Tablets
16. Alprostadil Urethral Suppository	17. Aminopropazine Fumarate and Neomycin Sulfate Tablets	18. Aminopropazine Fumarate Injection
19. Aminopropazine Fumarate Tablets	20. Aminopterin Sodium Tablets	21. Amiodarone Hydrochloride Injection
22. Amlodipine and Benazepril Hydrochloride Capsules ***(Received)***	23. Amphotericin B Injection	24. Anagrelide Hydrochloride Capsules ***(Received)***
25. Arsenic Trioxide Injection	26. Atovaquone and Proguanil Hydrochloride Tablets	27. Atovaquone Tablets

Small Molecules (Drug Products)—As of April 15, 2009 *(Continued)*

28. Auranofin Capsules	29. Azatadine Maleate and Pseudoephedrine Sulfate Extended-Release Tablets	30. Azelaic Acid Cream
31. Azithromycin for Injection ***(Received)***	32. Azithromycin Tablets ***(Received)***	33. Baclofen Injection
34. Beclomethasone Dipropionate Inhalation Aerosol	35. Beclomethasone Dipropionate Nasal Suspension	36. Benazepril Hydrochloride and Hydrochlorothiazide Tablets
37. Bentoquatam Topical Suspension	38. Benzocaine and Cetylpyridinium Chloride Lozenges	39. Benzocaine and Menthol Lotion
40. Benzphetamine Hydrochloride Tablets	41. Bivalirudin for Injection ***(Received)***	42. Brompheniramine Maleate, Dextromethorphan Hydrobromide and Pseudoephedrine HCl Oral Solution
43. Budesonide Inhalation Aerosol	44. Bupivacaine and Lidocaine Hydrochlorides Injection	45. Buprenorphine Hydrochloride Injection
46. Butalbital and Acetaminophen Capsules	47. Butalbital and Acetaminophen Tablets	48. Calcipotriene Cream
49. Calcipotriene Ointment	50. Calcipotriene Topical Solution	51. Calcitriol Capsules
52. Calcitriol Oral Solution	53. Calcium Acetate Capsules	54. Calcium Trisodium Pentetate Injection
55. Calfactant Intratracheal Suspension	56. Carbidopa and Levodopa Tablets For Oral Suspension ***(Received)***	57. Carbidopa, Levidopa, and Entacapone Tablets
58. Carmustine Implant	59. Cefdinir Tablets	60. Cefditoren Pivoxil Tablets
61. Ceftibuten Capsules	62. Ceftibuten For Oral Suspension	63. Ceftiofur Hydrochloride Oral Suspension
64. Cetirizine Hydrochloride Tablets ***(Received)***	65. Cetrorelix Injection	66. Cevimeline Hydrochloride Capsules
67. Chloroxine Cream	68. Chlorpromazine Hydrochloride Extended-Release Capsules	69. Choline and Magnesium Salicylates Oral Solution
70. Choline and Magnesium Salicylates Tablets	71. Choline Salicylate Oral Solution ***(Received)***	72. Ciclopirox Shampoo
73. Ciclopirox Topical Gel	74. Ciclopirox Topical Solution ***(Received)***	75. Cimetidine Oral Solution
76. Ciprofloxacin Extended-Release Tablets	77. Ciprofloxacin Hydrochloride and Hydrocortisone Otic Suspension	78. Ciprofloxacin Otic Solution
79. Cisplatin Injection	80. Citalopram Hydrobromide Oral Solution	81. Citric Acid, Gluconolactone, and Magnesium Carbonate Irrigation
82. Cladribine Injection	83. Clemastine Fumarate Syrup	84. Clobetasol Propionate Gel
85. Clorazepate Dipotassium Capsules	86. Clorazepate Dipotassium Extended-Release Tablets	87. Clotrimazole and Betamethasone Dipropionate Lotion
88. Compound Undecylenic Acid Cream	89. Compound Undecylenic Acid Topical Powder	90. Conjugated Estrogens and Medroxyprogesterone Acetate Tablets
91. Cyclosporine Modified Capsules	92. Cyclosporine Modified Oral Solution	93. Cyclosporine Ointment
94. Cyclosporine Topical Solution	95. Cysteamine Bitartrate Capsules	96. Cytarabine Liposome Injection
97. Dalfopristin and Quinupristin Injection	98. Dantrolene Sodium Oral Suspension	99. Dapiprazole for Ophthalmic Solution
100. Desirudin for Injection	101. Desonide Cream	102. Dexrazoxane for Injection
103. Dextroamphetamine Sulfate Extended-Release Capsules	104. Dextromethorphan Polistirex Extended-Release Oral Suspension	105. Diazepam Injectable Emulsion
106. Diclofenac Sodium Ophthalmic Solution	107. Diethylpropion Hydrochloride Extended-Release Tablets	108. Difenoxin Hydrochloride and Atropine Sulfate Tablets
109. Difloxacin Hydrochloride Tablets	110. Dihydroergotamine Mesylate Metered Spray	111. Diltiazem Hydrochloride Injection
112. Dinoprostone Vaginal Suppositories	113. Diphenhydramine Hydrochloride and Acetaminophen Tablets	114. **Divalproex Sodium Delayed-Release Capsules** ***(Received)***
115. Dorzolamide and Timolol Ophthalmic Solution	116. Dorzolamide Ophthalmic Solution	117. Doxepin Hydrochloride Cream
118. Doxycycline Oral Gel	119. Econazole Nitrate Cream	120. Edrophonium Chloride and Atropine Sulfate Injection
121. Enalapril Maleate and Felodipine Extended-Release Tablets	122. Entacapone Tablets ***(Received)***	123. Ephedrine Sulfate and Guaifenesin Tablets
124. Epirubicin Hydrochloride for Injection	125. Epirubicin Hydrochloride Injection	126. Epoprostenol for Injection
127. Epoprostenol Injection	128. Esmolol Hydrochloride Injection	129. Esomeprazole Magnesium Capsules

Small Molecules (Drug Products)—As of April 15, 2009 *(Continued)*

130. Estazolam Tablets ***(Received)***	131. Estramustine Phosphate Sodium Capsules	132. Ethanolamine Oleate Injection
133. Etidronate Disodium Injection Concentrate	134. Etomidate Injection ***(Received)***	135. Exemestane Tablets
136. Famotidine Orally Disintegrating Tablets	137. Felbamate Oral Suspension	138. Felbamate Tablets
139. Fentanyl Lozenges	140. Famciclovir Tablets	141. Fentanyl Transdermal System ***(Received)***
142. Ferrous Fumarate and Docusate Sodium Extended-Release Capsules	143. Fluconazole Oral Suspension	144. Flunisolide Inhalation Aerosol
145. Flunisolide Nasal Spray	146. Fluocinolone Acetonide Shampoo	147. Fluorescein Sodium Ophthalmic Solution
148. Fluorometholone Ointment	149. Fluticasone Propionate Inhalation Powder ***(Received)***	150. Fluticasone Propionate Pressurized Inhaler
151. Foscarnet Sodium Injection	152. Fosfomycin for Oral Solution	153. Gabapentin Oral Solution
154. Gadobenate Dimeglumine Injection	155. Gallium Nitrate Injection	156. Ganciclovir Capsules
157. Ganirelix Acetate Injection	158. Gatifloxacin Injection	159. Gatifloxacin Tablets
160. Gentamicin Sulfate Oral Solution	161. Gentamicin Sulfate Soluble Powder	162. Glipizide Extended-Release Tablets
163. Guaifenesin and Pseudoephedrine Hydrochloride Extended-Release Tablets	164. Guaifenesin and Salts of Dextromethorphan and Pseudoephedrine Oral Solution	165. Guanidine Hydrochloride Tablets
166. Halobetasol Propionate Cream	167. Halobetasol Propionate Ointment	168. Haloperidol Decanoate Injection
169. Haloperidol Lactate Injection	170. Haloperidol Lactate Oral Concentrate	171. Hydralazine Hydrochloride and Hydrochlorothiazide Capsules
172. **Hydrochlorothiazide Capsules** ***(Received)***	173. Hydrochlorothiazide Oral Solution	174. Hydrocodone Bitartrate and Acetaminophen Capsules
175. Hydrocodone Bitartrate and Acetaminophen Oral Solution	176. Hydrocodone Bitartrate and Aspirin Tablets	177. Hydrocodone Bitartrate and Guaifenesin Oral Solution
178. Hydrocodone Bitartrate and Homatropine Methylbromide Syrup	179. Hydrocortisone Acetate Dental Paste	180. Hydrocortisone Acetate Rectal Foam Aerosol
181. Hydrocortisone Butyrate Lotion	182. Hydroflumethiazide and Reserpine Tablets	183. Hydroquinone Lotion
184. Ibandronate Sodium Tablets	185. Ibuprofen Capsules	186. Idarubicin Hydrochloride Injection
187. Imipramine Pamoate Capsules	188. Imiquimod Topical Cream	189. Ipratropium Bromide Inhalation Aerosol
190. Ipratropium Bromide Inhalation Solution	191. Irinotecan Hydrochloride Injection	192. Isosulfan Blue Injection
193. Isradipine Extended-Release Tablets	194. Itraconazole Injection	195. Itraconazole Oral Solution
196. Ketoconazole Cream	197. Ketoconazole Shampoo	198. Ketoprofen Capsules ***(Received)***
199. Ketoprofen Extended-Release Capsules	200. Ketoprofen Tablets	201. Ketotifen Fumarate Ophthalmic Solution
202. Lactic Acid Lotion	203. Lamotrigine Tablets ***(Received)***	204. Latanoprost Ophthalmic Solution
205. Leucovorin Calcium for Injection	206. Levetiracetam Tablets ***(Received)***	207. Levocabastine Ophthalmic Suspension
208. Levofloxacin Solution	209. Lincomycin Hydrochloride and Spectinomycin Sulfate Soluble Powder	210. Liothyronine Injection
211. Lomustine Capsules ***(Received)***	212. Lopinavir and Ritonavir Solution	213. Lopinavir Capsules
214. Lopinavir Solution	215. Melphalan for Injection	216. Mesalamine Suppositories
217. Mesoridazine Besylate Concentrate	218. Metaraminol Bitartrate Injection	219. Methacholine Chloride for Inhalation Solution
220. Methadone Hydrochloride Oral Concentrate	221. Methocarbamol and Aspirin Tablets	222. Methoxsalen Softgels
223. Methyclothiazide and Deserpidine Tablets	224. Methylphenidate Hydochloride Chewable Tablets	225. Metipranolol Ophthalmic Solution
226. Metronidazole Cream	227. Metronidazole Extended-Release Tablets	228. Metronidazole Hydrochloride for Injection
229. Metronidazole Lotion	230. Miconazole Nitrate Topical Aerosol	231. Mifepristone Tablets

Small Molecules (Drug Products)—As of April 15, 2009 *(Continued)*

232. Miglitol Tablets	233. Milrinone Injection	234. Misoprostol Tablets ***(Received)***
235. Moexipril Hydrochloride and Hydrochlorothiazide Tablets	236. Moexipril Hydrochloride Tablets	237. Molindone Hydrochloride Oral Solution
238. Morphine Sulfate for Injection Concentrate	239. Morphine Sulfate Oral Solution	240. Morphine Sulfate Oral Solution Concentrate
241. Morphine Sulfate Tablets	242. Nalbuphine Hydrochloride Injection	243. Mycophenolate Mofetil Oral Solution
244. Nalmefene Hydrochloride Injection	245. Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution	246. Naproxen Sodium Extended-Release Tablets
247. Nedocromil Sodium Inhalation Aerosol	248. Neomycin Sulfate Oral Powder	249. Nicardipine Hydrochloride Capsules
250. Nilutamide Tablets	251. Nimodipine Capsules	252. Nisoldipine Extended-Release Tablets
253. Nitroglycerin Solution In Acrylic Adhesive	254. Nitroglycerin Transdermal System	255. Nizatidine Tablets
256. Ofloxacin In Dextrose Injection	257. Ofloxacin Injection	258. Olsalazine Sodium Capsules
259. Orphenadrine Citrate Extended-Release Tablets ***(Received)***	260. Orphenadrine Citrate, Aspirin, and Caffeine Tablets	261. Oxcarbazepine Suspension
262. Oxiconazole Cream	263. Pamidronate Disodium Injection	264. Pantoprazole Sodium for Injection
265. Pantoprazole Sodium Tablets	266. Paroxetine Hydrochloride Extended-Release Tablets	267. Paroxetine Oral Suspension
268. Pemirolast Potassium Ophthalmic Solution	269. Penicillin G Potassium Tablets for Oral Solution	270. Pentamidine Isethionate for Inhalation
271. Pentamidine Isethionate Injection ***(Received)***	272. Pentazocine Hydrochloride and Acetaminophen Tablets	273. Phendimetrazine Tartrate Extended-Release Capsules
274. Phenobarbital Capsules	275. Phentermine Resin Complex Capsules	276. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Capsules
277. Phenylephrine Hydrochloride, Chlorpheniramine Maleate, and Acetaminophen Extended-Release Tablets	278. Pilocarpine Hydrochloride Ophthalmic Gel	279. Pilocarpine Hydrochloride Ophthalmic Ointment
280. Pioglitazone Hydrochloride Tablets ***(Received)***	281. Piperonyl Butoxide and Pyrethrins Aerosol Foam	282. Pirbuterol Acetate Inhalation Aerosol
283. Poractant Alpha Suspension	284. Porfimer Sodium for Injection	285. Povacrylate Solution
286. Povacrylate-Iodine Topical Solution	287. Povidone-Iodine Gauze	288. Povidone-Iodine Swabsticks
289. Povidone-Iodine Topical Aerosol Foam	290. Povidone-Iodine Vaginal Suppositories	291. Pramipexole Dihydrochloride Tablets
292. Prednisolone Sodium Phosphate Oral Solution	293. Prochlorperazine Maleate Extended-Release Capsules	294. Progesterone Capsules
295. Propafenone Hydrochloride Tablets	296. Pseudoephedrine Hydrochloride and Brompheniramine Maleate Extended-Release Tablets	297. Pseudoephedrine Hydrochloride and Naproxen Sodium Extended-Release Tablets
298. Pseudoephedrine Hydrochloride, Chlorpheniramine Maleate, and Codeine Phosphate Oral Solution	299. Pseudoephedrine Hydrochloride, Guaifenesin, and Codeine Phosphate Oral Solution	300. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Extended-Release Tablets
301. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Oral Solution	302. Pseudoephedrine Sulfate, Dexbrompheniramine Maleate, and Acetaminophen Extended-Release Tablets	303. Pyrilamine Maleate Injection
304. Quinapril Hydrochloride and Hydrochlorothiazide Tablets	305. Quinidine Sulfate Injection	306. Ranitidine Capsules
307. Rauwolfia Serpentina and Endroflumethiazide Tablets	308. Reserpine and Polythiazide Tablets	309. Rimantadine Hydrochloride Oral Solution
310. Risperidone Orally Disintegrating Tablets ***(Received)***	311. Rivastigmine Tartrate Capsules ***(Received)***	312. Rivastigmine Tartrate Oral Solution ***(Received)***
313. Rocuronium Bromide Injection	314. Ropinirole Hydrochloride Tablets	315. Rosiglitazone Maleate Tablets
316. Salicylic Acid and Sulfur Cleansing Lotion	317. Salicylic Acid and Sulfur Lotion	318. Salicylic Acid and Sulfur Shampoo
319. Salicylic Acid Cream	320. Salicylic Acid Ointment	321. Salmeterol Inhalation Aerosol

Small Molecules (Drug Products)—As of April 15, 2009 *(Continued)*

322. Salmeterol Xinafoate Inhalation Powder	323. Scopolamine Transdermal System	324. Selegiline Hydrochloride Capsules
325. Sertraline Hydrochloride Oral Solution	326. Sibutramine Hydrochloride Capsules	327. Sodium Bicarbonate and Sodium Citrate for Oral Solution
328. Sodium Bicarbonate, Sodium Citrate, and Sodium Tartrate for Oral Suspension	329. Sodium Iodide Injection	330. Sodium Phenylbutyrate Oral Powder
331. Sodium Phenylbutyrate Tablets	332. Sodium Phosphates for Oral Suspension	333. Sodium Phosphates Tablets
334. Sodium Salicylate and Sulfur Shampoo	335. Sterile Talc Aerosol	336. Streptozocin for Injection
337. Sucralfate Oral Suspension	338. Sulconazole Nitrate Cream	339. Sulfacetamide Sodium and Fluorometholone Ophthalmic Suspension
340. Sulfacetamide Sodium and Prednisolone Sodium Phosphate Ophthalmic Solution	341. Sulfasalazine Oral Suspension	342. Sulisobenzone Lotion
343. Sumatriptan Injection	344. Tacrolimus Injection	345. Tacrolimus Ointment
346. Technetium Tc 99m Teboroxime Injection	347. Tenofovir Disoproxil Fumarate Tablets ***(Received)***	348. Terbinafine Hydrochloride Cream
349. Terbinafine Tablets ***(Received)***	350. Terbinafine Topical Solution	351. Terconazole Vaginal Cream
352. Terconazole Vaginal Suppositories	353. Testosterone Transdermal Gel	354. Testosterone Transdermal System
355. Tetracycline Hydrochloride Periodontal Fiber	356. Theophylline Extended-Release Tablets	357. Tioconazole Vaginal Ointment
358. Tiopronin Tablets	359. Tolnaftate Topical Aerosol Solution	360. Topiramate Capsules ***(Received)***
361. Torsemide Injection	362. Torsemide Tablets ***(Received)***	363. Trandolapril and Verapamil Hydrochloride Extended-Release Tablets
364. Trandolapril Tablets	365. Tranexamic Acid Injection	366. Tretinoin Capsules
367. Tretinoin Microsphere Gel	368. Triamcinolone Acetonide Nasal Suspension	369. Trifluridine Ophthalmic Solution
370. Trimetrexate for Injection	371. Trimipramine Maleate Capsules	372. Triprolidine and Pseudoephedrine Hydrochlorides and Codeine Phosphate Syrup
373. Trolamine Salicylate Cream	374. Trolamine Salicylate Gel	375. Trolamine Salicylate Topical Emulsion
376. Undecylenic Acid Topical Foam Aerosol	377. Urea Cream	378. Vecuronium Bromide for Injection
379. Venlafaxine Extended-Release Capsules ***(Received)***	380. Venlafaxine Tablets ***(Received)***	381. Verapamil Hydrochloride Capsules
382. Verapamil Hydrochloride Extended-Release Capsules	383. Voriconazole Injection	384. Voriconazole Oral Suspension
385. Voriconazole Tablets	386. Yttrium Y-90 Chloride Solution	387. Yttrium Y-90 Glass Microspheres
388. Yttrium Y-90 Microspheres Injection	389. **Zaleplon Capsules** ***(Received)***	390. Zidovudine and Lamivudine Tablets ***(Received)***
391. Zinc Acetate Capsules	392. Zinc Tridosium Pentetate Injection	393. Ziprasidone Hydrochloride Capsules
394. Zoledronic Acid for Injection	395. Zonisamide Capsules ***(Received)***	

Excipients—As of April 15, 2009

1. Acetone Sodium Bisulfite	2. Acetylated Monoglycerides	3. *N*-Acetyl-L-Methionine
4. Aconitic Acid (Achilleic Acid)	5. Acrylic Acid-Octyl Acrylate Copolymer	6. Albumin Colloidal
7. Aliphatic Polyesters	8. Allantoin-Sodium Pyrrolidone Carboxylate	9. Aluminum Ammonium Sulfate
10. Aluminum Lactate	11. Aluminum Oxide	12. Aluminum Potassium Sulfate
13. Aluminum Silicate	14. Aluminum Sodium Sulfate	15. Aluminum Stearate
16. Ammonium Bicarbonate	17. Ammonium Calcium Alginate	18. Ammonium Phosphate
19. Beeswax, Synthetic	20. Benzododecinium Bromide	21. Benzyl Chloride
22. Benzyl Nicotinate	23. Beta Naphthol	24. *N,N*-Bis(2-Hydroxyethyl)Stearamide

Excipients—As of April 15, 2009 *(Continued)*

25. Brominated Vegetable Oil	26. Butadiene-Styrene Rubber	27. Butyl Stearate ***(Received)***
28. Butylalcohol Monostearate	29. Butylated Hydromethylphenol	30. Butylene Glycol
31. Butylphthalyl Butylglycolate	32. Calcium Acid Pyrophosphate	33. Calcium Alginate
34. Calcium Alginate and Ammonium Alginate	35. Calcium Bromide	36. Calcium Chloride Solution
37. Calcium Phosphate Monobasic	38. Calcium Propionate	39. Calcium Pyrophosphate
40. Calcium Sorbate	41. Calcium Stearoyl Lactylate	42. Caldiamide Sodium
43. Calteridol Calcium	44. Capric Acid	45. Caprylic/Capric Diglyceryl Succinate
46. Carbon	47. Carboxymethyl Starch	48. Carboxymethylamylopectin Sodium
49. Carboxymethylcellulose Potassium	50. Cetostearyl Isononanoate	51. Chlorodifluoroethane
52. Cholic Acid	53. Cinnamaldehyde	54. Cocamide Diethanolamine
55. Cocamide Oxide	56. Cocoyl Caprylocaprate	57. Crystal Gum
58. Cutina	59. Cystine	60. Dammar Gum
61. Decanoic Acid	62. Decyl Oleate	63. Dextrin Palmitate
64. Dextrins Modified	65. Diacetyl Tartaric Acid Esters of Mono- and Diglycerides	66. Dicetyl Phosphate
67. Dichlorofluoromethane	68. Diethyl Sebacate ***(Received)***	69. Difluoroethane
70. Diglycol Stearate	71. Diisobutyl Adipate	72. Diisopropyl Adipate
73. Diisopropylbenzothiazyl-2-Sulfenamide	74. Dilauryl Thiodipropionate	75. Dimethyl Dicarbonate
76. Dimyristoyl Lecithin	77. Dimyristoyl Phosphatidylglycerol	78. Dipropylene Glycol
79. Disodium Edisylate	80. Disodium Guanylate	81. Disodium Inosinate
82. Disodium Monooleamide Sulfasuccinate	83. D-Mannose	84. Docusate Sodium/Sodium Benzoate
85. Erythrosine	86. Ethoxylated Mono- and Diglycerides	87. Ethoxyquin
88. Ethyl Hexanediol	89. Ethyl Linoleate	90. Ethyl Maltol ***(Received)***
91. Ethylene Dichloride	92. Ethylurea	93. Ferric Ammonium Citrate
94. Ferric Citrate	95. Ferric Oxide, Brown	96. Ferric Phosphate
97. Ferric Pyrophosphate	98. Ferrous Citrate	99. Ferrous Glycinate
100. Ferrous Lactate	101. Fluorochlorohydrocarbons	102. Formic Acid
103. Furcelleran	104. Gentistic Acid	105. Geraniol
106. Glutamic Acid Hydrochloride	107. Gluten	108. Glycerol Ester of Gum Rosin (Ester Gum)
109. Glyceryl Laurate	110. Glyceryl Palmitate	111. Glyceryl Ricinoleate
112. Glyceryl Tristearate	113. Glycine Hydrochloride	114. Glycofurol
115. Glycol Stearate	116. Heptafluoropropane	117. Heptylparaben
118. Hexadecyl Isostearate	119. Hexane	120. Hexanetriol(-1,2,6-)
121. Hydrocarbon Gel	122. Hydroxyethylmethylcellulose	123. Hydroxylated Lecithin
124. Indigotine	125. Iron Carbonyl	126. Iron Subcarbonate
127. Isobutylated-Isoprene Copolymer	128. Isooctylacrylate	129. Isopropyl Isostearate
130. Isopropyl Stearate	131. Isostearic Acid	132. Isostearyl Alcohol
133. Lactose Ferrin, Bovine	134. Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol	135. Lactylic Esters of Fatty Acids
136. Lanolin (Wool Fat), Hydrogenated	137. Lanolin Alcohols, Acetylated	138. Lanolin Hydrous
139. L-Ascorbyl Stearate	140. Lauramine Oxide	141. Lauric Myristic Diethanolamide
142. Lauric Acid	143. Lauric Diethanolamide	144. Lavender Oil
145. L-Cysteine Monohydrochloride	146. Lecithin, Hydroxylated	147. L-Glutamic Acid ***(Received)***
148. Linoleic Acid ***(Received)***	149. L-Leucine	150. Macrogol Sorbitan Tristearate
151. Macrogolglycerol Cocoates	152. Macrogolglycerol Triisostearate	153. Magnesium Aluminum Silicate Hydrate
154. Magnesium Aspartame Dihydrate	155. Magnesium Aspartate	156. Magnesium Phosphate Tribasic
157. Magnesium Phosphate, Dibasic, Trihydrate	158. Magnesium Tartrate	159. Malt Syrup
160. Maltitol Syrup	161. Maltol Isobutyrate	162. Manganese Chloride
163. Manganese Citrate	164. Manganese Glycerophosphate	165. Manganese Hypophosphite

Excipients—As of April 15, 2009 *(Continued)*

166. Medical Antifoam Emulsion C	167. Medronate Disodium	168. Medronic Acid
169. Methyl Chloride	170. Methylchloroisothiazolinone	171. Methylisothiazolinone
172. *N*-Methylpyrrolidone ***(Received)***	173. Microcrystalline Cellulose, Silicified ***(Received)***	174. Mineral Spirits
175. Monoisostearyl Glyceryl Ester	176. Monopotassium Glutamate Monohydrate	177. Monosodium Citrate
178. Mullein Leaf	179. Myristyl Gamma-Picolinium Chloride	180. Myristyl Lactate
181. Naphtha	182. Non-Pareil Seeds	183. Nutmeg Oil
184. Octanoic Acid	185. Oxystearin	186. Pentasodium Triphosphate
187. Pentetate Calcium Trisodium	188. Pentetate Pentasodium	189. Phenprobamate
190. Phenylmercuric Acetate	191. Phenylmercuric Nitrate	192. Pine Oil
193. Polacrilin	194. Polyglycerol Esters of Fatty Acids	195. Polyglycerol Polyricinoleic Acid
196. Polyoxyethylene Castor Oil (USP has 35)	197. Polyoxyl Stearate (USP has 40)	198. Polypropylene Oleate
199. Polypropylene Stearyl Ether	200. Polysorbate 65	201. Polyvinylacetal Diethylanoacetate
202. Polyvinylpolypyrrolidone	203. Polyvinylpyrrolidone Ethylcellulose	204. Potassium Acid Tartrate
205. Potassium Bromate	206. Potassium Carbonate Solution	207. Potassium Dichloroisocyanurate
208. Potassium Gibberellate	209. Potassium Glycerophosphate	210. Potassium Iodate
211. Potassium Nitrite	212. Potassium Phosphate	213. Potassium Phosphate Tribasic
214. Potassium Polymetaphosphate	215. Potassium Pyrophosphate	216. Potassium Stearate
217. Potassium Sulfate	218. Potassium Sulfite	219. Potassium Tripolyphosphate
220. Propyl Propionate	221. Propylene Glycol Diacetate	222. Propylene Glycol Mono- and Diesters
223. Rice Bran Wax	224. Rosin	225. Silicone
226. Sodium Acid Pyrophosphate	227. Sodium Aluminosilicate ***(Received)***	228. Sodium Aluminum Phosphate Acidic
229. Sodium Aluminum Phosphate Basic	230. Sodium Aspartate	231. Sodium Bisulfate
232. Sodium Bisulfite	233. Sodium Carbonate Hydrate	234. Sodium Carboxymethyl Betaglucan
235. Sodium Caseinate	236. Sodium Chlorate	237. Sodium Citrate, Dibasic
238. Sodium Citrate, Monobasic	239. Sodium Dehydroacetate	240. Sodium Diacetate
241. Sodium Erythorbate	242. Sodium Ferric Pyrophosphate	243. Sodium Ferrocyanide
244. Sodium Hypophosphite ***(Received)***	245. Sodium Laureth Sulfate	246. Sodium Lauroyl Sarcosinate
247. Sodium Lauryl Sulfoacetate	248. Sodium Magnesium Aluminosilicate	249. Sodium Magnesium Silicate
250. Sodium Malate	251. Sodium Metaphosphate, Insoluble	252. Sodium Metasilicate
253. Sodium Methylate	254. Sodium Polyphosphates Glassy	255. Sodium Potassium Tripolyphosphate
256. Sodium Pyrophosphate	257. Sodium Pyrrolidone Carboxylate	258. Sodium Sesquicarbonate
259. Sodium Sesquinoleate	260. Sodium Stearoyl Lactylate	261. Sodium Thiomalate
262. Sodium Trimetaphosphate	263. Sodium Trioleate	264. Sodium Tripolyphosphate
265. Soy Polysaccharides	266. Stannous Tartrate	267. Starch, Pregelatinized Corn
268. Starch, Pregelatinized Tapioca	269. Stearalkonium Chloride	270. Stearyl Citrate
271. Stearyl Monoglyceridyl Citrate	272. Succinylated Monoglycerides	273. Sucrose Acetate Isobutyrate
274. Sucrose Fatty Acid Esters	275. Sucrose Stearate ***(Received)***	276. Sugar Fruit Fine
277. Sulfobutyl Ether Beta Cyclodextrin ***(Received)***	278. Tallow	279. Tallow Glycerides
280. Tallow Oil	281. Tetrafluoroethane	282. Thioglycerol
283. Thyme Oil	284. Tribehenin	285. Tricetereth-4 Phosphate
286. Trichloroethylene	287. Trimyristin	288. Trisodium Citrate
289. Trolamine Lauryl Sulfate	290. Vegetable Oil	291. Wheat Flour
292. Wheat Germ Oil	293. Wheat Gluten ***(Received)***	294. Whey

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:
- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become effective before the effective date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The effective date for these revisions is stated on the next page.)

Readers should review this section to determine if they are affected by any of the changes.

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

Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the issue of a given *PF* volume.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements,* that does not accurately reflect the intended official requirements of the Council of Experts. Beginning with *PF* 35(2), Errata will be published both in the *Pharmacopeial Forum* and on the usp.org website. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to *USP 32–NF 27*. The page number indicates where the item is found in *USP–NF.* Errata are updated as necessary in each *Pharmacopeial Forum* issue and monthly on the usp.org website. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF.* The list of Errata has been relocated to www.usp.org, where updates will be posted monthly.

INTERIM REVISION ANNOUNCEMENT to *USP 32* and to *NF 27*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

Duane M. Kirking, Pharm.D., Ph.D., *Chair, USP Board of Trustees*

Roger L. Williams, M.D., *Executive Vice President, CEO,* and *Chairman, USP Council of Experts*

Susan de Mars, J.D., General Counsel and Chief, Documentary Standards Officer
William F. Koch, Ph.D., FACB, *Chief Metrology Officer*

Released July 1, 2009 **Official August 1, 2009**

All inquiries and comments regarding *USP 32* text and *NF 27* text should be addressed to the Executive Secretariat, *USP–NF,* 12601 Twinbrook Parkway, Rockville, MD 20852 (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 32* or *NF 27* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard.

USP 23-Epi-26-deoxyactein RS (January 1, 2009)
USP Actein RS (January 1, 2009)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 32* or *NF 27* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of February 6, 2009. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at http://www.uspcatalog.com.

USP Acarbose RS
USP Acarbose System Suitability Mixture RS
USP *S*-Adenosyl-L-homocysteine RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Fludeoxyglucose Related Compound B RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Alpha Lipoic Acid RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Propylene Glycol Dilaurate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin Related Compound A RS
USP Vasopressin RS

DIETARY SUPPLEMENTS—MONOGRAPHS

Hawthorn Leaf with Flower

Change to read:

Labeling—The label states the Latin binomial and, following the official name, the parts of the plant contained in the article. ••4

Powdered Hawthorn Leaf with Flower

Change to read:

Labeling—The label states the Latin binomial and, following the official name, the parts of the plant source from which the article was derived. ••4

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

USP32–NF27 **Page**	**Title**	**Section**	**Description**
310	⟨788⟩ *Particulate Matter in Injections*	*Introduction*	Second paragraph, line 2: Change ''of mobile undissolved particles,'' to: of extraneous mobile undissolved particles,
1178	*Butylated Hydroxytoluene*	*Related compounds*	Line 1 under *Potassium ferricyanide solution*: Change ''50 mg'' to: 500 mg Line 1 under *Ferric chloride solution*: Change ''105 mg'' to: 1050 mg
1855	*Cefprozil*	*Chemical names*	Change the first chemical name to read: 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-(1-propenyl)-, monohydrate, [6*R*-[6α,7β(*R**)]]- Change the second chemical name to read: (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate

USP32–NF27 **Page**	**Title**	**Section**	**Description**
1948	*Citalopram Hydrobromide*	*Related compounds*	*Table 1* referenced under TEST 1, *Procedure*, appears at the bottom of page 1950.
2906	*Metformin Hydrochloride Tablets*	*Dissolution* ⟨711⟩	Insert Test 3, official in Second IRA of 2007. TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3.* *Medium:* pH 6.8 phosphate buffer; 1000 mL. *Apparatus 1:* 100 rpm. *Time:* 60 minutes. Determine the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved by employing the following method. *0.05 M Sodium phosphate with 1-pentanesulfonic acid solution*—Dissolve 1.38 g of monobasic sodium phosphate in about 1800 mL of water. Add 3.484 g of 1-pentanesulfonic acid sodium salt, and mix. Adjust with diluted phosphoric acid to a pH of 3.00 ± 0.05. Add water to make 2000 mL, and mix. *Mobile phase*—Prepare a filtered and degassed mixture of *0.05 M Sodium phosphate with 1-pentanesulfonic acid solution* and acetonitrile (19:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). *Standard stock solution*—Transfer about 25 mg, accurately weighed, of USP Metformin Hydrochloride RS to a 100-mL volumetric flask, and add about 50 mL of *Medium*. Sonicate until dissolved, and dilute with *Medium* to volume. *Standard solution*—Transfer 10.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Medium* to volume. *Test solution*—Withdraw a portion of the solution under test, and pass through a 0.45-μm nylon filter. Dilute with *Medium,* if necessary, to obtain a concentration similar to that of the *Standard solution.* *Chromatographic system*—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; the column efficiency is not less than 1500 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%. *Procedure*—Separately inject equal volumes (about 40 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of metformin released by the formula: $$\frac{r_U \times C_S \times 900 \times 100}{r_S \times D \times LC}$$ in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of metformin in the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; *D* is the dilution factor of the *Test solution;* and *LC* is the Tablet label claim, in mg. *Tolerances*—Not less than 70% *(Q)* of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ is dissolved in 60 minutes.

USP32–NF27 **Page**	**Title**	**Section**	**Description**
3372	*Prednisolone Sodium Phosphate*	*Related compounds*	Line 1 under *Test solution*: Change ''Accurately weigh a known quantity of USP Prednisolone Sodium Phosphate RS'' to: Accurately weigh a known quantity of prednisolone sodium phosphate
3374	*Prednisolone Sodium Phosphate Injection*	*Identification*	Change: ''**B:** It responds to *Identification* test *A* under *Prednisolone Sodium Phosphate*.'' to: **B:** *Infrared Absorption* ⟨197K⟩— *Test specimen:* Place 5 mL of the *Assay preparation* obtained as directed in the *Assay,* in a glass-stoppered, 100-mL volumetric flask, mix with 5 mL of *Alkaline phosphatase solution* prepared as directed in the *Assay,* and add 50 mL of methylene chloride. Insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Filter the methylene chloride layer through a dry paper, and evaporate 25 mL of the filtrate to dryness. *Standard specimen*: Prepare as directed in *Infrared Absorption* ⟨197K⟩, using USP Prednisolone RS.

USP32–NF27 **Page**	**Title**	**Section**	**Description**
		Assay	Change ''*pH 9 Buffer with magnesium*—Prepare as directed in the *Assay* under *Prednisolone Sodium Phosphate.*'' to: *pH 9 Buffer with magnesium*—Mix 3.1 g of boric acid and 500 mL of water in a 1-L volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix. Change ''*Alkaline phosphatase solution*—Prepare as directed in the *Assay* under *Prednisolone Sodium Phosphate.*'' to: *Alkaline phosphatase solution*—Transfer 250 mg of alkaline phosphate enzyme to a 25-mL volumetric flask, dissolve by adding *pH 9 Buffer with magnesium* to volume, and mix. Prepare this solution fresh daily. Change: ''*Standard preparation*—Prepare as directed in the *Assay* under *Prednisolone Sodium Phosphate.*'' to: *Standard preparation*—Dissolve a suitable, accurately weighed quantity of USP Prednisolone RS in methylene chloride, and dilute quantitatively and stepwise with methylene chloride to obtain a solution having a known concentration of about 16 μg per mL. Pipet 100 mL of the solution into a glass-stoppered, 100-mL cylinder, and add 1.0 mL of *Alkaline phosphatase solution* and 1.0 mL of water. Allow to stand, with occasional gentle inversion, for 2 hours. Change: ''*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Prednisolone Sodium Phosphate.*'' to: *Procedure*—Pipet 1 mL of the *Assay preparation* into a glass-stoppered, 100-mL cylinder, add 1.0 mL of *Alkaline phosphatase solution* and about 50 mL of methylene chloride, insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Add methylene chloride to volume, mix, and allow to stand until the methylene chloride layer is clear (about 20 minutes). Concomitantly and without delay, determine the absorbances of the methylene chloride solution obtained from the *Assay preparation* and the *Standard preparation* at 241 nm, with a suitable spectrophotometer, using methylene chloride as the blank.

USP32–NF27 **Page**	**Title**	**Section**	**Description**
3375	*Prednisolone Sodium Phosphate Ophthalmic Solution*	*Identification*	Change: ''**Identification**—It responds to *Identification* test *A* under *Prednisolone Sodium Phosphate* and to *Identification* test *A* under *Prednisolone Sodium Phosphate Injection.*'' to: **Identification**— **A:** *Infrared Absorption* ⟨197K⟩ *Test specimen:* Place 5 mL of the *Assay preparation* obtained as directed in the *Assay,* in a glass-stoppered, 100-mL volumetric flask, mix with 5 mL of *Alkaline phosphatase solution* prepared as directed in the *Assay,* and add 50 mL of methylene chloride. Insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Filter the methylene chloride layer through a dry paper, and evaporate 25 mL of the filtrate to dryness. *Standard specimen:* Prepare as directed in *Infrared Absorption* ⟨197K⟩, using USP Prednisolone RS. **B:** Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of dilute sulfuric acid (3 in 5), add 5 mL of isopropyl alcohol, and mix. Heat 5 mL of this solution with 1 mL of *Assay preparation* (obtained as directed in the *Assay*) at 70° for 2 hours: a yellow color develops.
First Supplement to USP32–NF27			
4031	*Betamethasone Oral Solution*	*Assay*	Line 1 under *Standard stock preparation*: Change ''Dissolve an accurately weighed quantity of USP Betamethasone RS in alcohol,'' to: Dissolve an accurately weighed quantity of USP Betamethasone RS in dehydrated alcohol, Line 1 under *System suitability preparation*: Change ''Dissolve an accurately weighed quantity of betamethasone in alcohol,'' to: Dissolve an accurately weighed quantity of betamethasone in dehydrated alcohol,

PROPOSED INTERIM REVISION ANNOUNCEMENTS

This section includes proposals for *Interim Revision Announcements* (*IRAs*) that will be published as official *USP* or *NF* standards. There is a 60-day comment period for these proposals, beginning on the 15th of the first month of this *Pharmacopeial Forum*. The approved official text will be published in a future *Pharmacopeial Forum* and additionally in the ''New Official Text'' section of USP's web site (www.usp.org). Readers should review material in this section and provide comments to the Scientific Liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Expert Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

Proposed IRA

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision,* or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

> **Name of Item**, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:
>
> (DSI: D. Sarma.) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. Because of the redesign of monographs, any proposed new text with revisions for *USP 33–NF 28* and beyond will be set off from the current official text by shading where the symbols surround the text changes. Standards that become official as *Interim Revision Announcements (IRAs)* in *Pharmacopeial Forum* will continue to identify changed text in a larger font (print edition only). All *USP–NF* revisions use the following symbols that indicate the final destination of the official text: •new text• if slated for an *IRA*; ▲new text▲ if slated for *USP–NF*; and ■new text■ if slated for a *Supplement* to *USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as ••, or ■■, or ▲▲, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (*USP 32*) indicates that the proposed revision is slated for the *Second Supplement to USP 32*, and ▲*USP33* and ▲*NF28* indicates that the revisions are proposed for *USP 33* and *NF 28*, respectively.

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

MONOGRAPHS (USP)

BRIEFING

Medical Air, *USP 32* page 1434; **Oxygen,** *USP 32* page 3173; **Oxygen 93 Percent,** *USP 32* page 3173. It is proposed to revise the *Assay* to specify the use of a paramagnetic oxygen analyzer. The paramagnetic analyzer measures the concentration based on the paramagnetic response of the oxygen molecules. Oxygen is strongly paramagnetic, whereas most common gases such as nitrogen, helium, and nitrous oxide are diamagnetic. The *Identification* test proposed is limited to the presence of oxygen in the sample. USP is seeking feedback on the need for a procedure to provide positive identification of all components of Medical Air. The *Impurities*, *Packaging and Storage*, and *Labeling* sections have been revised as well. The *Assay* and *Impurities* sections refer to the proposed new *USP* general test chapters *Impurities Testing in Medical Gases* ⟨413⟩ and *Medical Gases Assay* ⟨415⟩, which appear elsewhere in this issue of *PF.*

(AER: K. Zaidi.) RTS—C61164

Medical Air

DEFINITION

Medical Air is a natural or synthetic mixture of gases consisting largely of nitrogen and oxygen. It contains NLT 19.5% and NMT 23.5%, by volume, of O_2.

IDENTIFICATION

Add the following:

■• When tested as directed in the Assay, the analytical result is NLT 19.5% and NMT 23.5%, by volume, of oxygen.■2S (*USP33*)

ASSAY

Change to read:

• **PROCEDURE**

~~**Analysis:**~~■2S (*USP33*) Determine the oxygen concentration of Medical Air using ~~an electrochemical cell analyzer readable to 0.1% of oxygen and calibrated with ambient air to an accuracy of ± 0.2% of oxygen.~~

~~[NOTE—The instrument uses the variations of electric current produced by the interaction of oxygen with an electrochemical cell to display the oxygen strength of a confined sample or an in-line flow of the gas. This current generates a signal proportional to the oxygen concentration, which is displayed on a meter.]~~■a paramagnetic analyzer for oxygen (see *Medical Gases Assay* ⟨415⟩).

Zero gas: Nitrogen certified standard

Span gas: 21% oxygen in nitrogen certified standard

Sample: Sample under test

[NOTE—See *Reagents, Indicators, and Solutions* for information on certified standards.]

Analysis: Determine the percentage of oxygen in the sample.■2S (*USP33*)

Acceptance criteria: ~~19.5%–23.5%~~■NLT 19.5% and NMT 23.5%, by volume, of O_2.■2S (*USP33*)

IMPURITIES

Change to read:

Inorganic Impurities

■See *Impurities Testing in Medical Gases* ⟨413⟩.

[NOTE—The various detector tubes called for in the respective tests are listed under *Reagents, Indicators, and Solutions.*]■2S (*USP33*)

• **CARBON DIOXIDE:** ~~Pass 1000 ± 50 mL through a carbon dioxide detector tube at the rate specified for the tube: the indicator change corresponds to NMT 500 ppm.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass through a carbon dioxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT 500 ppm.■2S (*USP33*)

• **CARBON MONOXIDE:** ~~Pass 1000 ± 50 mL through a carbon monoxide detector tube at the rate specified for the tube: the indicator change corresponds to NMT 10 ppm.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass through a carbon monoxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT 10 ppm.■2S (*USP33*)

• **SULFUR DIOXIDE:** ~~Pass 1000 ± 50 mL through a sulfur dioxide detector tube at the rate specified for the tube: the indicator change corresponds to NMT 5 ppm.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass through a sulfur dioxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT 5 ppm.■2S (*USP33*)

• **LIMIT OF NITRIC OXIDE AND NITROGEN DIOXIDE:** ~~Pass 1000 ± 50 mL through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to NMT 2.5 ppm.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT 2.5 ppm.■2S (*USP33*)

Organic Impurities

• **PROCEDURE: WATER AND OIL:** Support 1 container in an inverted position (with the valve at the bottom) for 5 min. Cautiously open the valve slightly, maintaining the container in an inverted position. Vent the gas with a barely audible flow against a stainless steel mirror for a few s: no liquid is discernible on the mirror.

SPECIFIC TESTS

• **ODOR:** Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in cylinders or in a low-pressure collecting tank. Containers used for Medical Air are not to be treated with any toxic, sleep-inducing, or narcosis-producing compounds, and are not to be treated with any compound that would be irritating to the respiratory tract when the Medical Air is used. [NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube to minimize contamination or change of the specimens.]~~

~~The various detector tubes called for in the respective tests are listed under *Reagents, Indicators, and Solutions—Reagent Specifications.*~~

In-Process Revision

■Preserve in pressurized containers. Container connections shall be appropriate for air. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.■2S (*USP33*)

Change to read:

• **LABELING:** Where it is piped directly from the collecting tank to the point of use, label each outlet "Medical Air".

■Where the medical air is a synthetic mixture, label as "Mixture of Oxygen USP and Nitrogen NF".

Where the medical air is a synthetic mixture of oxygen and nitrogen and where oxygen complies to Oxygen USP and Nitrogen complies to Nitrogen NF, then the *Impurities* tests are not required.■2S (*USP33*)

BRIEFING

Atropine Sulfate, *USP 32* page 1604. Atropine Sulfate is a racemic mixture. One of its enantiomer, the Hyoscyamine Sulfate monograph, was modernized via *In-Process Revision* in *PF* 31(4) [Jul.– Aug. 2005]. Similarly, this monograph is modernized and the following revisions are proposed:

1. Include an *Identification* test, based on the retention time of the major peak in the test for *Organic Impurities*.
2. Replace the current test for *Other Alkaloids* with a more specific test for *Organic Impurities* from the Hyoscyamine Sulfate monograph. The stability-indicating liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Aquasil C18 brand of L1 column. The typical retention time for hyoscyamine is about 9.8 min. The proposed method is very similar to *European Pharmacopoeia* monograph. The only difference is that the *European Pharmacopoeia* monograph uses a mixture of impurities whereas this proposal uses only one of the impurities. The chemical names for impurities are included in the proposal.
3. Omit the test for *Melting Range or Temperature* because the test for *Organic Impurities* provides sufficient information for potential impurities.
4. Replace the test for *Angular Rotation* with *Specific Rotation* to make it independent of concentration. Additionally, the specification is revised to be consistent with that in the *European Pharmacopoeia* monograph, *6th Edition*.
5. The test for *Acidity* is removed because the test for *Organic Impurities* provides enough information to determine the purity of the product.
6. Storage conditions consistent with that of the Hyoscyamine Sulfate monograph in *USP 32* and the *European Pharmacopoeia* monograph, 6th *Edition* is included.

(MD-PP: H. Ramanathan, R. Ravichandran.) RTS—C57193

Atropine Sulfate

$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ 694.83
$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ 676.83

Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo [3.2.1]oct-3-yl ester, *endo*-(±)-, sulfate (2:1) (salt), monohydrate;
1α*H*,5α*H*-Tropan-3-α-ol (±)-tropate (ester), sulfate (2:1) (salt) monohydrate [5908-99-6].
Anhydrous [55-48-1].

DEFINITION
Atropine Sulfate contains NLT 98.5% and NMT 101.0% of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.
[**CAUTION—**Handle Atropine Sulfate with exceptional care, because it is highly potent.]

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨**191**⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements

Add the following:

■• **C.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities, Procedure*.■2S (*USP33*)

ASSAY
• **PROCEDURE**
Sample: 1 g
Analysis: Dissolve in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 67.68 mg of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$.
Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.2%

Change to read:

Organic Impurities
~~• **OTHER ALKALOIDS**~~
~~**Sample:** 150 mg~~
~~**Analysis:** Dissolve in 10 mL of water. To 5 mL of the solution, add a few drops of platinic chloride TS: no precipitate is formed. To the remaining 5 mL of the solution, add 2 mL of 6 N ammonium hydroxide, and shake vigorously.~~
~~**Acceptance criteria:** A slight opalescence may develop but no turbidity is produced.~~

■• **PROCEDURE**
Buffer: 7.0 g/L of monobasic potassium phosphate, adjusted with 0.05 M phosphoric acid to a pH of 3.3
Solution A: Dissolve 3.5 g of sodium dodecyl sulfate in 606 mL of *Buffer*, and add 320 mL of acetonitrile.
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
20	70	30
20.1	95	5
25	95	5

Standard stock solution: 0.24 mg/mL of USP Hyoscyamine Sulfate RS in *Solution A*
Standard solution: 0.24 µg/mL of USP Hyoscyamine Sulfate RS from *Standard stock solution*, in *Solution A*
System suitability solution: 0.24 µg/mL of USP Hyoscyamine Related Compound A RS in *Standard stock solution*
Sample solution: 0.24 mg/mL of Atropine Sulfate from in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between hyoscyamine related compound A and hyoscyamine
Tailing factor: NMT 2.0, for hyoscyamine peak
Relative standard deviation: NMT 1.0%, for hyoscyamine peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Atropine Sulfate taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response from *Standard solution*
C_S = concentration of USP Hyoscyamine Sulfate RS in the *Standard solution* (µg/mL)
C_U = concentration of Atropine Sulfate in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
DL-Tropic acid[a]	0.2	0.2
7-Hydroxyhyoscyamine[b]	0.67	0.2
6-Hydroxyhyoscyamine[c]	0.72	0.2
Scopolamine[d]	0.8	0.2
Hyoscyamine related compound A[e]	0.9	0.3
Atropine	1.0	—
Littorine[f]	1.1	0.2
Apoatropine[g]	1.8	0.2
Any other individual impurity	—	0.1

[a] (2*RS*)-3-Hydroxy-2-phenylpropanoic acid.
[b] (1*S*,3*R*,5*S*,6*RS*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.
[c] (1*R*,3*S*,5*R*,6*RS*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.
[d] 6*β*,7*β*-Epoxy-1α*H*,5α*H*-tropan-3α-ol (-)-tropate (ester).
[e] (1*R*,3*R*,5*S*)-8-Azabicyclo[3.2.1]oct-3-yl(2*S*)-3-hydroxy-2-phenylpropanoate.
[f] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate.
[g] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropenoate.
■2S (*USP33*)

SPECIFIC TESTS

Delete the following:

■• **~~Melting Range or Temperature,~~** ~~*Class Ia* ⟨741⟩:~~ ~~Not lower than 187°, determined after drying at 120° for 4 h~~

~~[Note—Because anhydrous Atropine Sulfate is hygroscopic, determine its melting temperature promptly on a specimen placed in the capillary tube immediately after drying.]~~■2S (*USP33*)

Delete the following:

■• **~~Optical Rotation,~~** ~~*Angular Rotation* ⟨781A⟩:~~ ~~The observed rotation, in degrees, multiplied by 200, and divided by the length, in mm, of the polarimeter tube used, is between –0.60° and +0.05° (limit of hyoscyamine).~~
~~**Sample solution:** 1 g, in water to make a volume of 20 mL at 25°~~■2S (*USP33*)

Add the following:

■• **Optical Rotation,** *Specific Rotation* ⟨**781S**⟩**:** Between –0.50° and +0.05°
Cell length: 2 dm
Sample solution: 0.1 mg/mL in water■2S (*USP33*)

Delete the following:

■• **~~Acidity~~**
~~**Sample:** 1.0 g~~
~~**Analysis:** Dissolve *Sample* in 20 mL of water, and add 1 drop of *methyl red TS*. Titrate with 0.020 N sodium hydroxide.~~
~~**Acceptance criteria:** NMT 0.30 mL is required to produce a yellow color.~~■2S (*USP33*)

• **Water Determination,** *Method I* ⟨**921**⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in tight ■light-resistant■2S (*USP33*) containers.

Change to read:

• **USP Reference Standards** ⟨**11**⟩
USP Atropine Sulfate RS
■USP Hyoscyamine Sulfate RS
USP Hyoscyamine Related Compound A RS■2S (*USP33*)

BRIEFING

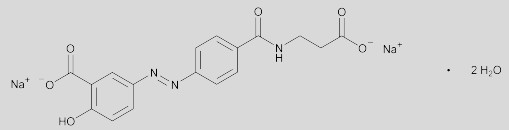
Balsalazide Disodium. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is proposed. The liquid chromatographic procedure in the *Organic Impurities* is based on analyses performed with the Zorbax Eclipse XDB-C18 brand of L1 column. The typical retention time for the balsalazide peak is about 17 min.

(MD-GRE: E. Gonikberg.) RTS—C70442

In-Process Revision

Add the following:

■Balsalazide Disodium



$C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ 437.31

Benzoic acid, 5-[[4-[[(2-carboxyethyl)amino]carbonyl]phenyl]azo]-2-hydroxy-, disodium salt, dihydrate, (*E*)-;
(*E*)-5-[[*p*-[(2-Carboxyethyl)carbamoyl]phenyl]azo]salicylic acid, disodium salt, dihydrate [150399-21-6].

DEFINITION

Balsalazide Disodium contains NLT 98.0% and NMT 102.0% of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$, calculated on the as is basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
  Sample solution: 10 µg/mL in water
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩

ASSAY

- **PROCEDURE**
  Sample: 219 mg
  Analysis: Add 80 mL of glacial acetic acid to the *Sample*, sonicate to dissolve, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$.
  Acceptance criteria: 98.0%–102.0% on the as is basis

IMPURITIES

Inorganic Impurities

- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE**
  Buffer: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of 6.00 ± 0.1.
  Diluent: Use *Buffer*.
  Solution A: Use *Buffer*.
  Solution B: Use acetonitrile.
  Standard solution: 0.5 µg/mL of USP Balsalazide Disodium RS, 0.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent* [NOTE—USP Balsalazide Related Compound A RS is the disodium salt of (*E*)-5-[(*p*-carboxyphenyl)azo]-2-salicylic acid. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]
  Sample solution: 1 mg/mL of Balsalazide Disodium in *Diluent*
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection size: 30 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5 between balsalazide and balsalazide related compound B
Relative standard deviation: NMT 5% for each peak
Tailing factor: NMT 1.8 for balsalazide peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

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

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for the corresponding impurity from the *Standard solution* [NOTE—For unspecified impurities, r_S is the peak response for the balsalazide peak from the *Standard solution*.]
C_S = concentration of the corresponding impurity in the *Standard solution* (mg/mL) [NOTE—For unspecified impurities, C_S is the concentration of balsalazide disodium in the *Standard solution*.]
C_U = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Reporting level for impurities: 0.03%
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	0.05
Balsalazide related compound A[a]	0.70	0.05
Balsalazide	1.00	—
Balsalazide related compound B[b]	1.2	0.05
Any other individual unspecified impurity	—	0.05

[a](*E*)-5-[(*p*-carboxyphenyl)azo]-2-salicylic acid.
[b](*E*)-5-({*m*-[(2-carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** 7.8%–8.8%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Balsalazide Disodium RS
  USP Balsalazide Related Compound A RS
  USP Balsalazide Related Compound B RS
  USP Salicylic Acid RS■2S *(USP33)*

In-Process Revision

BRIEFING

Balsalazide Disodium Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with the Supelco Hypersil BDS C18 brand of L1 column. The typical retention time for the balsalazide peak is about 5 min. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with the Zorbax Eclipse XDB-C18 brand of L1 column. The typical retention time for the balsalazide peak is about 17 min.

(MD-GRE: E. Gonikberg. BPC: M. Marques.) RTS—C70442

Add the following:

▪Balsalazide Disodium Capsules

DEFINITION

Balsalazide Disodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of balsalazide disodium ($C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION**

  Using a 0.2-cm cell, record the UV spectrum of the *Sample solution*, obtained in the *Assay*, in the range of 200–400 nm: it exhibits maxima at about 261 nm and 357 nm.

ASSAY

- **PROCEDURE**

  Buffer: Add 5 mL of triethylamine to 1000 mL of water, and adjust to a pH of 6.00 ± 0.1 with phosphoric acid.
  Mobile phase: Acetonitrile and *Buffer* (1:4)
  Diluent: Water
  Standard solution: 60 µg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication as necessary.]
  Sample stock solution: Transfer an equivalent to 150 mg of balsalazide disodium, from the Capsules contents, to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.
  Sample solution: 60 µg/mL of balsalazide disodium, from *Sample stock solution*, in *Diluent*. Pass a portion of this solution through a suitable filter, discarding the first 3 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 360 nm
  Column: 4.6-mm × 25-cm column; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 10,000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Sample: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ in the portion of Capsules taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of balsalazide disodium in the *Sample solution* (mg/mL)

  Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

- **PROCEDURE**

  Buffer: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust to a pH of 6.00 ± 0.1, using 10% potassium hydroxide solution.
  Diluent: Use water.
  Solution A: Use the *Buffer*.
  Solution B: Use acetonitrile.
  Sample solution: Transfer an amount of finely crushed powder equivalent to 100 mg of balsalazide disodium to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a 0.45-µm PVDF filter.
  Standard solution: 1.0 µg/mL of USP Balsalazide Disodium RS in *Diluent*
  System suitability solution: 1.0 µg/mL of USP Balsalazide Disodium RS, 1.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 238 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 45°
  Flow rate: 1 mL/min
  Injection size: 30 µL
  System suitability
  Sample: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 5 between balsalazide and balsalazide related compound B, from *System suitability solution*

In-Process Revision

Relative standard deviation: NMT 5.0%, from *Standard solution*
Tailing factor: NMT 1.5, from *Standard solution*
Analysis
Sample: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times (C_S/C_U) \times 100$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = the peak response for the balsalazide peak from the *Standard solution*
F = relative response factor (see *Impurity Table 1* for values)
C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of balsalazide disodium in the *Sample solution*, based on the label claim (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.15% of balsalazide related compound A; NMT 0.10% of any individual unspecified impurity
Reporting level for impurities: 0.05%
Total impurities: NMT 1.0% [NOTE—When reporting results for *Individual impurities* and *Total impurities*, disregard peaks corresponding to salicylic acid and balsalazide related compound B, as these impurities are controlled in the drug substance only.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor
Salicylic acid	0.37	—
Balsalazide related compound A[a]	0.70	1.3
Balsalazide	1.00	—
Balsalazide related compound B[b]	1.2	—
Any other individual unspecified impurity	—	1.0

[a](*E*)-5-[(*p*-carboxyphenyl)azo]-2-salicylic acid.
[b](*E*)-5-({*m*-[(2-carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.

PERFORMANCE TESTS
• **DISSOLUTION** ⟨**711**⟩
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm, with stainless steel wire helix sinkers
Time: 30 min
Detector: UV 357 nm, with background correction at 590 nm
Path length: 0.02-cm flow cell
Blank: *Medium*
Standard solution: 0.83 mg/mL of USP Balsalazide Disodium RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable 20-µm filter.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (900 mL)
L = label claim (mg/unit)

Tolerances: NLT 70% (Q) of the labeled amount of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

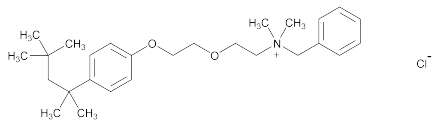
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Balsalazide Disodium RS
USP Balsalazide Related Compound A RS
USP Balsalazide Related Compound B RS
USP Salicylic Acid RS■2S *(USP33)*

BRIEFING

Benzethonium Chloride, *USP 32* page 1638. It is proposed to modernize this monograph by replacing two wet chemistry procedures, *Identification* tests *B* and *C*, with a single, more selective IR test.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C73680

Benzethonium Chloride



$C_{27}H_{42}ClNO_2$ 448.08
Benzenemethanaminium, *N*,*N*-dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride;
Benzyldimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride [121-54-0].

DEFINITION
Benzethonium Chloride contains NLT 97.0% and NMT 103.0% of $C_{27}H_{42}ClNO_2$, calculated on the dried basis.

IDENTIFICATION
• **A. PROCEDURE**
Sample solution: 10 mg/mL
Analysis: To 1 mL of the *Sample solution*, add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.
Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

Delete the following:

■• ~~**B.** A solution (1 in 100) forms precipitates with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.~~■2S *(USP33)*

Delete the following:

■• ~~**C. PROCEDURE**~~
~~**Sample solution:** 0.1 g in 1 mL of sulfuric acid~~
~~**Analysis:** Add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool, add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol~~

In-Process Revision

~~dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.~~

~~**Acceptance criteria:** The solution turns orange-red, and a brown precipitate may be formed.~~ ■2S *(USP33)*

Add the following:

■• **B. INFRARED ABSORPTION** ⟨197K⟩■2S *(USP33)*

ASSAY

• **PROCEDURE**

Sample: 0.3 g of Benzethonium Chloride

Analysis: Dissolve the *Sample* in 75 mL of water contained in a glass-stoppered, 250-mL flask. Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of $C_{27}H_{42}ClNO_2$.

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

Organic Impurities

• **PROCEDURE: LIMIT OF AMMONIUM COMPOUNDS:** To 5 mL of a solution (1 in 50), add 3 mL of 1 N sodium hydroxide, and heat to boiling: the odor of ammonia is not perceptible.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 158°–163°, the specimen having been dried previously

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 4 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Add the following:

■• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzethonium Chloride RS■2S *(USP33)*

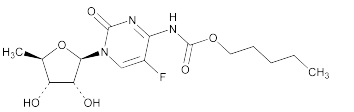
BRIEFING

Capecitabine, *USP 32* page 1774. On the basis of comments received, it is proposed to make the following changes:

1. In the *Assay*, specify the use of glacial acetic acid in *Solution A*.
2. In order to be consistent with ICH guidelines, in the test for *Organic Impurities*, delete the requirement for *Total unspecified impurities* in *Impurity Table 1*.

(MD-OOD: F. Mao, M. Waddell.) RTS—C74146

Capecitabine



$C_{15}H_{22}FN_3O_6$ 359.35

Carbamic acid, [1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]-, pentyl ester;

Pentyl 1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate [154361-50-9].

DEFINITION

Capecitabine contains NLT 98.0% and NMT 102.0% of $C_{15}H_{22}FN_3O_6$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Sample: 2 mg of sample in 300 mg of potassium bromide

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Diluent: Methanol, acetonitrile, and water (7:1:12)

Solution A: 0.1% mixture of ■glacial■2S *(USP33)* acetic acid in water

Solution B: Methanol, acetonitrile, and *Solution A* (7:1:12)

Solution C: Methanol, acetonitrile, and *Solution A* (16:1:3)

Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

System suitability solution: 0.6 µg/mL each of USP Capecitabine RS, USP Capecitabine Related Compound A RS, USP Capecitabine Related Compound B RS, and USP Capecitabine Related Compound C RS in *Diluent*. [NOTE—Sonicate if necessary.]

Standard solution: 0.6 mg/mL of USP Capecitabine RS in *Diluent*. [NOTE—Sonicate if necessary.]

Sample solution: 0.6 mg/mL of Capecitabine in *Diluent*. [NOTE—Sonicate if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1*. The relative retention times are measured with respect to capecitabine.]

In-Process Revision

Suitability requirements
Resolution: NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{22}FN_3O_6$ in the portion of Capecitabine taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = concentration of Capecitabine in the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Change to read:

Organic Impurities
• **PROCEDURE**
Diluent, Solution B, Solution C, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capecitabine taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for Capecitabine from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = concentration of Capecitabine in the *Sample solution* (mg/mL)
F = relative response factor for the impurity, from *Impurity Table 1*
Acceptance criteria
Individual impurities: See *Impurity Table 1.*
Total impurities: NMT 1.5%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +96.0° to +100.0°
Sample solution: 10 mg/mL, on the anhydrous and solvent-free basis, in methanol, at 20°
• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Capecitabine RS
USP Capecitabine Related Compound A RS
USP Capecitabine Related Compound B RS
USP Capecitabine Related Compound C RS

BRIEFING

Capecitabine Tablets, *USP 32* page 1775 and page 72 of *PF* 35(1) [Jan.–Feb. 2009]. On the basis of comments received, it is proposed to make the following changes:
1. Specify the use of glacial acetic acid in *Solution A* for the *Mobile phase* preparation in the *Assay*.
2. Delete the requirement for total unspecified impurities in the *Organic Impurities* test, to be consistent with ICH guidelines.
3. Modify *Impurity Table 1* to include known process impurities that are likely to be present in the *Sample solution.* Add a footnote to *Impurity Table 1* specifying that process impurities are not included in the *Total degradation products* requirement.

(MD-OOD: F. Mao, M. Waddell.) RTS—C72555

Capecitabine Tablets

DEFINITION
Capecitabine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of capecitabine ($C_{15}H_{22}FN_3O_6$).

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
Analytical wavelength: 1500–1760 cm^{-1}
Sample: Grind one Tablet to a fine powder with a mortar and pestle. Mix 1 mg of this sample with 300 mg of potassium bromide.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

Change to read:

• **PROCEDURE**
Diluent: Methanol, acetonitrile, and water (7:1:12)
Solution A: 0.1% mixture of ■glacial■2S *(USP33)* acetic acid in water
Solution B: Methanol, acetonitrile, and *Solution A* (7:1:12)
Solution C: Methanol, acetonitrile, and *Solution A* (16:1:3)
Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

System suitability solution: Includes 0.6 µg/mL of USP Capecitabine RS, 0.6 µg/mL of USP Capecitabine Related Compound A RS, 0.6 µg/mL of USP Capecitabine Related Compound B RS, and 0.6 µg/mL of USP Capecitabine Related Compound C RS in *Diluent.* [NOTE—Sonicate if necessary.]
Standard solution: 0.6 mg/mL of USP Capecitabine RS in *Diluent.* [NOTE—Sonicate if necessary.]
Sample solution: Equivalent to 0.6 mg/mL of Capecitabine, from powdered Tablets (NLT 20), in *Diluent.* [NOTE—Pass through a PVDF 0.45-µm membrane filter, and use the filtrate.] [NOTE—Sonicate if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)

In-Process Revision

Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Autosampler temperature: 5°
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1*. The relative retention times are measured with respect to capecitabine.]
Suitability requirements
Resolution: NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{22}FN_3O_6$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩
Medium: Water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min
Sample solution: *Sample* per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*. Pass a portion of the solution under test through a 0.45-µm fiberglass filter.
Standard solutions
For Tablets labeled to contain 150 mg: 17 mg of USP Capecitabine RS in 100 mL of *Medium*
For Tablets labeled to contain 500 mg: 28 mg of USP Capecitabine RS in 50 mL of *Medium*
Analysis: Determine the amount of $C_{15}H_{22}FN_3O_6$ dissolved by employing UV absorption at the wavelength of maximum absorbance at 304 nm ~~(for Tablets labeled to contain 150 mg) and at 325 nm (for Tablets labeled to contain 500 mg)~~▲▲*USP33* on portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with the appropriate *Standard solution*, using a 1-mm quartz cell. Calculate the percentage of $C_{15}H_{22}FN_3O_6$ dissolved in each Tablet:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of capecitabine in the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
L = label claim (mg/Tablet)
Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{22}FN_3O_6$ is dissolved.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	0.3
Capecitabine related compound B	0.19	0.81	0.3
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine	0.36	0.89	0.1
5′-Deoxy-5-fluoro-*N*4-(2-methyl-1-butyloxycarbonyl)cytidine + 5′-Deoxy-5-fluoro-*N*4-(3-methyl-1-butyloxycarbonyl)cytidine	0.95	1.01	0.5
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.06	1.00	0.3
[1-[5-Deoxy-2-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.09	1.00	0.2
Capecitabine related compound C	1.11	0.91	0.3
[1-[5-Deoxy-3-*O*-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.20	1.00	0.3
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-*N*4-(pentyloxycarbonyl)cytidine	1.37	0.85	0.1
Individual unspecified impurity	—	1.00	0.1
~~Total unspecified impurities~~	~~—~~	~~—~~	~~0.5~~■■2S *(USP33)*

In-Process Revision

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**

Diluent, Solution A, Solution B, Solution C, System suitability solution, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for capecitabine from the *Standard solution*

C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)

F = relative response factor for each impurity, from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total ~~impurities:~~ ■degradation products: ■2S (*USP33*) NMT 2.0%

~~Impurity Table~~

~~Name~~	~~Relative Retention Time~~	~~Relative Response Factor (RRF)~~	~~Acceptance Criteria, NMT (%)~~
~~Capecitabine related compound A~~	~~0.18~~	~~1.05~~	~~1.0~~
~~Capecitabine related compound B~~	~~0.19~~	~~0.81~~	~~1.0~~
~~Capecitabine~~	~~1.00~~	~~1.00~~	~~—~~
~~Capecitabine related compound C~~	~~1.11~~	~~0.91~~	~~0.5~~
~~Individual unspecified impurity~~	~~—~~	~~1.00~~	~~0.1~~
~~Total unspecified impurities~~	~~—~~	~~—~~	~~0.5~~

■Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	1.0
Capecitabine related compound B	0.19	0.81	1.0
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine*	0.36	0.89	—
5′-Deoxy-5-fluoro-*N*4-(2-methyl-1-butyloxycarbonyl)cytidine + 5′-Deoxy-5-fluoro-*N*4-(3-methyl-1-butyloxycarbonyl)cytidine*	0.95	1.01	—
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.06	1.00	—
[1-[5-Deoxy-2-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.09	1.00	—
Capecitabine related compound C	1.11	0.91	0.5
[1-[5-Deoxy-3-*O*-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.20	1.00	—
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-*N*4-(pentyloxycarbonyl)cytidine*	1.37	0.85	—
Individual unspecified degradation product	—	1.00	0.1
The impurities marked with an "*" are process impurities and are not included in the total degradation products.			

■2S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

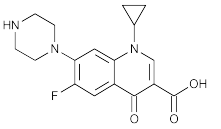
USP Capecitabine RS
USP Capecitabine Related Compound A RS
USP Capecitabine Related Compound B RS
USP Capecitabine Related Compound C RS

BRIEFING

Ciprofloxacin, *USP 32* page 1939. It is proposed to replace the current TLC identification procedure with the HPLC retention time agreement from the *Assay*.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C74255

Ciprofloxacin



$C_{17}H_{18}FN_3O_3$ 331.34

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-;

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [85721-33-1].

DEFINITION

Ciprofloxacin contains NLT 98.0% and NMT 102.0% of $C_{17}H_{18}FN_3O_3$, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption**

The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima at the same wavelengths as that of a similar preparation of USP Ciprofloxacin RS.

Change to read:

- **B. ~~Thin-Layer Chromatographic Identification Test~~**

~~**Standard solution:** 10.0 mg/mL of USP Ciprofloxacin RS in 6 N ammonium hydroxide~~

~~**Sample solution:** 10.0 mg/mL of Ciprofloxacin in 6 N ammonium hydroxide~~

~~**Developing solvent system:** Methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4.4.2.1)~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of silica gel mixture~~

~~**Application volume:** 5 µL~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Separately apply, as 1-cm bands to the thin-layer chromatographic plate, 5 µL of each solution to a suitable thin-layer chromatographic plate. Place the plate in an atmosphere of ammonia for about 15 min, then transfer the plate to a suitable unsaturated chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under both short- and long-wavelength UV light.~~

~~**Acceptance criteria:** The intensity and R_F value of the principal band from the *Sample solution* corresponds to that from the *Standard solution*.~~ ■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S (*USP33*)

ASSAY

- **Procedure**

Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.

Mobile phase: Acetonitrile and *Solution A* (13:87)

Standard solution: Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask. Add 0.1 mL of 7% phosphoric acid and dilute with *Mobile phase* to volume.

System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 30 ± 1°

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

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

[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

Tailing factor: NMT 2.5 for the ciprofloxacin peak, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{17}H_{18}FN_3O_3$ in the portion of Ciprofloxacin taken:

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

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **Residue on Ignition ⟨281⟩:** NMT 0.1%, except that where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is NMT 0.2%.
- **Chloride**

Sample solution: Add 30.0 mL of water to 0.5 g of Ciprofloxacin, shake for 5 min, and pass through chloride-free filter paper. Use the filtrate as the *Sample solution*.

Standard solution: 8.2 µg/mL of sodium chloride (5 µg/mL of chloride)

Analysis

Samples: *Sample solution* and *Standard solution*

Transfer 15.0 mL of the *Sample solution* to a 50-mL color-comparison tube, and transfer 10.0 mL of the *Standard solution* to a second matched 50-mL color-comparison tube, add 5.0 mL of water to the tube containing the *Standard solution*, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix.

Acceptance criteria: The turbidity exhibited by the *Sample solution* does not exceed that of the *Standard solution* (0.02%).

- **Sulfate**

Sample solution: Dissolve 0.5 g in 5.0 mL of 2 N acetic acid and 15.0 mL of water.

Standard solution: 18.1 µg/mL of potassium sulfate in 30% alcohol (10 µg/mL of sulfate)

Analysis

Samples: *Sample solution* and *Standard solution*

To each of two 50-mL matched color-comparison tubes transfer 1.50 mL of the *Standard solution*. To each tube add, successively and with continuous shaking, 1.0 mL of 250 mg/mL barium chloride solution, and allow to stand for 1 min. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Sample solution* and 0.5 mL of 30% acetic acid, and mix.

In-Process Revision

Acceptance criteria: The turbidity exhibited in the tube containing the *Sample solution* does not exceed that of the tube containing the *Standard solution* (0.04%).

- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: LIMIT OF FLUOROQUINOLONIC ACID**

Standard stock solution: Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide and dilute with water to volume.

Standard solution: Dilute 2.0 mL of the *Standard stock solution* with water to 10.0 mL.

Sample solution: 10.0 mg/mL of Ciprofloxacin in 0.1 N acetic acid

Developing solvent system: Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 5 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chamber in which is placed a beaker containing 50 mL of ammonium hydroxide. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spot from the *Sample solution*, at an R_F value corresponding to the principal spot from the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%).

- **PROCEDURE 2**

Solution A, Mobile phase, System suitability stock solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Prepare as directed in the *Assay*.

Analysis: Proceed as directed in the *Assay*. Calculate the percentage of each impurity peak in the chromatogram from the *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria

Ciprofloxacin ethylenediamine analog or any other individual impurity peak: NMT 0.2%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **CLARITY OF SOLUTION:** Dissolve 0.25 g in 10 mL of 0.1 N hydrochloric acid: a clear to slightly opalescent solution is obtained.
- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, the total microbial count does not exceed 1000 cfu/g, and the total combined molds and yeast count does not exceed 100 cfu/g. It also meets the requirement for absence of *Salmonella* species and *Escherichia coli*.
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry it in a vacuum at 120° for 6 h: it loses NMT 1.0% of its weight, except that where it is labeled as intended for use in preparing Ciprofloxacin for Oral Suspension, it loses between 10% and 20% of its weight.
- **STERILITY TESTS** ⟨**71**⟩**:** Where the label states that it is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where the label states that it is sterile or where the label states that Ciprofloxacin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursion permitted between 15° and 30°, and avoid excessive heat.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

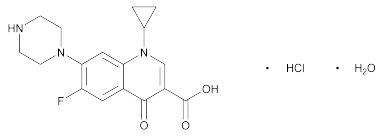
USP Ciprofloxacin RS
USP Ciprofloxacin Ethylenediamine Analog RS
USP Endotoxin RS
USP Fluoroquinolonic Acid RS

BRIEFING

Ciprofloxacin Hydrochloride, *USP 32* page 1940—See briefing under *Ciprofloxacin*.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C74255

Ciprofloxacin Hydrochloride



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$ 385.82

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-, monohydrochloride, monohydrate;
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride, monohydrate [86393-32-0].

DEFINITION

Ciprofloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{17}H_{18}FN_3O_3 \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩

Change to read:

- **B. ~~THIN-LAYER CHROMATOGRAPHY~~**

~~**Standard solution:** 10 mg/mL of USP Ciprofloxacin Hydrochloride RS in water~~

~~**Sample solution:** 10 mg/mL of Ciprofloxacin Hydrochloride in water~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨*621*⟩*, Thin-Layer Chromatography*.)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of silica gel mixture~~

~~**Application volume:** 5 µL~~

~~**Developing solvent system:** Methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1)~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Proceed as directed under the general test chapter. Separately apply, as 1-cm bands to the thin-layer chromatographic plate. Place the plate in an atmosphere of ammonia for about 15 min, then transfer the plate to a suitable unsaturated chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent~~

In-Process Revision

~~front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under both short- and long-wavelength UV light.~~

~~**Acceptance criteria:** The intensity and R_F value of the principal band of the *Sample solution* corresponds to that of the *Standard solution.*~~

■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*■2S (*USP33*)

- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩

ASSAY

- **PROCEDURE**

Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.

Mobile phase: Acetonitrile and *Solution A* (13:87)

Standard solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

System suitability solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: 0.5 mg/mL of Ciprofloxacin Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; packing L1

Temperature: 30 ± 1°

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak, *System suitability solution*

Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

Tailing factor: NMT 2.5 for the ciprofloxacin peak, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{17}H_{18}FN_3O_3$ in the portion of Ciprofloxacin Hydrochloride taken:

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

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A 375-mg portion shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.04%).
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: LIMIT OF FLUOROQUINOLONIC ACID**

Standard solution: Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide, add water to volume, and mix. Transfer 2.0 mL of this solution to a 10.0-mL volumetric flask, and dilute with water to volume.

Sample solution: 10 mg/mL of Ciprofloxacin Hydrochloride in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 5 µL

Developing solvent system: Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed under the general test chapter. Place the plate in a suitable chamber in which is placed a beaker containing 50 mL of ammonium hydroxide. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system*. Allow the chromatogram to develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spot of the *Sample solution*, at an R_F value corresponding to the principal spot of the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%).

- **PROCEDURE 2**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Prepare as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity peak in the chromatogram of the *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity peak
r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: NMT 0.2% for the ciprofloxacin ethylenediamine analog or any other individual impurity peak

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **pH** ⟨**791**⟩**:** 3.0–4.5, in a 25 mg/mL solution
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** 4.7%–6.7%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Ciprofloxacin Ethylenediamine Analog RS
USP Ciprofloxacin Hydrochloride RS
USP Fluoroquinolonic Acid RS

BRIEFING

Ciprofloxacin Injection, *USP 32* page 1942—See briefing under *Ciprofloxacin*.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C74255

In-Process Revision

Ciprofloxacin Injection

DEFINITION

Ciprofloxacin Injection is a sterile solution of Ciprofloxacin or Ciprofloxacin Hydrochloride in Water for Injection, in 5% Dextrose Injection, or in 0.9% Sodium Chloride Injection prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

Change to read:

- ~~**A. THIN-LAYER CHROMATOGRAPHY**~~

~~**Standard solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in water~~
~~**Sample solution:** 0.5 mg/mL of Ciprofloxacin from Injection in water~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography.*)~~
~~**Mode:** TLC~~
~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture~~
~~**Application volume:** 10 µL~~
~~**Developing solvent system:** Methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1)~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Proceed as directed under General Chapter. Separately apply, as 1-cm bands to the thin-layer chromatographic plate. Place the plate in an atmosphere of ammonia for about 15 min, then transfer the plate to a suitable unsaturated chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under both short- and long-wavelength UV light.~~
~~**Acceptance criteria:** The intensity and R_F value of the principal band from the *Sample solution* corresponds to that from the *Standard solution*.~~

■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S *(USP33)*

ASSAY

- **PROCEDURE**

Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.
Mobile phase: Acetonitrile and *Solution A* (13:87)
Standard solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*
System suitability solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.
Sample solution: Equivalent to 0.5 mg/mL of Ciprofloxacin from Injection diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30 ± 1°
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak, *System suitability solution*
Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*
Tailing factor: NMT 2.5 for the ciprofloxacin peak, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the label claim of $C_{17}H_{18}FN_3O_3$ in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin, *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0% of $C_{17}H_{18}FN_3O_3$

OTHER COMPONENTS

- **LACTIC ACID CONTENT**

Mobile phase: Acetonitrile and 0.005 N sulfuric acid (3:17)
Standard solution: 0.8 mg/mL of USP Sodium Lactate RS in water or 4 mg/mL where the Injection is labeled as being a concentrated form
Sample solution: Use the undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 208 nm
Column: 7.8-mm × 30-cm; packing L17
Temperature: 40 ± 1°
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: NMT 2.0%
[NOTE—After each analysis, rinse the column with a mixture of 0.01 N sulfuric acid and acetonitrile to elute the ciprofloxacin from the column. Promptly regenerate the column with 0.01 N sulfuric acid, and the column may be reused or stored.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration of lactic acid ($C_3H_6O_3$) in mg/mg of ciprofloxacin:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

r_U = peak response of lactic acid from the *Sample solution*
r_S = peak response of lactic acid from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lactic acid, 90.08
M_{r2} = molecular weight of sodium lactate, 112.07

Acceptance criteria: 0.288–0.352 mg of lactic acid for each mg of ciprofloxacin claimed on the label, except that where the Injection is labeled as being a concentrated form, it contains 0.335–0.409 mg of lactic acid for each mg of ciprofloxacin claimed on the label

- **DEXTROSE CONTENT** (if present)

Analysis

Sample solution: Undiluted Injection

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage (g/100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$A \times R \times (M_{r1}/M_{r2}) \times (100/F)$$

A = 100 mm divided by the length of the polarimeter tube (mm)
R = observed rotation (degrees)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16
F = midpoint of the specific rotation range for anhydrous dextrose (degrees), 52.9

Acceptance criteria: 4.75–5.25 g/100 mL

- **SODIUM CHLORIDE CONTENT** (if present)

Sample solution: Injection

Analysis: Transfer 10.0 mL of *Sample solution* to a suitable container, dilute with water to 150 mL, add 1.5 mL of potassium chromate TS, and titrate with 0.1 N silver nitrate TS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Acceptance criteria: 85.5–94.5 mg

IMPURITIES

Organic Impurities

- **PROCEDURE: LIMIT OF CIPROFLOXACIN ETHYLENEDIAMINE ANALOG**

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Proceed as directed for *Analysis* in the *Assay*. Calculate the percentage of ciprofloxacin ethylenediamine analog from the chromatogram obtained from the *Sample solution:*

$$\text{Result} = F \times r_A/(0.7r_A + r_C) \times 100$$

F = correction factor for ciprofloxacin ethylenediamine analog, 0.7
r_A = ciprofloxacin ethylenediamine analog peak response
r_C = ciprofloxacin peak response

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements
- **pH** ⟨791⟩**:** 3.5–4.6, except that where the Injection is labeled as being a concentrated form, its pH is 3.3–3.9
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **COLOR AND ACHROMICITY** ⟨631⟩ (where it is labeled as being a concentrated form)**:** It has no more color than a solution prepared by diluting 5.0 mL of *Matching Fluid O* with 95.0 mL of 0.12 N hydrochloric acid.
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections* ⟨1⟩*, Volume in Container*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and store in a cool place or at controlled room temperature. Avoid freezing and exposure to light.
- **LABELING:** The label indicates whether the vehicle is Sterile Water for Injection, 5% Dextrose Injection, or 0.9% Sodium Chloride Injection. Label the Injection that has Sterile Water for Injection as the vehicle to indicate that it is a concentrated form that must be diluted to appropriate strength (1–2 mg/mL) with 5% Dextrose Injection or 0.9% Sodium Chloride Injection before administration, and that the resulting solution is stable for up to 14 days when stored in a cool place or at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ciprofloxacin Ethylenediamine Analog RS
USP Ciprofloxacin Hydrochloride RS
USP Endotoxin RS
USP Sodium Lactate RS

BRIEFING

Ciprofloxacin Ophthalmic Solution, *USP 32* page 1944—See briefing under *Ciprofloxacin*.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C74255

Ciprofloxacin Ophthalmic Solution

DEFINITION

Ciprofloxacin Ophthalmic Solution is a sterile, aqueous solution of Ciprofloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

Change to read:

- ~~**A. THIN-LAYER CHROMATOGRAPHY**~~

~~**Standard solution:** 3.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in water~~

~~**Sample solution:** 3 mg/mL of ciprofloxacin from Ophthalmic Solution in water~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography.*)~~

~~**Mode:** TLC~~

~~**Adsorbent:** 0.25-mm layer of silica gel mixture~~

~~**Application volume:** 3 µL~~

~~**Developing solvent system:** Methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1)~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Proceed as directed under General Chapter. Separately apply, as 1-cm bands to the thin-layer chromatographic plate. Place the plate in an atmosphere of ammonia for about 15 min, then transfer the plate to a suitable unsaturated chromatographic chamber, and develop the chromatogram in developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under both short- and long-wavelength UV light.~~

~~**Acceptance criteria:** The intensity and R_f value of the principal band from the *Sample solution* corresponds to that from the *Standard solution*.~~

■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S *(USP33)*

ASSAY

- **PROCEDURE**

Solution A: 0.005 M tetrabutylammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Methanol and *Solution A* (1:3)

Standard solution: 0.14 mg/mL of USP Ciprofloxacin Hydrochloride RS in water

System suitability solution: 0.01 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*

In-Process Revision

Sample solution: Equivalent to 0.12 mg/mL of ciprofloxacin from Ophthalmic Solution, in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: Between ciprofloxacin ethylenediamine analog and ciprofloxacin is NLT 1.5, *System suitability solution*
Capacity factor: 1.5–6 for the ciprofloxacin peak, *Standard solution*
Column efficiency: NLT 500 theoretical plates, *Standard solution*
Tailing factor: 0.9–2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the label claim of $C_{17}H_{18}FN_3O_3$ in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (unit/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{17}H_{18}FN_3O_3$

SPECIFIC TESTS
- **PH ⟨791⟩:** 3.5–5.5
- **STERILITY TESTS ⟨71⟩:** It meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers protected from light, at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Ciprofloxacin Ethylenediamine Analog RS
USP Ciprofloxacin Hydrochloride RS

BRIEFING

Ciprofloxacin Tablets, *USP 32* page 1945. It is proposed to omit *Identification* test *B* from the monograph because the procedure is not orthogonal to *Identification* test *A*.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C68281

Ciprofloxacin Tablets

DEFINITION
Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

Change to read:

• **~~A.~~** ■2S (*USP33*) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Delete the following:

■• **~~B. THIN-LAYER CHROMATOGRAPHY~~**
~~**Standard solution:** 1.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in water~~
~~**Sample solution:** Place a number of Tablets, equivalent to about 1500 mg of ciprofloxacin, in a suitable flask containing 750 mL of water, and sonicate for about 20 min. Dilute with water to 1000 mL, and mix. Centrifuge a portion of this suspension, and use the clear supernatant.~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)~~
~~**Mode:** TLC~~
~~**Adsorbent:** 0.25-mm layer of silica gel mixture~~
~~**Application volume:** 10 µL~~
~~**Developing solvent system:** Methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4.4:2.1)~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Proceed as directed under General Chapter. Separately apply, as 1-cm bands to the thin-layer chromatographic plate. Place the plate in an atmosphere of ammonia for about 15 min, then transfer the plate to a suitable unsaturated chromatographic chamber, and develop the chromatogram in developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under both short- and long-wavelength UV light.~~
~~**Acceptance criteria:** The intensity and R_F value of the principal band from the *Sample solution* corresponds to that from the *Standard solution*.~~ ■2S (*USP33*)

ASSAY
- **PROCEDURE**
Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 2.0 ± 0.1.
Solution B: Acetonitrile and *Solution A* (13:87)
Solution C: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.
Mobile phase: Acetonitrile and *Solution C* (13:87)
System suitability solution: 0.05 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*
Standard solution: 0.2 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Solution B*
Sample solution: Transfer 5 Tablets to a 500-mL volumetric flask, add 400 mL of *Solution B*, and sonicate for about 20 min. Dilute with *Solution B* to volume, mix, and pass through a 0.45-µm membrane filter. Prepare nominally 0.20 mg/mL of ciprofloxacin from the filtrate with *Solution B*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30 ± 1°
Flow rate: 1.5 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The retention time is 6.4–10.8 min for ciprofloxacin. The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: Between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak is NLT 6, *System suitability solution*
Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*
Tailing factor: NMT 2.0 for the ciprofloxacin peak, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{18}FN_3O_3$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL), calculated on the anhydrous basis
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩**
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Sample solutions: Sample per ⟨711⟩ *Dissolution*. Dilute with *Medium* as needed.
Standard solution: USP Ciprofloxacin Hydrochloride RS in *Medium*
Spectrometric conditions
Mode: UV
Analytical wavelength: 276 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: An amount of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$) equivalent to NLT 80% (Q) of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is dissolved in 30 min.
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Ciprofloxacin Ethylenediamine Analog RS
USP Ciprofloxacin Hydrochloride RS

BRIEFING

Citalopram Tablets. *USP 32* page 1949. On the basis of comments received, it is proposed to modify the monograph as follows:

1. Include an alternative sample preparation procedure for *Identification* test *A*.
2. The sample solution preparation and final concentration in *Uniformity of Dosage Units* is identical to the *Assay*. The redundant procedure and calculations are deleted from the *Uniformity of Dosage Units* section.
3. The test for *Organic Impurities* is simplified by deleting the *Related compounds stock solution* and *Peak identification solution*.
4. The concentration of the *Standard stock solution* is modified so that the *System suitability solution* will have the appropriate concentration of citalopram hydrobromide.
5. The *System suitability solution* is modified to serve the peak identification purposes.
6. The system suitability parameters, *Capacity factor* and *Column efficiency,* have been deleted, because the method has four other system suitability parameters: *Resolution*, *Tailing factor*, *Relative standard deviation*, and *Signal-to-noise ratio*, which are adequate to ensure the suitability of the HPLC system.
7. Citalopram has been added to the impurity table to provide appropriate reference for calculations of the *Relative retention time*.

(MD-PP: R. Ravichandran.) RTS—C59574

Citalopram Tablets

DEFINITION

Citalopram Tablets contain an amount of Citalopram Hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base ($C_{20}H_{21}FN_2O$).

IDENTIFICATION

Change to read:

• **A. INFRARED ABSORPTION ⟨197K⟩**
Sample: Extract finely ground Tablet powder containing 200 mg of citalopram with 30 mL of water, and filter. Add 1 mL of 1 N sodium hydroxide to the filtrate, and extract with 50 mL of cyclohexane by shaking for 10 min. Pass the cyclohexane layer through a silicone-treated filter paper into a beaker. Reduce the filtrate down to 3 mL, using gentle heat as necessary. Transfer the hot solution to a small centrifuge tube. Induce crystallization while cooling by scratching the side of the test tube with a spatula. Centrifuge the mixture, and decant off the cyclohexane. Dry the residue under vacuum in a desiccator. ■[NOTE—If crystallization fails to occur in the above procedure, use the following alternative procedure. Extract finely ground Tablet powder containing about 50 mg of citalopram, mix with 10 mL of chloroform in a test tube, and sonicate for 1 min. Centrifuge for 10 min, and filter into a beaker. Evaporate to dryness with nitrogen and if necessary induce crystallization by etching the beaker.]■2S *(USP33)*
Mix approximately 2 mg of the residue with approximately 300 mg of potassium bromide, and record the IR spectrum.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE**

Solution A: 1.42 mg/mL of anhydrous dibasic sodium phosphate in water

Diluent: Methanol and *Solution A* (4:1)

Mobile phase: 0.77 mg/mL of dodecyltrimethylammonium bromide in *Diluent*

Internal standard solution: 0.25 mg/mL of USP Citalopram Related Compound F RS in *Diluent*

Standard stock solution: 1.25 mg/mL of USP Citalopram Hydrobromide RS in *Diluent*

Standard solution: Pipet 5.0 mL of the *Standard stock solution* and 5.0 mL of *Internal standard solution* into a 50-mL volumetric flask. Dilute with *Diluent* to volume.

Sample solution: Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Solution A*, and shake by mechanical means until disintegrated. Add 100 mL of methanol, and sonicate for about 5 min. Allow to cool to room temperature, then dilute with *Diluent* to volume. Allow to stand until the residue settles before taking an aliquot for dilution. Transfer a volume of the clear supernatant to a 50-mL volumetric flask to obtain a final nominal concentration between 0.090 and 0.10 mg/mL of citalopram. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume. Pass a portion through a filter (PTFE) having a 0.45-µm or finer porosity.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for citalopram related compound F and citalopram are about 1.36 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between citalopram and citalopram related compound F

Column efficiency: NLT 2000 theoretical plates, calculated from the citalopram peak

Relative standard deviation: NMT 1.5% for the citalopram peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of label claim of citalopram in the portion of the Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = ratio of the peak response of citalopram to the internal standard from the *Sample solution*

R_S = ratio of the peak response of citalopram to the internal standard from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of citalopram, 324.39

M_{r2} = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{20}H_{21}FN_2O$

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩

Buffer solution: pH 1.5 buffer (prepared by transferring 118 mL of 1 N hydrochloric acid and 82 mL of 1 N sodium hydroxide to a 1000-mL volumetric flask, diluting with water to volume, and adjusting with 1 N sodium hydroxide to a pH of 1.5

Medium: *Buffer solution;* 800 mL, deaerated

Apparatus 1: 100 rpm

Time: 30 min

Detector: UV 239 nm

Sample solutions: Sample per *Dissolution* ⟨711⟩. Pass through a 0.45-µm PVDF filter, dilute with *Medium* as needed.

Standard solution: 12 µg/mL of USP Citalopram Hydrobromide RS in *Medium*

Analysis: Calculate the amount of citalopram dissolved, as a percentage:

$$(A_U \times C_S \times M_{r1} \times D \times 800 \times 100)/(A_S \times M_{r2} \times L)$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

M_{r1} = molecular weight of citalopram, 324.39

M_{r2} = molecular weight of citalopram hydrobromide, 405.30

D = dilution factor of the *Sample solution*

800 = volume of *Medium* (mL)

L = Tablet label claim of citalopram (mg)

Tolerances: NLT 80% (Q) of the labeled amount of citalopram ($C_{20}H_{21}FN_2O$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

~~**Procedure for content uniformity**~~

~~**Solution A, Diluent, Internal standard solution, Mobile phase, Chromatographic system** and **System suitability:** Proceed as directed in the *Assay*.~~

~~**Sample solution:** Transfer 1 Tablet to a 100-mL volumetric flask, add 10 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 40 mL of methanol, and sonicate for about 5 min. Allow to cool to room temperature. Add sufficient volume of *Internal standard solution*, and dilute to prepare 0.1 mg/mL of citalopram and 0.025 mg/mL of internal standard with *Diluent*. Pass a portion of this solution through a membrane filter (PVDF) having a 0.45-µm or finer porosity, and use the filtrate.~~

~~**Standard solution:** Use the *Standard solution*, prepared as directed in the *Assay*.~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of $C_{20}H_{21}FN_2O$ in the portion of sample taken:~~

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$~~

~~r_U = ratio of the peak responses of citalopram to the internal standard from the *Sample solution*~~

~~r_S = ratio of the peak responses of citalopram to the internal standard from the *Standard solution*~~

~~C_S = concentration of the *Standard solution* (mg/mL)~~

~~C_U = concentration of the *Sample solution* (mg/mL)~~

~~M_{r1} = molecular weight of citalopram, 324.39~~

~~M_{r2} = molecular weight of citalopram hydrobromide, 405.30~~

■2S (*USP33*)

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**

Solution A: 3.15 mg/mL of potassium dihydrogen phosphate and 3.60 mg/mL of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) in water

Mobile phase: Methanol, acetonitrile, and *Solution A* (38:7:55). Adjust with phosphoric acid to a pH of 6.5.

In-Process Revision

Standard stock solution: ~~0.5 mg/mL~~■0.25 mg/mL■2S (USP33) of USP Citalopram Hydrobromide RS in *Mobile phase*

Standard solution: 0.625 µg/mL of citalopram hydrobromide from *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.05 µg/mL of citalopram hydrobromide from *Standard solution* in *Mobile phase*

~~**Related compounds stock solutions:** Separately prepare 0.1 mg/mL each of USP Citalopram Related Compound A RS, USP Citalopram Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E RS in *Mobile phase*.~~

~~**Peak identification solution:** 1 µg/mL each of USP Citalopram Related Compound A RS, USP Citalopram Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E RS, in *Standard stock solution*~~

~~**System suitability solution:** Dilute 0.5 mL of *Citalopram related compound C stock solution* and 25.0 mL of *Standard stock solution* with *Mobile phase* to 50 mL to obtain a solution containing 1 µg/mL of Citalopram related compound C and 0.25 mg/mL of citalopram hydrobromide.~~

■**System suitability solution:** 1 µg/mL each of USP Citalopram Related Compound A RS, USP Citalopram Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E RS, in *Standard stock solution*■2S (USP33)

Sample solution: Transfer 10 Tablets into a 200-mL volumetric flask, add 25 mL of *Solution A*, and shake by mechanical means until disintegrated. Add 100 mL of a mixture of methanol and water (1:1), mix, and sonicate for about 5 min. Allow to cool, dilute with a mixture of methanol and water (1:1) to volume, and mix thoroughly. Allow the excipients to settle. Dilute as necessary to obtain a final concentration of 0.5 mg/mL of citalopram. Pass a portion of this solution through a polytetrafluoroethylene (PTFE) membrane filter having a 0.45-µm or finer porosity, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 0.8 mL/min

Injection size: 20 µL

System suitability

~~**Sample:** *Standard solution*~~

~~**Suitability requirements**~~

~~**Capacity factor, k′:** NLT 3.5~~

~~**Column efficiency:** NLT 5000 theoretical plates~~

~~**Tailing factor:** NMT 1.5~~

~~The citalopram peak shows no shoulders or excessive tailing.~~

~~**Relative standard deviation:** NMT 5%~~

~~**Sample:** *Sensitivity solution*~~

~~**Suitability requirements**~~

~~**Signal-to-noise ratio:** At least 3~~

~~**Sample:** *System suitability solution*~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 3 between citalopram related compound C and citalopram~~

~~**Sample:** *Peak identification solution*~~

~~**Suitability requirements**~~

~~**Resolution:** The four related compound peaks are baseline resolved from each other and the citalopram peak.~~

~~[NOTE—For identification purposes, approximate relative retention times are given in *Impurity Table 1*.]~~

■**Samples:** *System suitabilty solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—The relative retention times are given in *Impurity Table 1*.]

Suitability requirements

Resolution: NLT 3 between citalopram related compound C and citalopram, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5%, *Standard solution*

Signal-to-noise ratio: NLT 3, *Sensitivity solution*■2S (USP33)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response for each citalopram related compound from the *Sample solution*

r_S = peak response of the corresponding peak in the *Standard solution*

C_S = concentration of citalopram hydrobromide in the *Standard solution* (mg/mL)

C_U = nominal concentration of citalopram hydrobromide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of citalopram, 324.39

M_{r2} = molecular weight of citalopram hydrobromide, 405.30

F = relative response factor for each impurity relative to citalopram (free base)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.8%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A	0.43	0.77	0.2
Citalopram related compound B	0.60	0.98	0.25
Citalopram related compound C	0.83	0.69	0.25
■Citalopram	1.0	—	—■2S (USP33)
Citalopram related compound E	1.32	0.91	0.1
Any other individual unidentified impurity	—	1.0	0.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citalopram Hydrobromide RS
  USP Citalopram Related Compound A RS
  USP Citalopram Related Compound B RS
  USP Citalopram Related Compound C RS
  USP Citalopram Related Compound E RS
  USP Citalopram Related Compound F RS

In-Process Revision

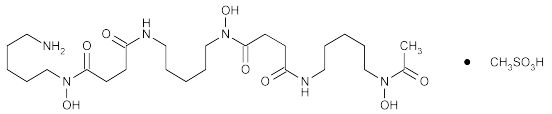
BRIEFING

Deferoxamine Mesylate, *USP 32* page 2062. As a part of monograph modernization, the following changes are proposed for this monograph:

1. Replace the nonspecific wet chemistry *Identification* test with a more specific IR spectroscopic test procedure, and add *Identification* test *B* based on the HPLC retention time agreement of the major peaks in the *Sample solution* and *Standard solution.*
2. Add a test for *Organic Impurities* based on the stability indicating HPLC procedure.
3. Replace the spectrophotometric *Assay* with a specific, stability-indicating HPLC procedure employed in the *Organic Impurities* test. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with the Zorbax SB C-18 or Zorbax Eclipse XDB-C18 brands of L1 column. The typical retention time for the deferoxamine peak is about 15 min.
4. Because the current nonspecific *Assay* does not differentiate between deferoxamine mesylate and its impurities, the introduction of the specific *Assay* procedure necessitates the change in the lower assay limit in the *Definition*, to reflect the actual content of deferoxamine mesylate present in the sample.
5. Add storage conditions to the *Packaging and Storage* section.

(MDGRE: E. Gonikberg, H. Ramanathan.) RTS—C66406

Deferoxamine Mesylate



$C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ 656.79

Butanediamide, *N*′-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxy-, monomethanesulfonate;
N-[5-[3-[(5-Aminopentyl)hydroxycarbamoyl]propionamido]pentyl]-3-[[5-(*N*-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid monomethanesulfonate (salt) [138-14-7].

DEFINITION

Change to read:

Deferoxamine Mesylate contains NLT ~~98.0%~~■95.0%■2S *(USP33)* and NMT 102.0% of $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$, calculated on the anhydrous basis.

IDENTIFICATION

Delete the following:

■• ~~**PROCEDURE**~~
~~**Sample:** 5 mg~~
~~**Analysis:** Dissolve *Sample* in 5 mL of water, add 2 mL of tribasic sodium phosphate solution (1 in 200), then add 10 drops of *β*-naphthoquinone-4-sodium sulfonate solution (1 in 40).~~
~~**Acceptance criteria:** A blackish brown color is produced.~~■2S *(USP33)*

Add the following:

■• **A. INFRARED ABSORPTION** ⟨197K⟩■2S *(USP33)*

Add the following:

■• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*■2S *(USP33)*

ASSAY

Change to read:

• **PROCEDURE**
~~**Solution A:** Dissolve 6.7 g of ferric chloride in dilute hydrochloric acid (1 in 100) in a 100-mL volumetric flask. Add dilute hydrochloric acid (1 in 100) to volume.~~
~~**Standard solution:** 1 mg/mL of USP Deferoxamine Mesylate RS~~
~~**Sample solution:** 1 mg/mL of Deferoxamine Mesylate~~
~~**Spectrometric conditions**~~
~~**Mode:** UV-Vis~~
~~**Analytical wavelength:** 485 nm~~
~~**Cell:** 1 cm~~
~~**Blank:** Water~~
~~**Analysis**~~
~~**Samples:** *Standard solution*, *Sample solution*, and *Blank*~~
~~Pipet 2 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* into separate 25-mL volumetric flasks. To each flask, add 3 mL of *Solution A*, and dilute with water to volume. Concomitantly determine the absorbances.~~
~~Calculate the percentage of $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ in the portion taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U = absorbance from the *Sample solution*~~
~~A_S = absorbance from the *Standard solution*~~
~~C_S = concentration of USP Deferoxamine Mesylate RS in the *Standard solution* (mg/mL)~~
~~C_U = concentration of Deferoxamine Mesylate in the *Sample solution* (mg/mL)~~
~~**Acceptance criteria:** 98.0%–102.0%~~

■**Solution A:** 1.32 g/L of dibasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and *Solution A* (1:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	80	20
35	57.5	42.5
35.1	88	12
40	88	12

Diluent: Acetonitrile and water (6:94)
Standard solution: 1.0 mg/mL of USP Deferoxamine Mesylate RS in *Diluent*
Sample solution: 1.0 mg/mL of Deferoxamine Mesylate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 7.5-cm column; 3.5-µm packing L1
Column temperature: 32°
Autosampler temperature: 5°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of deferoxamine mesylate ($C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$) in the portion of Deferoxamine Mesylate taken:

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

r_U = response of deferoxamine from the *Sample solution*
r_S = response of deferoxamine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous basis■2S (*USP33*)

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%, 2.0 g being used
- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A 1.2-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.012%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A 0.5-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 10 ppm

Add the following:

■**Organic Impurities**

- **PROCEDURE**

Solution A, Solution B, Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Standard stock solution: Use the *Standard solution,* prepared as directed in the *Assay.* [NOTE—USP Deferoxamine Mesylate RS contains Impurity A as a minor component.]
Standard solution: 0.01 mg/mL of USP Deferoxamine Mesylate RS in *Diluent,* from *Standard stock solution*
System suitability
Sample: *Standard stock solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between Impurity A and deferoxamine peaks, *Standard stock solution*
Relative standard deviation: NMT 5.0% for the deferoxamine peak, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Deferoxamine Mesylate taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of deferoxamine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1.*
Total impurities: NMT 5.0%
[NOTE—Reporting level for impurities is 0.04%.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity A*	0.85	1.5
Deferoxamine	1.0	—
Any unspecified impurity	—	1.0

*Des-methylene impurity (Desferrioxamine A_1).

■2S (*USP33*)

SPECIFIC TESTS

- **pH** ⟨**791**⟩**:** 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 2.0%
- **STERILITY TESTS** ⟨**71**⟩**:** Where the label states that Deferoxamine Mesylate is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where the label states that Deferoxamine Mesylate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ■Store in a cold place.■2S (*USP33*)

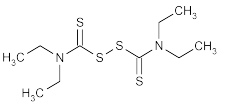
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Deferoxamine Mesylate RS
USP Endotoxin RS

BRIEFING

Disulfiram, *USP 32* page 2187. USP has received comments from a manufacturer stating that selenium is not used in the manufacturing process. In order to address this, a note is being added to the test for *Selenium* in the *Inorganic Impurities* section. In addition, to simplify the monograph, *Solution A* and *Solution B* in the *Assay* have been combined into a single *Buffer* solution.

(MD-PP: R. Ravichandran.) RTS—C74344

Disulfiram



$C_{10}H_{20}N_2S_4$ 296.54
Thioperoxydicarbonic diamide $[(H_2N)C(S)]_2S_2$, tetraethyl-; Bis(diethylthiocarbamoyl) disulfide [97-77-8].

DEFINITION

Disulfiram contains NLT 98.0% and NMT 102.0% of $C_{10}H_{20}N_2S_4$.

In-Process Revision

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

~~**Solution A:** 68 g/L of monobasic potassium phosphate~~
~~**Solution B:** Dilute 100 mL of *Solution A* with water to 1000 mL, and adjust with 45% potassium hydroxide solution to a pH of 7.0.~~
~~**Mobile phase:** Methanol and *Solution B* (7:3)~~
■**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide solution to a pH of 7.0.
Mobile phase: Methanol and *Buffer* (7:3)■2S *(USP33)*
Standard stock solution: 1 mg/mL of USP Disulfiram RS in alcohol [NOTE—Discard this solution after 5 days.]
Standard solution: 0.02 mg/mL of USP Disulfiram RS from *Standard stock solution* diluted with *Mobile phase*. [NOTE—Prepare the *Standard solution* fresh daily.]
Sample stock solution: Transfer 50 mg of Disulfiram to a 50-mL volumetric flask, add 40 mL of alcohol, sonicate for 5 min to completely dissolve, cool, and dilute with alcohol to volume. [NOTE—Discard this solution after 5 days.]
Sample solution: 0.02 mg/mL of Disulfiram from *Sample stock solution* diluted with *Mobile phase* [NOTE—Prepare the *Sample solution* fresh daily.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1800 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{10}H_{20}N_2S_4$ in the portion of Disulfiram taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Disulfiram RS in the *Standard solution* (µg/mL)
C_U = concentration of Disulfiram in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Change to read:

Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **SELENIUM** ⟨**291**⟩**:** 30 ppm ■[NOTE—Perform this test only if selenium is a known inorganic process impurity.]■2S *(USP33)*

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨**741**⟩**:** 69°–72°

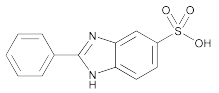
ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Disulfiram RS

BRIEFING

Ensulizole, *USP* 32 page 2257. It is proposed to replace the name of USP Phenylbenzimidazole Sulfonic Acid RS with USP Ensulizole RS to be consistent with the name used on the label for this Reference Standard. The *USP Reference Standards* section is being revised to reflect this change.

(MD-AA: H. Ramanathan, B. Davani.) RTS—C73290

Ensulizole



$C_{13}H_{10}N_2O_3S$ 274.30
1*H*-Benzimidazole-5-sulfonic acid-2-phenyl-;
2-Phenylbenzimidazole-5-sulfonic acid [27503-81-7].

DEFINITION
Ensulizole contains NLT 98.0% and NMT 102.0% of $C_{13}H_{10}N_2O_3S$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
Sample solution: 5 µg/mL
[NOTE—Transfer 100 mg of Ensulizole to a 100-mL volumetric flask, dissolve in 4 mL of 1 N sodium hydroxide, and dilute with water to volume. Transfer 0.5 mL of the solution to a 100-mL volumetric flask, and dilute with water to volume.]
Acceptance criteria: The absorptivities, calculated on the dried basis, at the wavelength of maximum absorbance at about 302 nm, do not differ by more than 3.0%.

ASSAY
- **PROCEDURE**
Sample solution: 48 mg/mL of Ensulizole in 0.5 N sodium hydroxide
Titrant: 0.5 N hydrochloric acid VS
Analysis: To the *Sample solution,* add phenolphthalein TS, and titrate the excess with *Titrant.* Perform a blank determination, and make any necessary corrections (see *Titrimetry* ⟨541⟩). Each mL of 0.5 N sodium hydroxide is equivalent to 137.15 mg of $C_{13}H_{10}N_2O_3S$.
Calculate the percentage of $C_{13}H_{10}N_2O_3S$ in the portion of Ensulizole taken:

$$\text{Result} = [(N \times (V_B - V_A))/(W \times (1 - \text{LOD}))] \times M_{r1} \times 100$$

N = actual normality of *Titrant*
V_B = volume of *Titrant* used for the blank (mL)
V_A = volume of *Titrant* used for the *Sample solution* (mL)
W = weight of Ensulizole taken for the *Sample solution* (mg)
LOD = percentage of loss on drying expressed as a decimal fraction, as determined in the test for *Loss on Drying*

In-Process Revision

M_{r1} = molecular weight of ensulizole, 274.30
Acceptance criteria: 98.0%–102.0% on the dried basis

SPECIFIC TESTS
• **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 4 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**
~~USP Phenylbenzimidazole Sulfonic Acid RS~~
■USP Ensulizole RS■2S (*USP33*)

BRIEFING

Helium, *USP 32* page 2550; **Nitrogen,** *NF 27* page 1288; **Nitrogen 97 Percent,** *NF 27* page 1288. The following changes are proposed: the *Assay* is modified to use a chromatographic method that has a higher level of accuracy, precision, and reliability than the official test. The new method also reduces the time of analyses. The *Identification* test is modified accordingly. The *Packaging and Storage* section has been revised, and a *Labeling* section has been added. Two new general test chapters referenced in this monograph appear elsewhere in this issue of *PF: Impurities Testing in Medical Gases* ⟨413⟩ and *Medical Gases Assay* ⟨415⟩.

(AER: K. Zaidi.) RTS—C61165

Helium

He 4.0026
Helium [7440-59-7].

DEFINITION
Helium contains NLT 99.0%, by volume, of He.

IDENTIFICATION

Delete the following:

~~■• **A:** The flame of a burning splinter of wood is extinguished when inserted into an inverted test tube filled with Helium. [NOTE—Use caution.]~~■2S (*USP33*)

Delete the following:

~~■• **B:** A small balloon filled with Helium shows decided buoyancy.~~■2S (*USP33*)

Add the following:

■• Introduce a Helium sample into a gas chromatograph, as directed in the *Assay*. Compare the chromatograms of an air–helium certified standard and the sample when each chromatogram is allowed to run four times longer than the retention time for the air peak. No other peak of more than 0.1% is observed.■2S (*USP33*)

ASSAY

Change to read:

• **PROCEDURE**
■**Sample gas:** Helium
Standard gas: Suitable 1% air–helium certified standard (see *Reagents, Indicators, and Solutions—Reagents*)■2S (*USP33*)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*; ■and *Medical Gases Assay* ⟨415⟩).■2S (*USP33*)
Mode: GC
Detector: Thermal conductivity
Column: ~~4-mm × 6-m, packed with porous polymer beads [NOTE—The column permits complete separation of nitrogen and oxygen from Helium, although the nitrogen and oxygen may not be separated from each other.]~~■2.1-mm × 3.6-m; packing 80/100 mesh S3■2S (*USP33*)
~~**Column temperature**~~■**Temperature:**■2S (*USP33*) Controlled
Carrier gas: Industrial grade helium (99.99%)
Flow rate: 30 mL/min
Injection type: Gas sampling valve
■**System suitability**
Sample: *Standard gas*
Suitability requirements
Resolution: NLT 2.0 between nitrogen and oxygen
Retention time: The retention time of the peak response for the *Sample gas* corresponds to that of the *Standard gas*.■2S (*USP33*)
Analysis
■**Samples:** *Sample gas* and *Standard gas*■2S (*USP33*)
Introduce the *Sample gas* into a gas sampling valve. ~~Select operating conditions of the chromatograph such that the standard peak signal corresponds to NLT 70% of the full-scale reading. The peak response produced by the assay specimen exhibits a retention time corresponding to that produced by an air-helium certified standard. (see *Reagents, Indicators, and Solutions—Reagents*), and indicates NMT 1.0% of air when compared to the peak response of the air-helium certified standard, and NLT 99.0%, by volume, of He.~~
■Calculate the percentage of Helium taken:

$$\text{Result} = 100 - (r_U/r_S)$$

r_U = peak response from the *Sample gas*
r_S = peak response from the *Standard gas*
[NOTE—The column might not separate nitrogen and oxygen; where separation occurs, the sum of the results of both peaks is used in the calculation.]■2S (*USP33*)
Acceptance criteria: NLT 99.0%

IMPURITIES

Change to read:

Inorganic Impurities
• **CARBON MONOXIDE**
■(See *Impurities Testing in Medical Gases* ⟨413⟩.)
Sample: Manufacturer's recommended gas volume (±5%)■2S (*USP33*)
Analysis: Pass ~~1000 ± 50 mL manufacturer's recommended gas volume "±5%"~~■2S (*USP33*) the *Sample* through a carbon monoxide detector tube (see *Reagents, Indicators, and Solutions—Reagents*) at the rate specified for the tube.
Acceptance criteria: The indicator change corresponds to NMT ~~0.001%~~■10 ppm.■2S (*USP33*)
• **AIR:** NMT 1.0% of air is present, determined as directed in the *Assay*.

In-Process Revision

SPECIFIC TESTS

Change to read:

• **ODOR**
Analysis: Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. ■[NOTE—Not applicable to liquid samples.]■2S (*USP33*)
Acceptance criteria: No appreciable odor is discernible.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in cylinders~~ [~~NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.~~]■Preserve Helium in pressurized containers. Container connections shall be appropriate for Helium. Adapters shall not be used to connect containers to patient-use supply system piping or equipment.■2S (*USP33*)

Add the following:

■• **LABELING:** Where Helium is piped directly from a container or storage tank to the point of use, label each outlet Helium.■2S (*USP33*)

BRIEFING

Hydromorphone Hydrochloride Oral Solution. Because there is no exisiting *USP* monograph for this article, a new monograph, based on validated methods, is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities* are based on analyses performed with a Waters Symmetry brand of L1 column. The typical retention times for hydromorphone in the *Assay* and in the test for *Organic Impurities* are about 5.0 and 20.0 min, respectively.

(MD-CCA: C. Anthony. MSA: R. Tirumalai.) RTS—C44494

Add the following:

■Hydromorphone Hydrochloride Oral Solution

DEFINITION
Hydromorphone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_{17}H_{19}NO_3 \cdot HCl$. It may contain suitable preservatives.

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
Blank: 0.01 N hydrochloric acid and water (8:2) containing the expected concentration of excipients
Standard solution: 0.04 mg/mL of USP Hydromorphone Hydrochloride RS, 2 mg/mL of glycerin, and 20 mg/mL of sucrose in 0.01 N hydrochloric acid and water (4:1)
Sample solution: Equivalent to 0.2 mg/mL of hydromorphone hydrochloride in water
Analysis: Pretreat a solid phase extraction column containing L1 packing serially with about 10 mL of methanol and about 10 mL of 0.01 N hydrochloric acid. Transfer 5.0 mL of *Sample solution* to the column, add about 19 mL of 0.01 N hydrochloric acid in at least two portions, and elute into a 25-mL volumetric flask. Dilute with 0.01 N hydrochloric acid to volume, and mix. Using a suitable cell, compare the spectrum of the *Sample solution* with that of the *Standard solution*, using the *Blank* to zero the instrument.
Acceptance criteria: The absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelength as that of the *Standard solution*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Phosphoric acid and water (1:1000)
Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:9). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1.
Solution B: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:1). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	85	15
24	5	95
25	85	15
30	85	15

Standard solution: 0.08 mg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*
Sample solution: Equivalent to 0.08 mg/mL of hydromorphone hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Temperature: 45°
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for hydromorphone peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{19}NO_3 \cdot HCl$ in the portion of Oral Solution taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

IMPURITIES
Organic Impurities
• **PROCEDURE**
Diluent, Solution A, and Solution B: Proceed as directed in the *Assay*.

In-Process Revision

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

Standard solution: 4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

System suitability solution: 0.8 mg/mL each of USP Hydromorphone Hydrochloride RS and USP Hydromorphone Related Compound A RS in *Diluent*. [NOTE—The solution should be kept in a cool place protected from light.]

Sample solution: Equivalent to 0.4 mg/mL of hydromorphone hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Temperature: 45°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.0 between hydromorphone related compound A and hydromorphone peaks, *System suitability solution*

Tailing factor: NMT 1.5 for hydromorphone peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each degradation product found, including those in *Impurity Table 1*, for the *Sample solution*

r_S = peak response of hydromorphone from the *Standard solution*

C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding individual specified or unspecified impurity from *Impurity Table 1*

Calculate the total degradation products by summing the percentage of all individual specified and unspecified degradation products determined to be at a level of 0.1% or greater, excluding the known process impurities, as indicated in *Impurity Table 1*.

Acceptance criteria: See *Impurity Table 1*. [NOTE—Disregard peaks corresponding to those from the *Diluent,* peaks that elute before a relative retention time of about 0.50, except for any peak with a relative retention time of about 0.34, and peaks that elute at the relative retention times of the process-related substances designated in *Impurity Table 1*.]

Impurity Table 1

Name	Relative Retention Time (RRT)	Limit (%)	Relative Response Factor
Unknown degradation product	0.34	NMT 0.2	1.0
8-Hydroxy-hydromorphone**[a]	0.50	—	—
Dihydromorphine (DHM)**[b]	0.61	—	—
Morphine**[c]	0.65	—	—
Hydromorphone *N*-oxide*[d]	0.79	NMT 0.2	0.87
Hydromorphone	1.0	—	—
2,2′-Bishydromorphone dihydrochloride*[e]	2.02	NMT 0.2	1.7
Individual unspecified degradation products	—	NMT 0.2	1.0
Total degradation products	—	1.0	—

*Degradation product.

**Process impurity.

[a] 4,5α-Epoxy-17-methylmorphinan-3,8-diol-6-one.

[b] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.

[c] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.

[d] 4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.

[e] 2,2′-Bihydromorphone.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 100 cfu/mL, and the total yeasts and molds count does not exceed 10 cfu/mL. Meets the requirements of the test for the absence of *Escherichia coli*.
- **pH** ⟨**791**⟩**:** Between 4.5 and 6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Identify in the product labeling any preservative used in the Oral Solution.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Hydromorphone Hydrochloride RS
  USP Hydromorphone Related Compound A RS■2S *(USP33)*

BRIEFING

Hydroxypropyl Cellulose Ocular System, *USP 32* page 2594. On the basis of comments received, it is proposed to specify the sample amount needed to prepare the *Standard solution* and the *Sample solution* in the *Assay*. Using the proposed sample weight minimizes weighing issues, as hydroxypropyl cellulose is both electrostatic and hygroscopic. It is also recommended to stir the *Standard solution* and the *Sample solution* overnight before diluting to volume to ensure complete dissolution.

(MD-OOD: H. Ramanathan, F. Mao.) RTS—C64389

In-Process Revision

Hydroxypropyl Cellulose Ocular System

DEFINITION
Hydroxypropyl Cellulose Ocular System contains NLT 85.0% and NMT 115.0% of the labeled amount of Hydroxypropyl Cellulose. It contains no other substance. It is sterile.

IDENTIFICATION
• **INFRARED ABSORPTION**
Sample solution: Prepare a 10 mg/mL solution in methanol, based on the labeled amount of Hydroxypropyl Cellulose. Evaporate 2 drops of the solution on a silver chloride plate so that it forms a thin film.
Acceptance criteria: The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroxypropyl Cellulose RS.

ASSAY

Change to read:

• **PROCEDURE**
~~**Standard solution:** 0.05 mg/mL of USP Hydroxypropyl Cellulose RS~~
~~[NOTE—Dissolve with agitation.]~~
~~**Sample solution:** 0.05 mg/mL of hydroxypropyl cellulose from a sufficient number of Ocular Systems to provide 25 mg of hydroxypropyl cellulose~~
~~[NOTE—Dissolve with agitation in 250 mL of water before diluting to volume.]~~
■**Standard stock solution:** 0.25 mg/mL of USP Hydroxypropyl Cellulose RS prepared as follows: Weigh 25 mg of USP Hydroxypropyl Cellulose RS into a 100-mL volumetric flask. Dissolve in 80 mL of water. Mix well by agitating on a mechanical shaker until completely dissolved. Add one drop of methanol to dispel the foam and dilute with water to volume. [NOTE—Stirring overnight before diluting to volume is recommended.]
Standard solution: 0.05 mg/mL of hydroxypropyl cellulose in water, from *Standard stock solution*
Sample stock solution: 0.25 mg/mL of hydroxypropyl cellulose prepared using the same procedure as the *Standard stock solution*
Sample solution: 0.05 mg/mL of hydroxypropyl cellulose in water, from *Sample stock solution*■2S *(USP33)*

Spectrometric conditions
Mode: UV-Vis
Analytical wavelength: 620 nm
■**Cell length:** 1.0 cm, quartz■2S *(USP33)*

Analysis
Samples: *Standard solution* and *Sample solution*
Separately pipet 2 mL of the *Standard solution*, the *Sample solution*, and water, to provide a blank, into individual 50-mL centrifuge tubes. Add to each tube, 6.0 mL of a 0.5 mg/mL solution of anthrone in sulfuric acid, and mix on a vortex mixer. ~~After 40 min, again mix~~■Let the centrifuge tubes cool for approximately 40 min and remix.■2S *(USP33)*
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*. ■[NOTE—Prepare anthrone in sulfuric acid solution just before use in low-actinic glassware, and mix well before adding to the tube. Use it within 12 h of preparation. Avoid contact between the glassware and the paper products during analysis; the cellulose in the paper will react with the sulfuric acid and alter the results.]■2S *(USP33)*
Calculate the percentage of hydroxypropyl cellulose in the Ocular System:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxypropyl cellulose in the *Sample solution* (mg/mL)
Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS
• **WEIGHT VARIATION**
Analysis: Determine the weight of each of a sufficient number of Ocular Systems.
Acceptance criteria: NMT 1 out of 20 Ocular Systems varies more than 25% from the average or, failing that, NMT 6 out of 60 (including the original 20) vary more than 25% (but none more than 35%) from the average weight.

SPECIFIC TESTS
• **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements

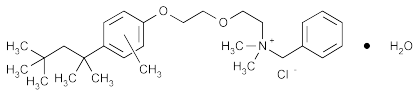
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, at a temperature not exceeding 30°.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Hydroxypropyl Cellulose RS

BRIEFING

Methylbenzethonium Chloride, *USP 32* page 2937. It is proposed to modernize this monograph by replacing three wet chemistry procedures, *Identification* tests *B*, *C*, and *D*, with a single, more selective IR test.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C73681

Methylbenzethonium Chloride



$C_{28}H_{44}ClNO_2 \cdot H_2O$ 480.12
Benzenemethanaminium, *N*,*N*-dimethyl-*N*-[2-[2-[methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride, monohydrate;
Benzyldimethyl[2-[2-[[4-(1,1,3,3-tetramethylbutyl)tolyl]-oxy]ethoxy]ethyl]ammonium chloride monohydrate [1320-44-1].
Anhydrous 462.12
[25155-18-4].

DEFINITION
Methylbenzethonium Chloride contains NLT 97.0% and NMT 103.0% of $C_{28}H_{44}ClNO_2$, calculated on the dried basis.

IDENTIFICATION
• **A. PROCEDURE**
Analysis: To 1 mL of solution (1 in 100), add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.
Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid and soluble in 6 N ammonium hydroxide, is formed.

Delete the following:

■~~• **B. PROCEDURE**~~
~~**Analysis:** Treat separate portions of a solution (1 in 100) with 2 N nitric acid and with mercuric chloride TS, respectively.~~
~~**Acceptance criteria:** Precipitates are formed that dissolve upon the addition of alcohol.~~■2S *(USP33)*

In-Process Revision

Delete the following:

~~■• **C. PROCEDURE**~~
~~**Analysis:** To 10 mL of a solution (1 in 20,000), add 0.1 g of sodium carbonate, 1 mL of bromophenol blue TS, and 10 mL of chloroform, and shake the mixture.~~
~~**Acceptance criteria:** The chloroform layer is blue.~~■2S *(USP33)*

Delete the following:

~~■• **D. PROCEDURE**~~
~~**Sample solution:** Dissolve 100 mg in 1 mL of sulfuric acid, add 1.0 g of potassium nitrate, and heat on a steam bath for 3 min.~~
~~**Analysis:** Cautiously dilute the *Sample solution* with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool, add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol di-potassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.~~
~~**Acceptance criteria:** The solution turns orange-red, and a brown precipitate may be formed.~~■2S *(USP33)*

Add the following:

■• **B. INFRARED ABSORPTION** ⟨197K⟩■2S *(USP33)*

ASSAY
• **PROCEDURE**
Sample solution: Transfer the equivalent to 500 mg of dried methylbenzethonium chloride, with the aid of 35 mL of water, to a glass-stoppered, 250-mL conical separator containing 25 mL of chloroform. Add 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper in the separator, shake, allow the layers to separate, and discard the chloroform layer. Wash the aqueous layer with three 10-mL portions of chloroform, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold hydrochloric acid to the flask, and mix.
Analysis: Titrate with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of chloroform, insert the stopper in the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the chloroform layer becomes colorless and the aqueous layer is clear yellow. Perform a blank determination, using 20 mL of water as the test specimen (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.05 M potassium iodate is equivalent to 46.21 mg of $C_{28}H_{44}ClNO_2$.
Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
Organic Impurities
• **PROCEDURE: LIMIT OF AMMONIUM COMPOUNDS**
Analysis: To 5 mL of a solution (1 in 50) add 3 mL of 1 N sodium hydroxide, and heat to boiling.
Acceptance criteria: The odor of ammonia is not perceptible.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 159°–163°, the specimen having been previously dried
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 4 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Add the following:

■• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylbenzethonium Chloride RS■2S *(USP33)*

BRIEFING

Mycophenolate Mofetil Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedure in the *Assay* and in the test for *Limit of Degradation Products* is based on analyses performed with a Phenomenex Luna C8 (2) brand of L7 column. The typical retention time for mycophenolate mofetil is about 4.2 min. The liquid chromatographic procedure in the test for *Limit of Z-Mycophenolate Mofetil* is based on analyses performed with an Agilent Technologies Zorbax SB C8 brand of L7 column. The typical retention times for mycophenolate mofetil and *Z*-mycophenolate mofetil are about 11.5 and 12.4, respectively.

(MD-OOD: F. Mao. BPC: M. Marques.) RTS—C69397

Add the following:

■Mycophenolate Mofetil Capsules

DEFINITION
Mycophenolate Mofetil Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION** ⟨197U⟩
Standard solution and **Sample solution:** Use the *Standard solution* and *Sample solution* as prepared in the test for *Dissolution*.
Acceptance criteria: The UV absorption spectrum of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ±3 nm.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Phosphoric acid solution: Phosphoric acid and water (3:50)
Triethylamine solution: Transfer 3 mL of triethylamine to 1000 mL of water. Adjust the pH to 5.3 with *Phosphoric acid solution*.
Mobile phase: Acetonitrile and *Triethylamine solution* (11:9)
Standard solution: 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with acetonitrile to volume. Pass

In-Process Revision

through a 0.45-µm nylon filter, and discard the first 5 mL of filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L7
Column temperature: 45°
Auto sampler temperature: 10° ±5°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨**711**⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 40 rpm with sinkers
Time: 20 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Standard solution: 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Detector: UV 250 nm
Path length: 0.1 cm
Blank: *Medium*
Calculate the percentage of mycophenolate mofetil dissolved:

$$(A_U/A_S) \times (C_S/L) \times 900 \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = 900 mL
L = tablet label claim (mg)
Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil is dissolved.

• **Uniformity of Dosage Units** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure 1: Limit of Degradation Products**
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.0625 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
[Note—The run time for the *Sample solution* is three times that of the retention time of the mycophenolate mofetil peak.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*
r_S = response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (See *Impurity Table 1*.)
Acceptance criteria
Individual degradation products: See *Impurity Table 1*. [Note—Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.]
Total degradation products: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Limit (%)
Mycophenolic acid[a]	0.6	1.4	0.7
Mycophenolate *N*-oxide analog[b]	0.8	1.0	0.2
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1

[a](*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b]2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.

• **Procedure 2: Limit of *Z*-Mycophenolate Mofetil** [Note—*Z*-mycophenolate mofetil is 2-morpholinoethyl (*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]
Triethylamine solution: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Triethylamine solution* (7:13)
Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a 0.45-µm nylon filter, and discard the first 2 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm column; 3.5-µm packing L7
Column temperature: 60°
Flow rate: 1.5 mL/min
Run time: 1.7 times the retention time of the mycophenolate mofetil peak
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[Note—The relative retention times for mycophenolate mofetil and *Z*-mycophenolate mofetil are 1.0 and 1.1, respectively.]

In-Process Revision

Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *Z*-mycophenolate mofetil in the portion of Capsules taken:

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

r_U = response of *Z*-mycophenolate mofetil from the *Sample solution*
r_S = response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria
***Z*-mycophenolate mofetil:** NMT 0.10%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed and light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Mycophenolate Mofetil RS■2S *(USP33)*

BRIEFING

Mycophenolate Mofetil Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedure in the *Assay* and in the test for *Limit of Degradation Products* is based on analyses performed with a Phenomenex Luna C8 (2) brand of L7 column. The typical retention time for mycophenolate mofetil is about 4.2 min. The liquid chromatographic procedure in the test for *Limit of Z-Mycophenolate Mofetil* is based on analyses performed with a Zorbax SB C8 brand of L7 column. The typical retention times for mycophenolate mofetil and *Z*-mycophenolate mofetil are about 11.5 and 12.4, respectively.

(MD-OOD: F. Mao. BPC: M. Marques.) RTS—C69397

Add the following:

■Mycophenolate Mofetil Tablets

DEFINITION
Mycophenolate Mofetil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION
- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
Standard solution and **Sample solution:** Use the *Standard solution* and *Sample solution* as prepared in the test for *Dissolution*.
Acceptance criteria: The UV absorption spectrum of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ±3 nm.
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Phosphoric acid solution: Phosphoric acid and water (3:50)
Triethylamine solution: Transfer 3 mL of triethylamine to 1000 mL of water. Adjust the pH to 5.3 with *Phosphoric acid solution*.
Mobile phase: Acetonitrile and *Triethylamine solution* (11:9)
Standard solution: 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with acetonitrile to volume. Pass through a 0.45-µm nylon filter, and discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L7
Column temperature: 45°
Auto sampler temperature: 10° ±5°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION ⟨711⟩**
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 5 and 15 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Standard solution: 0.55 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Detector: UV 304 nm
Path length: 0.1 cm
Blank: *Medium*
Calculate the percentage of mycophenolate mofetil dissolved:

$$(A_U/A_S) \times (C_S/L) \times 900 \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = 900 mL
L = tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of mycophenolate mofetil is dissolved in 5 min, and NLT 85% of the labeled amount of mycophenolate mofetil is dissolved in 15 min.

In-Process Revision

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE 1: LIMIT OF DEGRADATION PRODUCTS**

Mobile phase, Standard solution, Sample solution, and *Chromatographic system*: Proceed as directed in the *Assay*.

Sensitivity solution: 0.0625 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis [NOTE—The run time for the *Sample solution* is three times that of the retention time of the mycophenolate mofetil peak.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_S = response of mycophenolate mofetil from the *Standard solution*

C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

C_U = concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (See *Impurity Table 1*.)

Acceptance criteria

Individual degradation products: See *Impurity Table 1*. [NOTE—Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.]

Total degradation products: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Limit (%)
Mycophenolic acid[a]	0.6	1.4	0.7
Mycophenolate *N*-oxide analog[b]	0.8	1.0	0.2
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1

[a](*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

[b]2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.

• **PROCEDURE 2: LIMIT OF *Z*-MYCOPHENOLATE MOFETIL**

[NOTE—*Z*-mycophenolate mofetil is 2-morpholinoethyl (*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]

Triethylamine solution: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Triethylamine solution* (7:13)

Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a 0.45-µm nylon filter, and discard the first 2 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm column; 3.5-µm packing L7

Column temperature: 60°

Flow rate: 1.5 mL/min

Run time: 1.7 times the retention time of the mycophenolate mofetil peak

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for mycophenolate mofetil and mycophenolate *Z*-mycophenolate mofetil are 1.0 and 1.1, respectively.]

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *Z*-mycophenolate mofetil in the portion of Tablets taken:

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

r_U = response of *Z*-mycophenolate mofetil from the *Sample solution*

r_S = response of mycophenolate mofetil from the *Standard solution*

C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

C_U = concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria

***Z*-mycophenolate mofetil:** NMT 0.10%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed and light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Mycophenolate Mofetil RS■2S *(USP33)*

BRIEFING

Nevirapine Oral Suspension, *USP 32* page 3073. On the basis of comments received, it is proposed to clarify the *Acceptance criteria* for total impurities in the *Procedure* under *Organic Impurities* to specify only unknown impurities because the known process impurities are controlled in the drug substance.

(MD-AA: M. Puderbaugh; B. Davani.) RTS—C67480

Nevirapine Oral Suspension

DEFINITION

Nevirapine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 5 mg/mL of USP Nevirapine Anhydrous RS in chloroform

Sample solution: Transfer a volume of Oral Suspension, equivalent to 10 mg of nevirapine, to an 8-mL glass stoppered tube. Pipet 2.0 mL of chloroform into the tube. Shake the solution and allow the two phases to separate, and then using

a disposable glass Pasteur pipet, remove some of the organic layer from the bottom, and transfer to another container.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel 60 F254
Mode: TLC
Application volume: 5 µL
Developing solvent system: Ethyl acetate, isopropanol, and concentrated ammonium hydroxide (18:2:0.1)
Spray reagent: 1.35 g of ferric chloride in 25 mL of water and 1.64 g of potassium ferricyanide in 25 mL of water. Mix the two solutions immediately before use.

Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram in a chamber saturated with a solvent system until the solvent front has moved 6–7 cm from the point of application. Remove the plate from the chamber, mark the solvent front, and dry. Examine the chromatograms under UV light at 254 nm, and outline the spots with a soft pencil. Spray the plate with *Spray reagent.*

Acceptance criteria: The R_F value (approximately 0.4–0.5) of the principal blue spot under UV and after spraying, from the *Sample solution*, corresponds to that from the *Standard solution.*

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Diluent: Methanol and water (1:4)
Solution A: 13.6 g of monobasic potassium phosphate in 1900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.
Solution B: Acetonitrile and *Solution A* (3:97)
Solution C: Acetonitrile and *Solution A* (24:76)
Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
1	100	0
31	0	100
32	100	0
42	100	0

Standard stock solution: Dissolve 50 mg of USP Nevirapine Anhydrous RS in 20 mL of methanol in a 50-mL volumetric flask. Sonicate with intermittent swirling until the sample dissolves. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume. The concentration is 1 mg/mL of nevirapine.
Standard solution: 0.3 mg/mL of nevirapine from *Standard stock solution* diluted with *Diluent*
Stock impurity solution: 3 mg of USP Nevirapine Related Compound A RS and 3 mg of USP Nevirapine Related Compound B RS in 20 mL of methanol in a 100-mL volumetric flask. Sonicate to dissolve. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume.
System suitability solution: Transfer 15.0 mL of *Standard stock solution* and 2.0 mL of *Stock impurity solution* into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Weight determination: Using a 1- to 10-mL suitable pipet and a positive displacement tip, withdraw 5.0 mL of the Oral Suspension. The sample should be free of air bubbles. Dispense into a tared vial, and record the weight of the Oral Suspension to ±0.1 mg.
Sample solution: Equivalent to 60 mg of nevirapine is taken using a 1- to 10-mL suitable pipet and a positive displacement tip to withdraw the Oral Suspension. The sample should be free of air bubbles. Remove the excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip, and deliver the sample into a 200-mL tared volumetric flask. Record the sample weight to the nearest ±0.1 mg. Add 40 mL of methanol, and sonicate for 5 min with intermittent swirling. Add water to 1 cm below the meniscus. Do not shake the flask. Allow the solution to attain room temperature, and dilute with water to volume. Shake the flask gently, and allow to stand for 5 min.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L10
Guard column: 4.6-mm × 12.5-mm; 5-µm packing L10
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between nevirapine and nevirapine related compound A; NLT 1.7 between nevirapine and nevirapine related compound B
Tailing factor: NMT 1.5 for the nevirapine peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for the nevirapine peak. Calculate the quantity, in mg, of $C_{15}H_{14}N_4O$ in each mL of the Oral Suspension taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 25 rpm
Time: 45 min
Determine the amount of $C_{15}H_{14}N_4O$ dissolved by using the following method.
Diluent: Dehydrated alcohol and water (1:1)
Mobile phase: Acetonitrile and water (23:77)
System suitability solution: Dissolve 10 mg of USP Nevirapine Anhydrous RS and 15 mg of methylparaben in 2 mL of *Diluent*, and dilute with *Medium* to 250 mL.
Standard solution: Dissolve 28 mg of USP Nevirapine Anhydrous RS in 2 mL of *Diluent*, and sonicate for 1 min. Standard will not be completely dissolved at this point. Dilute with *Medium* to 500 mL, and visually examine the solution to ensure that the Standard is completely dissolved. The final concentration is 0.056 mg/mL of nevirapine.
Sample solution: For sample mixing, gently shake the bottle for approximately 10 s by inverting it slowly and rotating it from side to side. The sample should be free of air bubbles. Do not sonicate the sample. Using a 1–10 mL suitable positive displacement pipet set at 5 mL, withdraw the equivalent of 50 mg of nevirapine. Remove excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip. Introduce the sample into the dissolution vessel over a 1–2 s time period by immersing the tip of the pipet midway between the paddle and the side of the vessel, approximately 1 cm below the meniscus. Similarly dispense the Oral Suspension into the other vessels. At 45 min, withdraw 5 mL of the solution under test, and pass through a 0.45-µm nylon filter, discarding the first 2 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 214 nm
Guard column: 3.9-mm × 20-mm; packing L1
Column: 3.9-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between nevirapine and methylparaben
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for at least 14 min, and measure the responses for the nevirapine peaks.
Calculate the percentage of $C_{15}H_{14}N_4O$ dissolved:

$$\text{Result} = (r_U \times C_S \times V_1)/(r_S \times V_2 \times L) \times 100$$

r_U = peak response from the *Sample solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
V_1 = volume of the *Medium*, 900 mL
r_S = peak response from the *Standard solution*
V_2 = volume of Oral Suspension taken (mL)
L = label claim (mg/mL)
Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{14}N_4O$ is dissolved.

IMPURITIES

Change to read:

Organic Impurities
• **PROCEDURE**
Diluent, **Solution A**, **Solution B**, **Solution C**, and **Mobile phase:** Proceed as directed in the *Assay*.
Standard stock solution: Use the *Standard stock solution*, prepared as directed in the *Assay*.
Standard solution: 0.3 µg/mL of nevirapine from *Standard stock solution* diluted with *Diluent*
System suitability solution: Prepare as directed in the *Assay*.
Weight determination: Use the weight obtained as directed for *Weight determination* in the *Assay*.
Sample solution: Use the *Assay Sample solution*.
Chromatographic system: Proceed as directed in the *Assay*.
Injection size: 20 µL
Relative standard deviation: NMT 10.0%, for replicate injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unknown impurity in the portion of Oral Suspension taken:

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

r_U = peak response for each impurity in the *Sample solution*
r_S = peak response for nevirapine in the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nevirapine in the *Sample solution* (mg/mL)
Acceptance criteria
Individual unknown impurities: NMT 0.1%
Total ■unknown■2S (USP33) impurities: NMT 0.2%
[NOTE—The excipients and their degradation products should not be included in the determination of impurities.]

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** It meets the requirements of the tests for absence of *Escherichia coli*. The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 50 cfu/mL.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Nevirapine Anhydrous RS
USP Nevirapine Related Compund A RS
USP Nevirapine Related Compound B RS

BRIEFING

Nitrous Oxide, *USP 32* page 3099. It is proposed to change the procedure in the *Assay* to a chromatographic method that provides a higher level of accuracy, precision, and reliability. Time of analysis is also reduced with this modification. The *Identification* test is also modified accordingly. The *Packaging and Storage* section has been revised and a new section on *Labeling* has been added to the monograph.

(AER: K. Zaidi.) RTS—C73268

Nitrous Oxide

N_2O 44.01
Nitrogen oxide (N_2O);
Dinitrogen monoxide [10024-97-2].

DEFINITION

Change to read:

Nitrous Oxide contains NLT 99.0%, by volume, of N_2O.
~~[NOTE—The following tests are designed to reflect the quality of Nitrous Oxide in both the vapor and liquid phases that are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the samples for the tests with the least possible release of Nitrous Oxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination or change of the specimens. Perform tests in the sequence in which they are listed.~~
~~The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.]~~
■■2S (USP33)

IDENTIFICATION

Change to read:

• ~~**A:**~~ ~~With the container temperatures the same and maintained between 15° and 25°, concomitantly read the pressure of the Nitrous Oxide container and of a container of nitrous oxide certified standard (see *Reagents, Indicators, and Solutions—Reagents*).~~ ~~[NOTE—Do not use the nitrous oxide certified standard if it has been depleted to less than half of its full capacity.]~~ ~~The pressure of the Nitrous Oxide container is within 50 psi of that of the nitrous oxide certified standard~~■When tested as directed in the *Assay*, the result is NLT 99.0% Nitrous Oxide, by volume.■2S (USP33)

In-Process Revision

Delete the following:

~~■• **B.** Pass 100 ± 5 mL released from the vapor phase of the contents of the Nitrous Oxide container through a carbon dioxide detector tube at the rate specified for the tube: no color change is observed (distinction from carbon dioxide).■2S (USP33)~~

Delete the following:

~~■• **C.** Collect 100 mL of the gas under test in a 100-mL tube fitted at the top with a stopcock. Open the stopcock, and quickly add a freshly prepared solution of 500 mg of pyrogallol in 2 mL of water and a freshly prepared solution of 12 g of potassium hydroxide in 8 mL of water. Immediately close the stopcock, and mix: the gas is not absorbed, and the solution does not become brown (distinction from oxygen).■2S (USP33)~~

ASSAY

Change to read:

- **PROCEDURE**

Sample: Nitrous Oxide, taken from the liquid phase. ■[NOTE—Sampling can be from the vapor phase, although this may give a higher air/lower assay result. If a result of more than 1% air is obtained, a sample may be taken from the liquid phase and the analysis repeated. Care shall be taken to ensure that the sample is fully vaporized before injection into the gas chromatograph.]■2S (USP33)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability* ■and *Medical Gases Assay* ⟨415⟩.)

Sample gas: Sample under test

Standard gas: Suitable 1% air–helium certified standard (see *Reagents, Indicators, and Solutions—Reagents*.)■2S (USP33)

Mode: GC

Column: ~~6 m × 4 mm (i.d.); packed with porous polymer beads, which permit complete separation of N_2 and O_2 from N_2O, although the N_2 and O_2 may not be separated from each other~~■2.1-mm × 2.4-m; 80/100 mesh S3 packing■2S (USP33)

Detector: Thermal conductivity

■**Temperature:** Column, injector, and detector: 70°■2S (USP33)

Carrier gas: Industrial grade helium (99.99%)

■**Flow rate:** 30 mL/min

Injection type: Gas sampling loop

System suitability

Sample: *Standard gas*

System suitability requirements

Resolution: NLT 1.5 between air and nitrous oxide

Relative retention time: NLT 1.5■2S (USP33)

Analysis

Samples: *Sample gas* and *Standard gas*

~~Introduce the *Sample* as directed in the test for *Nitrogen Dioxide*, into a gas chromatograph. Select the operating conditions of the gas chromatograph such that the peak response resulting from the following procedure corresponds to NLT 70% of the full-scale reading. Preferably, use an apparatus corresponding to the general type, and control the column temperature.~~

■■2S (USP33)

Acceptance criteria: The air peak response produced by the *Sample* under test is equivalent to or less than 1.0% of air when compared to the peak response of the *Standard gas* (NLT 99.0%, by volume, of N_2O).

IMPURITIES

Change to read:

Inorganic Impurities

■(See *Impurities Testing in Medical Gases* ⟨413⟩.)

[NOTE—The various detector tubes called for in the respective tests are listed under *Reagents, Indicators, and Solutions*.]■2S (USP33)

- **AMMONIA** ~~Proceed with Nitrous Oxide as directed in the test for *Carbon Monoxide*, except to use an ammonia detector tube.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass the manufacturer's recommended gas volume of nitrous oxide drawn from the vapor phase through an ammonia detector tube at the rate specified for the tube.■2S (USP33)

Acceptance criteria: The indicator change corresponds to NMT ~~0.0025%~~■25 ppm.■2S (USP33)

- **NITRIC OXIDE** ~~1000 ± 50 mL~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass the manufacturer's recommended gas volume of nitrous oxide drawn from the vapor phase through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube.■2S (USP33)

Acceptance criteria: The indicator change corresponds to NMT 1 ppm.

- **NITROGEN DIOXIDE** ~~Arrange a container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube.~~

■**Sample:** Arrange a container so that when its valve is opened, a portion of the liquid phase of the contents is released through a heated regulator or piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide sufficient vaporized sample plus any excess necessary to ensure adequate flushing of air from the system.

Analysis: Pass the manufacturer's recommended volume (±5%) of vaporized nitrous oxide through a nitric oxide–nitrogen dioxide detector tube of the appropriate measurement range.■2S (USP33)

Acceptance criteria: The indicator change corresponds to NMT 1 ppm.

- **HALOGENS** ~~1000 ± 50 mL released from the vapor phase of the contents of the container, through a chlorine detector tube at the rate specified for the tube.~~

■**Sample:** Manufacturer's recommended gas volume (± 5%)

Analysis: Pass the manufacturer's recommended gas volume of nitrous oxide drawn from the vapor phase through a chlorine detector tube at the rate specified for the tube.■2S (USP33)

Acceptance criteria: The indicator change corresponds to NMT 1 ppm.

- **CARBON MONOXIDE** ~~1000 ± 50 mL released from the vapor phase of the contents of the container, through a carbon monoxide detector tube at the rate specified for the tube.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)

Analysis: Pass the manufacturer's recommended gas volume of nitrous oxide drawn from the vapor phase through a carbon monoxide detector tube at the rate specified for the tube.■2S (USP33)

Acceptance criteria: The indicator change corresponds to NMT ~~0.001%~~■10 ppm■2S (USP33)

In-Process Revision

• **CARBON DIOXIDE** ~~1000 ± 50 mL released from the vapor phase of the contents of the container, through a carbon dioxide detector tube at the rate specified for the tube.~~

■**Sample:** Manufacturer's recommended gas volume (±5%)
Analysis: Pass the manufacturer's recommended gas volume of nitrous oxide drawn from the vapor phase through a carbon dioxide detector tube at the rate specified for the tube.■2S (*USP33*)

Acceptance criteria: The indicator change corresponds to NMT ~~0.03%~~■300 ppm.■2S (*USP33*)

SPECIFIC TESTS

Change to read:

• **WATER** ~~It meets the requirements of the test for *Water* under *Carbon Dioxide*~~

■**Sample:** Manufacturer's recommended gas volume (±5%)
Analysis: Flush the regulator with 5 L or more of the gas specimen. Following the detector tube manufacturer's recommended procedure, pass nitrous oxide released from thevapor phase through a water vapor detector tube of the appropriate measurement range connected to the regulator with a minimum length of metal or polyethylene tubing. Measure the gas passing through the detector tube with a gas flowmeter set at a flow rate specified for the tube.
Acceptance criteria: The indicator change corresponds to NMT 150 mg/m^3.■2S (*USP33*)

• **AIR:** NMT 1.0% of air is present, determined as directed in the *Assay*.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in cylinders.~~■Preserve nitrous oxide in pressurized containers. Container connections shall be appropriate for nitrous oxide. Adapters shall not be used to connect containers to patient-use supply system piping or equipment.■2S (*USP33*)

Add the following:

■**Labeling:** Where Nitrous Oxide is piped directly from a remote location to the patient point of use, label the point of use outlet "Nitrous Oxide".■2S (*USP33*)

BRIEFING

Oxygen, *USP 32* page 3173—See briefing under *Medical Air*.

(AER: K. Zaidi.) RTS—C61157

Oxygen

O_2 32.00
Oxygen
Oxygen [7782-44-7].

DEFINITION
Oxygen contains NLT 99.0% of O_2, by volume.
[NOTE—Oxygen that is produced by the air-liquefaction process is exempt from the requirements of the tests for *Carbon Dioxide* and *Carbon Monoxide*.]

IDENTIFICATION

Change to read:

• ~~**PROCEDURE**~~■■2S (*USP33*)

When tested as directed in the *Assay*, ~~NMT 1.0 mL of gas remains~~■the result is NLT 99.0% of oxygen, by volume.■2S (*USP33*)

Delete the following:

■~~• **B. PROCEDURE**~~
~~**Analysis:** Pass 100 ± 5 mL released from the vapor phase of the contents of the Oxygen container or through a carbon dioxide detector tube at the rate specified for the tube.~~
~~**Acceptance criteria:** No color change is observed (distinction from carbon dioxide).~~■2S (*USP33*)

ASSAY

Change to read:

• **PROCEDURE**
~~**Sample:** Oxygen~~
~~**Analysis:** Place a sufficient quantity of ammonium chloride-ammonium hydroxide solution, prepared by mixing equal volumes of water and ammonium hydroxide and saturating with ammonium chloride at room temperature, in a test apparatus composed of a calibrated 100-mL buret, provided with a two-way stopcock, a gas absorption pipet, and a leveling bulb, both of suitable capacity and all suitably interconnected. Fill the gas absorption pipet with metallic copper in the form of wire coils, wire mesh, or other suitable configuration. Eliminate all gas bubbles from the liquid in the test apparatus. Activate the test solution by performing two or three tests that are not for record purposes. Fill the calibrated buret, all interconnecting tubing, both stopcock openings, and the intake tube with liquid. Draw 100.0 mL of *Sample* into the buret by lowering the leveling bulb. Open the stopcock to the absorption pipet, and force the *Sample* into the absorption pipet by raising the leveling bulb. Agitate the pipet to provide frequent and intimate contact of the liquid, gas, and copper. Continue agitation until no further diminution in volume occurs. Draw the residual gas back into the calibrated buret, and measure its volume.~~

■Determine the oxygen concentration using a paramagnetic analyzer for oxygen (see *Medical Gases Assay* ⟨415⟩).
[NOTE—See *Reagents, Indicators, and Solutions* for information on certified standards.]
Zero Gas: Nitrogen certified standard
Span Gas: Oxygen certified standard
Sample: Sample under test
Analysis: Determine the percentage of oxygen in the *Sample*.■2S (*USP33*)

Acceptance criteria: ~~NMT 1.0 mL of gas remains.~~■NLT 99.0% of oxygen, by volume of O_2■2S (*USP33*)

IMPURITIES

Change to read:

Inorganic Impurities
■(See *Impurities Testing in Medical Gases* ⟨413⟩.)
[NOTE—The various detector tubes called for in the respective tests below are listed under *Reagents, Indicators, and Solutions*.]■2S (*USP33*)

• **CARBON DIOXIDE**
Sample: ~~1000 ± 50 mL of Oxygen~~■Manufacturer's recommended gas volume (±5%).■2S (*USP33*)

In-Process Revision

Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube.
Acceptance criteria: The indicator change corresponds to NMT ~~0.03%~~■300 ppm■2S (USP33).

• **CARBON MONOXIDE**
Sample: ~~1000 ± 50 mL of Oxygen~~■Manufacturer's recommended gas volume (±5%).■2S (USP33)
Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube.
Acceptance criteria: The indicator change corresponds to NMT ~~0.001%~~■10 ppm■2S (USP33).

SPECIFIC TESTS

• **ODOR**
Sample: Oxygen
Analysis: Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose.
Acceptance criteria: No appreciable odor is discernible.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in cylinders or in a pressurized storage tank. Containers used for Oxygen must not be treated with any toxic, sleep-inducing, or narcosis-producing compounds, and must not be treated with any compound that will be irritating to the respiratory tract when the Oxygen is used.~~
~~[NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube to minimize contamination or change of the specimens.]~~
■Preserve Oxygen in pressurized containers. Container connections shall be appropriate for Oxygen. Adapters shall not be used to connect containers to patient-use supply system piping or equipment.■2S (USP33)

Change to read:

• **LABELING:** ~~Label it to indicate whether or not it has been produced by the air-liquefaction process. Where it is piped directly from the cylinder or storage tank to the point of use, label each outlet 'Oxygen.'~~■Labeling shall indicate if the Oxygen was produced by the air-liquefaction process. If the Oxygen is piped from a remote location to the patient point of use, label the point of use outlet "Oxygen".■2S (USP33)
~~[NOTE—The various detector tubes called for in the respective tests are listed under *Reagents* in the *Reagents, Indicators, and Solutions* section.]~~
■■2S (USP33)

BRIEFING

Oxygen 93 Percent, *USP 32* page 3173—See briefing under *Medical Air*.

(AER: K. Zaidi.) RTS—C61161

Oxygen 93 Percent

DEFINITION
Oxygen 93 Percent is Oxygen produced from air by the molecular sieve process. It contains NLT 90.0% and NMT 96.0%, by volume, of O_2, the remainder consisting mostly of argon and nitrogen.

IDENTIFICATION

Change to read:

• ~~**A. PROCEDURE**~~■■2S (USP33) When tested as directed in the *Assay*, ~~NMT 10.0 mL and NLT 4.0 mL of gas remains~~■the result is NLT 90.0% and NMT 96.0% of oxygen, by volume.■2S (USP33)

Delete the following:

■~~• **B. PROCEDURE**~~
~~**Analysis:** Pass 100 ± 5 mL released from the vapor phase of the contents of the Oxygen 93 Percent container or from the outlet at the point of use through a carbon dioxide detector tube at the rate specified for the tube.~~
~~**Acceptance criteria:** No color change is observed (distinction from carbon dioxide).~~■2S (USP33)

ASSAY

Change to read:

• **PROCEDURE**
Analysis: ~~Place a sufficient quantity of ammonium chloride-ammonium hydroxide solution, prepared by mixing equal volumes of water and ammonium hydroxide and saturating with ammonium chloride at room temperature, in a test apparatus composed of a calibrated 100-mL buret, provided with a two-way stopcock, a gas absorption pipet, and a leveling bulb, both of suitable capacity and all suitably interconnected. Fill the gas absorption pipet with metallic copper in the form of wire coils, wire mesh, or other suitable configuration. Eliminate all gas bubbles from the liquid in the test apparatus. Activate the test solution by performing two or three tests that are not for record purposes. Fill the calibrated buret, all interconnecting tubing, both stopcock openings, and the intake tube with liquid. Draw 100.0 mL of Oxygen 93 Percent into the buret by lowering the leveling bulb. Open the stopcock to the absorption pipet, and force the Oxygen 93 Percent into the absorption pipet by raising the leveling bulb. Agitate the pipet to provide frequent and intimate contact of the liquid, gas, and copper. Continue agitation until no further diminution in volume occurs. Draw the residual gas back into the calibrated buret, and measure its volume~~■Determine the oxygen concentration using a paramagnetic analyzer for oxygen (See *Medical Gases Assay* ⟨415⟩).
[NOTE—See *Reagents, Indicators, and Solutions* for information on certified standards.]
Zero gas: Nitrogen certified standard
Span gas: 93% oxygen certified standard
Sample: Sample under test
Analyses: Determine the percentage of oxygen in the *Sample*.■2S (USP33)
Acceptance criteria: ~~NMT 10.0 mL and NLT 4.0 mL of gas remains.~~■NLT 90.0% and NMT 96.0% of oxygen, by volume of O_2■2S (USP33)

IMPURITIES

Change to read:

■**Inorganic Impurities**
(See *Impurities Testing in Medical Gases* ⟨413⟩.)
[NOTE—The various detector tubes called for in the respective tests below are listed under *Reagents, Indicators, and Solutions*.]■2S (USP33)

In-Process Revision

• **CARBON DIOXIDE**

Sample: ~~Oxygen 93 percent~~■Manufacturer's recommended gas volume (±5%)■2S (*USP33*)

Analysis: ~~Pass 1000 ± 50 mL~~■Pass the *Sample*■2S (*USP33*) through a carbon dioxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT ~~0.03%~~■300 ppm.■2S (*USP33*)

• **CARBON MONOXIDE**

Sample: ~~Oxygen 93 percent~~■Manufacturer's recommended gas volume (±5%)■2S (*USP33*)

Analysis: ~~Pass 1000 ± 50 mL~~■Pass the *Sample*■2S (*USP33*) through a carbon monoxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT ~~0.001%~~■10 ppm.■2S (*USP33*)

SPECIFIC TESTS

• **ODOR**

Analysis: Carefully open the container valve or system outlet to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose.

Acceptance criteria: No appreciable odor is discernible.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in cylinders or in a low pressure collecting tank. Containers used for Oxygen 93 Percent must not be treated with any toxic, sleep-inducing, or narcosis-producing compounds, and must not be treated with any compound that will be irritating to the respiratory tract when the Oxygen 93 Percent is used~~■Preserve Oxygen 93 Percent in pressurized containers. Container connections shall be appropriate for Oxygen 93 Percent. Adapters shall not be used to connect containers to patient-use supply system piping or equipment.■2S (*USP33*).

Change to read:

• **LABELING:** ~~Where it is piped directly from the collecting tank to the point of use, label each outlet "Oxygen 93 Percent."~~

~~[NOTE—The various detector tubes called for in the respective tests are listed in *Reagents, Indicators, and Solutions—Reagents*.]~~

~~Where it is preserved in cylinders, reduce the pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.~~

■If the oxygen is piped from a remote location to the patient point of use, label the point of use outlet "Oxygen 93 Percent".■2S (*USP33*)

BRIEFING

Pantoprazole Oral Suspension. Because there is no existing *USP* monograph for this dosage form, the following new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is based on analyses performed with a 4.6-mm × 15-cm analytical column that contains 5-µm packing L1. USP has received data indicating that an Inertsil ODS-3 from MetaChem Technologies, Inc. is suitable. The typical retention time for the pantoprazole peak is about 2.6 min.

(CRX: R. Schnatz.) RTS—60335

Add the following:

■Pantoprazole Oral Suspension

DEFINITION

Pantoprazole Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of pantoprazole sodium. Prepare Pantoprazole Oral Suspension, 2 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).[1]

Pantoprazole Sodium	200 mg
Sodium Bicarbonate 8.4% Injection	A sufficient quantity
To make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Remove the trademark imprint from the Tablets by gently rubbing on a paper towel that has been dampened with Alcohol, USP. Allow the Tablets to air-dry for a few min. Triturate the Tablets to a coarse powder by using a mortar and pestle, and transfer to a calibrated bottle. Add 50 mL of Sodium Bicarbonate 8.4% Injection and agitate until the coating is dissolved. Add sufficient Sodium Bicarbonate 8.4% Injection to bring the final volume to 100 mL and mix well until the powder is uniformly suspended.

[NOTE—If the imprint is not properly removed, the pharmaceutical elegance of the final product will be compromised by the presence of flecks of dark material.]

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 50 mM dibasic potassium phosphate (40:60). Adjust the pH to 7.0 with phosphoric acid. Make adjustments if necessary.

Standard stock solution: 1.0 mg/mL of USP Pantoprazole Sodium RS in water

Standard solution: Transfer 1.5 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume in order to obtain a solution containing about 15 µg/mL of pantoprazole sodium. Pass through a 0.22-µm filter.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 3.75 mL of Oral Suspension to a 50-mL volumetric flask. Add 25 mL of water to the flask, and place on an orbital shaker for 20 min. Dilute with water to volume. Take a 5-mL portion of the diluted sample and further dilute with water to 50 mL to obtain a solution with a nominal concentration of 15 µg/mL of pantoprazole sodium. Pass through a 0.22-µm filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[1] This formula is frequently used for home health patients with feeding tubes who have been discharged from hospitals. The goal of the drug is to neutralize the acidity of the stomach.

Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.6 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for the pantoprazole peak is about 2.6 min.]
Suitability requirements
Relative standard deviation: NMT 1.0% for the replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{14}F_2N_3NaO_4S \cdot 1.5\ H_2O$ in the volume of Oral Suspension taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (µg/mL)
C_U = concentration of pantoprazole sodium in the *Sample solution* (µg/mL)
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH ⟨791⟩:** Between 7.9 and 8.3

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored at controlled cold temperature
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Pantoprazole Sodium RS■2S (*USP33*)

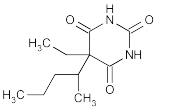
BRIEFING

Pentobarbital, *USP 32* page 3249. On the basis of comments received, the following revisions are proposed:

1. Delete *Identification* test *B* by UV because the remaining two *Identification* tests by IR and HPLC retention time match are sufficient to establish the identity of the drug substance.
2. Delete the *Capacity factor* sytem suitability requirement from both the *Assay* and the test for *Organic Impurities* because it contributes no additional value in establishing the suitability of the HPLC system. The remaining three parameters namely, theoretical plates, tailing factor, and relative standard deviation are adequate to ensure the suitability of the HPLC system.
3. Revise the calculation formula under the *Organic Impurities* test to be consistent with the redesigned format.
4. Delete the test for *Melting Range or Temperature* because it does not contribute any additional value in establishing the quality of the drug substance. This test was included in the original monograph when there was no selective method to quantify the impurities. The currently official monograph contains a selective stability-indicating HPLC method for the *Assay* and the *Organic Impurities* test both of which together provide sufficient information about the purity of the drug substance.

(MD-PP: R. Ravichandran.) RTS—C64602

Pentobarbital



$C_{11}H_{18}N_2O_3$ 226.27
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methylbutyl)-, (±)-; (±)-5-Ethyl-5-(1-methylbutyl)barbituric acid [76-74-4].

DEFINITION
Pentobarbital contains NLT 98.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis. Where the material is labeled as intended solely for veterinary use, Pentobarbital contains NLT 97.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197S⟩**
Sample solution: 7 in 100
Medium: Chloroform

Delete the following:

~~■• **B. ULTRAVIOLET ABSORPTION ⟨197U⟩**~~
~~**Sample solution:** 16 µg/mL~~
~~**Medium:** 0.1 N sodium hydroxide~~■2S (*USP33*)

Change to read:

• **~~C.~~■B.**■2S (*USP33*) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
Mobile phase: 0.01 M monobasic potassium phosphate and acetonitrile (65:35). Adjust the pH to 3.5.
Standard solution: 0.1 mg/mL of USP Pentobarbital RS in *Mobile phase*
Sample stock solution: 1 mg/mL of Pentobarbital in *Mobile phase* (sonicate until dissolved)
Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 15000 theoretical plates
Tailing factor: NMT 1.5
~~**Capacity factor, k′:** NLT 2.5~~■■2S (*USP33*)
Relative standard deviation: NMT 2.0% for pentobarbital
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{11}H_{18}N_2O_3$ in the portion of Pentobarbital taken:

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

In-Process Revision

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis; 97.0%–102.0% on the dried basis, where the material is labeled as intended solely for veterinary use

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 20 ppm

Change to read:

Organic Impurities

- **PROCEDURE**

Mobile phase: Prepare as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Pentobarbital RS in *Mobile phase*
Sample solution: 1 mg/mL of Pentobarbital in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 15000 theoretical plates
Tailing factor: NMT 1.5
~~**Capacity factor, k′:** NLT 2.5~~ ■2S (*USP33*)
Relative standard deviation: NMT 15.0% for pentobarbital
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Pentobarbital taken:

~~$\text{Result} = (r_U/r_S) \times (C_S/W) \times (10{,}000/F)$~~

~~r_U = peak area for any impurity in the *Sample solution*~~
~~r_S = peak area for pentobarbital in the *Standard solution*~~
~~C_S = concentrationof USP Pentobarbital RS in the *Standard solution* (mg/mL)~~
~~W = weight of Pentobarbital, on the dried basis, in the *Sample solution* (mg)~~
~~F = relative response factor of the impurity according to *Impurity Table 1*~~

■

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for any impurity in the *Sample solution*
r_S = peak area for pentobarbital in the *Standard solution*
C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)
F = relative response factor of the impurity (see *Impurity Table 1*)

■2S (*USP33*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Imino-5-ethyl-5-(1-methyl butyl) barbituric acid	0.39	1.5	0.2
5-Ethyl-5-(1-ethyl-propyl) barbituric acid[a]	0.93	1.0	0.1
Pentobarbital	1.0	—	—
5-Ethyl-5-(1,3-dimethylbutyl) barbituric acid	1.5	0.9	0.3
Unknown impurities	—	1.0	0.1
Total	—	—	0.5

[a] Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

SPECIFIC TESTS

Delete the following:

~~■• **MELTING RANGE OR TEMPERATURE,** *Class I* **⟨741⟩:** 127°–133°~~ ■2S (*USP33*)

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

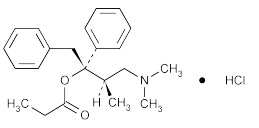
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Pentobarbital RS

BRIEFING

Propoxyphene Hydrochloride, *USP 32* page 3417. The current official methods for *Organic Impurities* and the *Assay* use a Microbondapak C18 column, which is not well-suited for basic compounds. It is proposed to replace the column with a Zorbax XDB Eclipse C18 brand of L1 column. Under the new chromatographic conditions, propoxyphene related compound A, propoxyphene related compound B, and propoxyphene have typical retention times of about 5.7, 8.1, and 9.5 min, respectively. The new method requires the *Definition* to be revised from 98.0–101.0% to 98.0–102.0%, based on approved acceptance criteria. The revised *Organic Impurities* method allows the inclusion of limits for specified and unspecified impurities. It is also proposed to delete the *Melting Range* test, since it contributes no additional value in establishing the quality of the drug substance as a selective HPLC method has been added via the proposed revision.

(MD-CCA: C. Anthony.) RTS—C39791

Propoxyphene Hydrochloride



$C_{22}H_{29}NO_2 \cdot HCl$ 375.93

Benzeneethanol, α-[2-(dimethylamino)-1-methylethyl]-α-phenyl-, propanoate (ester), hydrochloride, [*S*-(*R**,*S**)]-; (2*S*,3*R*)-(+)-4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-butanol propionate (ester) hydrochloride [1639-60-7].

DEFINITION

Change to read:

Propoxyphene Hydrochloride contains NLT 98.0% and NMT ~~101.0%~~■102.0%■2S (USP33) of $C_{22}H_{29}NO_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197S⟩
  Sample solution: 50 mg/mL in chloroform
- **B. PROCEDURE**
  Sample solution: 17 mg/mL of Propoxyphene Hydrochloride in Purified Water
  Analysis: Treat 3 mL of *Sample solution* with 1 mL of 6 N ammonium hydroxide to precipitate the propoxyphene base. Filter to remove the precipitate, acidify the filtrate with 2 mL of nitric acid, and add 1 mL of silver nitrate TS.
  Acceptance criteria: A white, curdy precipitate that is soluble in an excess of 6 N ammonium hydroxide confirms the presence of silver chloride.

ASSAY

Change to read:

- **PROCEDURE**

Solution A ■(0.02 M KH_2PO_4):■2S (USP33) ~~11.5 g of monobasic ammonium phosphate and 1.0 mL of triethylamine in 1000 mL of water. Adjust with 10% sodium hydroxide to a pH of 6.3 ± 0.05 (0.1 M Monobasic ammonium phosphate buffer, pH 6.3)~~■2.72 g/L monobasic potassium phosphate■2S (USP33)

Mobile phase: ~~Methanol and *Solution A* (67:33)~~■Acetonitrile, triethylamine, and *Solution A* (53:0.1:47). Adjust the pH to 8.0 with 85.0% phosphoric acid. [NOTE—Mobile phase should be discarded after 30 days and should be examined prior to use for particulates and clarity.]■2S (USP33)

Standard solution: ~~5.0~~■0.11■2S (USP33) mg/mL of USP Propoxyphene Hydrochloride RS in *Mobile phase*. ■Sonicate to dissolve if necessary.■2S (USP33)

Sample solution: ~~5~~■0.11■2S (USP33) mg/mL of Propoxyphene Hydrochloride in *Mobile phase*. ■Sonicate to dissolve if necessary.■2S (USP33)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV ~~254 nm~~■214 nm■2S (USP33)
Column: ~~3.9-mm × 30-cm; packing L1~~■4.6 mm × 15 cm; 3.5-µm packing L1■2S (USP33)
Flow rate: ~~1.5~~■1■2S (USP33) mL/min
Injection size: ~~50~~■10■2S (USP33) µL

System suitability
Sample: *Standard solution*
~~[NOTE—The retention time for propoxyphene hydrochloride is about 9 min.]~~■■2S (USP33)
Suitability requirements
Tailing factor: NMT ~~3.5~~■1.5■2S (USP33) for the propoxyphene hydrochloride peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{29}NO_2 \cdot HCl$ in the portion of Propoxyphene Hydrochloride taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Propoxyphene Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propoxyphene hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–~~101.0%~~■102.0% on the dried basis■2S (USP33)

IMPURITIES

Change to read:

Organic Impurities

- **PROCEDURE**

Mobile phase: ~~and **Chromatographic system**~~■2S (USP33) Proceed as directed in the *Assay*.

~~**Standard stock solution:** 10 mg each of USP Propoxyphene Related Compound A RS and USP Propoxyphene Related Compound B RS, dissolved in 2 mL of methanol, and diluted with *Mobile phase* to 50 mL~~■■2S (USP33)

Standard solution: ~~*Mobile phase* and *Standard stock solution* (9:1)~~■3 µg/mL of USP Propoxyphene Related Compound A RS, 2 µg/mL of USP Propoxyphene Related Compound B RS, and 1 µg/mL of USP Propoxyphene Hydrochloride RS in *Mobile phase*. Sonicate to dissolve if necessary.■2S (USP33)

Sample solution: ~~Use the *Sample solution* as directed in the *Assay*.~~■1.1 mg/mL of Propoxyphene Hydrochloride in *Mobile phase*. Sonicate to dissolve if necessary. Discard after 14 days.■2S (USP33)

System suitability solution: ~~4.5 mg/mL of USP Propoxyphene Hydrochloride RS in *Standard solution*. [NOTE—The solution also contains 0.02 mg/mL of USP Propoxyphene Related Compound A RS and 0.02 mg/mL of USP Propoxyphene Related Compound B RS.]~~■Use the *Standard solution*.
Chromatographic system: Proceed as directed in the *Assay*, with the exception that the chromatographic run time is six times the retention time of propoxyphene.■2S (USP33)

System suitability
Sample: ~~*System Suitability*~~ ■*Standard solution*■2S (USP33)
~~[NOTE—The relative retention times for propoxyphene related compound A, propoxyphene related compound B, and propoxyphene hydrochloride are 0.63, 0.78, and 1.0, respectively.]~~■■2S (USP33)
Suitability requirements
Resolution: NLT 2.0 between propoxyphene related compound A and propoxyphene related compound B, ■and NLT 2.0 between propoxyphene related compound B and propoxyphene■2S (USP33)

In-Process Revision

Relative standard deviation: NMT ~~2.0%~~■15%■2S (*USP33*)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of propoxyphene related compound A in the portion of Propoxyphene Hydrochloride taken:

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

r_U = peak response of propoxyphene related compound A from the *Sample solution*
r_S = peak response of propoxyphene related compound A from the *Standard solution*
C_S = concentration of USP Propoxyphene Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Propoxyphene Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: ~~NMT 0.5%~~■See *Impurity Table 1*.■2S (*USP33*).

Calculate the percentage of propoxyphene related compound B as the hydrochloride in the portion of Propoxyphene Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of propoxyphene related compound B from the *Sample solution*
r_S = peak response of propoxyphene related compound B from the *Standard solution*
C_S = concentration of USP Propoxyphene Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propoxyphene related compound B in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of propoxyphene related compound B as the hydrochloride, 361.93
M_{r2} = molecular weight of propoxyphene related compound B, 325.45

Acceptance criteria: ~~NMT 0.6%.~~ ■See *Impurity Table 1*.

Calculate the percentage of any other specified and unspecified impurity in the portion of Propoxyphene Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any specified or unspecified impurity of the *Sample solution*
r_S = peak response of propoxyphene hydrochloride of the *Standard solution*
C_S = concentration of USP Propoxyphene Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Propoxyphene Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding individual impurity from *Impurity Table 1*

Acceptance criteria: See *Impurity Table 1*. [NOTE—Disregard any impurity less than 0.05%.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Acceptance Criteria, NMT (%)
Propoxyphene related compound A[a]	0.6	—	0.5
Propoxyphene related compound B[b]	0.85	—	0.6
Propoxyphene	1.0	—	—
E-Aminostilbene[c]	1.3	1.5	0.2
Butyroxyphene[d]	1.6	1.0	0.2
Z-Aminostilbene[e]	2.0	1.3	0.2
Ethylidene bibenzyl[f]	3.5	1.9	0.2
Any individual unspecified impurity	—	1.0	0.1

[a] α-*d*-4-Dimethylamino-1,2-diphenyl-3-methyl-2-butanol hydrochloride.
[b] α-*d*-2-Acetoxy-4-dimethylamino-1,2-diphenyl-3-methylbutane hydrochloride.
[c] (*S*)-1-Dimethylaminopropan-2-yl-(*E*)-stilbene.
[d] (2*S*,3*R*)-4-(Dimethylamino)-3-methyl-1,2-diphenylbutan-2-yl butyrate.
[e] (*S*)-1-Dimethylaminopropan-2-yl-(*Z*)-stilbene.
[f] (*E*)-1,2-Diphenylbut-2-ene.

■2S (*USP33*)

SPECIFIC TESTS

Delete the following:

■• **MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** ~~163.5°–168.5°, but the range between beginning and end of melting does not exceed 3°~~■2S (*USP33*)

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +52° to +57°
  Sample solution: 10 mg/mL in water, freshly prepared
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Propoxyphene Hydrochloride RS
  USP Propoxyphene Related Compound A RS
  USP Propoxyphene Related Compound B RS

BRIEFING

Ramipril Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and in the tests for *Dissolution* and *Organic Impurities* were validated using a Zorbax SB 5-µm column of packing L1. The typical retention time for ramipril under the gradient conditions for the *Assay* and the test for *Organic Impurities* is about 26 minutes. The typical retention time under the isocratic conditions for the *Dissolution* test is about 2.6 minutes.

(MDCV: S. Ramakrishna. BPC: M. Marques.) RTS—C65921

In-Process Revision

Add the following:

▪Ramipril Capsules

DEFINITION

Ramipril Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{23}H_{32}N_2O_5$.

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
Phosphoric acid solution: 30 mL of phosphoric acid in 1 L of water
Diluent: Acetonitrile and *Phosphoric acid solution* (2:3)
Standard solution: 0.2 mg/mL of USP Ramipril RS in *Diluent*. Sonicate for 1 min, if necessary, for complete dissolution.
Sample solution: Use the *Sample solution* prepared as directed in the *Assay*.
Wavelength range: 200–400 nm

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**
Buffer: Dissolve 17 g of monobasic potassium phosphate and 11.2 g of sodium perchlorate in 750 mL of water in a 1-L flask. Dilute with water to volume. Adjust with phosphoric acid to a pH of 2.3.
Solution A: Acetonitrile, *Buffer*, and water (1:2:2). [NOTE—Do not filter *Solution A*.]
Solution B: Acetonitrile, *Buffer*, and water (9:10:6). [NOTE—Do not filter *Solution B*.]
Mobile phase: Use the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
50	0	100
51	0	100
51.1	100	0
60	100	0

Phosphoric acid solution: Prepare as directed in *Identification* test *A*.
Diluent: Prepare as directed in *Identification* test *A*.
Standard solution: 0.2 mg/mL of USP Ramipril RS and 0.002 mg/mL of USP Ramipril Related Compound A RS in *Diluent*
Sample stock solution: Transfer the contents of 8 Capsules into a suitable flask as described in *Table 1*. Add Capsule shells into the flask. Add acetonitrile per *Table 1* and swirl to agitate the contents. Sonicate for 15 min and mechanically shake for 10 min. Dilute with acetonitrile to volume for Capsule strengths 5.0 and 10 mg only. For 1.25 and 2.5 mg use the solution as is without further dilution. [NOTE—Extracts from the vial cap may result in extraneous peaks.]

Table 1

Strength of Capsule (mg)	Volumetric Flask Size (mL)	Acetonitrile (mL)
1.25	50	25
2.5	100	50
5.0	100	70
10	200	140

Sample solution: 0.2 mg/mL of ramipril in *Phosphoric acid solution* from *Sample stock solution*. Pass through a 0.20-µm nylon filter, and discard the first 2 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1 with a guard column, packing L1
Temperature: 60°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between ramipril and ramipril related compound A
Tailing factor: NMT 2.5 for the ramipril peak
Relative standard deviation: NMT 2.0% for the ramipril peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{32}N_2O_5$, based on the label claim, in the portion of Capsules taken:

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

r_U = response of the ramipril peak from the *Sample solution*
r_S = response of the ramipril peak from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩**
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is catalog number CAPWHT-02 available from www.QLA-LLC.com.]
Time: 30 min
Standard solution: 0.01 mg/mL of USP Ramipril RS in *Medium*.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Phosphoric acid solution: Prepare as directed in *Identification* test *A*.
Mobile phase: Acetonitrile and *Phosphoric acid solution* (2:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 25 µL
Suitability requirements
Sample: *Standard solution*
Tailing factor: NMT 2.0 for the ramipril peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ramipril dissolved by the formula:

$$(r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
L = capsule label claim (mg)
V = 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ramipril is dissolved.

In-Process Revision

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements
Procedure for content uniformity
Phosphoric acid solution: Prepare as directed in *Identification* test *A*.
Mobile phase: Acetonitrile and *Phosphoric acid solution* (2:3). Pass through a 0.45-µm nylon filter.
Standard solution: 0.03 mg/mL of USP Ramipril RS in *Mobile phase*. Sonicate for 1 min, if not dissolved completely.
Sample solution: Transfer the contents of 1 Capsule into a suitable flask as described in *Table 2*. Add (about 50% of total volume) *Mobile phase*, and sonicate for 25 min. Mechanically shake for 10 min and dilute with *Mobile phase* to volume. Further dilute samples as shown in *Table 2* with *Mobile phase*. Pass through a 0.20-µm nylon filter, and discard the first 2 mL of the filtrate.

Table 2

Strength of Capsule (mg)	Volumetric Flask Size (mL)	Dilution Volume (mL)	Volumetric Flask (mL)
1.25	50	—	—
2.5	100	—	—
5.0	200	—	—
10	50	6.0	50

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Proceed as directed in the test for *Dissolution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{32}N_2O_5$, based on the label claim, in the portion of Capsules taken:

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

r_U = response of the ramipril peak from the *Sample solution*
r_S = response of the ramipril peak from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
C_U = concentration of ramipril in the *Sample solution* (mg/mL)

IMPURITIES
Organic Impurities
• **PROCEDURE**
Buffer, Solution A, Solution B, Phosphoric acid solution, Diluent, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.
Sensitivity solution: Dilute the *Standard solution* in *Diluent* to prepare a solution having a concentration of 0.1 µg/mL of ramipril.
Chromatographic system: Prepare as directed in the *Assay*.
Suitability requirements
Samples: *Standard solution* and *Sensitivity solution*
Resolution: NLT 2.5 between ramipril and ramipril related compound A, *Standard solution*
Tailing factor: NMT 2.5 for the ramipril peak, *Standard solution*
Signal-to-noise ratio: NLT 10 for each peak, *Sensitivity solution*
Relative standard deviation: NMT 2.0% for the ramipril peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100 \times (1/F)$$

r_U = response of each individual impurity from the *Sample solution*
r_S = response of ramipril from the *Standard solution*
C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
F = relative response factor (see *Table 3*)
Acceptance criteria
Individual impurities: Impurities meet the limits in *Table 3*.
Total impurities: NMT 6.0% for Capsule strengths 1.25 mg and 2.5 mg, and NMT 3.0 for Capsule strengths 5 mg and 10 mg. [NOTE—Total impurities include the sum of individual specified and unspecified degradants. Disregard any peak below 0.05%.]

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Ramipril RS
USP Ramipril Related Compound A RS■2S *(USP33)*

Table 3

Name	Relative Retention Time	Relative Response Factor	Limit (%) for 1.25-mg and 2.5-mg Capsules	Limit (%) for 5-mg and 10-mg Capsules
Ramipril diacid	0.24	0.41	0.2	—
Ramipril related compound A[1]	0.72	—	—	—
Ramipril diacid impurity [1]	0.85	—	—	—
Ramipril	1	—	—	—
Ramipril related compound B[1]	1.31	—	—	—
Ramipril related compound C[1]	1.68	—	—	—
Ramipril related compound D[2]	1.84	1	5.5	2.5
Any other individual unspecifed degradant	—	—	0.2	0.2

[1] Disregard this impurity as it is process related and is controlled in the drug substance.
[2] Ramipril diketopiperazine (6,7,8-trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one).

BRIEFING

Risperidone Oral Solution. Because there is no existing *USP* monograph for this dosage form, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities* were validated with a Hypersil BDS brand of L1 column, in which risperidone elutes at about 9 min.

(MD-PP: R. Ravichandran.) RTS—C51559

Add the following:

▪Risperidone Oral Solution

DEFINITION

Risperidone Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$). It may contain a suitable preservative.

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 5.0 g/L ammonium acetate in water

Mobile phase: Acetonitrile:*Buffer* (11:39)

Standard stock solution: 1 mg/mL of USP Risperidone RS, in methanol

Standard solution: 0.2 mg/mL of USP Risperidone RS prepared from *Standard stock solution* as follows: transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask. Add 5.0 mL of water followed by 12.5 mL of *Buffer*, and allow to cool to room temperature. Dilute with methanol to volume.

Sample solution: Nominal concentration of 0.2 mg/mL risperidone prepared from Oral Solution as follows: transfer an amount of Oral Solution, equivalent to 5 mg of risperidone to a 25-mL volumetric flask, add 12.5 mL of *Buffer*, fill with methanol almost to volume, and mix well. Allow to cool to room temperature, and dilute with methanol to volume.

System suitability solution: 0.25 mg/mL of USP Risperidone Related Compounds Mixture RS prepared as follows: weigh USP Risperidone Related Compounds Mixture RS into a suitable volumetric flask. Dissolve first in 20% of the flask volume of methanol. Add 20% of the flask volume of water followed by 50% of the flask volume of *Buffer*, and allow to cool to room temperature. Dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: 2 times the retention time of risperidone

System suitability

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

Relative retention times: See *Impurity Table 1*.

Suitability requirements

Resolution: NLT 1.5 between bicyclorisperidone and *Z*-oxime, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of $C_{23}H_{27}FN_4O_2$ in the portion of risperidone taken:

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

r_U = peak response for risperidone from the *Sample solution*

r_S = peak response of risperidone from the *Standard solution*

C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of risperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME ⟨698⟩:** Meets the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Buffer, Mobile phase, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay*

Standard stock solution: 5 µg/mL of USP Risperidone RS, in methanol

Standard solution: 1 µg/mL of USP Risperidone RS from *Standard stock solution* prepared as follows: transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask. Add 5.0 mL of water followed by 12.5 mL of *Buffer*, and allow to cool to room temperature. Dilute with methanol to volume.

Chromatographic system

Prepare as directed in the *Assay*.

System suitability

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

Relative retention times: See *Impurity Table 1*.

Suitability requirements

Resolution: NLT 1.5 between bicyclorisperidone and *Z*-oxime, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of risperidone taken:

$$\text{Result} = (r_I/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_I = response for each individual impurity from the *Sample solution*

r_S = response for risperidone from the *Standard solution*

C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of risperidone in the *Sample solution* (mg/mL)

F = relative response factor from *Impurity Table 1*

In-Process Revision

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Risperidone *cis N*-oxide[1]	0.33	0.97	0.50
Bicyclorisperidone[2]	0.43	0.67	0.50
Z-oxime[3]	0.53	—	—
Risperidone	1.0	—	—
Any unspecified degradation product	—	1.0	0.20

[1]*cis*-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one,*N*-oxide monohydrate.
[2]3-(4-Fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-a]pyrimidin-3-yl)ethyl]-1-aza-2-azoniabicyclo[2.2.2]oct-2-ene salt.
[3](*Z*)-3-[2-[4-(2,4-Difluorophenyl)(hydroxyimino)methyl]-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one.
[NOTE—Process impurity, not to be quantified in drug product: it is used to establish system suitability only.]

SPECIFIC TESTS
- **pH ⟨791⟩:** 2.0–4.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in light-resistant containers, at controlled room temperature. Do not freeze.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Risperidone RS
  USP Risperidone Related Compounds Mixture RS■2S *(USP33)*

BRIEFING

Sumatriptan Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on validated methods. The liquid chromatographic procedures in the *Assay* and test for *Organic Impurities*, *Procedure* are based on analyses performed using a Waters Spherisorb ODS-1 brand of L1 column. The typical retention time for sumatriptan is about 6 min in the *Assay* and about 13 min in the test for *Organic Impurities*, *Procedure.*

(MD-PP: R. Ravichandran. BPC: M. Marques.) RTS—C73613

Add the following:

■Sumatriptan Tablets

DEFINITION
Sumatriptan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$).

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩:** The IR spectrum exhibits main bands at or near (± 1) wave numbers (cm^{-1}) 1708, 1567, 1339, 1300, 1235, 1207, 989, 844, and 767.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 2.93 g/L of monobasic sodium phosphate, 1.3 mL/L of dibutylamine, and 0.4 mL/L of phosphoric acid in water, adjusted to a pH of 6.5 with 10 N sodium hydroxide solution
  Mobile phase: Acetonitrile and *Solution A* (1:3)
  Solution B: 3.9 g/L of monobasic sodium phosphate, adjusted to a pH of 6.5 with 10 N sodium hydroxide solution prior to dilution
  Diluent: Acetonitrile and *Solution B* (1:3)
  Standard solution: Equivalent to 0.1 mg/mL of sumatriptan from USP Sumatriptan Succinate RS in *Diluent*
  Sample solution: Equivalent to 0.1 mg/mL of sumatriptan in *Diluent* [NOTE—Sonicate if necessary.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 282 nm
  Column: 4.6-mm × 25-cm column; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection size: 10 µL
  Run time: 10 min
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{21}N_3O_2S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)
  C_U = concentration of the *Sample solution* (mg/mL)
  M_{r1} = molecular weight of sumatriptan, 295.4
  M_{r2} = molecular weight of sumatriptan succinate, 413.5
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION ⟨711⟩**
  Medium: 0.01 N hydrochloric acid; 900 mL
  Apparatus 2: 30 rpm
  Time: 15 min
  Standard solution: 0.025 mg/mL of USP Sumatriptan Succinate RS in *Medium*
  Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute with *Medium*, if necessary.
  Mode: Spectrophotometry
  Detection: UV 282 nm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity of $C_{14}H_{21}N_3O_2S$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times (M_{r1}/M_{r2}) \times 100$$

  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  L = tablet label claim (mg)
  D = dilution factor of the *Sample solution*
  V = volume of *Medium*, 900 mL
  M_{r1} = molecular weight of sumatriptan, 295.4
  M_{r2} = molecular weight of sumatriptan succinate, 413.5
  Tolerances: NLT 80% (Q) of the labeled amount of sumatriptan is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Solution A: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile and *Solution A* (3:17)

System suitability solution: 0.1 mg/mL each of USP Sumatriptan Succinate RS and USP Sumatriptan Succinate Related Compound C RS in *Mobile phase*

Standard solution: 3 µg/mL of USP Sumatriptan Succinate RS in *Mobile phase*

Sample solution: Equivalent to 3 mg/mL of sumatriptan in *Mobile phase* [NOTE—Sonicate if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 282 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: Four times the retention time of sumatriptan

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between sumatriptan and sumatriptan succinate related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for the sumatriptan peak, *Standard solution*

Relative standard deviation: NMT 5.0% for the sumatriptan peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the impurity peak from the *Sample solution*

r_S = peak response of the sumatriptan peak from the *Standard solution*

C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)

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

F = relative response factor (see *Impurity Table 1*)

M_{r1} = molecular weight of sumatriptan, 295.4

M_{r2} = molecular weight of sumatriptan succinate, 413.5

Acceptance criteria

[NOTE—Reporting limit is 0.5 times the response of the principal peak in the *Standard solution* (0.05%).]

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT %
Sumatriptan amino[a, e]	0.42	1.0	—
Sumatriptan monomethyl[b,e]	0.62	0.8	—
Sumatriptan *N*-oxide[c]	0.78	1.0	0.5
Sumatriptan succinate related compound C[d,e]	0.87	0.7	—
Sumatriptan	1.0	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] [3-[2-Aminoethyl)]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.

[b] *N*-Methyl-1-{3-[2-(methylamino)ethyl]-1*H*-indol-5-yl}methanesulfonamide.

[c] [3-[2-(Dimethylamino-*N*-oxide)ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.

[d] [3-[2-(Dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.

[e] This is a process impury, which is controlled in the drug substance; and it is listed here for information only.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Sumatriptan Succinate RS

USP Sumatriptan Succinate Related Compound C RS■2S (*USP33*)

BRIEFING

Terazosin Capsules. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedure in the *Assay* is validated using a Burdick and Jackson OD5 5-µm column containing packing L1. The test for *Organic Impurities* is validated using a Symmetry Shield RP18 5-µm column containing packing L1. The typical retention time for terazosin hydrochloride is about 11.5 min for the *Assay* conditions and about 8.1 min for the *Organic Impurities* test conditions.

(MD-CV: S. Ramakrishna.) RTS—C65483

Add the following:

■Terazosin Capsules

DEFINITION

Terazosin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{19}H_{25}N_5O_4 \cdot HCl$, calculated as a free base.

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**

[NOTE—Discard the first 5 mL from the filtered *Diluent*, *Standard solution*, and *Sample solution* for use.]

Diluent: 0.1 N hydrochloric acid

Standard solution: 0.005 mg/mL of USP Terazosin Hydrochloride RS in *Diluent*. Sonicate for 10 min for complete dissolution. Pass the solution through a 0.45-µm nylon filter.

Sample solution: Combine the contents from 20 Capsules and transfer 10 mg into a 100-mL flask, and fill with *Diluent* to 50% of the volume of the flask. Sonicate the flask for 10 min. Allow it to cool to room temperature. Dilute with *Diluent*

In-Process Revision

to volume. Further dilute 5 mL of this solution with *Diluent* to 100 mL, and mix well. Pass 10 mL of this preparation through a 0.45-µm PTFE filter.
Blank: Pass the *Diluent* through a 0.45-µm PTFE filter.
Analytical wavelength: 200–400 nm
Flow cell: 1 cm
Acceptance criteria: The UV spectrum of the *Sample solution* exhibits maxima and minima at the same wavelength as that of the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE** [NOTE—Use all glass syringe.]

Hydrochloric acid solution: Prepare 0.1 N methanolic HCL by adding 0.85 mL of hydrochloric acid in 1 L methanol
Diluent: *Hydrochloric acid solution*:water (2:3)
Mobile phase: Mix 700 mL of acetonitrile with 300 mL of water. Pass through a 0.45-µm nylon filter. Add 10.00 mL of glacial acetic acid, and degas. After degassing, pipet 0.20 mL of diethylamine into the solution, and mix.
Standard stock solution: 0.55 mg/mL of USP Terazosin Hydrochloride RS in *Diluent.* Sonication for 5 min may be necessary for complete dissolution.
Standard solution: Use the *Standard stock solution* to prepare 0.055 mg/mL of Terazosin Hydrochloride in *Diluent.* Pass through a 0.45-µm PTFE filter and discard NLT the first 8 mL of filtrate.
Sample solution: Combine the contents of NLT 20 Capsules and weigh a quantity equivalent to 10 mg of terazosin into a 200-mL flask. Add 100 mL of *Diluent* and sonicate for NLT 10 min. Shake the flask mechanically for NLT 10 min. Repeat until the sample is well dispersed. Allow the solution to cool, and dilute with *Diluent* to volume. Pass through a 0.45-µm PTFE filter and discard the first 8 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 2.5 mL/min
Run time: NLT twice the retention time of the terazosin peak
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8 for the terazosin peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of $C_{19}H_{25}N_5O_4$, based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100 \times (M_{r1}/M_{r2})$$

r_U = response of the terazosin peak from the *Sample solution*
r_S = response of the terazosin peak from the *Standard solution*
C_S = concentration of Terazosin Hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of terazosin, 387.44
M_{r2} = molecular weight of terazosin hydrochloride, 423.90

Acceptance criteria: 90.0%–110.0% as a free base

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩**:** [To come]
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

- **PROCEDURE**

Buffer: Dissolve 4.1 g of monobasic potassium phosphate and 1.1 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water. Adjust the pH to 3.0 ± 0.10 with phosphoric acid. Dilute with water to 1000 mL. Pass through a 0.45-µm nylon filter.
Mobile phase: Acetonitrile:*Buffer* (6:19)
Standard solution: 0.003 mg/mL of USP Terazosin Hydrochloride RS in *Mobile phase*
Sample solution: Transfer 15 mg of terazosin from the contents of 20 Capsules into a 50-mL volumetric flask. Dilute with *Mobile phase* to approximately half the volume of the flask. Sonicate for NLT 10 min, and shake the flask for NLT 20 min. Dilute with *Mobile phase* to volume, and pass through a 0.45-µm nylon or Teflon filter, discarding the first 5 mL of filtrate. The final concentration of the *Sample solution* is 0.3 mg/mL.
Sensitivity solution: Dilute the *Standard solution* with *Mobile phase* to prepare 0.15 µg/mL of USP Terazosin Hydrochloride RS.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: NLT 4.5 times the retention time of terazosin in the *Sample solution* and NLT 1.2 times the retention time of terazosin for the *Standard solution* and *Sensitivity solution*
System suitability
Sample: *Standard solution* and *Sensitivity solution*
Capacity factor: NLT 1.0 for the terazosin peak
Relative standard deviation: NLT 2.0%, *Standard solution*
Tailing factor: NMT 2.0 for the terazosin peak, *Standard solution*
Signal to noise: NLT 10 for the terazosin peak from the *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_I/r_S) \times (C_S/C_U) \times 100 \times (1/F) \times (M_{r1}/M_{r2})$$

r_I = response of each individual impurity from the *Sample solution*
r_S = response of terazosin from the *Standard solution*
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
C_S = concentration of terazosin hydrochloride in the *Standard solution* (mg/mL)
F = relative response factor as given in *Impurity Table 1*
M_{r1} = molecular weight of terazosin, 387.44
M_{r2} = molecular weight of terazosin hydrochloride, 423.90

Acceptance criteria
Impurity limits: Meets the impurity limits specified in *Impurity Table 1*

In-Process Revision

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Acceptance Criteria, NMT (%)
Piperazinyl-ADMQ[1]	0.52	1.1	0.4
Chloro ADMQ[2]	1.37	1.2	0.4
Bis-ADMQ-piperazine[3]	3.85	1.0	0.4
Any other individual impurity	—	—	0.2
Total impurities	—	—	1.2

[1] *N*-(4-amino-6,7 dimethoxy-2-quinazolinyl)piperazine.
[2] 2-Chloro-4-amino-6,7 dimethoxy-2-quinazoline.
[3] *N,N*-Bis-(4-amino-6,7 dimethoxy-2-quinazolinyl)piperazine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers protected from moisture and light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Terazosin Hydrochloride RS■2S *(USP33)*

BRIEFING

Terazosin Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on validated data. The liquid chromatographic procedure in the *Assay* is validated using a Burdick and Jackson C18 5-µm column containing packing L1. The test for *Organic Impurities* is validated using a Symmetry Shield RP18 5-µm column containing packing L1. The typical retention time for terazosin hydrochloride is about 2.3 min for the *Assay* conditions and about 8.1 min for the *Organic Impurities* test conditions.

(MD-CV: S. Ramakrishna.) RTS—C65484

Add the following:

■Terazosin Tablets

DEFINITION

Terazosin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{19}H_{25}N_5O_4 \cdot HCl$, calculated as a free base.

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** ⟨197U⟩
  [NOTE—Discard the first 5 mL from the filtered *Standard solution* and *Sample solution* for use.]
  Diluent: 0.1 N hydrochloric acid
  Standard solution: 0.005 mg/mL in *Diluent*. Sonicate for 10 min for complete dissolution. Pass the solution through a 0.45-µm nylon filter.
  Sample solution: Transfer 10 mg from the pool of ground Tablets (NLT 20) into a 100-mL flask. Dilute with *Diluent* to 50% of the volume of the flask. Sonicate the flask for 10 min. Allow it to cool to room temperature, and dilute with *Diluent* to volume. Further dilute 5 mL of this solution with *Diluent* to 100 mL, and mix well. Pass 20 mL of this preparation through a 0.45-µm PTFE filter.
  Analytical wavelength: 200–400 nm
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE** [NOTE—Use all glass syringe.]
  Hydrochloric acid solution: Prepare 0.01 N methanolic HCl by adding 0.85 mL of hydrochloric acid to 1 L of methanol.
  Diluent: *Hydrochloric acid solution*:water (2:3)
  Mobile phase: Acetonitrile:water (7:3). Add 10.00 mL/L of glacial acetic acid, and degas. Pass through a 0.45-µm nylon filter. Pipet 0.20 mL of diethylamine into the solution and mix.
  Standard stock solution: 0.55 mg/mL of USP Terazosin Hydrochloride RS in *Diluent*. Sonicate for 5 min for complete dissolution.
  Standard solution: Use *Standard stock solution* to prepare 0.055 mg/mL of terazosin hydrochloride in *Diluent*. Pass through a 0.45-µm PTFE filter and discard the first few mL of filtrate.
  Naproxen standard solution: 0.5 mg/mL of USP Naproxen RS solution. Dissolve by sonication USP Naproxen RS in acetonitrile (25% of the volume of the flask), and dilute with water to volume.
  System suitability solution: Use the *Standard solution* and *Naproxen standard solution* to prepare 0.05 mg/mL of naproxen and 0.055 mg/mL of terazosin hydrochloride. Pass through a 0.45-µm PTFE filter, and discard the first few mL of filtrate.
  Sample solution: 0.05 mg/mL of terazosin in *Diluent*. Combine the contents of NLT 20 Tablets, and weigh a quantity equivalent to 10 mg of terazosin into a 200-mL flask. Add 100 mL of *Diluent*, and sonicate for NLT 10 min. Shake the flask mechanically for NLT 10 min. Repeat until the sample is well dispersed. Allow the solution to cool, and dilute with *Diluent* to volume. Pass through a 0.45-µm PTFE filter, and discard the first few mL of filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 15-cm column; 5-µm packing L1
  Flow rate: 2.5 mL/min
  Run time: NLT 1.8 times the retention time of the terazosin peak
  Injection size: 25 µL
  System suitability
  Sample: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 2.0 between the naproxen and terazosin peaks, *System suitability solution*
  Tailing factor: NMT 1.8 for the terazosin peak, *System suitability solution*
  Relative standard deviation: NMT 2.0, *Standard solution*
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Calculate the percentage of $C_{19}H_{25}N_5O_4$, based on the label claim, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100 \times (M_{r1}/M_{r2})$$

r_U = response of the terazosin peak from the *Sample solution*
r_S = response of the terazosin peak from the *Standard solution*
C_S = concentration of terazosin hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of terazosin, 387.44
M_{r2} = molecular weight of terazosin hydrochloride, 423.90

In-Process Revision

Acceptance criteria: 90.0%–110.0% as a free base

PERFORMANCE TESTS
- **DISSOLUTION ⟨711⟩:** [To come]
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES
Organic Impurities
- **PROCEDURE**

Buffer: Dissolve 4.1 g of monobasic potassium phosphate and 1.1 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water. Adjust the pH to 3.0 ± 0.10 with phosphoric acid. Dilute to 1 L with water. Pass through a 0.45-µm nylon filter.
Mobile phase: Acetonitrile:*Buffer* (6:19)
Standard solution: 0.003 mg/mL of USP Terazosin Hydrochloride RS in *Mobile phase*
Sample solution: Transfer 15 mg of the powder from the crushed Tablets (NLT 20) into a 50-mL volumetric flask. Dilute with *Mobile phase* to approximately half the volume of the flask. Sonicate for NLT 10 min and shake the flask for NLT 20 min. Dilute with *Mobile phase* to volume, and pass through a 0.45-µm nylon or Teflon filter, discarding the first 5 mL of filtrate. The final concentration of the *Sample solution* is 0.3 mg/mL.
Sensitivity solution: Dilute the *Standard solution* with *Mobile phase* to prepare 0.15 µg/mL of USP Terazosin Hydrochloride RS.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Column temperature: Ambient
Injection size: 10 µL
Run time: NLT 1.2 times the retention time of terazosin in the *Standard solution*; NLT 4.5 times the retention time of terazosin in the *Sample solution*
System suitability
Sample: *Standard solution* and *Sensitivity solution*
Capacity factor: NLT 1.0 for the terazosin peak, *Standard solution*
Relative standard deviation: NLT 2.0%, *Standard solution*
Tailing factor: NMT 2.0 for the terazosin peak, *Standard solution*
Signal to noise: NLT 10 for the terazosin peak from the *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_I/r_S)\,(C_S/C_U) \times 100 \times (1/F) \times (M_{r1}/M_{r2})$$

r_I = response of each individual impurity from the *Sample solution*
r_S = response of terazosin from the *Standard solution*
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
C_S = concentration of terazosin hydrochloride in the *Standard solution* (mg/mL)
F = relative response factor as given in *Impurity Table 1*
M_{r1} = molecular weight of terazosin, 387.44
M_{r2} = molecular weight of terazosin hydrochloride, 423.90

Acceptance criteria
Impurity limits: Meets the impurity limits specifed in *Impurity Table 1*

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Acceptance Criteria, NMT (%)
Piperazinyl-ADMQ[1]	0.52	1.1	0.4
Chloro ADMQ[2]	1.37	1.2	0.4
Bis-ADMQ-piperazine[3]	3.85	1.0	0.4
Any other individual impurity	—	—	0.2
Total impurities	—	—	1.2

[1] *N*-(4-amino-6,7 dimethoxy-2-quinazolinyl)piperazine.
[2] 2-Chloro-4-amino-6,7 dimethoxy-2-quinazoline.
[3] *N,N*-Bis-(4-amino-6,7 dimethoxy-2-quinazolinyl)piperazine.

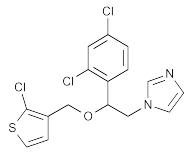
ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers protected from moisture and light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Naproxen RS
USP Terazosin Hydrochloride RS■2S *(USP33)*

BRIEFING

Tioconazole, *USP 32* page 3751. It is proposed to revise the *Assay* to remove the dimensions of the precolumn which are not critical to the method.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C73797

Tioconazole



$C_{16}H_{13}Cl_3N_2OS$ 387.71
1*H*-Imidazole, 1-[2-[(2-chloro-3-thienyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-;
1-[2,4-Dichloro-[*β*-(2-chloro-3-thenyl)-oxy]phenethyl]imidazole [65899-73-2].

DEFINITION
Tioconazole contains NLT 97.0% and NMT 103.0% of $C_{16}H_{13}Cl_3N_2OS$.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197M⟩**
- **B. PROCEDURE**

Standard solution: 50 mg/mL of USP Tioconazole RS in methanol
Sample solution: 50 mg/mL of Tioconazole in methanol
Developing solvent system: Chloroform, methanol, and glacial acetic acid (40:5:1)
Visualizing solution: Dissolve 0.85 g of bismuth subnitrate in 10 mL of glacial acetic acid, and dilute with water to 50 mL. Mix 10 mL of this solution, 50 mL of potassium iodide solution (2 in 25), and 20 mL of glacial acetic acid, and dilute to 100 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber and locate the spots on the plate by viewing under short- and long-wavelength UV light after drying the plate at 80° for 5 min. Spray the plate with *Visualizing solution*, air-dry for 2 min, and overspray with sodium nitrite solution (1 in 20). Air-dry the plate for 5 min, and examine it for brown spots on a pale yellow background.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **C.** The retention time of the major peak for tioconazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (44:40:28). Degas the solution, and add 2.0 mL of ammonium hydroxide. [NOTE—Prepare the *Mobile phase* fresh daily.]
Standard solution: 200 µg/mL of USP Tioconazole RS in methanol
Sample solution: 200 µg/mL of Tioconazole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 219 nm
Column: 5-mm × 25-cm; packing L1 with a ~~4-mm × 10-cm~~ 2S *(USP33)* precolumn containing packing L4 installed between the pump and the injector. [NOTE—Replace the precolumn daily.]
Flow rate: Adjust to obtain a retention time of between 12 and 17 min for tioconazole.
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates, determined from the analyte peak
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{13}Cl_3N_2OS$ in the Tioconazole taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tioconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 97.0%–103.0%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.2%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A 0.7-g portion dissolved in methanol shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.05%).
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 50 ppm

Organic Impurities
- **PROCEDURE**

Mobile phase: Prepare as directed in the *Assay*.
Standard solution: Dissolve 1 mg each of USP Tioconazole Related Compound A RS, USP Tioconazole Related Compound B RS, and USP Tioconazole Related Compound C RS in 15.0 mL of methanol, and shake until the contents are completely dissolved.
Sample solution: Dissolve 100 mg of Tioconazole in 15.0 mL of methanol, and shake until the substance is completely dissolved.
Chromatographic system: Use the system as directed in *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate, in turn, the percentages of 1-[2,4-dichloro-β-[(3-thenyl)-oxy]phenethyl]imidazole hydrochloride (tioconazole related compound A), 1-[2,4-dichloro-β-[(2,5-dichloro-3-thenyl)-oxy]phenethyl]imidazole hydrochloride (tioconazole related compound B), and 1-[2,4-dichloro-β-[(5-bromo-2-chloro-3-thenyl)-oxy]phenethyl]imidazole hydrochloride (tioconazole related compound C) in the portion of Tioconazole taken:

$$\text{Result} = (r_U/r_S) \times (W_I/W_U) \times 100$$

r_U = peak response for the related compound from the *Sample solution*
r_S = peak response for the related compound from the *Standard solution*
W_I = weight of the respective USP Reference Standard taken to prepare the *Standard solution* (mg)
W_U = weight of Tioconazole taken to prepare the *Sample solution* (mg)
Acceptance criteria: The limit of each related compound is NMT 1.0%.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Tioconazole RS
USP Tioconazole Related Compound A RS
USP Tioconazole Related Compound B RS
USP Tioconazole Related Compound C RS

BRIEFING

Ursodiol Tablets, *USP 32* page 3833. On the basis of comments received, it is proposed to specify the developing distance in the TLC test for *Organic Impurities*. In addition, it is proposed to use the term "ursodiol" consistently throughout the monograph, and replace the term "ursodeoxycholic acid" with "ursodiol" in the *Sample solution* under *Assay*.

(MD-GRE: E. Gonikberg.) RTS—C72722

Ursodiol Tablets

DEFINITION
Ursodiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ursodiol ($C_{24}H_{40}O_4$).

In-Process Revision

IDENTIFICATION

Change to read:

• **THIN-LAYER CHROMATOGRAPHY**
Standard solution: 1 mg/mL of USP Ursodiol RS in methanol
Sample solution: Equivalent to 1 mg/mL of ursodiol, from powdered Tablets, in methanol [NOTE—Mix for 20 min and centrifuge for 10 min at 4000 rpm. Use the clear supernatant.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture activated for at least 4 h at 105°
Application volume: 25 µL
Developing solvent system: Chloroform, acetone, and acetic acid (7:2:1)
Spray reagent: 2.5 g of phosphomolybdic acid in 50 mL of glacial acetic acid. Add 2.5 mL of concentrated sulfuric acid, and mix well.
Analysis: Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. ■Allow the chromatogram to develop until the solvent front has moved about three-fourths of the length of the plate.■2S (USP33) Spray the plate lightly with *Spray reagent*. Dry the plate by heating at 105° for about 7 min.
Acceptance criteria: The principal indigo-colored spot of the *Sample solution* corresponds in color and in R_F value to that of the *Standard solution*.

ASSAY

Change to read:

• **PROCEDURE**
Mobile phase: Methanol, phosphoric acid, and water (77:0.6:23)
Internal standard solution: 3.75 mg/mL of propylparaben in *Mobile phase*
Standard solution: 3.75 mg/mL of USP Ursodiol RS in *Internal standard solution*
Sample solution: Transfer an equivalent to about 37.5 mg of ~~ursodexoycholic acid~~■ursodiol■2S (USP33), from powdered Tablets (20), to a glass-stoppered conical flask. Add 10.0 mL of *Internal standard solution*, and shake by mechanical means for 15 min. Sonicate at 40° for an additional 15 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Column: 4.6-mm × 25-cm; packing L7
Temperature: Detector, 40°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for propylparaben and ursodiol are 0.73 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ursodiol and propylparaben
Column efficiency: NLT 1600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{40}O_4$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (100/L)$$

R_U = ratio of peak responses from the *Sample solution*
R_S = ratio of peak responses from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (Tablet/mL)
L = label claim (mg/Tablet)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨**711**⟩
Medium: Simulated intestinal fluid TS, prepared without pancreatin and adjusted with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid to a pH of 8.0; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Mobile phase: Methanol, phosphoric acid, and water (77:0.6:23)
Standard solution: USP Ursodiol RS in *Medium*
Sample solutions: Sample per *Dissolution* ⟨711⟩. [NOTE—Use a filtered portion of the solution under test.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Column: 4.6-mm × 25-cm; packing L7
Temperature: Detector, 40°
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{40}O_4$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES
Organic Impurities
• **PROCEDURE**
Standard solution A: 20 µg/mL of USP Ursodiol RS in methanol
Standard solution B: 10 µg/mL of lithocholic acid in methanol
Standard solution C: 300 µg/mL of chenodeoxycholic acid in methanol
Sample solution: Prepare as directed under *Identification*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, activated for at least 4 h at 105°
Application volume: 25 µL of *Standard solutions A*, *B*, and *C*; 50 µL of *Sample solution*
Developing solvent system: Chloroform, acetone, and acetic acid (7:2:1)
Spray reagent: 2.5 g of phosphomolybdic acid in 50 mL of glacial acetic acid. Add 2.5 mL of concentrated sulfuric acid, and mix well.
Analysis: Proceed as directed in the chapter. Spray the plate lightly with *Spray reagent*. Dry the plate by heating at 105° for about 7 min.
Acceptance criteria: The spot due to lithocholic acid from the *Sample solution*, if present, is not greater in size and intensity than that from *Standard solution B* (0.05%). The spot due to chenodeoxycholic acid from the *Sample solution*, if present, is not greater in size and intensity than that from *Standard solution C* (1.5%). No other unidentified spot in the *Sample solution* is greater in size and intensity than the spot from *Standard solution A* (0.1%).

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at a temperature between 20° and 25°.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Ursodiol RS

In-Process Revision

BRIEFING

Valacyclovir Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is proposed based on the validated methods. The proposed liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities* are performed using a Diacel Chiral Phase Crownpack, 5-µm column containing packing L66. The liquid chromatographic procedure in the test for *Dissolution* is performed using a Hypersil BDS or MOS 5-µm column containing packing L1. The typical retention time for valacyclovir is about 4.5 min under the specified conditions for the *Assay*.

(MD-AA: S. Ramakrishna; B. Davani. BPC: M. Marques.)
RTS—C44129; C59195

Add the following:

▪Valacyclovir Tablets

DEFINITION

Valacyclovir Tablets contain an amount of Valacyclovir Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution,* as obtained in the *Assay.*
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Diluent: 0.1% (v/v) phosphoric acid in water
Mobile phase: Methanol and *Diluent* (5:95)
Standard solution: 0.1 mg/mL of USP Valacyclovir Hydrochloride RS in *Diluent*. [NOTE—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D-valacyclovir.]
Sample solution: Transfer NLT 5 Tablets into a suitable volumetric flask, and add 0.1 M hydrochloric acid (approximately 80% of the volume of volumetric flask). Mechanically shake the sample until the Tablets disintegrate into a fine suspension (60 min), and sonicate for 10 min. Cool to ambient temperature, dilute with 0.1 M hydrochloric acid to volume, and mix to obtain a solution having a concentration of 2.5 mg/mL. Dilute a portion of the sample with *Diluent* to obtain a nominal concentration of 0.1 mg/mL of valacyclovir, and mix. Pass a portion of this solution through a 0.45-µm or finer porosity membrane filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 15-cm column; 5-µm, packing L66
Column temperature: 10°
Flow rate: 0.75 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution NLT 1.3 between the D-valacyclovir and valacyclovir peaks
Tailing factor: NMT 2.0 for the valacyclovir peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{13}H_{20}N_6O_4$, based on the label claim, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of valacyclovir hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Diluent: Prepare as directed in the *Assay.*
Buffer: Dissolve about 1.15 g of ammonium dihydrogen orthophosphate in 950 mL of water, add 2 mL of triethylamine, and adjust with *o*-phosphoric acid to a pH of 2.5 ± 0.05. Add 10 mL of acetonitrile, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Diluent* (5:95)
Standard solution: Prepare a solution in *Diluent* containing USP Valacyclovir Hydrochloride RS equivalent to 0.044 mg/mL of valacyclovir free base.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute with *Diluent* to obtain a final concentration of about 0.044 mg/mL considering complete dissolution of the Tablet label claim.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6 mm x 5-cm, 5-µm packing L1
Flow rate: 2.0 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valacyclovir dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S) \times (M_{r1}/M_{r2}) \times (1/L) \times 100 \times 900$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of valacyclovir in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80
L = Tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of valacyclovir is released in 45 min.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meets the requirements [NOTE—All of the concentrations are expressed as valacyclovir free base.]

Diluent: Prepare as directed in the *Assay.*
Mobile phase: Acetonitrile and *Diluent* (5:95)
Standard solution: Prepare a solution of USP Valacyclovir Hydrochloride RS, equivalent to 0.04 mg/mL of valacyclovir in *Diluent*.
Sample solution: Transfer one Tablet into a suitable volumetric flask. Add *Diluent* (approximately 60% of the volume of the

In-Process Revision

flask), and mechanically shake the samples until the Tablets disintegrate into a fine suspension, and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Dilute a portion of each sample with *Diluent* to obtain a nominal concentration of 0.04 mg/mL of valacyclovir. Pass a portion of each sample through a 0.45-µm membrane filter, and use the filtrate.

Chromatographic system and **System suitability:** Proceed as directed under *Dissolution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{13}H_{20}N_6O_4$, based on the label claim, in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of valacyclovir in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)

IMPURITIES

Organic Impurities

• **PROCEDURE**

Diluent, Mobile phase, Chromatographic system, Standard solution and **Sample solution:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of D-valacyclovir and the percentage of acyclovir in the portion of Tablets taken:

$$\text{Result} = (r_i/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_i = peak responses of D-valacyclovir or acyclovir from the *Sample solution*
r_S = peak response of valacyclovir from the *Standard solution*
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
C_S = concentration of valacyclovir hydrochloride in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80
F = relative response factor as given in *Impurity Table 1*

Acceptance criteria

Impurity limits: Meets the impurity limits specifed in *Impurity Table 1*

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
D-Valacyclovir [a]	0.82	1.0	3.2
Acyclovir [b]	0.56	1.4	2.5

[a] D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

[b] 2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (acyclovir).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Valacyclovir Hydrochloride RS■2S *(USP33)*

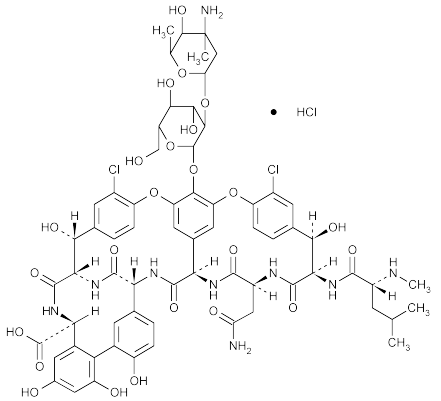
BRIEFING

Vancomycin Hydrochloride, page 3845 of *USP 32* and page 111 of *PF* 34(1). On the basis of comments received, the following revisions are being proposed:

- The *Identification* test is revised to delete the word "undried," which is redundant since the label for USP Vancomycin Hydrochloride RS indicates that this material should not be dried.
- A test for *Heavy Metals* is added to the monograph to reflect approved acceptance criteria. Vancomycin Hydrochloride is soluble in water, but *Heavy Metals, Method II* ⟨231⟩ is specified to avoid interference from solution color.
- The retention times specified in the test for *Limit of Monodechlorovancomycin* are deleted, as these values do not ensure optimal resolution for the peaks of interest.
- The term "elution time" in the *Analysis* subsection of the test for *Composition of Vancomycin* is revised to "retention time," since the latter is more commonly used by chromatographers. The *Acceptance criteria* for vancomycin B and any other peak in this test are revised on the basis of comments received regarding the *PF* 34(1) proposal and on supporting data from marketed products.

(MD-ANT: A. Wise.) RTS—C62988

Vancomycin Hydrochloride



$C_{66}H_{75}Cl_2N_9O_{24} \cdot HCl$ 1485.71

Vancomycin, monohydrochloride;
Vancomycin monohydrochloride
(S_a)-(3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,36*R*,38a*R*)-44-[[2-*O*-(3-Amino-2,3,6-trideoxy-3-C-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2*R*)-4-methyl-2-(methylamino)]valeramido]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-26-carboxylic acid, monohydrochloride;
[3*S*-[3*R**,6*S**(*S**),7*S**,22*S**,23*R**,26*R**,36*S**,38a*S**]]-3-(2-Amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-C-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[[4-methyl-2-(methylamino)-1-oxopentyl]amino]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-26-carboxylic acid, monohydrochloride [1404-93-9].

In-Process Revision

DEFINITION

Vancomycin Hydrochloride is the hydrochloride salt of a kind of vancomycin, a substance produced by the growth of *Streptomyces orientalis* (Fam. Streptomycetaceae), or a mixture of two or more such salts. It has a potency equivalent to NLT 900 µg of vancomycin/mg, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **INFRARED ABSORPTION** ⟨197K⟩**:** ~~Undried~~■■2S (*USP33*)

ASSAY

• **PROCEDURE:** Proceed with Vancomycin Hydrochloride as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: NLT 900 µg/mg on the anhydrous basis

IMPURITIES

Add the following:

■Inorganic impurities

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm■2S (*USP33*)

Change to read:

Organic Impurities

• **PROCEDURE: LIMIT OF MONODECHLOROVANCOMYCIN**

[NOTE—The *System suitability solution*, *Sample solution*, and *Standard solution* should be refrigerated immediately after preparation and during analysis. The solutions are stable for 4 days when refrigerated.]

Mobile phase: Dissolve 2.2 g of 1-heptanesulfonic acid in about 500 mL of water, add 125 mL of acetonitrile and 10 mL of acetic acid, and dilute with water to 1 L.

Rinse solution: 10% acetonitrile in water. [NOTE—Use to rinse needle and column.]

System suitability solution: 1 mg/mL of vancomycin B from USP Vancomycin B with Monodechlorovancomycin RS in water

Standard solution: 50 µg/mL of vancomycin B, from *System suitability solution*

Sample solution: 1 mg/mL of Vancomycin Hydrochloride in water

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6 mm × 25 cm; packing L1

Temperature: 60°

Autosampler temperature: 5°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

[NOTE—This procedure is sensitive to temperature changes. Sufficient tubing should be placed in the column oven to ensure that the samples have reached 60° before separation.]

System suitability

Samples: *System suitability solution*, *Standard solution*, *Sample solution*, and *Blank*

[NOTE—*Blank* and *Standard solution* run times are about 90 min. *System suitability solution* and *Sample solution* run times are about 120 min.]

[NOTE—~~The retention time of vancomycin B is 32–42 min. The relative retention time of monodechlorovancomycin is 1.1.~~■The relative retention times for vancomycin B and monodechlorovancomycin are 1.0 and 1.1, respectively.■2S (*USP33*)]

Suitability requirements

Selectivity: The chromatogram for the *Blank* does not contain peaks that interfere with vancomycin B or monodechlorovancomycin.

Retention times: ± 3.0% between monodechlorovancomycin in the *Sample solution* and the mean of the monodechlorovancomycin peaks in the *Standard solution*

Resolution: NLT 1.5 between vancomycin B and monodechlorovancomycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of monodechlorovancomycin in the portion of Vancomycin Hydrochloride taken:

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

r_U = peak area of monodechlorovancomycin in the *Sample solution*

r_S = average peak area of the vancomycin peak in the *Standard solution*

C_S = concentration of USP Vancomycin B with Monodechlorovancomycin RS in the *Standard solution* (mg/mL)

C_U = concentration of Vancomycin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of Vancomycin in USP Vancomycin B with Monodechlorovancomycin RS (mg/mg)

Acceptance criteria: NMT 4.7% of monodechlorovancomycin is found.

SPECIFIC TESTS

Add the following:

■• **STERILITY TESTS** ⟨**71**⟩**:** Where the label states that Vancomycin Hydrochloride is sterile, it meets the requirements when tested as directed for *Test for Sterility of Product to Be Examined, Membrane Filtration*, except to dissolve the specimen in water, instead of in *Fluid A*.■2S (*USP32*)

Add the following:

■• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where the label states that Vancomycin Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of vancomycin.■2S (*USP32*)

• **pH** ⟨**791**⟩**:** 2.5–4.5, 50 mg/mL in water

• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 5.0%

Change to read:

• **COMPOSITION OF VANCOMYCIN**

Solution A: Triethylamine and water (1:500). Adjust the pH to 3.2 with phosphoric acid.

Solution B: Acetonitrile, tetrahydrofuran, and *Solution A* (7:1:92)

Solution C: Acetonitrile, tetrahydrofuran, and *Solution A* (29:1:70)

Mobile phase: See the gradient table below. [NOTE—Make adjustments, if necessary, changing the acetonitrile proportion in *Solution B* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.]

Time (min)	Solution B (%)	Solution C (%)
0	100	0
12	100	0

In-Process Revision

Time (min)	Solution B (%)	Solution C (%)
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: 0.5 mg/mL of USP Vancomycin Hydrochloride RS in water. Heat at 65° for 48 h, and allow to cool.
Sample solution A: 10 mg/mL of Vancomycin Hydrochloride in *Solution B*
Sample solution B: 0.4 mg/mL of Vancomycin Hydrochloride, from *Sample solution A* in *Solution B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The elution order is Compound 1, vancomycin B, and Compound 2. Compound 2 elutes 3–6 min after the start of the period when the percentage of *Solution C* is increasing from 0%–100%.]
Suitability requirements
Resolution: NLT 3.0 between Compound 1 and vancomycin B
Column efficiency: NLT 1500 theoretical plates, calculated from the vancomycin B peak
Analysis
Samples: *Sample solution A* and *Sample solution B*
[NOTE—Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between the peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.]
[NOTE—Correct any peak observed in the chromatograms obtained from *Sample solution A* and *Sample solution B* by subtracting the area response of any peak observed in the chromatogram of *Solution B* at the corresponding ~~elution~~■retention■2S *(USP33)* time.]
Calculate the percentage of vancomycin B in the portion of Vancomycin Hydrochloride taken:

$$\text{Result} = [(D \times r_B)/((D \times r_B) + r_A)] \times 100$$

D = dilution factor between *Sample solution A* and *Sample solution B*, 25
r_B = corrected area response of the main peak of *Sample solution B*
r_A = sum of the corrected area responses of all the peaks, other than the main peak, from *Sample solution A*

Calculate the percentage of each other peak in the portion of Vancomycin Hydrochloride taken:

$$\text{Result} = [(r_I/(D \times r_B) + r_A)] \times 100$$

r_I = corrected area response of any individual peak, other than the main peak of *Sample solution A*
D = dilution factor between *Sample solution A* and *Sample solution B*, 25
r_B = corrected area response of the main peak of *Sample solution B*
r_A = sum of the corrected area responses of all the peaks, other than the main peak from *Sample solution A*

Acceptance criteria: NLT ~~80.0%~~▲~~93.0%~~▲*USP33*■85.0%■2S *(USP33)* of vancomycin B; NMT ~~9.0%~~▲~~4.0%~~▲*USP33*■5.0%■2S *(USP33)* of any peak other than the main peak

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Add the following:

■• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.■2S *(USP32)*

Change to read:

• **USP REFERENCE STANDARDS** ⟨**11**⟩
■USP Endotoxin RS■2S *(USP32)*
USP Vancomycin Hydrochloride RS
USP Vancomycin B with Monodechlorovancomycin RS

BRIEFING

Vancomycin Hydrochloride for Injection, *USP 32* page 3847 and page 992 of *PF* 34(4). On the basis of comments received, the following revisions are proposed:
- Since there is a proposal in this *PF* to add a requirement for *Heavy Metals* to the *Vancomycin Hydrochloride* monograph, this requirement is no longer necessary in the *Vancomycin Hydrochloride for Injection* monograph and will be deleted.
- On the basis of comments received regarding the *PF* 34(1) proposal to replace the monograph for *Sterile Vancomycin Hydrochloride* with the monograph for *Vancomycin Hydrochloride for Injection*, the *Acceptance criteria* for vancomycin B and any other peak in the test for *Composition of Vancomycin* have been revised to reflect the limits for marketed products.

(MD-ANT: A. Wise.) RTS—C62988

Vancomycin Hydrochloride for Injection

DEFINITION

Change to read:

Vancomycin Hydrochloride for Injection is a sterile dry mixture of Vancomycin Hydrochloride and ■may contain■2S *(USP32)* a suitable stabilizing agent. It ■■2S *(USP32)* contains NLT 90.0% and NMT 115.0% of the labeled amount of vancomycin.

In-Process Revision

IDENTIFICATION

Add the following:

■• **INFRARED ABSORPTION** ⟨**197K**⟩■2S *(USP32)*

ASSAY

Change to read:

• **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨**81**⟩■■2S *(USP32)*

Sample solution ■**1**■2S *(USP32)* (where it is represented as being in a single-dose container)**:** Constitute a container of Vancomycin Hydrochloride for Injection in water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Dilute to an equivalent of 1 mg/mL of vancomycin with water.

■**Sample solution 2** (where it is packaged for dispensing)**:** Dissolve the contents of 1 container of Vancomycin Hydrochloride for Injection in water and dilute with water to obtain a solution having a concentration of 1 mg of vancomycin/mL.■2S *(USP32)*

Sample solution 3 (where the label states the quantity of vancomycin in a given volume of constituted solution)**:** Constitute a container of Vancomycin Hydrochloride for Injection in water corresponding to the volume of diluent specified in the labeling. Dilute a portion to obtain a final concentration equivalent to 1 mg/mL of vancomycin in water.

Analysis: Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩. [NOTE—Use a measured volume of the appropriate *Sample solution*, diluted quantitatively with *Buffer No. 4* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.]

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

Add the following:

■• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meets the requirements■2S *(USP32)*

IMPURITIES

Delete the following:

■~~**Inorganic Impurities**~~

~~• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm~~■2S *(USP33)*

SPECIFIC TESTS

• **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements under small-volume injections

• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** NMT 0.33 USP Endotoxin Unit/mg of vancomycin

• **STERILITY TESTS** ⟨**71**⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except to dissolve the specimen in water instead of in *Fluid A*.

Add the following:

■• **pH** ⟨**791**⟩**:** 2.5–4.5, 50 mg/mL in water■2S *(USP32)*

Add the following:

■• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 5.0%■2S *(USP32)*

• **INJECTIONS,** *Constituted Solutions* ⟨**1**⟩**:** At the time of use, it meets the requirements.

Change to read:

• **COMPOSITION OF VANCOMYCIN**

Solution A: Triethylamine and water (1:500). Adjust the pH to 3.2 with phosphoric acid.

Solution B: Acetonitrile, tetrahydrofuran, and *Solution A* (7:1:92)

Solution C: Acetonitrile, tetrahydrofuran, and *Solution A* (29:1:70)

Mobile phase: See the gradient table below.

[NOTE—Make adjustments if necessary, changing the acetonitrile proportion in *Solution B* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.]

Time (min)	Solution B (%)	Solution C (%)
0	100	0
12	100	0
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: 0.5 mg/mL of USP Vancomycin Hydrochloride RS. Heat at 65° for 48 h, and allow to cool.

Sample solution A: 10 mg/mL of Vancomycin Hydrochloride for Injection in *Solution B*

Sample solution B: 0.4 mg/mL of vancomycin hydrochloride from *Sample solution A* in *Solution B*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period, when the percentage of *Solution C* is increasing from 0% to 100%.]

Suitability requirements

Resolution: NLT 3.0 between compound 1 and vancomycin B

Column efficiency: NLT 1500 theoretical plates, calculated from the vancomycin B peak

Analysis

Samples: *Sample solution A* and *Sample solution B*

[NOTE—Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.]

Measure the area responses for all of the peaks.

[NOTE—Correct any peak observed in the chromatograms obtained from *Sample solution A* and *Sample solution B* by subtracting the area response of any peak observed in the chromatogram of *Solution B* at the corresponding elution time.]

In-Process Revision

Calculate the percentage of vancomycin B in the specimen tested:

$$\text{Result} = [D \times r_B/((D \times r_B) + r_A)] \times 100$$

D = dilution factor, *Sample solution A* to *Sample solution B*, 25
r_B = corrected area response of the main peak from *Sample solution B*
r_A = sum of the corrected area responses of all the peaks, other than the main peak, from *Sample solution A*

Calculate the percentage of each other peak taken:

$$\text{Result} = [r_I/(D \times r_B) + r_A] \times 100$$

r_I = corrected area response of any individual peak, other than the main peak, from *Sample solution A*
D = dilution factor between *Sample solution A* and *Sample solution B* (25)

Acceptance criteria: NLT ~~88.0%~~▲~~86.0%~~▲*USP33*■80.0%■2S *(USP33)* of vancomycin B; NMT ~~4.0%~~■9.0%■2S *(USP33)* of any peak other than the main peak

Change to read:

• **OTHER REQUIREMENTS:** ■It meets the requirements under *Injections* ⟨1⟩.■2S *(USP32)*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Injections* ⟨1⟩, *Containers for Sterile Solids*.

Add the following:

■• **LABELING:** Meets the requirements under *Injections* ⟨1⟩■2S *(USP32)*

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Endotoxin RS
USP Vancomycin Hydrochloride RS

BRIEFING

Sterile Water for Inhalation, *USP 32* page 3871. On the basis of comments received, it is proposed to update the *Definition* of this monograph to specify that no added antimicrobial agents are contained.

(PW: A. Hernandez-Cardoso.) RTS—C73472

Sterile Water for Inhalation

DEFINITION

Change to read:

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

Sterile Water for Inhalation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no ~~antimicrobial agents, except where used in humidifiers or other similar devices and where liable to contamination over a period of time, or other added substances~~ ■added antimicrobial agents.■2S *(USP33)*

[NOTE—Do not use Sterile Water for Inhalation for parenteral administration or for other sterile compendial dosage forms.]

SPECIFIC TESTS
• **OXIDIZABLE SUBSTANCES**
Sample: 100 mL
Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Inhalation in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
Acceptance criteria: The pink color does not completely disappear.
• **WATER CONDUCTIVITY,** *Packaged Water* ⟨**645**⟩**:** Meets the requirements
• **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements
• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Less than 0.5 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
• **LABELING:** Label it to indicate that it is for inhalation therapy only and that it is not for parenteral administration.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Endotoxin RS

BRIEFING

Zolpidem Tartrate Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with a 4.6-mm × 15-cm Spherisorb ODS1 C 18 brand of 5-µm, L1 column. The typical retention time for zolpidem is about 9.8 min.

(MD-PP: R. Ravichandran. BPC: M. Marques.) RTS—C47817

Add the following:

■Zolpidem Tartrate Tablets

DEFINITION
Zolpidem Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩**:** The spectrum of the *Sample solution* from the test for *Dissolution* matches that of the *Standard solution.*
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 3.4 g/L of monobasic potassium phosphate in water, adjusted with ammonium hydroxide to a pH of 5.5
Mobile phase: Acetonitrile, methanol, and *Solution A* (3:2:5)
Standard stock solution: 0.8 mg/mL of USP Zolpidem Tartrate RS in 0.01 M hydrochloric acid

In-Process Revision

Standard solution: 0.16 mg/mL of USP Zolpidem Tartrate RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Transfer NLT 20 Tablets to a suitable volumetric flask to obtain a solution having a concentration of 0.4 mg/mL of zolpidem tartrate. Add 40% of the flask volume of 0.125 N hydrochloric acid. Mix well until the Tablets disintegrate, then add 50% of the flask volume of *Mobile phase*. Dilute with water to volume, and stir for 30 min using a magnetic stirrer. Allow solid particles to settle, and pass the supernatant through a suitable filter (e.g. Whatman No. 40 filter or equivalent).
Sample solution: 0.16 mg/mL of zolpidem tartrate from filtered *Sample stock solution* and *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.0 for zolpidem
Relative standard deviation: NMT 2.0% for zolpidem
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{42}H_{48}N_6O_8$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩
Medium: 0.01 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Standard solution: L/1000 mg/mL of USP Zolpidem tartrate RS in *Medium*, where L is the Tablet label claim in mg
Detection: UV 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_{42}H_{48}N_6O_8$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim per Tablet (mg)
V = 900 mL
Tolerance: NLT 80% (Q) of the labeled amount of $C_{42}H_{48}N_6O_8$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**
Solution A, Mobile phase and **Standard stock solution:** Proceed as directed in the *Assay*.
System suitability solution: 2 mg/mL of USP Zolpidem Impurities Mixture RS, prepared by dissolving the weighed amount of USP Zolpidem Impurities Mixture RS in 10% of the flask volume of 0.01 N hydrochloric acid, and diluting with *Mobile phase* to volume.
Standard solution: 8 µg/mL of USP Zolpidem Hydrochloride RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Use the *Sample solution* as prepared in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Prepare as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between zolpidem related compound B and zolpidem related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for the zolpidem peak, *Standard solution*
Relative standard deviation: NMT 10.0% for zolpidem peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for zolpidem from the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
C_T = concentration of zolpidem tartrate in the *Sample solution* (mg/mL)
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zolpidem acid[a]	0.23	0.3
Zolpidem related compound B[b]	0.58	0.3
Zolpidem related compound C[c]	0.70	0.3
Zolpidem tartrate	1.0	—
Zolpidem carbaldehyde[d]	1.45	0.3
Any individual unspecified degradation product	—	0.3

[a] 2-(6-Methyl-2-*p*-tolylimidazo[1,2-α]pyridin-3-yl)acetic acid.
[b] *N*,*N*-Dimethyl-2-(6-methyl-2-*p*-tolylimidazo[1,2-a]pyridin-3-yl)-2-oxoacetamide.
[c] 4-Methyl-*N*-(5-methylpyridin-2-yl)benzamide.
[d] 6-Methyl-2-*p*-tolylimidazo[1,2-α]pyridine-3-carbaldehyde.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Zolpidem Impurities Mixture RS
USP Zolpidem Tartrate RS■2S *(USP33)*

In-Process Revision

DIETARY SUPPLEMENTS—MONOGRAPHS

BRIEFING

Ashwagandha. A new *USP* Dietary Supplement monograph is proposed. The liquid chromatographic procedure in the test for *Content of Withanolides* is based on analyses performed with the Luna C18(2) brand of L1 column, with 5-µm packing. Typical retention times for withanoside IV, physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanolide A, withanone, and withanolide B are about 15.5, 16.6, 17.6, 19.6, 19.6, 20.2, 21.2, 22.1, 22.3, and 25.2 min, respectively.

(DSB: M. Sharaf.) RTS—C56130

Add the following:

▪Ashwagandha

DEFINITION

Ashwagandha is the dried mature roots of *Withania somnifera* (L.) Dunal (Fam. Solanaceae). It contains NLT 0.3% of withanolides, calculated on the dried basis as the sum of withanolide aglycones, calculated as withanolide A, and withanolide glycosides, calculated as withanoside IV.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Standard solution: About 200 mg of USP Powdered Ashwagandha Extract RS in 10 mL of methanol. Heat gently for 10–15 min, centrifuge, and use the supernatant. [NOTE—Save the remaining volume of the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Transfer about 5.0 g of Ashwagandha, finely powdered, to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 10–15 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum to about 40 mL, and adjust the volume to 50.0 mL using methanol. [NOTE—Save the remaining volume of the *Sample solution* for use in the test for *Content of Withanolides*.]

Developing solvent system: A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Spray reagent: Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order given.

Application volume: 25 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop until the solvent front has moved about 90% of the length of the plate. Dry the plate, spray with *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The *Sample solution* exhibits five main grayish-blue bands with R_F values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the main bands in the *Standard solution*. Other less intense bands are observed for the *Sample solution* and the *Standard solution*.

• **B.** The chromatogram of the *Sample solution* in the test for *Content of Withanolides* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A* and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used. The *Sample solution* shows additional peaks corresponding to some of the following withanolides: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B.

COMPOSITION

• **CONTENT OF WITHANOLIDES**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Solution B: Filtered and degassed acetonitrile

Standard solution A: Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.

Standard solution B: Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.

Standard solution C: Dilute 5 mL of the *Standard solution* prepared in *Identification* test *A* with methanol to 10 mL, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity.

Sample solution: Use the *Sample solution* prepared in *Identification* test *A*. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first few mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm, end-capped; packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

Column temperature: 27 ± 1°

System suitability

Samples: *Standard solution A* and *Standard solution C*

[NOTE—Using the chromatogram of *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used, identify the retention times of the peaks corresponding to the various withanolide aglycones and glycosides. The approximate relative retention times of the withanolide aglycones and glycosides are provided in the following table.]

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-hydroxywithanone	0.80
Withanoside V	0.89

Analyte	Relative Retention Time
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements

[NOTE—The chromatogram for *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used.]

Resolution: NLT 1.0 between the withanolide A and withanone peaks, *Standard solution C*; NLT 3.0 between the withaferin A and coeluting withanoside V and withanoside VI peaks, *Standard solution C*

Tailing factor: NMT 1.5, withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Calculate the percentage of withanolide aglycones in the portion of Ashwagandha taken:

$$5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B in the *Sample solution*

r_S = peak response for withanolide A in *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

W = weight of Ashwagandha taken to prepare the *Sample solution* (g)

Calculate the percentage of withanolide glycosides in the portion of Ashwagandha taken:

$$5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V, and withanoside VI in the *Sample solution*

r_S = peak response for USP Withanoside IV in *Standard solution B*

C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)

W = weight of Ashwagandha taken to prepare the *Sample solution* (g)

Acceptance criteria: The sum of the percentages of withanolide aglycones and withanolide glycosides is NLT 0.3%, calculated on the dried basis. [NOTE—Because of inherent variations, some of the withanolides mentioned in this test may be present in minor quantities or may be totally absent. The sample will be deemed compliant if the sum of the total withanolides is NLT 0.3%.]

IMPURITIES

Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩**:** NMT 1.0%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matter* ⟨**561**⟩**:** NMT 2.0%
- **PROCEDURE 2: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

Macroscopic: Primary roots are not branched and are straight, conical, or fingerlike in shape and variable in thickness with age; some carry a crown, consisting of a number of remains of stem base; the outer surface is buff to grayish-yellow with longitudinal wrinkles; facture is short and uneven; secondary roots are thin and fibrous.

Histology

Transverse section of roots: It shows a narrow band of yellowish crumpled cork, moderate-size cortex and a wide wood. The cork cells are rectangular, radially flattened, nonlignified, and filled with starch grains and reddish brown content; cork cambium is 2–4 diffused rows of cells; secondary cortex is formed of 20–25 rows of thin-wall parenchymatous cells, filled with starch grains, and shows occasional microsphenoidal crystals of calcium oxalate; phloem consists of sieve tubes, companion cells, and phloem parenchyma; vascular cambium consists of tangentially elongated parenchymatous cells; vessels and tracheids are in radial rows toward the periphery of the wood; medullary rays are uniseriate to 2- to 3-seriate, and are filled with starch grains; scattered vessels in groups are embedded in the parenchyma; vessels have pitted and scalariform thickening, and generally the end walls are perforated; and a few fibers with thick lignified walls are also found scattered in the wood.

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of finely powdered Ashwagandha at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 7.0%, determined on 1.0 g of finely powdered Ashwagandha
- **ARTICLES OF BOTANICAL ORIGIN,** *Alcohol-Soluble Extractives, Method 2* ⟨**561**⟩**:** NLT 10.0%
- **MICROBIAL ENUMERATION TESTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*
- **ARTICLES OF BOTANICAL ORIGIN,** *Aflatoxins* ⟨**561**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Powdered Ashwagandha Extract RS
USP Withanolide A RS
USP Withanoside IV RS■2S (*USP33*)

BRIEFING

Powdered Ashwagandha. A new *USP* Dietary Supplement monograph is being proposed. See briefing under *Ashwagandha*, published elsewhere in this issue of *PF*.

(DSB: M. Sharaf.) RTS—C56131

Add the following:

■Powdered Ashwagandha

DEFINITION

Powdered Ashwagandha is Ashwagandha reduced to a fine or very fine powder.

In-Process Revision

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Standard solution: Heat gently, for 10–15 min, about 200 mg of USP Powdered Ashwagandha Extract RS in 10 mL of methanol, centrifuge, and use the supernatant. [NOTE—Save the remaining volume of the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Transfer about 5.0 g of Powdered Ashwagandha to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 10–15 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum to about 40 mL, and adjust the volume to 50.0 mL using methanol. [NOTE—Save the remaining volume of the *Sample solution* for use in the test for *Content of Withanolides*.]

Adsorbent: 0.25-mm layer of chromatographic silica gel

Developing solvent system: A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)

Spray reagent: Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order mentioned.

Application volume: 25 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop the chromatograms until the solvent front has moved about 90% of the plate. Remove the plate from the chamber, dry, spray with the *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits five main grayish-blue bands with R_F values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the main bands in the chromatogram of the *Standard solution*. Other less intense bands are observed in the chromatograms of the *Sample solution* and *Standard solution*.

• **B.** The chromatogram of the *Sample solution* obtained in the test for *Content of Withanolides* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A* and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS. The *Sample solution* shows additional peaks corresponding to some of the following withanolides: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B.

COMPOSITION

• **CONTENT OF WITHANOLIDES**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Solution B: Filtered and degassed acetonitrile

Standard solution A: Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.

Standard solution B: Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.

Standard solution C: Dilute 5 mL of the *Standard solution* prepared in *Identification* test *A* to 10 mL with methanol, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity.

Sample solution: Use the *Sample solution* prepared in *Identification* test *A*. Before injection, filter through a membrane filter having a 0.45-µm or finer porosity, discarding the first few mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm, end-capped; packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

Column temperature: 27 ± 1°

System suitability

Samples: *Standard solution A* and *Standard solution C*

[NOTE—Using the chromatogram of *Standard solution C* and the Reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS, identify the retention times of the peaks corresponding to the different withanolide aglycones and glycosides. The approximate relative retention times of the withanolide aglycones and glycosides are provided in the following table.]

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements

[NOTE—The chromatogram of *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS.]

Resolution: NLT 1.0 for the withanolide A and withanone peaks, and NLT 3.0 between the peak corresponding to withaferin A and the peak corresponding to the coeluting withanoside V and withanoside VI, *Standard solution C*

Tailing factor: NMT 1.5 for the withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha taken:

$$5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B in the *Sample solution*

r_S = peak response for withanolide A in *Standard solution A*

C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)

W = weight of Powdered Ashwagandha taken to prepare the *Sample solution* (g)

In-Process Revision

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha taken:

$$5(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V, and withanoside VI in the *Sample solution*
r_S = peak response for USP Withanoside IV RS in *Standard solution B*
C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)
W = weight of Powdered Ashwagandha taken to prepare the *Sample solution* (g)

Acceptance criteria: Add the percentages of withanolide aglycones and withanolide glycosides: NLT 0.3% is found, calculated on the dried basis. [NOTE—Due to inherent variations, some of the withanolides mentioned in the above test may be present in minor quantities or may be totally absent. The sample will be deemed compliant as long as the sum of the total withanolides is NLT 0.3%.]

IMPURITIES

Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩**:** NMT 1.0%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

Macroscopic: It is a dusty white or grey to light brown powder with a characteristic odor and a mucilagenous, bitter, acrid taste.

Histology

Microscopic examination: It shows collapsed cork cells filled with starch grains and reddish-brown content; thin-walled cortex parenchyma cells filled with starch grains and occasional microsphenoidal crystals of calcium oxalate; vessels, with pitted and scalariform thickening, and generally with end walls perforated; a few fibers with thick lignified walls and simple pits; abundant starch grains, mostly simple, sometimes compound, spherical, reniform-oval with central hilum.

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 7.0%, determined on 1.0 g of Powdered Ashwagandha
- **ARTICLES OF BOTANICAL ORIGIN,** *Alcohol-Soluble Extractives, Method 2* ⟨**561**⟩**:** NLT 10.0%
- **MICROBIAL ENUMERATION TESTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **ARTICLES OF BOTANICAL ORIGIN,** *Aflatoxins* ⟨**561**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Powdered Ashwagandha Extract RS
USP Withanolide A R
USP Withanoside IV RS■2S *(USP33)*

BRIEFING

Powdered Ashwagandha Extract. A new *USP* Dietary Supplement monograph is proposed. See the briefing under *Ashwagandha*, published elsewhere in this issue.

(DSB: M. Sharaf.) RTS—C56006

Add the following:

■Powdered Ashwagandha Extract

DEFINITION

Powdered Ashwagandha Extract is prepared from Ashwagandha using methanol, alcohol, water, or mixtures of these solvents. It contains NLT 2.5% of withanolides, calculated on the dried basis as the sum of withanolide aglycones and withanolide glycosides.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩

Standard solution: Heat gently for 10–15 min about 200 mg of USP Powdered Ashwagandha Extract RS in 10 mL of methanol, centrifuge, and use the supernatant. [NOTE—Save the remaining volume of the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Heat gently for 10–15 min about 200 mg of Powdered Ashwagandha Extract in 10 mL of methanol, centrifuge, and use the supernatant.

Adsorbent: 0.25-mm layer of chromatographic silica gel

Developing solvent system: A mixture of ethyl acetate, toluene, and acetic acid (45:55:3)

Spray reagent: Mix 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid in the order mentioned.

Application volume: 25 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands to a suitable plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop the chromatograms until the solvent front has moved about 90% of the length of the plate. Remove the plate from the chamber, dry, spray with the *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The *Sample solution* exhibits five main grayish-blue bands with R_F values of approximately 0.12, 0.29, 0.47, 0.67, and 0.73 that are similar in position and color to the main bands for the *Standard solution*. Other less intense bands are observed for the *Sample solution* and the *Standard solution*.

- **B.** The *Sample solution* in the test for *Content of Withanolides* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV for *Standard solution A* and *Standard solution B*, respectively. Identify other withanolide peaks in the *Sample solution* by comparison with *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used. The *Sample solution* shows additional peaks corresponding to some of the following withanolides: physagulin D, 27-hydroxywithanone, withanoside V, withanoside VI, withaferin A, withastramonolide, withanone, and withanolide B.

COMPOSITION

- **CONTENT OF WITHANOLIDES**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

In-Process Revision

Solution B: Filtered and degassed acetonitrile
Standard solution A: Dissolve, using gentle heat, a quantity of USP Withanolide A RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.
Standard solution B: Dissolve, using gentle heat, a quantity of USP Withanoside IV RS in methanol to obtain a solution having a known concentration of about 0.1 mg/mL.
Standard solution C: Dilute 5 mL of the *Standard solution* prepared in *Identification* test *A* with methanol to 10 mL, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity.
Sample solution: Transfer about 100 mg of Powdered Ashwagandha Extract, weighed, to a 10-mL volumetric flask, add about 7 mL of methanol, heat gently on a water bath for 15–20 min, dilute with methanol to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first few mL of the filtrate.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm, end-capped; packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
Column temperature: 27 ± 1°
System suitability
Samples: *Standard solution A* and *Standard solution C*
[NOTE—Using the chromatogram of *Standard solution C* and the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used, identify the retention times of the peaks corresponding to the various withanolide aglycones and glycosides. The approximate relative retention times of the withanolide aglycones and glycosides are provided in the following table.]

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

Suitability requirements
[NOTE—The chromatogram for *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Ashwagandha Extract RS being used.]
Resolution: NLT 1.0 for the withanolide A and withanone peaks, and NLT 3.0 between the peak corresponding to withaferin A and the peak corresponding to coeluting withanoside V and withanoside VI, *Standard solution C*
Tailing factor: NMT 1.5, withanolide A peak, *Standard solution A*
Relative standard deviation: NMT 2.0% for replicate injections, withanolide A peak, *Standard solution A*

Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Calculate the percentage of withanolide aglycones in the portion of Powdered Ashwagandha Extract taken:

$$(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withaferin A, withastramonolide, withanolide A, withanone, and withanolide B in the *Sample solution*
r_S = peak response for withanolide A in *Standard solution A*
C_S = concentration of USP Withanolide A RS in *Standard solution A* (mg/mL)
W = weight of Powdered Ashwagandha Extract taken to prepare the *Sample solution* (g)

Calculate the percentage of withanolide glycosides in the portion of Powdered Ashwagandha Extract taken:

$$(r_T/r_S)(C_S/W)$$

r_T = sum of the peak responses for withanoside IV, withanoside V and withanoside VI in the *Sample solution*
r_S = peak response for USP Withanoside IV in *Standard solution B*
C_S = concentration of USP Withanoside IV RS in *Standard solution B* (mg/mL)
W = weight of Powdered Ashwagandha Extract taken to prepare the *Sample solution* (g)

Acceptance criteria: The sum of the percentages of the withanolide aglycones and withanolide glycosides is NLT 2.5%, calculated on the dried basis. [NOTE—Because of inherent variations, some of the withanolides mentioned in this test may be present in minor quantities or may be totally absent. The sample will be deemed compliant if the sum of the total withanolides is NLT 2.5%.]

IMPURITIES
Inorganic Impurities
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities
- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 2.0 g at 105° for 3 h: it loses NMT 6.0% of its weight.
- **MICROBIAL ENUMERATION TESTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*
- **ARTICLES OF BOTANICAL ORIGIN,** *Aflatoxins* ⟨**561**⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements of the test for *Residual Solvents* under *Botanical Extracts* ⟨565⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Powdered Ashwagandha Extract RS
  USP Withanolide A R
  USP Withanoside IV RS■2S (*USP33*)

In-Process Revision

BRIEFING

Boswellia Serrata; Boswellia Serrata Extract. New monographs are proposed. The liquid chromatographic procedure in *Identification* test *B* and the test for *Content of Keto-Derivatives of B-Boswellic Acids* is based on analyses performed with the Alltech Prevail C18 brand of L1 column. The typical retention times observed for 11-keto-*B*-boswellic acid, 3-acetyl-11-keto-*B*-boswellic acid, *B*-boswellic acid, and 3-acetyl-*B*-boswellic acid are about 7.1, 9.6, 20.6, and 35.1 min, respectively. Similar results and shorter retention times were obtained on the Merck LiChrospher, 4.0-mm × 25-cm, 5-µm brand of L1 column.

(DSB: M. Sharaf.) RTS—C56127

Add the following:

▪Boswellia Serrata

DEFINITION

Boswellia Serrata is the oleogum resin obtained by incision or produced by spontaneuos exudation from the stem and branches of *Boswellia serrata* Roxb. (Fam. Burseraceae). It contains NLT 1.0% of the keto derivatives of *B*-boswellic acid, calculated on the dried basis as the sum of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Adsorbent: 0.25-mm layer of chromatographic silica gel

Standard solution: Treat a quantity of USP Boswellia Serrata Extract RS with gentle heating in methanol to obtain a solution having a known concentration of 30 mg/mL, cool, centrifuge, and use the supernatant.

Sample solution: Use the *Sample solution*, prepared as directed in the test for *Content of Keto-Derivatives of B-Boswellic Acids*, concentrate to 10% of the volume.

Developing solvent system: A mixture of hexane and ethyl acetate (6:4)

Dipping reagent: Prepare a solution of 10% sulfuric acid in methanol. [NOTE—Prepare fresh immediately before use.]

Application volume: 10 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the samples as bands to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber, dry, examine under UV light at 254 nm, dip in the *Dipping reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: Under UV light at 254 nm, the chromatogram of the *Sample solution* exhibits two main zones due to 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid at R_F values of about 0.30 and 0.36, respectively, corresponding to zones in the chromatogram from the *Standard solution*. Under visible light, the chromatogram of the *Sample solution* exhibits two additional zones due to *B*-boswellic acid and 3-acetyl-*B*-boswellic acid at R_F values of about 0.49 and 0.58, respectively, corresponding to zones in the chromatogram from the *Standard solution*. Other less intense zones are observed in the chromatograms of the *Sample solution* and *Standard solution*.

- **B.** The 210-nm chromatogram of the *Sample solution*, in the test for *Content of Keto-Derivatives of B-Boswellic Acids*, exhibits peaks for 11-keto-*B*-boswellic acid, 3-acetyl-11-keto-*B*-boswellic acid, *B*-boswellic acid, and 3-acetyl-*B*-boswellic acid at retention times that correspond to those in the 210-nm chromatogram of *Standard solution B* and the 210-nm Reference Chromatogram provided with the USP Boswellia Serrata Extract RS.

COMPOSITION

- **CONTENT OF KETO-DERIVATIVES OF *B*-BOSWELLIC ACIDS**

Standard solution A: Dissolve a quantity of USP 3-acetyl-11-keto-*B*-Boswellic Acid RS in methanol to obtain a solution having a known concentration of 0.1 mg/mL.

Standard solution B: Treat a quantity of USP Boswellia Serrata Extract RS with gentle heating in methanol to obtain a solution having a known concentration of 10 mg/mL. Before injection, pass through a filter having a 0.45-µm porosity.

Sample solution: Transfer about 2.0 g of crushed Boswellia Serrata into a round-bottom flask and reflux in 50 mL of methanol in a water bath for 15 min, stirring with a magnetic stirrer. Repeat till the last extract turns colorless. Evaporate the combined extracts to about 50 mL, transfer to a 100-mL volumetric flask, and complete to volume with methanol. Before injection, pass through a filter having a 0.45-µm porosity and discard the first few mL of the filtrate.

Mobile phase: Prepare a a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (900:100:0.1). Make adjustments if necessary.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: See the gradient table below.

Time (min)	Flow Rate (mL/min)
0	1
5	1.5
10	2
30	2
32	1
45	1

Injection size: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

[NOTE—The relative retention times for 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid are about 1.0 and 1.4, respectively.]

Suitability requirements: The chromatogram of *Standard solution B* is similar to the 254-nm Reference Chromatogram provided with the USP Boswellia Serrata Extract RS.

Relative standard deviation: NMT 2.0% of the 3-acetyl-11-keto-*B*-boswellic acid peak response in replicate injections, *Standard solution A*

Tailing factor: NMT 1.5, 11-keto-*B*-boswellic acid peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatogram of *Standard solution B* and the 254-nm Reference Chromatogram provided with the lot of USP Boswellia Serrata Extract RS being used, identify the retention times of the peaks of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid in the *Sample solution* chromatogram.

Separately calculate the percentages of the two analytes in the portion of Boswellia Serrata taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times 10F$$

r_U = peak area of each analyte in the *Sample solution*

r_S = peak area of 3-acetyl-11-keto-*B*-boswellic acid in *Standard solution A*

In-Process Revision

C_S = concentration of USP 3-Acetyl-11-keto-*B*-Boswellic Acid RS in *Standard solution A* (mg/mL)
W = weight of Boswellia Serrata taken to prepare the *Sample solution* (g)
F = conversion factor for each analyte (0.93 for 11-keto-*B*-boswellic acid and 1.0 for 3-acetyl-11-keto-*B*-boswellic acid)

Acceptance criteria: Add the percentages calculated for 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid: NLT 1.0% is found, on the dried basis.

IMPURITIES

Inorganic Impurities

- **PROCEDURE 1: ARTICLES OF BOTANICAL ORIGIN,** *Acid-insoluble Ash* ⟨**561**⟩**:** NMT 0.5%
- **PROCEDURE 2: HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matters* ⟨**561**⟩**:** NMT 2.0%
- **PROCEDURE 2: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**
  Macroscopic: It occurs as small ovoid tears, sometimes form agglomerated masses up to 5-cm long and 2-cm thick; whitish to golden yellow; fracture is brittle, fractured surface is waxy and translucent; characteristic aromatic odor, and an aromatic, slightly mucilagenous taste.
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of finely powdered Boswellia Serrata at 105° for 2 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 2.0%, determined on 2.0 g of finely powdered Boswellia Serrata
- **ARTICLES OF BOTANICAL ORIGIN,** *Alcohol-soluble Extractives, Method 2* ⟨**561**⟩**:** NLT 56%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
- **LABELING:** The label states the Latin binomial of the species of *Boswellia* from which the oleogum resin was obtained.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP 3-Acetyl-11-keto-*B*-Boswellic Acid RS
  USP Boswellia Serrata Extract RS■2S *(USP33)*

BRIEFING

Boswellia Serrata Extract. See briefing under *Boswellia Serrata*.

(DSB: M. Sharaf.) RTS—C40936

Add the following:

■Boswellia Serrata Extract

DEFINITION

Boswellia Serrata Extract is prepared from pulverized Boswellia Serrata using suitable solvents such as isopropanol, alcohol, methanol, hexanes, or mixtures of these solvents. The ratio of starting plant material to Extract is approximately 6:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of Extract, calculated, on the dried basis, as the sum of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid; it may contain suitable added substances.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Standard solution: Treat a quantity of USP Boswellia Serrata Extract RS with gentle heating in methanol to obtain a solution having a known concentration of 30 mg/mL, cool, centrifuge, and use the supernatant.
  Sample solution: Treat a quantity of Extract with gentle heating in methanol to obtain a solution having a known concentration of 30 mg/mL, cool, centrifuge, and use the supernatant.
  Developing solvent system: A mixture of hexane and ethyl acetate (6:4)
  Dipping reagent: Prepare a solution of 10% sulfuric acid in methanol. [NOTE—Prepare fresh immediately before use.]
  Application volume: 10 µL
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Apply the samples as bands to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber, dry, examine under UV light at 254 nm, dip in the *Dipping reagent*, heat for 5–10 min at 100°, and examine under visible light.
  Acceptance criteria: Under UV light at 254 nm, the chromatogram of the *Sample solution* exhibits two main zones due to 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid at R_F values of about 0.30 and 0.36, respectively, corresponding to zones in the chromatogram from the *Standard solution*. Under visible light, the chromatogram of the *Sample solution* exhibits two additional zones due to *B*-boswellic acid and 3-acetyl-*B*-boswellic acid at R_F values of about 0.49 and 0.58, respectively, corresponding to zones in the chromatogram from the *Standard solution*. Other less intense zones are observed in the chromatograms of the *Sample solution* and *Standard solution*.
- **B.** The 210-nm chromatogram of the *Sample solution*, in the test for *Content of Keto-Derivatives of B-Boswellic Acids*, exhibits peaks for 11-keto-*B*-boswellic acid, 3-acetyl-11-keto-*B*-boswellic acid, *B*-boswellic acid, and 3-acetyl-*B*-boswellic acid at retention times that correspond to those in the 210-nm chromatogram of *Standard solution B* and the 210-nm Reference Chromatogram provided with the USP Boswellia Serrata Extract RS.

COMPOSITION

- **CONTENT OF KETO-DERIVATIVES OF *B*-BOSWELLIC ACIDS**
  Standard solution A: Dissolve a quantity of USP 3-acetyl-11-keto-*B*-Boswellic Acid RS in methanol to obtain a solution having a known concentration of 0.1 mg/mL.
  Standard solution B: Treat a quantity of USP Boswellia Serrata Extract RS with gentle heating in methanol to obtain a solution having a known concentration of 10 mg/mL. Before injection, pass through a filter having a 0.45-µm porosity.
  Sample solution: Treat a quantity of Extract with gentle heating in methanol to obtain a solution having known concentration of 10 mg/mL. Before injection, pass through a filter having a 0.45-µm porosity and discard the first few mL of the filtrate.
  Mobile phase: Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (900:100:0.1). Make adjustments if necessary.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: See the gradient table below.

Time (min)	Flow Rate (mL/min)
0	1
5	1.5
10	2
30	2
32	1
45	1

Injection size: 20 µL
System suitability
Samples: *Standard solution A* and *Standard solution B*
[NOTE—The relative retention times for 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid are about 1.0 and 1.4, respectively.]
Suitability requirements: The chromatogram of *Standard solution B* is similar to the 254-nm Reference Chromatogram provided with the USP Boswellia Serrata Extract RS.
Relative standard deviation: NMT 2.0% of the 3-acetyl-11-keto-*B*-boswellic acid peak response in replicate injections, *Standard solution A*
Tailing factor: NMT 1.5, 11-keto-*B*-boswellic acid peak, *Standard solution A*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Using the chromatogram of *Standard solution B* and the 254-nm Reference Chromatogram provided with the lot of USP Boswellia Serrata Extract RS being used, identify the retention times of the peaks of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid in the *Sample solution* chromatogram.
Separately calculate the percentages of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid in the portion of Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S V/W) \times 100F$$

r_U = peak area of each analyte in the *Sample solution*
r_S = peak area of 3-acetyl-11-keto-*B*-boswellic acid in *Standard solution A*
C_S = concentration of USP 3-Acetyl-11-keto-*B*-Boswellic Acid RS in *Standard solution A* (mg/mL)
V = final volume of the *Sample solution* (mL)
W = weight of Extract taken to prepare the *Sample solution* (mg)
F = conversion factor for each analyte (0.93 for 11-keto-*B*-boswellic acid and 1.0 for 3-acetyl-11-keto-*B*-boswellic acid)

Acceptance criteria: Add the percentages of the two analytes. It contains NLT 90.0% and NMT 110.0% of the labeled amount of Extract, on the dried basis, as the sum of 11-keto-*B*-boswellic acid and 3-acetyl-11-keto-*B*-boswellic acid.

IMPURITIES
Inorganic Impurities
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
Organic Impurities
• **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of Extract at 105° for 2 h: it loses NMT 5.0% of its weight.
• **MICROBIAL ENUMERATION TESTS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
• **ABSENCE OF SPECIFIED MICROORGANISMS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
• **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP 3-Acetyl-11-keto-*B*-Boswellic Acid RS
USP Boswellia Serrata Extract RS■2S *(USP33)*

BRIEFING

Crypthecodinium Cohnii Oil. Because there is no existing *USP* monograph for this article, the following new monograph is being proposed. The *Content of DHA* is conducted using the same chromatographic procedure described in the section *Omega-3 Fatty Acids Determination and Profile* in the *USP* general test chapter *Fats and Fixed Oils* ⟨401⟩ that has been published in the *First Supplement* to *USP 32–NF 27* to become official on August 1, 2009.

(DSN: C. Phinney.) RTS—C55685

Add the following:

■Crypthecodinium Cohnii Oil

DEFINITION
Crypthecodinium Cohnii Oil is obtained from the fermentation and extraction of algae of the species *Crypthecodinium cohnii* and contains NLT 40.0% (w/w) of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22: 6 n-3), as the only significant polyunsaturated fatty acid present. Suitable antioxidants in appropriate concentration may be added.

IDENTIFICATION
• **FATTY ACID PROFILE:** Proceed as directed in the test for *Content of DHA*.
Analysis: Determine the peak area for all peaks with retention times longer than that of methyl laurate.
Acceptance criteria: The retention time of the peaks of the docosahexaenoic acid methyl ester and the eicosapentanoic acid methyl ester from the *Test Solution* corresponds to that from *Standard Solution 2*, as obtained in the test for *Content of EPA and DHA*. The area percent for the methyl esters of the fatty acids from the *Test Solution* in the test for *Content of EPA and DHA* meet the requirements for each fatty acid indicated in the table below.

Fatty Acid	Shorthand Notation	Lower Limit (area %)	Upper Limit (area %)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	0	1.0
Eicosapentanoic acid	20:5 n-3	0	0.1
Docosapentaenoic acid	22:5 n-6	0	0.1
Docosahexanoic acid	22:6 n-3	39.0	47.0

In-Process Revision

COMPOSITION

• **CONTENT OF DHA**

Analysis: Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.

Acceptance criteria: NLT 40.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

• **LIMIT OF ARSENIC**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: Transfer 1 g of ultrapure palladium metal into a Teflon beaker. Add 20 mL of water and 10 mL of nitric acid, and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate into a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (3:2:5). A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed under *Arsenic* ⟨211⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of arsenic.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of arsenic.

Sample solution: For preparation of the *Sample solution*, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [**CAUTION—**Wear proper eye protection and protective clothing and gloves.] Transfer approximately 500 mg of Crypthecodinium Cohnii Oil, weighed to the nearest 0.1 mg, into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [NOTE—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with water to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min; char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of Crypthecodinium Cohnii Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration as obtained above
W = weight of Crypthecodinium Cohnii Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of nitric acid and 40 mL of water to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: 1 g of ultrapure magnesium nitrate, to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Lead Nitrate Stock Solution*, prepared as directed under *Heavy Metals* ⟨231⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of lead.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of lead.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic*.

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2100°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for lead. Determine the peak area at the lead emission line at

283.3 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of lead, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of lead in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of lead in the portion of Crypthecodinium Cohnii Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Crypthecodinium Cohnii Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF CADMIUM**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid to volume (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution A: 0.1372 mg/mL of cadmium nitrate

Standard stock solution B: *Standard stock solution A*, nitric acid, and water (2:1:97). This solution contains 0.10 µg/mL of cadmium.

[NOTE—Before make up to final volume dissolve in a portion of water and nitric acid.]

Standard solutions: Dilute *Standard stock solution B* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of cadmium.

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic.*

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for cadmium. Determine the peak area at the cadmium emission line at 228.8 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of cadmium, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of cadmium in each mL of the *Sample solution* by interpolation from the regression line. Calculate the content of cadmium in the Crypthecodinium Cohnii Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Crypthecodinium Cohnii Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF MERCURY:** Proceed as directed under *Mercury* ⟨261⟩, *Method IIa*, except to use a *Standard Mercury Solution* having the equivalent of 0.1 µg/mL of mercury.

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic* combining the two duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution.*

Acceptance criteria: NMT 0.1 µg/g

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0
- **FATS AND FIXED OILS,** *Acid Value (Free Fatty Acids)* ⟨**401**⟩**:** The free fatty acids in 10 g require for neutralization NMT 1.42 mL of 0.1 N sodium hydroxide.
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26, calculated:

$$\text{Result} = (2 \times PV) + AV$$

PV = peroxide value
AV = anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 3.5%
- **SPECIFIC GRAVITY** ⟨**841**⟩**:** 0.91–0.93

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **LABELING:** The label states the content of docosahexaenoic acid in mg/g. It also states the name and concentration of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Docosahexaenoic Acid Ethyl Ester RS
USP Eicosapentaenoic Acid Ethyl Ester RS
USP Methyl Tricosanoate RS■2S *(USP33)*

BRIEFING

Schizochytrium Oil. Because there is no existing *USP* monograph for this dietary supplement ingredient, a new monograph is being proposed. The test for the *Content of DHA* references the gas chromatographic procedure described under USP general test chapter *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile* that has been published on page 3941 of the *First Supplement* to *USP 32*, to become official on August 1, 2009.

(DSN: C. Phinney.) RTS—C55687

Add the following:

■Schizochytrium Oil

DEFINITION

Schizochytrium Oil is obtained by fermentation and extraction of algae of the genus *Schizochytrium* and contains NLT 30.0% (w/w) of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22:6 n–3), as the main polyunsaturated fatty acid. Suitable antioxidants in appropriate concentration may be added.

In-Process Revision

IDENTIFICATION

• **LONG CHAIN UNSATURATED FATTY ACID PROFILE**

Analysis: Proceed as directed in *Composition*, *Content of DHA*. Determine the peak areas of all the peaks corresponding to fatty acids with retention times longer than that of lauric acid methyl ester.

Acceptance criteria: The retention times of the peaks of the docosahexaenoic acid methyl ester and the eicosapentanoic acid methyl ester of the *Test Solution* correspond to those of *Standard Solution 2*, as obtained in the test for the *Content of EPA and DHA* under *Fats and Fixed Oils* ⟨401⟩*, Omega-3 Fatty Acids Determination and Profile*. The area percentage for the methyl esters of the fatty acids from the chromatograph of the *Test Solution* in the test for the *Content of EPA and DHA* meet the requirements for each fatty acid indicated in the table below.

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Dihomo-gamma-linolenic acid	20:3 n–6	1.7	2.8
Arachidonic acid	20:4 n–6	0.6	1.3
Eicosapentaenoic acid (EPA)	20:5 n–3	1.3	3.9
Docosapentaenoic acid (DPA n–6)	22:5 n–6	10.5	16.5
Docosahexanoic acid (DHA)	22:6 n–3	30.0	40.0

COMPOSITION

• **CONTENT OF DHA**

Analysis: Proceed as directed under *Fats and Fixed Oils* ⟨401⟩*, Omega-3 Fatty Acids Determination and Profile*.

Acceptance criteria: NLT 30.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

• **LIMIT OF ARSENIC**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: Transfer 1 g of ultrapure palladium metal to a Teflon beaker. Add 20 mL of water and 10 mL of nitric acid, and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (3:2:5). A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed under *Arsenic* ⟨211⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of arsenic.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of arsenic.

Sample solution: For preparation of the *Sample solution*, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [**CAUTION—**Wear proper eye protection and protective clothing and gloves.] Transfer approximately 500 mg of Schizochytrium Oil, weighed to the nearest 0.1 mg, to a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [NOTE—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests to 25-mL volumetric flasks, and dilute with water to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min; char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of Schizochytrium Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration as obtained above
W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of nitric acid and 40 mL of water to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the

solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Lead Nitrate Stock Solution*, prepared as directed under *Heavy Metals* ⟨231⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of lead.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of lead.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2100°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for lead. Determine the peak area at the lead emission line at 283.3 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of lead, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of lead in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of lead in the portion of Schizochytrium Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF CADMIUM**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A, Solution B*, and 2% nitric acid to volume (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution A: 0.1372 mg/mL of cadmium nitrate

Standard stock solution B: *Standard stock solution A*, nitric acid, and water (2:1:97). This solution contains 0.10 µg/mL of cadmium. [NOTE—Before make up to final volume dissolve in a portion of water and nitric acid.]

Standard solutions: Dilute *Standard stock solution B* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of cadmium.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for cadmium. Determine the peak area at the cadmium emission line at 228.8 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of cadmium, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of cadmium in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of cadmium in the portion of Schizochytrium Oil taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Schizochytrium Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF MERCURY**

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic*, combining the two duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution* (see *Reagents* under *Mercury* ⟨261⟩, *Method IIa*).

Analysis: Proceed as directed under *Mercury* ⟨261⟩, *Method IIa*, except to use a *Standard Mercury Solution* having the equivalent of 0.1 µg/mL of mercury.

Acceptance criteria: NMT 0.1 µg/g

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0
- **FATS AND FIXED OILS,** *Free Fatty Acids* ⟨**401**⟩**:** The free fatty acids in 10 g require NMT 1.42 mL of 0.1 N sodium hydroxide for neutralization.
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26, calculated:

$$\text{Result} = (2 \times PV) + AV$$

PV = peroxide value
AV = anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 4.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **LABELING:** The label states the content of docosahexaenoic acid in mg/g. It also states the name and concentration of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Docosahexaenoic Acid Ethyl Ester RS
USP Eicosapentaenoic Acid Ethyl Ester RS
USP Methyl Laurate RS
USP Methyl Tricosanoate RS■2S *(USP33)*

In-Process Revision

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 27* page 1143 and page 318 of *PF* 35(2) [Mar.–Apr. 2009]. It is proposed to add *Carmellose* to the *Suspending and/or Viscosity-Increasing Agent* category; *Lactobionic Acid* to the *Antioxidant* category; and *Methacrylic Acid and Ethyl Acrylate Copolymer* and *Methacrylic Acid and Methyl Methacrylate Copolymer* to the *Coating Agent* and *Film-Forming Agent* categories, to complement the proposed new monographs for *Carmellose, Lactobionic Acid, Methacrylic Acid and Ethyl Acrylate Copolymer*, and *Methacrylic Acid and Methyl Methacrylate Copolymer,* which appear elsewhere in this issue of *PF.*

(EM1; EM2) RTS—C65791; C71283; C73593

Change to read:

Antimicrobial Preservative

Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzethonium Chloride
Benzoic Acid
Benzyl Alcohol
Butylparaben

▲Calcium Propionate▲*NF28*

Cetrimonium Bromide
Cetylpyridinium Chloride
Chlorobutanol
Chlorocresol
Cresol
Dehydroacetic Acid
▲Erythorbic Acid▲*NF27*
Ethylparaben
Methylparaben
Methylparaben Sodium
Phenol
Phenoxyethanol
Phenylethyl Alcohol
Phenylmercuric Acetate
Phenylmercuric Nitrate
Potassium Benzoate
Potassium Sorbate
Propylparaben
Propylparaben Sodium
Sodium Benzoate
Sodium Dehydroacetate
Sodium Propionate
Sorbic Acid
Thimerosal
Thymol

Change to read:

Antioxidant

Ascorbic Acid
Ascorbyl Palmitate
Butylated Hydroxyanisole
Butylated Hydroxytoluene
Stannous Chloride
▲Erythorbic Acid▲*NF27*
Hypophosphorous Acid

■Lactobionic Acid■2S (*NF28*)

Monothioglycerol
Potassium Metabisulfite
Propyl Gallate
Sodium Bisulfite
Sodium Formaldehyde Sulfoxylate
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Sulfur Dioxide
Tocopherol
Tocopherols Excipient

Change to read:

Buffering Agent

Acetic Acid
Adipic Acid
Ammonium Carbonate
Ammonium Phosphate
Boric Acid
Citric Acid, Anhydrous
Citric Acid Monohydrate

▲Alpha-Lactalbumin▲*NF28*

Lactic Acid
Phosphoric Acid
Potassium Citrate
Potassium Metaphosphate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Citrate
Sodium Lactate Solution
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Succinic Acid

Change to read:

Bulking Agent for Freeze-Drying

Creatinine

▲Alpha-Lactalbumin▲*NF28*

Mannitol
Polydextrose
Pullulan

■Trehalose■2S (*NF27*)

Change to read:

Coating Agent

Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carboxymethylcellulose, Sodium

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Cellaburate

Cellacefate (formerly Cellulose Acetate Phthalate)
Cellulose Acetate
Cellulose Acetate Phthalate (see Cellacefate)

▲Chitosan▲*NF28*

Coconut Oil

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone
Corn Syrup Solids
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Ethylcellulose Aqueous Dispersion

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S (*NF28*)

Gelatin
Glaze, Pharmaceutical
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hydroxypropyl Methylcellulose Phthalate (see Hypromellose Phthalate)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate
Hypromellose Phthalate (formerly Hydroxypropyl Methylcellulose Phthalate)

▲Alpha-Lactalbumin▲*NF28*

Maltodextrin

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S (*NF28*)

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S (*NF28*)

Methacrylic Acid Copolymer
Methacrylic Acid Copolymer Dispersion
Methylcellulose
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Glycol

▲Polyvinyl Acetate▲*NF28*

▲Polyvinyl Acetate Dispersion▲*NF28*

Polyvinyl Acetate Phthalate
Pullulan
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Shellac
Starch, Pregelatinized Modified
Sucrose
Titanium Dioxide
Wax, Carnauba
Wax, Microcrystalline
Zein

Change to read:

Complexing Agent

Edetate Calcium Disodium
Edetate Disodium
Edetic Acid

▲Alpha-Lactalbumin▲*NF28*

Oxyquinoline Sulfate

Change to read:

Desiccant

Calcium Chloride
Calcium Sulfate

▲Polyvinyl Acetate▲*NF28*

Silicon Dioxide

Change to read:

Emulsifying and/or Solubilizing Agent

Acacia
Carbomer Copolymer
Carbomer Interpolymer
Cholesterol
Stannous Chloride
Coconut Oil

▲Desoxycholic Acid▲*NF28*

Diethanolamine (Adjunct)
Diethylene Glycol Stearates
Ethylene Glycol Stearates
Gamma Cyclodextrin
Glyceryl Distearate
Glyceryl Monolinoleate
Glyceryl Monooleate
Glyceryl Monostearate

▲Alpha-Lactalbumin▲*NF28*

Lanolin Alcohols
Lecithin
Mono- and Di-glycerides
Monoethanolamine (Adjunct)
Oleic Acid (Adjunct)
Oleyl Alcohol (Stabilizer)
Oleyl Oleate
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

Poloxamer
Polyoxyethylene 50 Stearate
Polyoxyl 10 Oleyl Ether
Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polyoxyl Lauryl Ether
Polyoxyl Stearyl Ether
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Propylene Glycol Monostearate
Superglycerinated Fully Hydrogenated Rapeseed Oil

Sodium Cetostearyl Sulfate
Sodium Lauryl Sulfate
Sodium Stearate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Stearic Acid

■Sucrose Stearate■1S *(NF28)*

Trolamine
Wax, Emulsifying

Add the following:

Film-Forming Agent

▲Chitosan▲*NF28*

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S *(NF28)*

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S *(NF28)*

Change to read:

Humectant

Corn Syrup Solids
Erythritol
Glycerin
Hexylene Glycol
Inositol
Maltitol
Polydextrose
Propylene Glycol
Sorbitol
Sorbitol Sorbitan Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Tagatose

Change to read:

Stiffening Agent

Castor Oil, Hydrogenated
Cetostearyl Alcohol
Cetyl Alcohol
Cetyl Esters Wax
Cetyl Palmitate
Hard Fat

▲Alpha-Lactalbumin▲*NF28*

Paraffin
Synthetic Paraffin
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Stearyl Alcohol
Wax, Emulsifying
Wax, White
Wax, Yellow

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

▲Carmellose▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

▲Chitosan▲*NF28*

▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

▲Alpha-Lactalbumin▲*NF28*

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin
Polyethylene Oxide
Polyvinyl Alcohol
Povidone
Propylene Glycol Alginate
Pullulan
Hydrophobic Colloidal Silica
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca
Starch, Wheat

■Sucrose Palmitate■1S *(NF28)*

Tragacanth
Xanthan Gum

Change to read:

Sweetening Agent

Acesulfame Potassium
Aspartame
Aspartame Acesulfame
▲Corn Syrup▲*NF27*
Corn Syrup Solids
High Fructose Corn Syrup
Dextrates
Dextrose
Dextrose Excipient
Erythritol
Fructose
Galactose
Maltitol
Maltose
Mannitol
Saccharin
Saccharin Calcium
Saccharin Sodium
Sorbitol
Sorbitol Solution

▲Hydrogenated Starch Hydrolysate▲*NF28*

Sucralose
Sucrose
Sugar, Compressible
Sugar, Confectioner's
Syrup
Tagatose

■Trehalose■2S (*NF27*)

Change to read:

Tablet Binder

Acacia
Alginic Acid
Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Sodium
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

■Hydrogenated Coconut Oil■1S (*NF27*)

Copovidone
▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S (*NF28*)

Gelatin
Glucose, Liquid
Guar Gum
Low-Substituted Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate

▲Alpha-Lactalbumin▲*NF28*

Maltodextrin
Maltose
Methylcellulose

■Hydrogenated Palm Oil■1S (*NF27*)

Polyethylene Oxide

▲Polyvinyl Acetate▲*NF28*

Povidone
Pullulan
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Syrup

■Trehalose■2S (*NF27*)

Change to read:

Tablet and/or Capsule Diluent

Calcium Carbonate
Calcium Phosphate, Dibasic
Calcium Phosphate, Tribasic
Calcium Sulfate
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

Cellulose, Powdered
▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrates
Dextrin
Dextrose Excipient
Fructose
Kaolin

▲Alpha-Lactalbumin▲*NF28*

Lactitol
Lactose, Anhydrous
Lactose, Monohydrate
Maltitol
Maltodextrin
Maltose
Mannitol
Propylene Glycol Monocaprylate
Pullulan
Sorbitol
Starch
Starch, Corn

▲Hydrogenated Starch Hydrolysate▲*NF28*

▲Starch, Pea▲*NF28*

In-Process Revision

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Sucrose
Sugar, Compressible
Sugar, Confectioner's

■Trehalose■2S *(NF27)*

Change to read:

Tablet Disintegrant

Alginic Acid
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Croscarmellose Sodium
Crospovidone
Low-Substituted Hydroxypropyl Cellulose
Maltose
Polacrilin Potassium
Pullulan
Sodium Starch Glycolate
Starch
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat

■Trehalose■2S *(NF27)*

Change to read:

Tablet and/or Capsule Lubricant

■Behenoyl Polyoxylglycerides■2S *(NF27)*

Calcium Stearate

■Hydrogenated Coconut Oil■1S *(NF27)*

Glyceryl Behenate
Magnesium Stearate
Mineral Oil, Light

■Hydrogenated Palm Oil■1S *(NF27)*

Polyethylene Glycol
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Sodium Lauryl Sulfate
Sodium Stearyl Fumarate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Starch
Stearic Acid
Stearic Acid, Purified

■Sucrose Stearate■1S *(NF28)*

Talc
Vegetable Oil, Hydrogenated, Type I
Zinc Stearate

Change to read:

Vehicle

FLAVORED AND/OR SWEETENED

Aromatic Elixir
Benzaldehyde Elixir, Compound
Corn Syrup Solids
Dextrose

■Ethyl Maltol■2S *(NF27)*

Peppermint Water
Sorbitol Solution
Syrup

■Trehalose■2S *(NF27)*

OLEAGINOUS

Alkyl (C12-15) Benzoate
Almond Oil
Canola Oil
Corn Oil
Cottonseed Oil
Ethyl Oleate
Hydrogenated Polydecene
Isopropyl Myristate
Isopropyl Palmitate
Mineral Oil
Mineral Oil, Light
Octyldodecanol
Olive Oil
Peanut Oil

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Safflower Oil
Sesame Oil
Soybean Oil
Squalane

SOLID CARRIER

▲Chitosan▲*NF28*

Corn Syrup Solids

▲Alpha-Lactalbumin▲*NF28*

Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Sugar Spheres

STERILE

▲rAlbumin Human▲*NF27*
Sodium Chloride Injection, Bacteriostatic
Water for Injection, Bacteriostatic

Change to read:

Wetting and/or Solubilizing Agent

Benzalkonium Chloride
Benzethonium Chloride
Cetylpyridinium Chloride
Docusate Sodium
Nonoxynol 9
Octoxynol 9
Poloxamer
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Pullulan
Sodium Lauryl Sulfate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Tyloxapol

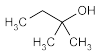
MONOGRAPHS (NF)

BRIEFING

Amylene Hydrate. *NF 27* page 1166. On the basis of comments received, it is proposed to replace *Identification* test *A, Identification Tests—General, Acetate* ⟨191⟩ and *Identification* test *B* with a more definitive infrared absorption test. The existing *Identification* test *C* will be renamed *Identification* test *B*.

(EM1: R. Lafaver.) RTS—C63260

Amylene Hydrate



$C_5H_{12}O$ 88.15
2-Butanol, 2-methyl-;
tert-Pentyl alcohol [75-85-4].

DEFINITION
Amylene Hydrate contains NLT 99.0% and NMT 100.0% of $C_5H_{12}O$.

IDENTIFICATION

Change to read:

• **A. ~~IDENTIFICATION TESTS—GENERAL,~~** *Acetate* ⟨**~~191~~**⟩
~~Sample:~~ ~~2 mL~~
~~Analysis:~~ ~~Mix the *Sample* with 15 mL of water, 5 mL of sulfuric acid, and 10 g of potassium dichromate. Heat the mixture under a reflux condenser for 2 h, and then distill, collecting and reserving the first 2 mL of the distillate. Continue the distillation until most of the water has distilled over, render this distillate alkaline with 1 N sodium hydroxide, add 2 N sulfuric acid dropwise until the solution is neutral to litmus, and carefully evaporate to dryness.~~
~~Acceptance criteria:~~ ~~Meets the requirements~~
■*Infrared Absorption* ⟨197F⟩■2S (*NF28*)

Change to read:

• **B. PROCEDURE**
~~Sample:~~ ~~1 mL of the reserved distillate obtained in *Identification* test *A*~~
~~Analysis:~~ ~~To the *Sample* add 5 drops of sodium nitroferricyanide TS and 2 mL of 1 N sodium hydroxide, then add a slight excess of 6 N acetic acid.~~
~~Acceptance criteria:~~ ~~A deep red liquid, which develops a violet tint when diluted with several volumes of water, is produced.~~
■**Sample solution:** 1 in 10
Analysis: To 10 mL of *Sample solution* quickly add 5 mL of a 1-in-100 solution of vanillin in sulfuric acid.
Acceptance criteria: A violet-red color is produced.■2S (*NF28*)

Delete the following:

■• ~~**C. PROCEDURE**~~
~~**Sample solution:** 1 in 10~~
~~**Analysis:** To 10 mL of *Sample solution* quickly add 5 mL of a 1-in-100 solution of vanillin in sulfuric acid.~~
~~**Acceptance criteria:** A violet-red color is produced.~~ ■2S (*NF28*)

ASSAY
• **PROCEDURE**
Sample solution: Amylene Hydrate
Chromatographic system
Mode: GC
Detector: Thermal conductivity
Column: 4-mm × 2-cm glass column; packed with chromatographic support S2 (under typical conditions)
Temperature
Column: 190°
Injection port: 200°
Detector block: 200°
Carrier gas: Helium
Flow rate: 50 mL/min
Injection size: 0.4 µL
Analysis
Samples: *Sample solution*
From the area under the curve, calculate the percentage (a/a) of $C_5H_{12}O$ in the Amylene Hydrate taken.
Acceptance criteria: 99.0%–100.0%

IMPURITIES
Inorganic Impurities
• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 10 mL
Analysis: Evaporate the *Sample* in a tared porcelain dish on a steam bath to a volume of about 1 mL, and allow it to evaporate spontaneously to dryness while protected from dust.
Acceptance criteria: The residue, if any, is colorless, and when dried at 105° for 1 h, weighs NMT 2 mg (0.02%).
• **HEAVY METALS,** *Method I* ⟨**231**⟩
Sample solution: Evaporate 5.0 mL (4 g) on a steam bath to dryness, warm the residue gently with 1 mL of dilute hydrochloric acid (1 in 120), add water to make 25 mL, and filter, if necessary.
Acceptance criteria: NMT 5 ppm
Organic Impurities
• **PROCEDURE**
Sample solution: 1 in 20
Analysis: To 10 mL of *Sample solution* add 0.10 mL of 0.10 N potassium permanganate.
Acceptance criteria: The pink color does not completely disappear within 10 min.

SPECIFIC TESTS
• **SPECIFIC GRAVITY** ⟨**841**⟩**:** 0.803–0.807
• **DISTILLING RANGE,** *Method I* ⟨**721**⟩**:** It distills completely between 97° and 103°, a correction factor of 0.037°/mm being applied as necessary.
• **WATER:** The relative retention time of water on the gas chromatographic column used in the *Assay* is approximately 0.2 times that of Amylene Hydrate. From the area under the curve obtained in the *Assay,* calculate the percentage (a/a) of water in the Amylene Hydrate taken: NMT 0.5% is found.
• **ALDEHYDE**
Sample solution: 1 in 20
Analysis: To 10 mL of *Sample solution* add 1 mL of silver-ammonium nitrate TS, and heat the mixture on a water bath at 60° for 10 min.

Acceptance criteria: No darkening occurs.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

BRIEFING

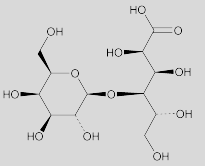
Lactobionic Acid. Because there is no existing *NF* monograph for this excipient, a new monograph, based on the monograph appearing in the *European Pharmacopoeia* 6.3, is being proposed. Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver. NOM: A. Wilk.) RTS—C65791

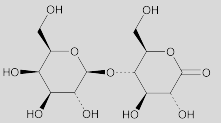
Add the following:

▪Lactobionic Acid

$C_{12}H_{22}O_{12}$ (acid form) 358.3
[96-82-2].



$C_{12}H_{20}O_{11}$ (δ-lactone) 340.3
[5965-65-1].



4-*O*-β-galactopyranosyl-D-gluconic acid.

DEFINITION
Lactobionic Acid is a mixture in variable proportions of 4-*O*-β-D-galactopyranosyl-D-gluconic acid and 4-*O*-β-D-galactopyranosyl-D-glucono-1,5-lactone. It contains NLT 98.0% and NMT 102.0%, on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩:** [NOTE—If the spectra obtained show differences, dissolve the test substance and USP Lactobionic Acid RS separately in water, dry at 105°, and record new spectra using the residues.]
- **B. THIN-LAYER CHROMATOGRAPHY ⟨621⟩**
  Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 µm (TLC plates)
  Sample solution: 10 mg/mL of Lactobionic Acid
  Standard solution: 10 mg/mL of USP Lactobionic Acid RS
  Developing solvent: Methanol, ethyl acetate, ammonium hydroxide, water (2:1:1:1)
  Application volume: 5 µL
  Spray reagent: Slowly add 10 mL of sulfuric acid to about 40 mL of water. Mix, and allow to cool. Dilute with water to 100 mL, and mix. Add 2.5 g of ammonium molybdate and 1 g of ceric sulfate, and shake for 15 min to dissolve.
  Analysis: Develop the chromatograms until the solvent front has moved about three-fourths the length of the plate, and allow to dry. Spray the plate with *Spray reagent*, and allow to dry. Repeat two more times, heat at 110° for 15 min, and examine.
  Acceptance criteria: The principal spot from the *Sample solution* is similar in position and color to the principal spot from the *Standard solution*.

ASSAY
- **PROCEDURE**
  Sample solution: Dissolve 0.350 g of Lactobionic Acid in 50 mL of carbon dioxide-free water, previously heated to 30°.
  Analysis: Immediately titrate with 0.1 M sodium hydroxide and determine the two equivalence points potentiometrically. Calculate the percentage of lactobionic acid in the portion of Lactobionic Acid taken:

$$\text{Result} = V_1 + (V_2 - V_1)$$

V_1 = 1 mL of 0.1 M sodium hydroxide, equivalent to 35.83 mg of $C_{12}H_{22}O_{12}$ (corresponds to the acid form of lactobionic acid)
V_2 = 1 mL of 0.1 M sodium hydroxide, equivalent to 34.03 mg of $C_{12}H_{20}O_{11}$ (corresponds to the δ-lactone form)

  Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
Inorganic Impurities
- **HEAVY METALS ⟨231⟩**
  Thioacetamide reagent: To 0.2 mL of thioacetamide TS, add 1 mL of a mixture of 5 mL of water, 15 mL of 1 M sodium hydroxide, and 20 mL of glycerin. Heat in a water bath for 20 s. [NOTE—Prepare immediately before use.]
  Lead nitrate stock solution: Prepare as directed for *Special Reagents* under *Heavy Metals*.
  Standard lead solution: On the day of use, dilute 2.0 mL of *Lead nitrate stock solution* (10 ppm Pb) in water to 30 mL.
  Sample solution: Dissolve 1 g of Lactobionic Acid in water to 30 mL.
  Prepare the filtration apparatus by adapting the barrel of a 50-mL syringe without its piston to a support containing, on the plate, a membrane filter (pore size 3 µm) and above it a prefilter.
  Transfer the *Sample solution* into the syringe barrel, put the piston in place, and then apply an even pressure on it until the whole of the liquid has been filtered. When opening the support and removing the prefilter, check that the membrane filter remains uncontaminated with impurities. If this is not the case replace it with another membrane filter and repeat the operation under the same conditions.
  Analysis: To the prefiltrate, add 2 mL of *pH 3.5 Acetate Buffer*. Mix, and add 1.2 mL of thioacetamide reagent. Mix immediately and allow to stand for 10 min, and again filter as described above, but inverting the order of the filters, the liquid passing first through the membrane filter before passing through the prefilter. The filtration must be carried out slowly and uniformly by applying moderate and constant pressure to the piston of the syringe. After complete filtration, open the support, remove the membrane filter, and dry using filter paper. In parallel, treat the reference solution in the same manner as the *Sample solution.*
  Acceptance criteria: The color of the spot from the *Sample solution* is not more intense than that from the reference solution (NMT 20 ppm).

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ia* **⟨921⟩**
  Sample solution: 0.50 g in a mixture of methanol and formamide (2:1)
  Acceptance criteria: NMT 5.0%
- **APPEARANCE OF SOLUTION**
  Sample solution: 120 mg/mL of Lactobionic Acid
  Standard stock solution: Pipet 24.0 mL of ferric chloride CS and 6.0 mL of cobaltous chloride CS into a 100-mL volumetric flask. Dilute with 1% (w/v) hydrochloric acid to volume.

In-Process Revision

Reference solution: Pipet 12.5 mL of *Standard stock solution* into a 100-mL volumetric flask. Dilute with 1% (w/v) hydrochloric acid to volume.
Acceptance criteria: The solution is clear and not more intensely colored than the *Reference solution*.

- **OPTICAL ROTATION** ⟨**781S**⟩
Sample solution: 10 mg/mL of Lactobionic Acid. Allow to stand for 24 h.
Acceptance criteria: +23.0° to +29.0° (anhydrous substance)
- **REDUCING SUGARS**
Dissolve 5.0 g of Lactobionic Acid in 25 mL of water with the aid of gentle heat. Cool, and add 20 mL of cupric citrate TS and a few glass beads. Heat so that boiling begins after 4 min, and maintain boiling for 3 min. Cool rapidly, and add 100 mL of a 2.4% solution of glacial acetic acid and 20.0 mL of 0.025 M iodine VS.
Analysis: With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water and, when the precipitate has dissolved, titrate the excess of iodine with 0.05 M sodium thiosulfate VS using 1 mL of starch TS, added towards the end of the titration, as an indicator.
Acceptance criteria: NLT 12.8 mL of 0.05 M sodium thiosulfate VS is required, corresponding to NMT 0.2% of reducing sugars, as glucose.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Lactobionic Acid RS■2S *(NF28)*

BRIEFING

Methacrylic Acid Copolymer, *NF* 27 page 1279. On the basis of comments and data received, the proposed revision in *PF* 33(6) has been cancelled; and this monograph is being split into two individual monographs, which are proposed elsewhere in this issue of *PF*:
- *Methacrylic Acid and Methyl Methacrylate Copolymer*
- *Methacrylic Acid and Ethyl Acrylate Copolymer*

The newly proposed *Methacrylic Acid and Methyl Methacrylate Copolymer* monograph contains the procedures and limits outlined for Methacrylic Acid Copolymer Types A and B. The newly proposed *Methacrylic Acid and Ethyl Acrylate Copolymer* monograph contains the procedures and limits outlined for Methacrylic Acid Copolymer Type C.

The two new monographs are proposed for inclusion in the *Second Supplement* to *USP* 33-*NF* 28, with an official date of December 1, 2010. However, use of the names (1) Methacrylic Acid and Methyl Methacrylate Copolymer and (2) Methacrylic Acid and Ethyl Acrylate Copolymer will be permitted as of December 1, 2010, but their use will not be mandatory until December 1, 2015. The 60-month extension will provide the time deemed necessary for labeling changes to be made for the article and the numerous preparations in which it is an ingredient, as well as for practitioners, consumers, and regulatory agencies to become familiar with the new terminology. After December 1, 2015, the *Methacrylic Acid Copolymer* monograph will be discontinued.

The following changes in the *Methacrylic Acid Copolymer* monograph are being proposed to make it consistent with the two new proposed monographs.
1. The chemical structure, chemical names, and CAS numbers are added.
2. The *Definition* is updated.
3. The *Identification* section is updated.
4. The *Assay* is updated.
5. The method in the test for *Limit of Monomers* is improved, employing the approach currently used in the *NF* monographs *Ammonio Methacrylate Copolymer* and *Ammonio Methacrylate Copolymer Dispersion*. This method includes precipitation of the polymer before analysis in order to avoid clogging of the column and other separation problems. The HPLC chromatographic procedures in the test for *Limit of Monomers* are based on analyses performed with a Nucleosil 100 C18 column with 7-µm L1 packing. With this system, methacrylic acid, ethyl acrylate, and methyl methacrylate elute at approximately 2.9, 7.4, and 8.2 min, respectively.
6. The *Analysis* in the test for *Viscosity* indicates the shear rate conditions. This is based on validation data demonstrating that the test solution behaves as a Newtonian fluid under the test conditions for a shear rate of NLT 1 s^{-1} and NMT 100 s^{-1}.
7. Storage conditions are specified in the *Packaging and Storage* section.
8. Added surface-active agents are indicated in the *Labeling* section for all the types.

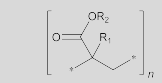
(EM2: H. Wang.) RTS—C65736

Methacrylic Acid Copolymer

(***Any article currently titled Methacrylic Acid Copolymer, Type A or Type B, will be officially titled Methacrylic Acid and Methyl Methacrylate Copolymer after December 1, 2015. Any article currently titled Methacrylic Acid Copolymer, Type C, will be officially titled Methacrylic Acid and Ethyl Acrylate Copolymer after December 1, 2015. After December 1, 2015, the Methacrylic Acid Copolymer monograph will no longer be valid.***)

Add the following:

■



Type	R_1	R_2	Ratio
A	CH_3	H or CH_3	1:1
B	CH_3	H or CH_3	1:2
C	CH_3	H	1:1
	or H	C_2H_5	

Type A or Type B: Poly(methacrylic acid, methyl methacrylate); Methacrylic acid–methyl methacrylate copolymer [25086-15-1].
Type C: Poly(methacrylic acid, ethyl acrylate); Methacrylic acid–ethyl acrylate copolymer [25212-88-8].■2S *(NF28)*

DEFINITION

Change to read:

~~Methacrylic Acid Copolymer is a fully polymerized copolymer of methacrylic acid and an acrylic or methacrylic ester. Type C may contain suitable surface-active agents. The assay and viscosity requirements differ for the several types, as described in the table below.~~■Methacrylic Acid Copolymer consists of methacrylic acid and methyl methacrylate monomers arranged in a random distribution or consists of methacrylic acid and ethyl acrylate monomers arranged in a random distribution. It may contain suitable surface-active agents. The *Assay* and *Viscosity* requirements differ for the three types, as described in the table below.■2S *(NF28)*

In-Process Revision

Type	Methacrylic Acid Units, Dried Basis (%)		Viscosity (~~cps~~■mPa·s■2S (NF28))	
	Min.	Max.	Min.	Max.
A	46.0	50.6	~~50~~■60■2S (NF28)	~~200~~■120■2S (NF28)
B	27.6	30.7	50	200
C	46.0	50.6	100	200

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Change to read:

• **B. PROCEDURE:**

Sample: ~~A few mL of the solution prepared for the *Viscosity* test~~

Analysis: ~~Pour the *Sample* onto a glass plate, and allow the solvent to evaporate.~~

Acceptance criteria: ~~A clear, brittle film results.~~ ■It meets the requirements of the test for *Assay*.■2S (NF28)

ASSAY

Change to read:

• **PROCEDURE**

Sample solution: ~~1 g of Methacrylic Acid Copolymer, previously dried, in 100 mL of neutralized acetone. Add 1 drop of phenolphthalein TS.~~

Analysis: ~~Titrate the *Sample solution* with 0.1 N sodium hydroxide VS until a pink color persists for 15 s. Each mL of 0.1 N sodium hydroxide is equivalent to 8.609 mg of methacrylic acid ($C_4H_6O_2$) units.~~

■**Sample:** 1 g, previously dried

Analysis: Dissolve the *Sample* in 100 mL of neutralized acetone and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 8.609 mg of methacrylic acid ($C_4H_6O_2$) units. ■2S (NF28)

Acceptance criteria

Type A: 46.0%–50.6%

Type B: 27.6%–30.7%

Type C: 46.0%–50.6%

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1% for Types A and B; NMT 0.4% for Type C

• **HEAVY METALS,** *Method II* ⟨231⟩**:** NMT 20 ppm

Change to read:

Organic Impurities

• **PROCEDURE: LIMIT OF MONOMERS**

~~pH 2.0 Phosphate buffer, 0.025 M:~~ ~~Prepare an aqueous solution containing 3.550 g of anhydrous dibasic sodium phosphate (Na_2HPO_4) and 3.400 g of monobasic potassium phosphate (KH_2PO_4)/L. Adjust with phosphoric acid to a pH of 2.0.~~

~~Mobile phase:~~ ~~700 mL of *pH 2.0 Phosphate buffer, 0.025 M*/L, in methanol~~

~~Standard solution:~~ ~~Prepare a solution in methanol to contain a concentration of 2.4 µg/mL each of methacrylic acid and either methyl methacrylate (for Type A and Type B) or ethyl acrylate (for Type C). To 50.0 mL of this solution, add 25.0 mL of water.~~

~~Sample solution:~~ ~~Dissolve 40 mg of Methacrylic Acid Copolymer in 50.0 mL of methanol, and add 25.0 mL of water.~~

~~Chromatographic system~~

~~(See *Chromatography, System Suitability* ⟨621⟩.)~~

~~Mode:~~ ~~LC~~

~~Detector:~~ ~~UV 202 nm~~

~~Column:~~ ~~4 mm × 12.5 cm; 5 µm packing L1~~

~~Flow rate:~~ ~~2.5 mL/min~~

~~Injection size:~~ ~~50 µL~~

~~System suitability~~

~~Sample:~~ ~~*Standard solution*~~

~~Suitability requirements~~

~~Resolution:~~ ~~NLT 2.0 of each pair of analytes~~

~~Capacity factors, k′:~~ ~~1.7, 4.3, and 4.8 for methacrylic acid, ethyl acrylate, and methyl methacrylate, respectively~~

~~Relative standard deviation:~~ ~~NMT 2%, determined from each analyte~~

~~Analysis~~

~~Samples:~~ ~~*Sample solution* and *Standard solution*~~

~~Calculate the quantity, in µg, of each monomer in the portion of Methacrylic Acid Copolymer taken:~~

$$\text{~~Result} = (r_U/r_S) \times C \times 75\text{~~}$$

~~r_U = peak response for the monomer from the *Sample solution*~~

~~r_S = peak response for the monomer from the *Standard solution*~~

~~C = concentration of the monomer in the *Standard solution* (µg/mL)~~

~~Acceptance criteria:~~ ~~NMT 0.05%~~

■**Phosphate buffer:** Prepare an aqueous solution containing 17.8 g/L of anhydrous dibasic sodium phosphate and 17.0 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0. This buffer has a concentration of 0.125 M.

Sodium perchlorate solution: 35 mg/mL of sodium perchlorate. This solution has a concentration of 0.25 M.

Mobile phase: Add phosphoric acid dropwise to water to obtain a solution having a pH of 2.0. Prepare a mixture of this acidified water and methanol (80:20), and degas.

Standard solution for Type A or Type B: Dissolve 0.05 g of methacrylic acid and 0.05 g of methyl methacrylate in 5 mL of butanol, and add methanol to exactly 100 mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume. Mix 3.0 mL of this solution with 10.0 mL of *Phosphate buffer*. This solution contains 1.15 µg/mL each of methacrylic acid and methyl methacrylate.

Standard solution for Type C: Dissolve 0.01 g of methacrylic acid and 0.01 g of ethyl acrylate in 5 mL of butanol, and add methanol to exactly 100 mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume. Mix 5.0 mL of this solution with 5.0 mL of *Sodium perchlorate solution*. This solution contains about 0.5 µg/mL each of methacrylic acid and ethyl acrylate.

Sample solution for Type A or Type B: Transfer 1 g of Methacrylic Acid Copolymer, Type A or Type B, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Add 3 mL of this solution dropwise, while continuously stirring, to a beaker that contains 10.0 mL of *Phosphate buffer*. Remove the precipitated polymer to obtain a clear supernatant by centrifugation (e.g., NLT 5000 × *g* for NLT 5 min). Use the clear supernatant.

Sample solution for Type C: Transfer 3 g of Methacrylic Acid Copolymer, Type C, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Add 5 mL of this solution dropwise, while continuously stirring, to a beaker that contains 5.0 mL of *Sodium perchlorate solution*. Remove the precipitated polymer to obtain a clear supernatant by centrifugation (e.g., NLT 5000 × *g* for NLT 5 min). Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 202 nm

Column: 4.0-mm × 12.5-cm analytical column; 7-µm packing L1

In-Process Revision

Flow rate: 2 mL/min
Injection size: 20 µL

For Type A or Type B

System suitability

Sample: *Standard solution for Type A or Type B*

[NOTE—The relative retention times for methacrylic acid and methyl methacrylate are 1.0 and 2.8, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methacrylic acid and methyl methacrylate

Relative standard deviation: NMT 2.0%.

Analysis

Samples: *Standard solution for Type A or Type B* and *Sample solution for Type A or Type B*

Calculate the percentage of each monomer (methacrylic acid or methyl methacrylate) in the portion of Methacrylic Acid Copolymer Type A or Type B taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times V_F \times D \times F \times 100$$

r_U = monomer (methacrylic acid or methyl methacrylate) peak response from the *Sample solution for Type A or Type B*

r_S = monomer (methacrylic acid or methyl methacrylate) peak response from the *Standard solution for Type A or Type B*

C = concentration of the monomer (methacrylic acid or methyl methacrylate) in the *Standard solution for Type A or Type B* (µg/mL)

W = weight of Methacrylic Acid Copolymer Type A or Type B taken to prepare the *Sample solution for Type A or Type B* (g)

V_F = final volume of the *Sample solution for Type A or Type B*, 13 mL

D = dilution factor for preparation of the *Sample solution for Type A or Type B*, 16.7

F = conversion factor, 10^{-6} g/µg

Acceptance criteria: NMT 0.05% for the total amount of monomers

For Type C

System suitability

Sample: *Standard solution for Type C*

[NOTE—The relative retention times for methacrylic acid and ethyl acrylate are 1.0 and 2.6, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methacrylic acid and ethyl acrylate

Relative standard deviation: NMT 2.0%.

Analysis

Samples: *Standard solution for Type C* and *Sample solution for Type C*

Calculate the percentage of each monomer (methacrylic acid or ethyl acrylate) in the portion of Methacrylic Acid Copolymer Type C taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times V_F \times D \times F \times 100$$

r_U = monomer (methacrylic acid or ethyl acrylate) peak response from the *Sample solution for Type C*

r_S = monomer (methacrylic acid or ethyl acrylate) peak response from the *Standard solution*

C = concentration of the monomer (methacrylic acid or ethyl acrylate) in the *Standard solution for Type C* (µg/mL)

W = weight of Methacrylic Acid Copolymer Type C taken to prepare the *Sample solution for Type C* (g)

V_F = final volume of the *Sample solution for Type C*, 10 mL

D = dilution factor for preparation of the *Sample solution for Type C*, 10

F = conversion factor, 10^{-6} g/µg

Acceptance criteria: NMT 0.01% for the total amount of monomers.■2S (*NF28*)

SPECIFIC TESTS

Change to read:

- **VISCOSITY** ⟨**911**⟩

Analysis: ~~Place 254.6 g of isopropyl alcohol and 7.9 g of water in a conical flask having a ground-glass joint. Add a quantity of Methacrylic Acid Copolymer equivalent to 37.5 g of solids on the dried basis, while stirring by means of a magnetic stirrer. Close the flask, and continue stirring until the polymer has dissolved completely. Adjust the temperature to 20 ± 0.1°. Equip a suitable rotational viscosimeter with a spindle having a cylinder 1.88 cm in diameter and 6.25 cm high attached to a shaft 0.32 cm in diameter, the distance from the top of the cylinder to the lower tip of the shaft being 0.75 cm, and the immersion depth being 8.15 cm (No. 1 spindle). With the spindle rotating at 30 rpm, immediately observe and record the scale reading. Convert the scale reading to centipoises by multiplying the reading by the constant for the viscosimeter spindle and speed employed.~~■Place 254.6 g of isopropyl alcohol and 7.9 g of water in a test flask. Add a quantity of Methacrylic Acid Copolymer, equivalent to 37.5 g of solids on the dried basis, while stirring by means of a magnetic stirrer. Close the flask, and continue stirring until the polymer has dissolved completely. Adjust the temperature to 20 ± 0.1°. Equip a rotational viscometer with an accessory.[1] The shear rate under the test condition is NLT 1 s^{-1} and NMT 100 s^{-1}. Follow the instrument manufacturer's directions to measure the apparent viscosity.■2S (*NF28*)

Acceptance criteria

~~**Type A:** 50–200 cps~~
~~**Type B:** 50–200 cps~~
~~**Type C:** 100–200 cps~~

■**Type A:** 60–120 mPa · s
Type B: 50–200 mPa · s
Type C: 100–200 mPa · s.■2S (*NF28*)

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry it at 110° for 6 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. ■Store at controlled room temperature.■2S (*NF28*)

Change to read:

- **LABELING:** Label it to state whether it is Type A, B, or C. ■The labeling also indicates the name and quantity of any added surface-active agent.■2S (*NF28*)
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Methacrylic Acid Copolymer, Type A RS
  USP Methacrylic Acid Copolymer, Type B RS
  USP Methacrylic Acid Copolymer, Type C RS

BRIEFING

Methacrylic Acid and Ethyl Acrylate Copolymer. See Briefing under *Methacrylic Acid Copolymer.* The newly proposed

[1] A suitable accessory is available from Brookfield Engineering as the LV1 spindle, a cylindrical spindle 1.9 cm in diameter and 6.5 cm high attached to a shaft 0.3 cm in diameter. The spindle rotates at 30 rpm at an immersion depth of 8.15 cm.

In-Process Revision

NF monograph shown here contains the procedures and limits outlined for Methacrylic Acid Copolymer Type C.
In this monograph:

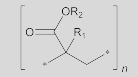
- The test for *Limit of Methacrylic Acid and Ethyl Acrylate* includes precipitation of the polymer before analysis in order to avoid clogging of the column and other separation problems. The HPLC chromatographic procedure in the test is based on analyses performed with a Nucleosil 100 C18 column with 7-µm L1 packing. With this system, methacrylic acid and ethyl acrylate elute at approximately 2.9 and 7.4 min, respectively.
- The *Analysis* in the test for *Viscosity* indicates the shear rate conditions. These are based on validation data demonstrating that the test solution behaves as a Newtonian fluid under the test conditions for a shear rate of NLT 1 s^{-1} and NMT 100 s^{-1}.

(EM2: H. Wang.) RTS—C73593

Add the following:

▪Methacrylic Acid and Ethyl Acrylate Copolymer

(***Title for this new monograph—to become official December 1, 2015***)
(***Prior to December 1, 2015, the current practice of labeling the article of commerce with the name Methacrylic Acid Copolymer, Type C, may be continued. Use of the name Methacrylic Acid and Ethyl Acrylate Copolymer will be permitted as of December 1, 2010, but the use of this name will not be mandatory until December 1, 2015. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.***)



R_1	R_2
CH_3	H
or H	C_2H_5

Poly(methacrylic acid, ethyl acrylate);
Methacrylic acid–ethyl acrylate copolymer [25212-88-8].

DEFINITION

Methacrylic Acid and Ethyl Acrylate Copolymer consists of methacrylic acid and ethyl acrylate monomers arranged in a random distribution. Methacrylic acid units in Methacrylic Acid and Ethyl Acrylate Copolymer are NLT 46.0% and NMT 50.6%, calculated on the dried basis. It may contain suitable surface-active agents.

IDENTIFICATION

- **A. Infrared Absorption ⟨197K⟩**
  Use USP Methacrylic Acid and Ethyl Acrylate Copolymer (1:1) RS for Methacrylic Acid and Ethyl Acrylate Copolymer having a range of 46.0%–50.6% for methacrylic acid units.
- **B.** It meets the requirements of the *Assay*.

ASSAY

- **Procedure**
  Sample: 1 g, previously dried
  Analysis: Dissolve the *Sample* in 100 mL of neutralized acetone, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 8.609 mg of methacrylic acid ($C_4H_6O_2$) units.
  Acceptance criteria: 46.0%–50.6% for Methacrylic Acid and Ethyl Acrylate Copolymer on the dried basis

IMPURITIES

Inorganic Impurities

- **Residue on Ignition ⟨281⟩:** NMT 0.4%
- **Heavy Metals,** *Method II* **⟨231⟩:** NMT 20 ppm

Organic Impurities

- **Procedure: Limit of Methacrylic Acid and Ethyl Acrylate**
  Sodium perchlorate solution: 35 mg/mL of sodium perchlorate. This solution has a concentration of 0.25 M.
  Mobile phase: Add phosphoric acid dropwise to water to obtain a solution having a pH of 2.0. Prepare a mixture of this acidified water and methanol (80:20) and degas.
  Standard solution: Dissolve 0.01 g of methacrylic acid and 0.01 g of ethyl acrylate in 5 mL of butanol and add methanol to exactly 100 mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume. Mix 5.0 mL of this solution with 5.0 mL of *Sodium perchlorate solution*. This solution contains about 0.5 µg/mL each of methacrylic acid and ethyl acrylate.
  Sample solution: Transfer about 3 g of Methacrylic Acid and Ethyl Acrylate Copolymer to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Add 5 mL of this solution dropwise, while continuously stirring, to a beaker that contains 5.0 mL of *Sodium perchlorate solution*. Remove the precipitated polymer to obtain a clear supernatant by centrifugation (e.g. NLT 5000 × *g* for NLT 5 min). Use the clear supernatant.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 202 nm
  Column: 4.0-mm × 12.5-cm analytical column; 7-µm packing L1
  Flow rate: 2 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  [Note—The relative retention times for methacrylic acid and ethyl acrylate are 1.0 and 2.6, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between methacrylic acid and ethyl acrylate
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each monomer (methacrylic acid or ethyl acrylate) in the portion of Methacrylic Acid and Ethyl Acrylate Copolymer taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times V_F \times D \times F \times 100$$

r_U	= monomer (methacrylic acid or ethyl acrylate) peak response from the *Sample solution*
r_S	= monomer (methacrylic acid or ethyl acrylate) peak response from the *Standard solution*
C	= concentration of the monomer (methacrylic acid or ethyl acrylate) in the *Standard solution* (µg/mL)
W	= weight of Methacrylic Acid and Ethyl Acrylate Copolymer taken to prepare the *Sample solution* (g)
V_F	= final volume of the *Sample solution*, 10 mL
D	= dilution factor for preparation of the *Sample solution*, 10
F	= conversion factor, 10^{-6} g/µg

  Acceptance criteria: NMT 0.01% for the total amount of monomers

SPECIFIC TESTS

- **Viscosity ⟨911⟩:** Place 254.6 g of isopropyl alcohol and 7.9 g of water in a test flask. Add a quantity of Methacrylic Acid and Ethyl Acrylate Copolymer, equivalent to 37.5 g of solids on the dried basis, while stirring by means of a magnetic stirrer. Close the flask, and continue stirring until the polymer has dissolved

In-Process Revision

completely. Adjust the temperature to 20 ± 0.1°. Equip a rotational viscometer with an accessory.[1] The shear rate under the test condition is NLT 1 s^{-1} and NMT 100 s^{-1}. Follow the instrument manufacturer's directions to measure the apparent viscosity.

Acceptance criteria: 100–200 mPa · s for Methacrylic Acid and Ethyl Acrylate Copolymer, with a range of 46.0%–50.6% for methacrylic acid units.

- **LOSS ON DRYING ⟨731⟩:** Dry it at 110° for 6 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate the range of methacrylic acid units. The labeling also indicates the name and quantity of any added surface-active agent.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Methacrylic Acid and Ethyl Acrylate Copolymer (1:1) RS (USP Methacrylic Acid Copolymer, Type C RS).■2S *(NF28)*

BRIEFING

Methacrylic Acid and Methyl Methacrylate Copolymer. See Briefing under *Methacrylic Acid Copolymer.* The newly proposed *NF* monograph shown here contains the procedures and limits outlined for Methacrylic Acid Copolymer Types A and B. In this monograph:

- The test for *Limit of Methacrylic Acid and Methyl Methacrylate* includes precipitation of the polymer before analysis in order to avoid clogging of the column and other separation problems. The HPLC chromatographic procedure in the test is based on analyses performed with a Nucleosil 100 C18 column with 7-µm L1 packing. With this system, methacrylic acid and methyl methacrylate elute at approximately 2.9 and 8.2 min, respectively.
- The *Analysis* in the test for *Viscosity* indicates the shear rate conditions. These are based on validation data demonstrating that the test solution behaves as a Newtonian fluid under the test conditions for a shear rate of NLT 1 s^{-1} and NMT 100 s^{-1}.

(EM2: H. Wang.) RTS—C73593

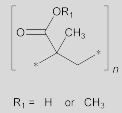
Add the following:

■Methacrylic Acid and Methyl Methacrylate Copolymer

(***Title for this new monograph—to become official December 1, 2015***)

(***Prior to December 1, 2015, the current practice of labeling the article of commerce with the name Methacrylic Acid Copolymer, Type A or Type B, whichever is appropriate, may be continued. Use of the name Methacrylic Acid and Methyl Methacrylate Copolymer will be permitted as of December 1, 2010, but the use of this name will not be mandatory until December 1, 2015. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.***)

[1] A suitable accessory is available from Brookfield Engineering as the LV1 spindle, a cylindrical spindle 1.9 cm in diameter and 6.5 cm high attached to a shaft 0.3 cm in diameter. The spindle rotates at 30 rpm at an immersion depth of 8.15 cm.



(Ratio of H to CH_3 is either 1:1 or 1:2)

Poly(methacrylic acid, methyl methacrylate);
Methacrylic acid–methyl methacrylate copolymer [25086-15-1].

DEFINITION

Methacrylic Acid and Methyl Methacrylate Copolymer consists of methacrylic acid and methyl methacrylate monomers arranged in a random distribution. Methacrylic acid units in Methacrylic Acid and Methyl Methacrylate Copolymer are NLT 27.6% and NMT 50.6%, calculated on the dried basis. It may contain suitable surface-active agents.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
  Use USP Methacrylic Acid and Methyl Methacrylate Copolymer (1:1) RS for Methacrylic Acid and Methyl Methacrylate Copolymer, with a range of 46.0%–50.6% for methacrylic acid units.
  Use USP Methacrylic Acid and Methyl Methacrylate Copolymer (1:2) RS for Methacrylic Acid and Methyl Methacrylate Copolymer, with a range of 27.6%–30.7% for methacrylic acid units.
- **B.** It meets the requirements of the *Assay*.

ASSAY

- **PROCEDURE**
  Sample: 1 g, previously dried
  Analysis: Dissolve the *Sample* in 100 mL of neutralized acetone, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 8.609 mg of methacrylic acid ($C_4H_6O_2$) units.
  Acceptance criteria
  46.0%–50.6% for Methacrylic Acid and Methyl Methacrylate Copolymer (1:1) on the dried basis
  27.6%–30.7% for Methacrylic Acid and Methyl Methacrylate Copolymer (1:2) on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 20 ppm

Organic Impurities

- **LIMIT OF METHACRYLIC ACID AND METHYL METHACRYLATE**
  Phosphate buffer: Prepare an aqueous solution containing 17.8 g/L of anhydrous dibasic sodium phosphate and 17.0 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0. This buffer has a concentration of 0.125 M.
  Mobile phase: Add phosphoric acid dropwise to water to obtain a solution having a pH of 2.0. Prepare a mixture of this acidified water and methanol (80:20), and degas.
  Standard solution: Dissolve 0.05 g of methacrylic acid and 0.05 g of methyl methacrylate in 5 mL of butanol, and add methanol to exactly 100 mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume. Mix 3.0 mL of this solution with 10.0 mL of *Phosphate buffer*. This solution contains 1.15 µg/mL each of methacrylic acid and methyl methacrylate.
  Sample solution: Transfer 1 g of Methacrylic Acid and Methyl Methacrylate Copolymer to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Add 3 mL of this solution dropwise, while continuously stirring, to a beaker that contains 10.0 mL of *Phosphate buffer*. Remove the precipitated polymer to obtain a clear supernatant by centrifugation (e.g., NLT 5000 × *g* for NLT 5 min). Use the clear supernatant.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 202 nm
Column: 4.0-mm × 12.5-cm analytical column; 7-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methacrylic acid and methyl methacrylate are 1.0 and 2.8, respectively.]
Suitability requirements
Resolution: NLT 2.0 between methacrylic acid and methyl methacrylate
Relative standard deviation: NMT 2.0%.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each monomer (methacrylic acid or methyl methacrylate) in the portion of Methacrylic Acid and Methyl Methacrylate Copolymer taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times V_F \times D \times F \times 100$$

r_U = monomer (methacrylic acid or methyl methacrylate) peak response from the *Sample solution*
r_S = monomer (methacrylic acid or methyl methacrylate) peak response from the *Standard solution*
C = concentration of the monomer (methacrylic acid or methyl methacrylate) in the *Standard solution* (µg/mL)
W = weight of Methacrylic Acid and Methyl Methacrylate Copolymer taken to prepare the *Sample solution* (g)
V_F = final volume of the *Sample solution*, 13 mL
D = dilution factor for preparation of the *Sample solution*, 16.7
F = conversion factor, 10^{-6} g/µg

Acceptance criteria: NMT 0.05% for the total amount of monomers

SPECIFIC TESTS

- **VISCOSITY ⟨911⟩:** Place 254.6 g of isopropyl alcohol and 7.9 g of water in a test flask. Add a quantity of Methacrylic Acid and Methyl Methacrylate Copolymer, equivalent to 37.5 g of solids on the dried basis, while stirring by means of a magnetic stirrer. Close the flask, and continue stirring until the polymer has dissolved completely. Adjust the temperature to 20 ± 0.1°. Equip a rotational viscometer with an accessory.[1] The shear rate under the test condition is NLT 1 s^{-1} and NMT 100 s^{-1}. Follow the instrument manufacturer's directions to measure the apparent viscosity.
  Acceptance criteria
  60–120 mPa · s for Methacrylic Acid and Methyl Methacrylate Copolymer having a range of 46.0%–50.6% for methacrylic acid units
  50–200 mPa · s for Methacrylic Acid and Methyl Methacrylate Copolymer having a range of 27.6%–30.7% for methacrylic acid units
- **LOSS ON DRYING ⟨731⟩:** Dry it at 110° for 6 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate the range of methacrylic acid units. The labeling also indicates the name and quantity of any added surface-active agent.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Methacrylic Acid and Methyl Methacrylate Copolymer (1:1) RS (USP Methacrylic Acid Copolymer, Type A RS)
  USP Methacrylic Acid and Methyl Methacrylate Copolymer (1:2) RS (USP Methacrylic Acid Copolymer, Type B RS)■2S (NF28)

[1] A suitable accessory is available from Brookfield Engineering as the LV1 spindle, a cylindrical spindle 1.9 cm in diameter and 6.5 cm high attached to a shaft 0.3 cm in diameter. The spindle rotates at 30 rpm at an immersion depth of 8.15 cm.

BRIEFING

Nitrogen, *NF 27* page 1288—See briefing under *Helium*.

(AER: K. Zaidi.) RTS—C73267

Nitrogen

N_2 28.01
Nitrogen;
Nitrogen [7727-37-9].

DEFINITION

Nitrogen contains NLT 99.0%, by volume, of N_2.

IDENTIFICATION

Change to read:

- ~~The flame of a burning wood splinter is extinguished when inserted into a test tube filled with Nitrogen~~

[~~NOTE—Exercise caution.~~]■The retention time of the nitrogen peak from the *Sample* and from 1.0% Oxygen in nitrogen certified standard differ by NMT 10%.■2S (NF28)

ASSAY

Change to read:

- **PROCEDURE**

Sample: ~~Nitrogen~~■Sample under test■2S (NF28)

Standard: ~~Oxygen helium~~■1.0% Oxygen in nitrogen■2S (NF28) certified standard
[NOTE—See *Reagents, Indicators, and Solutions*.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability* ■and *Medical Gases Assay* ⟨415⟩.)■2S (NF28)
Mode: GC
Detector: Thermal conductivity
Column: ~~3-m in length × 4-mm in inside diameter: molecular sieve prepared from a synthetic alkali metal aluminosilicate capable of absorbing molecules having diameters of up to 0.5 nm and completely separating oxygen from nitrogen~~■2.1-mm × 2.4-m, packed with 80/100 mesh S3 packing■2S (NF28)
Carrier gas: Helium (99.99%)
Flow rate: 30 mL/min
Column temperature: Thermostatically controlled ■at 70°■2S (NF28)
~~**Peak signal of standard:** NLT 70% of the full-scale reading~~
■**System suitability**
Samples: *Standard* and *Sample*
System suitability requirements
Resolution: NLT 2.0 between nitrogen and oxygen, *Standard*
Retention time: The oxygen peak response from the *Sample* exhibits a retention time corresponding to that from the *Standard*.■2S (NF28)

Analysis
Samples: *Standard* and *Sample*
Introduce the *Samples* separately into the gas chromatograph by means of a gas sampling valve.
Acceptance criteria: ~~The peak response produced by the *Sample* exhibits a retention time corresponding to that produced by the *Standard* and is equivalent to~~■■2S (NF28) NMT 1.0% of oxygen when compared to the peak response of the *Standard*, indicating NLT 99.0%, by volume, of N_2

In-Process Revision

IMPURITIES

Inorganic Impurities

(See *Impurities Testing in Medical Gases* ⟨413⟩.)

- **CARBON MONOXIDE**

■[NOTE—The detector tube called for in this test is listed under *Reagents, Indicators, and Solutions*.]■2S (*NF28*)

Sample: ~~1000 ± 50 mL~~■Manufacturer's recommended gas volume (±5%)■2S (*NF28*)

Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube.

Acceptance criteria: The indicator change corresponds to NMT ~~0.001%~~■10 ppm.■2S (*NF28*)

- **LIMIT OF OXYGEN:** NMT 1.0% of oxygen is present, determined as directed in the *Assay*.

SPECIFIC TESTS

Change to read:

- **ODOR:** Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.

[NOTE—~~Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.~~■Not applicable to liquid samples■2S (*NF28*)]

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** ~~Preserve in cylinders~~■Preserve Nitrogen in pressurized containers. Container connections shall be appropriate for Nitrogen. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.■2S (*NF28*)

Add the following:

■• **LABELING** Where Nitrogen is piped directly from the container or storage tank to the point of use, label each outlet "Nitrogen".■2S (*NF28*)

BRIEFING

Nitrogen 97 Percent, *NF 27* page 1288—See briefing under *Helium*.

(AER: K. Zaidi.) RTS—C61146

Nitrogen 97 Percent

DEFINITION

Change to read:

Nitrogen 97 Percent is Nitrogen produced from air by physical separation methods. It contains NLT 97.0%, by volume, of N_2.

[~~NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.The various detector tubes called for in the respective tests are listed under *Reagents, Indicators, and Solutions*~~]■■2S (*NF28*)

IDENTIFICATION

Change to read:

- ~~The flame of a burning wood splinter is extinguished when inserted into a test tube filled with Nitrogen 97 Percent.~~

[~~NOTE—Exercise caution.~~]■The retention time of the nitrogen peak from the *Sample* under test and from 3.0% Oxygen in nitrogen certified standard differ by NMT 10%.■2S (*NF28*)

ASSAY

Change to read:

- **PROCEDURE**

Sample: ~~Nitrogen 97 Percent~~■*Sample* under test■2S (*NF28*)

Standard: ~~Oxygen–helium~~■3.0% Oxygen in nitrogen■2S (*NF28*) certified standard

[NOTE—See *Reagents, Indicators, and Solutions*.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability* ■and *Medical Gases Assay* ⟨415⟩.)■2S (*NF28*)

Mode: GC

Detector: Thermal conductivity

Column: ~~3-m in length × 4-mm in inside diameter: molecular sieve prepared from a synthetic alkali metal aluminosilicate capable of absorbing molecules having diameters of up to 0.5 nm and completely separating oxygen from nitrogen~~■2.1-mm × 2.4-m, packed with 80/100 mesh S3 packing■2S (*NF28*)

Carrier gas: Helium (99.99%)

Column temperature: Thermostatically controlled ■at 70°■2S (*NF28*)

~~**Peak signal of standard:** NLT 70% of the full-scale reading~~

■**System suitability**

Samples: *Standard* and *Sample*

Suitability requirements

Resolution: NLT 2.0 between nitrogen and oxygen

Retention time: The oxygen peak response from the *Sample gas* exhibits a retention time corresponding to that from the *Standard gas*.■2S (*NF28*)

Analysis

Samples: ■*Standard* and *Sample*■2S (*NF28*)

Introduce separately into the gas chromatograph by means of a gas sampling valve.

Acceptance criteria: ~~The peak response produced by the *Sample* exhibits a retention time corresponding to that produced by the *Standard* and is equivalent to~~■■2S (*NF28*) NMT 3.0% of oxygen when compared to the peak response of the *Standard*, indicating NLT 97.0%, by volume, of N_2

IMPURITIES

Change to read:

Inorganic Impurities

■(See *Impurities Testing in Medical Gases* ⟨413⟩.)

[NOTE—The various detector tubes called for in the respective tests are listed under *Reagents, Indicators, and Solutions*.]■2S (*NF28*)

In-Process Revision

- **CARBON DIOXIDE**

  Sample: ~~1000 ± 50 mL~~■Manufacturer's recommended gas volume (±5%)■2S (*NF28*)

  Analysis: Pass through a carbon dioxide detector tube at the rate specified for the tube.

  Acceptance criteria: The indicator change corresponds to NMT 300 ppm.

- **CARBON MONOXIDE**

  Sample: ~~1000 ± 50 mL~~■Manufacturer's recommended gas volume (±5%)■2S (*NF28*)

  Analysis: Pass through a carbon monoxide detector tube at the rate specified for the tube.

  Acceptance criteria: The indicator change corresponds to NMT 10 ppm.

- **SULFUR DIOXIDE**

  Sample: ~~1000 ± 50 mL~~■Manufacturer's recommended gas volume (±5%)■2S (*NF28*)

  Analysis: Pass through a sulfur dioxide detector tube at the rate specified for the tube.

  Acceptance criteria: The indicator change corresponds to NMT 5 ppm.

- **LIMIT OF NITRIC OXIDE AND NITROGEN DIOXIDE**

  Sample: ~~1000 ± 50 mL~~■Manufacturer's recommended gas volume (±5%)■2S (*NF28*)

  Analysis: Pass through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube.

  Acceptance criteria: The indicator change corresponds to NMT 2.5 ppm.

- **LIMIT OF OXYGEN:** NMT 3.0% of oxygen is present, determined as directed in the *Assay*.

SPECIFIC TESTS

- **ODOR:** Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** ~~Preserve in cylinders or in a low-pressure collecting tank.~~■Preserve nitrogen in pressurized containers. Container connections shall be appropriate for Nitrogen 97 Percent. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.■2S (*NF28*)
- **LABELING:** Where it is piped directly from the collecting tank to the point of use, label each outlet "Nitrogen 97 Percent".

In-Process Revision

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

〈**11**〉 **USP Reference Standards,** *USP 32* page 35 and page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 1161 of *PF* 32(4) [July–Aug. 2006], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], page 144 of *PF* 35(1) [Jan.–Feb. 2009], page 330 of *PF* 35(2) [Mar–Apr. 2009], and page 612 of *PF* 35(3) [May–June 2009].

(HDQ) RTS—C44494; C47817; C51559; C56006; C65791; C70442; C71283; C73290; C73593; C73680; C73681

Add the following:

▪**USP Powdered Ashwagandha Extract RS.** ■2S (*USP33*)

Add the following:

▪**USP Balsalazide Disodium RS**. ■2S (*USP33*)

Add the following:

▪**USP Balsalazide Related Compound A RS [**(*E*)-5-[(*p*-carboxyphenyl)azo]-2-salicylic acid, disodium salt**]** ($C_{14}H_8N_2O_5Na_2$ ✧330.12).■2S (*USP33*)

Add the following:

▪**USP Balsalazide Related Compound B RS [**(*E*)-5-({*m*-[(2-c arboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid**]** ($C_{17}H_{15}N_3O_6$ ✧357.17).■2S (*USP33*)

Add the following:

▪**USP Benzethonium Chloride RS**. ■2S (*USP33*)

Add the following:

▲**USP Carmellose RS**. ▲*USP33*

Add the following:

▪**USP Ensulizole RS**. ■2S (*USP33*)

Add the following:

▪**USP Hydromorphone Related Compound A RS [**morphinone hydrochloride**].**■2S (*USP33*)

Add the following:

▪**USP Lactobionic Acid RS**. ■2S (*NF28*)

Add the following:

▪**USP Methacrylic Acid and Ethyl Acrylate Copolymer (1 : 1) RS** (USP Methacrylic Acid Copolymer, Type C RS). ■2S (*NF28*)

Add the following:

▪**USP Methacrylic Acid and Methyl Methacrylate Copolymer (1 : 1) RS** (USP Methacrylic Acid Copolymer, Type A RS). ■2S (*NF28*)

Add the following:

▪**USP Methacrylic Acid and Methyl Methacrylate Copolymer (1 : 2) RS** (USP Methacrylic Acid Copolymer, Type B RS). ■2S (*NF28*)

Add the following:

▪**USP Methylbenzethonium Chloride RS**. ■2S (*USP33*)

Change to read:

USP Phenylbenzimidazole Sulfonic Acid RS

▪—(NAME CHANGE) See *USP Ensulizole RS*. ■2S (*USP33*)

In-Process Revision

Add the following:

▪USP Risperidone Related Compounds Mixture RS—Contains a 98.9 : 0.5 : 0.30 : 0.3 (area %) mixture of four compounds.

98.9% of *Risperidone*

0.5% of *Risperidone cis-N-oxide:* **[***cis*-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one]

0.3% of *Bicyclorisperidone:* **[**3-(4-fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-*a*]pyrimidin-3-yl)ethyl]-1-aza-2-azoniabicyclo[2.2.2]oct-2-ene iodide**]**

0.3% of *Z-oxime:* **[**(*Z*)-3-[2-[4-(2,4-difluorophenyl)(hydroxyimino)methyl]-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one**]**.▪2S (*USP33*)

Add the following:

▪USP Withanolide A RS. ▪2S (*USP33*)

Add the following:

▪USP Withanoside IV RS. ▪2S (*USP33*)

Add the following:

▪USP Zolpidem Impurities Mixture RS—Contains at least 98.5% of zolpidem tartrate; 0.2% of zolpidem tartrate related compound B (*N,N,*6-trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-(2-oxoacetamide)); and 0.2% of zolpidem tartrate related compound C (5-methyl-2-(4-methylbenzamido)pyridine).▪2S (*USP33*)

BRIEFING

⟨92⟩ Growth Factors and Cytokines Used in Cell Therapy Manufacturing. Because there is no information in *USP* on this subject, it is proposed to include this new general test chapter to provide general considerations and requirements for growth factors and cytokines used in cell manufacturing.

(BB CGT: F. Atouf.) RTS—C72116

Add the following:

■⟨92⟩ GROWTH FACTORS AND CYTOKINES USED IN CELL THERAPY MANUFACTURING

DEFINITION

Qualification of reagents, source materials, and control of the manufacturing process are key elements that ensure the quality and safety of cell therapies. Growth factors and cytokines are important for the maintenance, growth, selection, and purification of cultures of cell therapy products. This chapter describes the accepted tests, procedures, and acceptance criteria for growth factors and cytokines that may be involved in the manufacturing of cell therapy products.

RECOMBINANT HUMAN INTERLEUKIN 4 (rhIL-4)

MHKCDITLQE	IIKTLNSLTE	QKTLCTELTV	TDIFAASKNT
TEKETFCRAA	TVLRQFYSHH	EKDTRCLGAT	AQQFHRHKQL
IRFLKRLDRN	LWGLAGLNSC	PVKEANQSTL	ENFLERLKTI
MREKYSKCSS			

$C_{658}H_{1071}N_{193}O_{197}S_8$ 15,096 Da

rhIL-4 is a single-chain polypeptide of 130 amino acid residues expressed in *Escherichia coli*. It is produced as a lyophilized powder and contains NLT 0.5×10^7 USP Units of IL-4/mg of total protein. Process specific host-cell DNA impurities in IL-4 with limits of less than 1 ng/mg are determined as described in *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)* ⟨1130⟩. Neither manufacturing license nor market approval is required for IL-4 intended for use as an ancillary material during manufacturing. Following are typical IL-4 quality attributes.

IDENTIFICATION

- **A.** Amino-terminal sequence analysis of at least eight amino acids is performed with an automated sequencer, as described in *Biotechnology-Derived Articles* ⟨1045⟩. Stepwise-released phenylthiohydantoin amino acids are identified with on-line reversed-phase high-performance liquid chromatography, on the basis of their elution times.
- **B.** Use the electrophoresis method followed by western blotting analysis to visualize the IL-4 protein. The method is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), described in the tests for *Purity*.

Phosphate buffered saline: Proceed as directed in the test for *Purity* in the *Assay*.
Laemmli sample buffer, reducing: Proceed as directed in the test for *Purity* in the *Assay*.
Laemmli sample buffer, nonreducing: Proceed as directed in the test for *Purity* in the *Assay*.
Standard stock solution: 50 µg/mL of reconstituted USP rHuman Interleukin 4 RS in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]
Standard solution: 20 µg/mL of IL-4, from *Standard stock solution*, in *Phosphate buffered saline*
Standard solution, reducing: Combine 20 µL of *Standard solution* and 5 µL of *Laemmli sample buffer, reducing*.
Standard solution, nonreducing: Combine 20 µL of *Standard solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Sample stock solution: 50 µg/mL of reconstituted IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]
Sample solution: 20 µg/mL of IL-4, from *Sample stock solution*, in *Phosphate buffered saline*
Sample solution, reducing: Combine 20 µL of *Sample solution* and 5 µL of *Laemmli sample buffer, reducing*.
Sample solution, nonreducing: Combine 20 µL of *Sample solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Analysis
Samples: *Standard solution, reducing; Standard solution, nonreducing; Sample solution, reducing;* and *Sample solution, nonreducing*
Western blotting: After electrophoresis, the proteins are transferred onto a polyvinylidene fluoride (PVDF) membrane using standard procedures. Incubate the membrane for 1 h at room temperature with *Phosphate buffered saline* containing 0.1% Tween 20 and 5% skim milk powder. The membrane is then incubated with an anti-IL-4 antibody[1] (diluted appropriately in *Phosphate buffered saline*), followed by incubation with a secondary antibody at room temperature under gentle agitation for 1 h for each of the antibodies. The IL-4 protein band is identified by developing the membrane using a suitable detection system.[2]
Acceptance criteria: The developed Western blot should give a positive signal equivalent to the USP rHuman Interleukin 4 RS.

ASSAY

- **PURITY:** [NOTE—Purity is determined on the bulk material.] SDS-PAGE is performed as described under *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩ under reducing and nonreducing conditions.

Molecular weight marker: Use a suitable molecular weight marker containing protein bands between 10 and 200 kDa.
Phosphate buffered saline: 2.67 mM of potassium chloride, 1.47 mM of potassium phosphate (KH_2PO_4), 137.93 mM of sodium chloride, and 8.06 mM of dibasic sodium phosphate in water. Adjust to a pH of 7.0 to 7.3.
Laemmli sample buffer, nonreducing: 100 mM TRIS-HCl, pH 6.8, 50% glycerol, 0.25% bromophenol blue indicator, and 10% sodium lauryl sulfate in water
Laemmli sample buffer, reducing: Add 2.5 µL mercaptoethanol to 50 µL of *Laemmli sample buffer, nonreducing*.
Sample stock solution: 400 µg/mL of bulk IL-4 in *Phosphate buffered saline*
Sample solution 1: Combine 20 µL of *Sample stock solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Sample solution 2: Combine 20 µL of *Sample stock solution* and 5 µL of *Laemmli sample buffer, reducing*.
Control A stock solution: 4 µg/mL of IL-4, from *Sample stock solution*, in *Phosphate buffered saline*. [NOTE—*Control A solutions* are run in triplicates in both reducing and nonreducing conditions.]
Control A solution 1: Combine 20 µL of *Control A stock solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Control A solution 2: Combine 20 µL of *Control A stock solution* and 5 µL of *Laemmli sample buffer, reducing*.
Control B stock solution: 12 µg/mL of IL-4, from *Sample stock solution*, in *Phosphate buffered saline*. [NOTE—*Control B solutions* are run in duplicates in both reducing and nonreducing conditions.]
Control B solution 1: Combine 20 µL of *Control B stock solution* and 5 µL of *Laemmli sample buffer, nonreducing*.

[1] A suitable anti-IL-4 antibody can be obtained from commercial sources (e.g., Dianova Inc.).
[2] A suitable detection system can be obtained from commercial sources (e.g., Pierce/Perbio Science).

In-Process Revision

Control B solution 2: Combine 20 µL of *Control B stock solution* and 5 µL of *Laemmli sample buffer, reducing*.

Electrophoretic conditions

(See *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.)

Mode: Discontinuous PAGE gel

Stacking gel: 4% acrylamide

Resolving gel: 12% acrylamide

Run conditions: 10 min at 100 V; then 30 min at 200 V

Protein detection: Silver stain

Analysis

Samples: *Sample solution 1, Sample solution 2, Control A solution 1, Control A solution 2, Control B solution 1*, and *Control B solution 2*

Incubate 25 µL of each *Sample solution* and *Control solution* under nonreducing conditions for 5 min at 60°, and load onto the gel. Incubate 20 µL of each *Sample solution* and *Control solution* under reducing conditions for 5 min at 60°, and load onto the gel. After silver staining and scanning the whole gel, determine the intensity of all detectable protein bands by densitometry, and calculate the percentage of each detectable protein band, in the *Sample solution*, twice by comparing the pixel intensity of each contaminating band with the mean value of *Control solutions A* and *B*, respectively, by the formulas:

$$(A_{100}) \times 1/(A_1) \text{ and}$$

$$(A_{100}) \times 3/(A_3)$$

A_{100} = intensity of one contaminating band of the *Sample solution*

A_1 = mean intensity of all detectable bands of *Control A solution*

A_3 = mean intensity of all detectable bands of *Control B solution*

IL-4 control solutions analysis should yield one detectable band with an apparent molecular weight of approximately 15 kDa. If values calculated by means of *Control A solution* are different from those revealed by comparison with *Control B solution*, the value corresponding to the highest amount of impurity should be taken. If the intensity of one of the contaminating bands is lower than the value of *Control A solution* (corresponding to 1%), the value of this contamination is set to 1%. The purity of the sample solution is then calculated:

$$\text{Result} = 100 - \Sigma\, C_n$$

C = percentage of each contamination given in rounded whole numbers

n = number of contaminants of the IL-4 *Sample solution*

Acceptance criteria: The purity of IL-4 is NLT 97%, as determined by SDS-PAGE.

• **PROTEIN CONTENT:** [NOTE—Protein content is determined on the basis of the packaged product.]

Sample solution: 50 µg/mL of IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]

Blank: *Phosphate buffered saline* as described in the test for *Purity* in the *Assay*.

Spectroscopic conditions

(See *Spectrometry and Light Scattering* ⟨851⟩.)

Mode: UV

Pathlength: 1 cm

Analytical wavelength: 280 nm

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein concentration:

$$C = A_{280}/0.63$$

C = IL-4 concentration of the *Sample solution* (mg/mL)

A_{280} = absorbance at 280 nm

SPECIFIC TESTS

• **BIOIDENTITY:** [NOTE—The biological activity measurement is determined on the basis of the packaged product.]

RPMI 1640 medium with L-glutamine: Prepare a mixture of the ingredients in the quantities shown in sufficient water to obtain 1 L of medium, and sterilize by filtration:

Material	Quantity
Calcium Nitrate ($Ca(NO_3)\cdot 4H_2O$)	100 mg
Magnesium Sulfate ($MgSO_4\cdot 7H_2O$)	100 mg
Potassium Chloride	400 mg
Sodium Chloride	6000 mg
Sodium Phosphate, Dibasic Anhydrous	800 mg
Sodium Bicarbonate	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic Acid	20 mg
L-Polyvinylidene Fluoride L-Cystine Dihydrochloride	20 mg
L-Glutamic Acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg
L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine Hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine Disodium Salt Dihydrate	20 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline Chloride	3 mg
D-Calcium Pantothenate	0.25 mg
Folic Acid	1 mg
i-Inositol	35 mg
Niacinamide	1 mg
Para-Aminobenzoic Acid	1 mg
Pyridoxine Hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine Hydrochloride	1 mg
Vitamin B_{12}	0.005 mg
D-Glucose (Dextrose)	2000 mg
Glutathione (Reduced)	1 mg
Phenol Red	5 mg

In-Process Revision

Growth medium: Using aseptic procedures, prepare the following tissue culture medium:

RPMI-1640 with L-Glutamine	500 mL
Sodium Pyruvate 100 mM	5 mL
Fetal Bovine Serum	50 mL
Human rGM-CSF[3]	3×10^4 International Units

[3]Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is added extemporaneously.

Sterilize by filtration, and store at between 2° to 8°. Use within one month. Add GM-CSF immediately prior to use.

Assay medium: Use *Growth medium* containing no GM-CSF.

Phosphate buffered saline: Proceed as directed in the test for *Purity* in the *Assay*.

Resazurin solution: 0.11 mg of resazurin in *Phosphate buffered saline*. [NOTE—Sterile filter and store solution protected from light at 4°. *Resazurin solution* is stable for at least six months if treated under sterile conditions.]

[NOTE—For all *Standard* and *Sample solutions*, IL-4 concentration is determined by photometry at 280 nm using an extinction coefficient (ε) of 0.63 $mg^{-1}cm^{-1}$.]

Standard stock solution: 50 µg/mL of USP rHuman Interleukin 4 RS in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]

Standard solutions: 36, 12, 4, 1.33, 0.44, 0.15, 0.05, 0.016, 0.006 ng/mL of IL-4, from *Standard stock solution* in *Assay medium*

Sample stock solution: 50 µg/mL of IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]

Sample solutions: 36, 12, 4, 1.33, 0.44, 0.15, 0.05, 0.016, 0.006 ng/mL of IL-4, from *Sample stock solution* in *Assay medium*

Control solution: Use the *Assay medium*.

Cell culture preparation: Prepare cell cultures of the human factor-dependent TF-1 cell line (ATCC No. CRL-2003), following the protocol described in the ATCC information sheet. Passage the cultures every 2–3 days, using 1:3 subcultures of the cells for up to 1 month. Seed density should be 0.5×10^6 cells/mL, and maximal density should be 3×10^6 cells/mL. Viability of the cells should be >90%. Maximal passage number is 24, and maximal cultivation time from thawing is 28 days. After 28 days, initiate a new culture. Cells are propagated using *Growth medium* at 37°, supplemented with air and 5% carbon dioxide.

Analysis

Samples: *Standard solutions, Sample solutions,* and *Control solution*

The activity of the *Sample solution* is determined in duplicate. Wash the cells three times in *Phosphate buffered saline*. Plate 2×10^4 TF1 cells resuspended in 100 µL of *Assay medium* per well in 96-well, flat-bottom microplates. Incubate for 72 h at 37° and 5% CO_2 atmosphere in a humidified incubator in the presence or absence of various concentrations of *Standard solution, Sample solution*, or *Control solution* by adding 100 µL of the corresponding solution to each well. Add 30 µL of *Resazurin solution* to each well and incubate for another 24 h. Determine the fluorescence intensity per well by reading the plate with a microplate reader using 544 nm (excitation) and 590 nm (emission). Convert the fluorescence intensity in each well to a percentage of the maximum fluorescence intensity. For the *Sample solution* and *Standard solution*, plot the percentage of fluorescence intensity versus the concentration of the respective solution. By using the least squares method of regression analysis, compute the ED50 in ng/mL of the *Sample solution* and the *Standard solution*. The coefficient of determination for curve regression should be ≥ 0.98. Calculate the potency in USP Interleukin 4 Units/mg:

$$\text{Result} = A \times E_S/E_U$$

A = activity of USP rHuman Interleukin 4 RS (USP units/mg)

E_S = determined ED_{50} of *Standard solution* (ng/mL)

E_U = determined ED_{50} of *Sample solution* (ng/mL)

Acceptance criteria: NLT 0.5×10^7 USP IL-4 Units/mg is found.

- **STERILITY TESTS ⟨71⟩:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 50 USP Endotoxin Units/mg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at –80°.
- **LABELING:** Material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Endotoxin RS
  USP rHuman Interleukin 4 RS■2S *(USP33)*

BRIEFING

⟨228⟩ Ethylene Oxide and Dioxane. This proposed new general test chapter is applicable to products prepared from ethylene oxide. Currently two test procedures are included in this chapter. *Method I* is used, among others, in a series of official and proposed monographs for polyoxyl-containing substances; *Method II* is used in the *Polyethylene Glycol* monograph proposed for harmonization and in the harmonized *Polysorbate 80* monograph. With the addition of this chapter, the detailed procedures for tests of ethylene oxide and dioxane, as well as cross-references to them, will be removed from the monographs where they occur and will be replaced by a cross-reference to this chapter.

(EGC; EM2: H. Wang.) RTS—C69591

Add the following:

■⟨228⟩ Ethylene Oxide and Dioxane

The following procedure is used to determine the contents of residual ethylene oxide and dioxane in the products prepared from ethylene oxide.

Method I

[**CAUTION**—*Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate at a temperature between 4° and 8°.*]

[NOTE—Before using the polyethylene glycol 200 in this test, remove any volatile components from it by placing 500 mL of polyethylene glycol 200 in a 1000-mL round-bottom flask and attaching the flask to a rotary evaporator maintained at 60° and under a vacuum of 10–20 mm Hg for 6 h.]

Acetaldehyde solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]

Ethylene oxide stock solution: 2.5 mg/g of ethylene oxide. Prepare as follows: Tare a glass-stoppered conical flask, add 50 mL of polyethylene glycol 200, and reweigh the flask. Transfer 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in a mixture of sodium chloride and ice (1:3). Transfer 300 µL (corresponding to 250 mg) of liquid ethylene oxide to the polyethylene glycol 200, and swirl gently to mix. Replace the stopper, reweigh the flask, and determine the amount of ethylene oxide absorbed by weight difference. Adjust the weight of the mixture with polyethylene glycol 200 to 100.0 g, replace the stopper, and swirl gently to mix.

[NOTE—Fill a chilled pressure bottle with liquid ethylene oxide, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Use adequately chilled apparatus where appropriate. Prepare this stock solution immediately prior to use, and store in a refrigerator after preparation.]

Ethylene oxide solution: Tare a glass-stoppered conical flask, and chill it in a refrigerator. Add 35 mL of polyethylene glycol 200, and reweigh the flask. Transfer 1 g of chilled *Ethylene oxide stock solution* to the tared conical flask. Adjust the weight of the solution with polyethylene glycol 200 to 50.0 g, replace the stopper, and swirl gently to mix. Transfer 10 g of this solution to a 50-mL volumetric flask. Add 30 mL of water, and mix. Dilute with water to volume, and mix to obtain a solution containing 10 µg/mL of ethylene oxide. [NOTE—Use adequately chilled apparatus where appropriate. Prepare immediately before use.]

Dioxane solution: 500 µg/mL of dioxane

Standard solution A: Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial. [NOTE—Other sizes such as 22-mL may be used, depending on operating conditions; however, the same size must be used for *Standard solution A*, *Standard solution B*, and the *Sample solution*.] Add 0.1 mL of *Acetaldehyde solution* and 0.1 mL of *Dioxane solution*, seal the vial, and mix.

Standard solution B: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, add 0.1 mL of *Ethylene oxide solution*, 0.1 mL of *Dioxane solution*, and 1.0 mL of *N*,*N*-dimethylacetamide. Seal the vial, and mix.

Sample solution: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, and add 1.0 mL of *N*,*N*-dimethylacetamide and 0.2 mL of water. Seal the vial, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Headspace GC

Detector: Flame ionization

Column: 0.32-mm × 30-m glass or quartz capillary; 1.0-µm layer of phase G1

Temperature

Injection port: 150°

Detector: 250°

Column: See the column temperature table below.

Temperature (°)	Rate (°/min)	Hold Time (min)
50	—	5
50→180	5	—
180→230	30	—
230	—	5

Carrier gas: Helium

Linear velocity: 20 cm/s

Injection volume: 1 mL (the gaseous headspace)

Injection type: Split ratio 20:1

Headspace sampler

Temperature equilibration time: 45 min

Equilibration temperature

70° for *Standard solution A*

90° for *Standard solution B*

90° for *Sample solution*

Transfer line temperature: 150°

Pressurization time: 1 min

Injection time: 12 s

System suitability

Sample: *Standard solution A*

[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.94 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between acetaldehyde and ethylene oxide

Signal-to-noise ratio: NLT 5, determined from the dioxane peak

Relative standard deviation: NMT 15%

Analysis

Samples: *Standard solution B* and *Sample solution*

[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 2.5, respectively.]

Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_E \times r_U / [(r_S \times W_U) - (r_U \times W_S)]$$

A_E = quantity of ethylene oxide added to *Standard solution B* (µg)

r_U = ethylene oxide peak responses from the *Sample solution*

r_S = ethylene oxide peak responses from *Standard solution B*

W_U = weight of test substance taken to prepare the *Sample solution* (g)

W_S = weight of test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_D \times r_U / [(r_S \times W_U) - (r_U \times W_S)]$$

A_D = quantity of dioxane added to *Standard solution B* (µg)

r_U = dioxane peak responses from the *Sample solution*

r_S = dioxane peak responses from *Standard solution B*

W_U = weight of the test substance taken to prepare the *Sample solution* (g)

W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Method II

Ethylene oxide standard solution: Dilute 0.5 mL of ethylene oxide in methylene chloride (50 mg/mL)[1] with water to 50.0 mL. [NOTE—The solution is stable for 3 months if stored in vials with polytetrafluoroethylene (polytef)-coated silicon membrane crimped caps at –20°.] Allow to reach room temperature. Dilute 1.0 mL with water to 250.0 mL to obtain a solution having a concentration of 2 µg/mL of ethylene oxide. [NOTE—Use this solution immediately after preparation.]

Dioxane standard solution: 0.05 µL/mL of dioxane

Acetaldehyde standard solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]

Resolution solution: Add 2.0 mL of *Acetaldehyde standard solution* and 2.0 mL of *Ethylene oxide standard solution* to a 10-mL headspace vial. Seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Standard solution A: 0.48 µg/mL of ethylene oxide, from *Ethylene oxide standard solution*, and 0.005 µL/mL of dioxane, from *Dioxane standard solution*, in water

Standard solution B: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of *Standard solution A*, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Sample solution: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of water, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Headspace GC

Detector: Flame ionization

Column: 0.53-mm × 50-m fused-silica capillary column; 5.0-µm layer of phase G27

[1] This is a commercially available solution.

In-Process Revision

Temperature
Injection port: 85°
Detector: 250°
Column: See the column temperature table below.

Temperature (°)	Rate (°/min)	Hold Time (min)
70→250	10	—
250	—	5

Carrier gas: Helium
Flow rate: 4 mL/min
Injection volume: 1 mL (gaseous headspace)
Injection type: Split ratio 3.5 : 1
Headspace sampler
Temperature equilibration time: 30 min
Equilibration temperature: 80°
System suitability
Sample: *Resolution solution*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between acetaldehyde and ethylene oxide
Analysis
Samples: *Standard solution B* and *Sample solution*
[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 1.9, respectively.]
Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_E \times V \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_E = concentration of ethylene oxide in the *Standard solution A* (µg/mL)
V = volume of *Standard solution A* added to *Standard solution B*, 2.0 mL
r_U = ethylene oxide peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of test substance taken to prepare the *Sample solution* (g)
W_S = weight of test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_D \times V \times \rho \times F \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_D = concentration of dioxane in *Standard solution A* (µL/mL)
V = volume of *Standard solution A* added to *Standard solution B*, 2.0 mL
ρ = density of dioxane, 1.03 g/mL = 1.03 mg/µL
F = conversion factor (1000 µg/mg)
r_U = dioxane peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of test substance taken to prepare the *Sample solution* (g)
W_S = weight of test substance taken to prepare the *Standard solution B* (g)■2S *(USP33)*

In-Process Revision

BRIEFING

Amphetamine Assay, *USP 32* page 141. It is proposed to delete this general test chapter from *USP* as it is no longer referenced in any *USP* monograph.

(MD-PP: R. Ravichandran.) RTS—C74660

Delete the following:

■⟨~~331~~⟩ ~~AMPHETAMINE ASSAY~~

~~**USP Reference Standards** ⟨11⟩ —*USP Dextroamphetamine Sulfate RS.*~~

~~**Standard Preparation**— Dissolve a suitable quantity of USP Dextroamphetamine Sulfate RS, accurately weighed, in 2 N sulfuric acid (saturated with chloroform), and dilute quantitatively with the same solvent to obtain a solution having a known concentration of about 0.5 mg of dextroamphetamine sulfate per mL.~~

~~**Assay Preparation**— Prepare as directed in the individual monograph.~~

~~**Preparation of Chromatographic Column** (see *Chromatography* ⟨621⟩)— Pack a pledget of fine glass wool in the base of a 25 × 300 mm chromatographic tube. Place 2 g of purified siliceous earth in a 100 mL beaker, add 1 mL of 0.1 N hydrochloric acid, and mix until a fluffy mixture is obtained. Transfer the mixture to the column, and tamp moderately to compress the material into a uniform mass. Transfer the *Assay Preparation* to the column, dry rinse the beaker with 1 g of purified siliceous earth, and transfer to the column. Tamp a pledget of fine glass wool into place at the top of the column.~~

~~**Procedure**— Wash the column with 100 mL of chloroform previously saturated with water, and discard the washings. Place under the column, as a receiver, a 125 mL separator containing 10.0 mL of 2 N sulfuric acid previously saturated with chloroform. Pass through the column 35 mL of ammoniacal chloroform, prepared by equilibrating 2 mL of ammonium hydroxide and 100 mL of chloroform, and complete the elution with 70 mL of chloroform previously saturated with water. Remove the separator, shake vigorously for 1 minute, allow the layers to separate, discard the chloroform layer, and use the 10.0 mL acid solution of the sulfate salt of the amphetamine as the *Assay Solution*. Concomitantly determine the absorbance of the solution from the *Standard Preparation* and that of the *Assay Solution* in 1 cm cells at 280 nm and at the wavelength of maximum absorbance at about 257 nm, with a suitable spectrophotometer, using 2 N sulfuric acid previously saturated with chloroform as the blank. Record the absorbance of the solution from the *Standard Preparation* as A_u and that of the *Assay Solution* as A_s, and calculate as directed in the individual monograph.~~ ■2S (*USP33*)

BRIEFING

⟨**413**⟩ **Impurities Testing in Medical Gases.** This new general test chapter is being developed to support the medical gases monographs. The chapter outlines the gas sampling procedure from high-pressure containers specifically for determining impurities using various detector tubes.

(AER: K. Zaidi) RTS—C64174

Add the following:

■⟨413⟩ IMPURITIES TESTING IN MEDICAL GASES

INTRODUCTION

This general test chapter defines the safe and proper means to sample high-pressure gas containers of different medical gas compositions using the manufacturer's suggested total gas volume for the purpose of conducting detector tube analysis to satisfy the *USP* monographs. See *Reagents* in the section *Reagents, Indicators, and Solutions* for information on each referenced detector tube.

There are two types of detector tubes currently manufactured, those to be used with a manual hand pump of fixed volume (e.g., 100 mL/pump stroke), and those used in a continuous flow system that can be set to pass a volume of gas through the detector tube at approximately one (1) atmosphere. It is important to match the appropriate detector tube type to the mode of gas volume exchange.

To ensure that the required gas volume has passed through the detector tube, measure the gas volume at the time of the analysis either by using a hand pump or using a flowmeter that is calibrated to the subject gas or corrected via a calibration chart. Flowmeter manufacturers generally provide a chart of the gas volume flow of

common gases for each of the flow tubes identified in this general chapter. [NOTE—See general chapter *Medical Gases Assay* ⟨415⟩ for sampling.]

Continous Flow System

Identify the gas contained in the container and select the appropriate gas regulator. Secure the regulator to the gas container. Do not apply lubricant or Teflon tape to the container-to-regulator connections. Purge the regulator with the gas under test. Select float setting to achieve total gas volume as recommended by the manufacturer. Attach the detector tube, then adjust the flow rate to the required level as indicated by the charts that accompany the flowmeter and the detector tube. Time the gas flow to achieve the desired total gas volume ±10 s. At the allotted time close the regulator valve, then the main container valve. Observe the tube while it is still attached to determine the degree of color change and record the result. Remove the tube, and disconnect the apparatus, vent the regulator gas pressure to atmosphere, and remove the regulator from the container. Dispose of the tube after use.

Hand Pump—Fixed Volume

The alternative approach is to apply the gas detector hand pump. The system draws a consistent volume with each pump stroke. To ensure the accuracy of the total gas volume, the user must follow the manufacturer's suggested recommendation about how to pump.■2S (*USP33*)

BRIEFING

⟨**415**⟩ **Medical Gases Assay.** This new general test chapter is being developed to support the medical gases monographs. The chapter includes information on sampling and qualification for conducting medical gases assay testing using gas chromatography and paramagnetic analyzers. It also covers validation and calibration of these instruments.

(AER: K. Zaidi) RTS—C64173

Add the following:

■⟨415⟩ MEDICAL GASES ASSAY

INTRODUCTION

The evaluation of the purity of a gas used for medical treatment or as a component of a pharmaceutical process is the purpose of a *USP* medical gas monograph. The purity generally is evaluated by an assay for the content of the article and by analyses for trace impurities. The application of gas chromatography, paramagnetic analysis, and detector tubes to medical gases is somewhat different from traditional procedures used for analytes in the liquid phase and therefore warrants a separate description. This general test chapter focuses on the assay for content tests. Sampling for impurities is addressed in general chapter *Impurities Testing in Medical Gases* ⟨413⟩.

This chapter includes sampling and qualification aspects of gas chromatographic and paramagnetic analyses of medical gases. In addition, it includes a description of the initial set-up, validation, and calibration of these instruments. The specific assay procedures are defined in the specific monograph for that gas.

The basic definitions of instrumental qualification and validation are included in general information chapters *Analytical Instrument Qualification* ⟨1058⟩ and *Validation of Compendial Methods* ⟨1225⟩, respectively, and will not be repeated. However, when variations of the materials presented in these chapters are necessary due to the character of the analyte, this chapter will define those variations.

METHODS

Gas Chromatography (GC)

See *Chromatography* ⟨621⟩.

Detectors for Medical Gases Assay

The two most common detectors used in the analyses of medical gases are the Thermal Conductivity Detector (TCD) and Flame Ionization Detector (FID).

The TCD will detect any gas or vapor that has a thermal conductivity (TC) that differs significantly from the high TC of the reference gas, usually helium, therefore it is virtually universal. However, the generally accepted lower detection limit for the TCD is 50 ppm v/v. This represents a limitation for the evaluation of trace impurities in medical gases.

The FID is also used for the evaluation of trace impurities in medical gases, because it is more sensitive to organic compounds but does not produce a signal for most common medical gases.

QUALIFICATION

Installation Qualification (IQ)—The IQ requirements ensure the gas chromatograph hardware and software (or readout device) is installed safely and in accordance with the manufacturer's instructions.

Consideration should be given to the following as applicable:

- Suitability of the sample system (including connections);
- Leakage (should be leak free);
- Representative sampling;
- Sample flow rates;
- Response time;
- Correct output signals;
- Power supply (including voltage regulation); and
- Appropriate environmental conditions of the instrument and of the sample itself (e.g., temperature and pressure).

Operational Qualification (OQ)—OQ verifies that the GC performs as intended within its anticipated operating range. For medical gas final product testing, the GC is tested to ensure repeatability (verification that relative standard deviation is consistent with claims) for each analyte of interest. Due to the specific nature of medical gas testing and the limited number of analytes, routine calibration and challenge testing of a GC instrument and the testing procedure may be used in place of initial or periodic OQ. When an instrument is used for a broader range of analytes, the replacement of OQ with calibration and challenge testing is inappropriate.

Performance Qualification (PQ)— For medical gas final product testing, the GC is periodically checked at appropriate intervals during analytical runs with a calibration gas (i.e., verifying that the results are consistent with a named concentration within acceptable accuracy and precision ranges after a specific number of sample injections).

Paramagnetic Oxygen Measurement

Theory—The paramagnetic analyzer measures the displacement of a diamagnetic gas (nitrogen) by a paramagnetic gas (oxygen), in a strong magnetic field. A measuring cell typically employs a glass dumbbell with nitrogen-filled spheres that is suspended on a torsion strip between magnets that concentrate the flux around the dumbbell. When oxygen molecules enter the measuring cell, the dumbbell is deflected by the force exerted by the oxygen molecules that are attracted to the strongest part of the magnetic field. By using optical sensors, a feedback coil, and suitable electronics, analysts measure an output that is directly proportional to the partial pressure of oxygen.

Oxygen is the only paramagnetic gas present above trace levels in the atmosphere. However, paramagnetic analyzers can be affected by the magnetic susceptibility of the background gas. Therefore changes to background gases in *USP* monographs should be avoided.

Design Considerations

The design considerations for the purchase of new instruments may include the following parameters.

Drift—A change of the output of the instrument for a given concentration over a stated period of time under constant conditions and without any adjustments being made to the instrument by external means. Drift is the summation of two components, zero drift and span drift. Drift determines the frequency of instrument calibration.

Zero Drift—A change in the output when zero gas being measured.

Span Drift—Change in the output at the level of oxygen concentration that is being measured.

Operating Temperature—The ambient temperature range for which the stated performance specification of the instrument will remain valid. A larger temperature coefficient will indicate that a smaller change in ambient temperature is permitted before recalibration is required.

Operating Pressure—The instrument should operate at the inlet pressures of the samples to be tested.

Qualification Aspects

Installation Qualification (IQ)—The IQ requirements ensure the oxygen analyzer hardware and software (or readout device) is installed safely and in accordance with the manufacturer's instructions.

Consideration should be given to the following as applicable:

- Suitability of the sample system (including connections);
- Leakage (should be leak free);
- Representative sampling;
- Sample flow rates;
- Response time;
- Correct output signals;
- Power supply (including voltage regulation); and
- Appropriate environmental conditions of the instrument and of the sample itself (e.g., temperature and pressure).

Operational Qualification (OQ)—The OQ requirements verify that the paramagnetic analyzer performs as intended within its anticipated operating range and is suitable for the actual conditions of use. Instruments and apparatus should be calibrated and used in accordance with the manufacturer's instructions. Because of the specific nature of the instrument, routine calibration may be used in place of initial or periodic OQ testing.

Performance Qualification (PQ)—The PQ requirements verify that the paramagnetic analyzer performs as intended in its normal operating environment. For medical gas final product testing, the paramagnetic analyzer is initially calibrated (zeroed and spanned using a certified standard) in accordance with the manufacturer's instructions and is periodically recalibrated to ensure continued acceptable performance.

Zeroing the Instrument (establishing the lower limit)—Using the certified standard defined in the monograph, establish a zero setting on the analyzer by passing the zero gas into the analyzer at the manufacturer's suggested flow rate. Maintain the flow until a stable reading is observed on the instrument. As necessary, adjust the zero setting to a value of 0.0% according to manufacturer's instructions. Confirm the reading is stable. [NOTE—Depending on the intended use of the instru-

ment, zeroing to a setting other than 0.0% is an acceptable alternative to this procedure if it provides greater measurement precision.]

Spanning the Range of Use—Establish the upper limit (span) with a span gas defined in the monograph and appropriate for the range of use. Pass the span gas through the instrument at the manufacturer's suggested flow rate. Confirm the reading is stable. Adjust the span setting to the certified value of the reference standard according to the manufacturer's instructions. Confirm the reading is stable.

VALIDATION

Validation of this instrument is generally completed during the (IQ/OQ) process. Routine verification is performed as described in the OQ/PQ sections of this chapter and therefore specific information on instrument validation is unnecessary.

PROCEDURE

For Off-line Instrument—Before analysis, the instrument is calibrated by zeroing and spanning as described in the PQ section. [NOTE—The calibration need not be run concomitantly with the test samples.] Connect the sample gas to the instrument, and establish a constant flow into the analyzer at the manufacturer's suggested flow rate. Maintain the flow until a constant reading is observed on the instrument. The definition of a constant reading is included in the manufacturer's instructions or in the user's instrument qualification documentation.

For On-line Instrument—The calibration intervals are defined by the manufacturer, by past history, or by statistical means. Establish a constant flow into the analyzer at the manufacturer's suggested flow rate.

SAMPLING

Sampling from Liquid Phase—Cylinders containing a dip tube allow a liquid sample to be obtained from the valve outlet with the cylinder in the upright position. If a dip tube is not present, the cylinder should be placed in an inverted position with the cylinder and main valve safely supported (so the liquid phase is in contact with the valve).

Sampling of medical gases should always be conducted using the required regulator. Regulators should be purged with the gas that will be sampled. When necessary, the flow to the analyzer should be measured using a calibrated flow-measuring device.

Sampling from Gaseous Phase—Cylinders that do not contain a dip tube allow a gaseous sample to be obtained from the valve outlet with the cylinder in the upright position. If a dip tube is present, the cylinder should be in an inverted position with the cylinder and main valve safely supported (so the gaseous phase is in contact with the end of the dip tube). Sampling of medical gases should always be conducted using the required regulator.

CERTIFIED STANDARDS FOR MEDICAL GAS ANALYSIS

USP monographs for medical gases require tests that use certified standards for instrument calibration and analytical determinations. Such compendial testing may be conducted using reference materials that are traceable to the U.S. National Institute of Standards and Technology.■2S (*USP33*)

Physical Tests and Determinations

BRIEFING

〈**741**〉 **Melting Range or Temperature,** *USP 32* page 294. On the basis of comments received, it is proposed to incorporate specific procedures for Class Ia, Apparatus II and Class Ib, Apparatus II. Also, because of the eventual inclusion of two new USP Melting Point Reference Standards, the number "six" corresponding to the current number of available Reference Standards has been removed.

(GC: A. Hernandez-Cardoso.) RTS—C75258

Change to read:

For Pharmacopeial purposes, the melting range, melting temperature, or melting point is defined as those points of temperature within which, or the point at which, the first detectable liquid phase is detected to the temperature at which no solid phase is apparent, except as defined otherwise for *Classes II* and *III* below. A melting transition may be instantaneous for a highly pure material, but usually a range is observed from the beginning to the end of the process. Factors influencing this transition include the sample size, the particle size, the efficiency of heat diffusion, and the heating rate, among other variables, that are controlled by procedure instructions. In some articles, the melting process is accompanied by simultaneous decomposition, which is visually evidenced as a side event like darkening of the material, charring, bubbling, or other incident. The visual impact of this side reaction frequently obscures the end of the melting process, which it may be impossible to accurately determine. In those circumstances, only the beginning of the melting can be accurately established; and it is to be reported as the melting temperature. The accuracy of the apparatus to be used as described below should be checked at suitable intervals by the use of one or more of the ~~six~~

■available■2S *(USP33)*
USP Melting Point Reference Standards, preferably those that melt nearest the melting temperatures of the compounds being tested (see *USP Reference Standards* 〈11〉).

Five procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for *Class Ia* for crystalline or amorphous substances and the procedure for *Class II* for waxy substances.

The procedure known as the mixed-melting point determination, whereby the melting range or temperature of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, e.g., the corresponding USP Reference Standard, if available, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture constitutes reliable evidence of chemical identity.

Change to read:

Procedure for Class I, Apparatus II—Prepare the substance under test and charge the capillary tube as directed for *Class I, Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 30° below the expected melting point. Insert the capillary tube into the heating block, and continue heating at a rate of temperature increase of about 1° to 2° per minute until melting is complete.

The temperature at which the detector signal first leaves its initial value indicates the beginning of melting, and the temperature at which the detector signal reaches its final value corresponds to the end of melting, or the melting point. The two temperatures fall within the limits of the melting range. ~~In the event of dispute, only the melting range or temperature obtained as directed for *Class I, Apparatus I*, is definitive.~~

■■2S *(USP33)*
If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting is within the range specified. In the event of dispute, only the melting range or temperature obtained as directed for *Class I, Apparatus I,* is definitive.

Add the following:

■**Procedure for Class Ia, Apparatus II—**Prepare the test substance and charge the capillary as directed for *Class I*, *Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 10° below the expected melting point and is rising at a rate of 1 ± 0.5° per minute. Insert the capillary as directed under *Class I*, *Apparatus I* when the temperature is about 5° below the lower limit of the expected melting range, and continue heating until melting is complete. Record the melting range as directed for *Class I*, *Apparatus I*. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting is within the range specified. In the event of dispute, only the melting range or temperature obtained as directed for *Class Ia*, *Apparatus I*, is definitive■2S *(USP33)*

Add the following:

■**Procedure for Class Ib, Apparatus II—**Place the test substance in a closed container and cool to 10°, or lower, for at least 2 hours. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I*, *Apparatus I*, then immediately place the charged tube in a vacuum desiccator and dry at a pressure not exceeding 20 mm of mercury for 3 hours. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable pro-

ceed with the determination of the melting range as follows: operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 10 ± 1° below the expected melting range, then introduce the charged tube, and heat at a rate of rise of 3 ± 0.5° per minute until melting is complete. Record the melting range as directed for *Class I*, *Apparatus I*.

If the particle size of the material is too large for the capillary, pre-cool the test substance as above directed, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube. In the event of dispute, only the melting range or temperature obtained as directed for *Class Ib*, *Apparatus I*, is definitive.■2S (*USP33*)

BRIEFING

⟨795⟩ Pharmaceutical Compounding—Nonsterile Preparations, *USP 32* page 31 4; **⟨1075⟩ Good Compounding Practices,** *USP 32* page 523. As part of USP's efforts to modernize and improve monographs and general chapters, the Compounding Pharmacy Expert Committee proposes to delete the contents in the existing general test chapter ⟨795⟩ and replace the content with completely new text. It is also proposed to delete general information chapter ⟨1075⟩.

(CRX: R. Schnatz) RTS—C71288

Change to read:

⟨795⟩ PHARMACEUTICAL COMPOUNDING—NONSTERILE PREPARATIONS

~~For the purposes of this chapter, the pharmacist or other licensed health care professional responsible for preparing the compounded preparations is referred to as "compounder".~~

~~Compounding is an integral part of pharmacy practice and is essential to the provision of health care. The purpose of this chapter and applicable monographs on formulation is to help define what constitutes good compounding practices and to provide general information to enhance the compounder's ability in the compounding facility to extemporaneously compound preparations that are of acceptable strength, quality, and purity.~~

~~Compounding is different from manufacturing, which is guided by GMPs (see *Good Manufacturing Practices for Bulk Pharmaceutical Excipients* ⟨1078⟩). Some of the characteristics or criteria that differentiate compounding from manufacturing include the existence of specific practitioner–patient–compounder relationships; the quantity of medication prepared in anticipation of receiving a prescription or a prescription order; and the conditions of sale, which are limited to specific prescription orders.~~

~~The pharmacist's responsibilities in compounding drug preparations are to dispense the finished preparation in accordance with a prescription or a prescriber's order or intent and to dispense those preparations in compliance with the requirements established by the Boards of Pharmacy and other regulatory agencies. Compounders must be familiar with statutes and regulations that govern compounding because these requirements vary from state to state.~~

~~The compounder is responsible for compounding preparations of acceptable strength, quality, and purity with appropriate packaging and labeling in accordance with good compounding practices (see *Good Compounding Practices* ⟨1075⟩), official standards, and relevant scientific data and information. Compounders engaging in compounding should have to continually expand their compounding knowledge by participating in seminars, studying appropriate literature, and consulting colleagues.~~

~~RESPONSIBILITY OF THE COMPOUNDER~~

~~The compounder is responsible for ensuring that the quality is built into the compounded preparations of products, with key factors including at least the following general principles. (See also *Good Compounding Practices* ⟨1075⟩.)~~

1. ~~Personnel are capable and qualified to perform their assigned duties.~~
2. ~~Ingredients used in compounding have their expected identity, quality, and purity.~~
3. ~~Compounded preparations are of acceptable strength, quality, and purity, with appropriate packaging and labeling, and prepared in accordance with good compounding practices, official standards, and relevant scientific data and information.~~
4. ~~Critical processes are validated to ensure that procedures, when used, will consistently result in the expected qualities in the finished preparation.~~
5. ~~The compounding environment is suitable for its intended purpose.~~
6. ~~Appropriate stability evaluation is performed or determined from the literature for establishing reliable beyond-use dating to ensure that the finished preparations have their expected potency, purity, quality, and characteristics, at least until the labeled beyond-use date.~~
7. ~~There is assurance that processes are always carried out as intended or specified and are under control.~~
8. ~~Compounding conditions and procedures are adequate for preventing errors.~~
9. ~~Adequate procedures and records exist for investigating and correcting failures or problems in compounding, testing, or in the preparation itself.~~

~~COMPOUNDING ENVIRONMENT~~

~~Facilities~~

~~Areas designated for compounding have adequate space for the orderly placement of equipment and materials to prevent mixups between ingredients, containers, labels, in-process materials, and finished preparations. The compounding area is also to be designed, arranged, used, and maintained to prevent adventitious cross-contamination. Areas used for sterile preparations are to be separated and distinct from the nonsterile compounding area (see *Environmental Quality and Control* under *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩). The entire compounding area is to be well lighted. Heating,~~

ventilation, and air conditioning systems are to be controlled to avoid decomposition of chemicals (see *Storage Temperature* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements* and the manufacturers' labeled storage conditions). Storage areas provide an environment suitably controlled to ensure quality and stability of bulk chemicals and finished preparations.

Potable water is to be supplied for hand and equipment washing. This water meets the standards prescribed in the EPA's National Primary Drinking Water Regulations (40 CFR Part 141). *Purified Water* must be used for compounding nonsterile drug preparations when formulations indicate the inclusion of water. *Purified Water* must also be used for rinsing equipment and utensils. In those cases when a water is used to prepare a sterile preparation, *Water for Injection, Sterile Water for Injection,* or *Bacteriostatic Water for Injection* must be used (see *Water for Pharmaceutical Purposes* ⟨1231⟩ and *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Compounding areas are to be maintained in a clean and sanitary condition. Adequate washing facilities are to be provided, including hot and cold water, soap or detergent, and air driers or single service towels. Sewage, trash, and other refuse in the compounding area is to be disposed of in a safe, sanitary, and timely manner. Equipment is to be thoroughly cleaned promptly after use to avoid cross contamination of ingredients and preparations. Special precautions are to be taken to clean equipment and compounding areas meticulously after compounding preparations that contain allergenic ingredients (e.g., sulfonamides or penicillins).

Equipment

Equipment is to be of appropriate design and size for compounding and suitable for the intended uses. The types and sizes of equipment will depend on the dosage forms and the quantities compounded (see *Weights and Balances* ⟨41⟩, *Prescription Balances and Volumetric Apparatus* ⟨1176⟩, and equipment manufacturers' instruction manuals). All equipment is to be constructed so that surfaces that contact pharmaceutical components, in process materials, or finished preparations are not reactive, additive, or adsorptive to avoid altering the safety, identity, strength, quality, or purity of the preparation. The use of micropipets, electronic or analytical balances, or triturations or dilutions shall be considered when needed quantities are too small to accurately measure with standard equipment required by a state Board of Pharmacy. Equipment and accessories used in compounding are to be inspected, maintained, cleaned, and validated at appropriate intervals to ensure the accuracy and reliability of their performance.

STABILITY OF COMPOUNDED PREPARATIONS

"Stability" is defined as the extent to which a preparation retains, within specified limits, and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of compounding. See the table *Criteria for Acceptable Levels of Stability* under *Stability Considerations in Dispensing Practice* ⟨1191⟩.

The compounder must avoid formulation ingredients and processing conditions that would result in a potentially toxic or ineffective preparation. The compounder's knowledge of the chemical reactions by which drugs degrade provides a means for establishing conditions under which the rate of degradation is minimized. The factors that influence the stability of compounded preparations are generally the same as those for manufactured drug products (see *Factors Affecting Product Stability* and *Responsibility of the Pharmacist* under *Stability Considerations in Dispensing Practice* ⟨1191⟩).

Primary Packaging

Compounded preparations should be packaged in containers meeting USP standards (see *Containers* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements, Containers—Plastics* ⟨661⟩, and *Containers—Performance Testing* ⟨671⟩). The container used depends on the physical and chemical properties of the compounded preparation. Container–drug interaction is to be considered with substances such as phenolic compounds and sorptive materials (e.g., polypeptides and proteins).

Sterility

Assurance of sterility in a compounded sterile preparation is mandatory. Compounding and packaging of sterile drugs, such as ophthalmic solutions, will require strict adherence to guidelines presented in the general test chapter *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ and in the manufacturers' labeling instructions.

Stability Criteria and Beyond-Use Dating

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

Compounders are to consult and apply drug-specific and general stability documentation and literature when available, and are to consider the nature of the drug and its degradation mechanism, the container in which it is packaged, the expected storage conditions, and the intended duration of therapy when assigning a beyond-use date (see *Expiration Date and Beyond-Use Date* under *Labeling* in the *General Notices and Requirements*). Beyond-use dates are to be assigned conservatively. When using manufactured solid dosage forms to prepare a solution or aqueous suspension, the compounder is also to consider factors such as hydrolysis and the freeze-thaw property of the final preparation before assigning a beyond-use date. In assigning a beyond-use date for a compounded drug preparation, in addition to using all available stability information, the compounder is also to use his or her pharmaceutical education and experience.

When a manufactured product is used as the source of active ingredient for a nonsterile compounded preparation, the product expiration date cannot be used to extrapolate directly a beyond-use date for the compounded preparation. However, a compounder may refer to the literature or to the manufacturer for stability information. The compounder may also refer to applicable publications to obtain stability, compatibility, and degradation information on ingredients. All stability data must be carefully interpreted in relation to the actual compounded formulation.

At all steps in the compounding, dispensing, and storage process, the compounder is to observe the compounded drug preparation for signs of instability. For more specific details of some of the common physical signs of deterioration, see *Observing Products for Evidence of Instability* under *Stability Considerations in Dispensing Practice* ⟨1191⟩. However, excessive chemical degradation and other drug concentration loss due to reactions may be invisible more often than they are visible.

In the absence of stability information that is applicable to a specific drug and preparation, the following maximum beyond-use dates are recommended for nonsterile compounded drug preparations[1] that are packaged in tight, light-resistant

[1] For guidelines applicable to dating sterile compounded preparations, see Storage and Beyond-Use Dating under Pharmaceutical Compounding—Sterile Preparations 797.

containers and stored at controlled room temperature unless otherwise indicated (see *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements*).

For Nonaqueous Liquids and **Solid Formulations**—

Where the Manufactured Drug Product is the Source of Active Ingredient—The beyond-use date is not later than 25% of the time remaining until the product's expiration date or 6 months, whichever is earlier.

Where a USP or NF Substance is the Source of Active Ingredient—The beyond-use date is not later than 6 months.

For Water-Containing Formulations (prepared from ingredients in solid form)—The beyond-use date is not later than 14 days for liquid preparations when stored at cold temperatures between 2° and 8° (36° and 46° F).

For All Other Formulations—The beyond-use date is not later than the intended duration of therapy or 30 days, whichever is earlier. These beyond-use date limits may be exceeded when there is supporting valid scientific stability information that is directly applicable to the specific preparation (i.e., the same drug concentration range, pH, excipients, vehicle, water content, etc.). See also the beyond-use dating information in the *Labeling* section under *Repackaging Into Single-Unit Containers and Unit-Dose Containers for Nonsterile Solid and Liquid Dosage Forms* ⟨681⟩.

Beyond-Use Labeling

Federal law requires that manufactured drug products be labeled with an expiration date. Some state laws may require a beyond-use date. The label on the container or package of an official compounded preparation must bear a beyond-use date. Good compounding practice dictates beyond-use labeling for all compounded preparations.

DEFINITIONS

For purposes of this chapter, the following terms shall have these meanings.

PREPARATION is a drug dosage form, a dietary supplement, or a finished device. It is the finished or partially finished preparation of one or more substances formulated for use on or for the patient or consumer (see *General Notices and Requirements*).

OFFICIAL SUBSTANCE includes an active drug entity, a dietary supplement, or a pharmaceutic ingredient (see also *NF 23*) or a component of a finished device.

ACTIVE INGREDIENT usually refers to chemicals, substances, or other components of articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of diseases in humans or other animals or for use as dietary supplements.

ADDED SUBSTANCES are ingredients that are necessary to prepare the preparation but are not intended or expected to cause a human pharmacologic response if administered alone in the amount or concentration contained in a single dose of the compounded preparation. The term *added substances* is usually used synonymously with the terms *inactive ingredients, excipients,* and *pharmaceutic ingredients.*

INGREDIENT SELECTION

Sources

Official compounded preparations are prepared from ingredients that meet requirements of the compendial monograph for those individual ingredients for which monographs are provided.

A USP or an NF grade substance is the preferred source of ingredients for compounding all other preparations. If that is not available, or when food, cosmetics, or other substances are or must be used, then the use of another high-quality source, such as analytical reagent (AR), certified American Chemical Society (ACS), or Food Chemicals Codex (FCC) grade, is an option for professional judgment. For any substance used in compounding not purchased from a registered drug manufacturer, the compounder must establish purity and safety by reasonable means, which may include lot analysis, manufacturer reputation, or reliability of source.

A manufactured drug product may be a source of active ingredient. Only manufactured drugs from containers labeled with a batch control number and a future expiration date are acceptable as a potential source of active ingredients. When compounding with manufactured drug products, the compounder must consider all ingredients present in the drug product relative to the intended use of the compounded preparation.

A compounder may not compound a drug preparation that appears on the FDA list of drug products withdrawn or removed from the market for safety reasons.

Compounding Nondrug Requirements

If the preparation is intended for use as a dietary or nutritional supplement (to supplement the diet) or cosmetic (e.g., to beautify), then the compounder must adhere to *Good Compounding Practices* ⟨1075⟩ and to this chapter, and must comply with any federal and state requirements.

CHECKLIST FOR ACCEPTABLE STRENGTH, QUALITY, AND PURITY

The following questions are to be considered carefully before compounding.

1. Have the physical and chemical properties and medicinal, dietary, and pharmaceutical uses of the drug substances been reviewed?
2. Are the quantity and quality of each active ingredient identifiable?
3. Will the active ingredients be effectively absorbed, locally or systemically according to the prescribed purpose, from the preparation and route of administration?
4. Are there added substances (see *Definitions*), confirmed or potentially present from manufactured products, that may be expected to cause an allergic reaction, irritation, toxicity, or undesirable organoleptic response from the patient? Are there added substances (see *Definitions*), confirmed or potentially present, that may be unfavorable (e.g., unsuitable pH or inadequate solubility)?
5. Were all calculations and measurements confirmed to ensure that the preparation will be compounded accurately (see *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩)?

COMPOUNDED PREPARATIONS

The term *compounded preparations* includes the terms *compounded dosage forms, compounded drugs,* and *compounded formulations,* and means finished forms that are prepared by or under the direct supervision of a licensed compounder.

When controlled substances are used, check with state and federal authorities concerning their policies. Unless otherwise indicated or appropriate, compounded preparations are to be prepared to ensure that each preparation shall contain not less than 90.0% and not more than 110.0% of the theoretically calculated and labeled quantity of active ingredient per unit weight or volume and not less than 90.0% and not more than 110.0% of the theoretically calculated weight or volume per unit of the preparation. Compounded preparations include, but are not restricted to, the following pharmaceutical dosage forms described under *Pharmaceutical Dosage Forms* ⟨1151⟩.

Chromatographic Reagents

Capsules, Powders, Lozenges, and Tablets

When compounding these dosage forms, the compounder is to prepare an amount of the total formulation sufficient to allow the prescribed amount or quantity to be accurately dispensed. Selected practices and precautions for compounding these dosage forms include the following:

- reducing solid ingredients to the smallest reasonable particle size;
- implementing appropriate checks to ensure that all ingredients are blended to achieve a homogeneous mixture;
- monitoring humidity if moisture might cause hydrolysis, dosage form adhesion to containers, or softening or partial dissolution of capsule shells;
- accurately performing weighings to ensure that each unit shall be not less than 90% and not more than 110% of the theoretically calculated weight for each unit [NOTE—Preparations classified as dietary supplements are required by the U.S. Food and Drug regulations to be not less than 100% of the declared potency.]; and
- packaging dosage units according to container specifications for capsules and tablets of the specific active ingredient unless specified otherwise in individual monographs (see *Containers—Glass* ⟨660⟩ and *Containers—Plastic* ⟨661⟩).

Emulsions, Solutions, and Suspensions

When compounding these dosage forms, the compounder is to prepare a 2% to 3% excess amount of the total formulation to allow the prescribed amount to be accurately dispensed. Selected practices and precautions for compounding these dosage forms include the following.

- For single-unit containers, the weight of each filled container, corrected for tare weight, shall be the equivalent of not less than 100% and not more than 110% of the labeled volume.
- Aqueous suspensions are prepared by levigating the powder mixture to a smooth paste with an appropriate wetting agent. This paste is converted to a free-flowing fluid by adding adequate vehicle. Successive portions of the vehicle are used to wash the mortar, or other vessel, to transfer the suspension quantitatively to a calibrated dispensing bottle or graduate. The preparation may be homogenized to ensure a uniform final dispersion.
- Reducing solid ingredients to the smallest reasonable particle size.
- Solutions shall contain no visible undissolved matter when dispensed. [NOTE—An exception may occur with supersaturated solutions such as *Potassium Iodide Oral Solution*.]
- Emulsions and suspensions are labeled, "Shake well before using."

Suppositories

When compounding suppositories, the compounder is to prepare an excess amount of total formulation to allow the prescribed quantity to be accurately dispensed. Selected practices and precautions for compounding these dosage forms include the following:

- not using ingredients that are caustic or irritating, and thoroughly comminute solids that are abrasive to the mucous membranes;
- selecting a base that allows active ingredients to provide the intended local or systemic therapeutic effect;
- reducing solid ingredients to the smallest reasonable particle size; and
- weighing a representative number of suppositories to ensure that each is not less than 90% and not more than 110% of the average weight of all suppositories in the batch.

Creams, Topical Gels, Ointments, and Pastes

When compounding semisolid dosage forms, the compounder is to prepare an excess amount of total formulation to allow the prescribed quantity to be accurately dispensed. Selected practices and precautions for compounding these dosage forms include the following:

- not using ingredients that are caustic, irritating, or allergenic to the skin or other application sites unless they are necessary for a treatment;
- selecting a base or vehicle that allows active ingredients to provide the intended local or systemic therapeutic effect;
- reducing solid ingredients to the smallest reasonable particle size;
- geometrically incorporating the active ingredients with the added substances to achieve a uniform liquid or solid dispersion in the dosage form; and
- observing the uniformity of the dispersion by spreading a thin film of finished formulation on a flat transparent surface (e.g., clear glass ointment slab).

COMPOUNDING PROCESS

The compounders are to consider using the following steps to minimize error and maximize the prescriber's intent.

1. Judge the suitability of the prescription to be compounded in terms of its safety and intended use. Determine what legal limitations, if any, are applicable.
2. Perform necessary calculations to establish the amounts of ingredients needed (see *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩).
3. Identify equipment needed.
4. Don the proper attire and wash hands.
5. Clean the compounding area and needed equipment.
6. Only one prescription should be compounded at one time in a specified compounding area.
7. Assemble all necessary materials to compound the prescription.
8. Compound the preparation following the formulation record or prescription (see *Compounding Records and Documents* below), according to the art and science of pharmacy.
9. Assess weight variation, adequacy of mixing, clarity, odor, color, consistency, and pH as appropriate.
10. Annotate the compounding log, and describe the appearance of the formulation.
11. Label the prescription containers to include the following items: a) the name of the preparation; b) the internal identification number; c) the beyond-use date (see *Beyond-Use Labeling*); d) the initials of the compounder who prepared the label; e) any storage requirements; and f) any other statements required by law.
12. Sign and date the prescription affirming that all procedures were carried out to ensure uniformity, identity, strength, quantity, and purity.
13. Thoroughly and promptly clean all equipment, and store properly.

COMPOUNDING RECORDS AND DOCUMENTS

All compounders who dispense prescriptions must comply with the record-keeping requirements of their individual states. If the compounder compounds a preparation according to the manufacturer's labeling instructions, then further documentation is not required. All other compounded preparations require further documentation. Such compounding documents are to list the ingredients and the quantity of each in the order of the compounding process.

The objective of the documentation is to allow another compounder to reproduce the identical prescription at a future date. The formulation record provides a consistent source document for preparing the preparation (recipe), and the compounding record documents the actual ingredients in the preparation and the person responsible for the compounding

activity. These records are to be retained for the same period of time that is required for any prescription under state law. The record may be a copy of the prescription in written or machine readable form that includes a formulation record, a compounding record, and a Material Safety Data Sheets (MSDS) file.

Formulation Record

The formulation record is a file of individually compounded preparations. This record must list the name, strength, and dosage form of the preparation compounded, all ingredients and their quantities, equipment needed to prepare the preparation, when appropriate, and mixing instructions. Mixing instructions should include the order of mixing, mixing temperatures or other environmental controls, such as the duration of mixing, and other factors pertinent to the replication of the preparation as compounded. The formulation record must include an assigned beyond use date, the container used in dispensing, the storage requirements, and any quality control procedures.

Compounding Record

The compounding record contains documentation of the name and strength of the compounded preparation, the formulation record reference for the preparation, and the sources and lot numbers of ingredients. The compounding record also includes information on the total number of dosage units compounded, the name of the person who prepared the preparation and the name of the compounder who approved the preparation, the date of preparation, the assigned internal identification number or the prescription number and an assigned beyond use date, and the prescription number. For all compounded preparations, results of quality control procedures are to be recorded (e.g., weight range of filled capsules). When compounding problems occur with preparations prepared according to USP compounding monographs, the compounder must complete a USP Monograph Experience Reporting Form, and submit the form to USP for evaluation.

MSDS File

MSDS are to be readily accessible to all employees working with drug substances or bulk chemicals located on the compounding facility premises. Employees are to be instructed on how to retrieve and interpret needed information.

QUALITY CONTROL

The safety, quality, and performance of compounded preparations depend on correct ingredients and calculations, accurate and precise measurements, appropriate formulation conditions and procedures, and prudent pharmaceutical judgment. As a final check, the compounder is to review each procedure in the compounding process. To ensure accuracy and completeness, the compounder is to observe the finished preparation to ensure that it appears as expected and is to investigate any discrepancies and take appropriate corrective action before the prescription is dispensed to the patient (see the *Checklist for Acceptable Strength, Quality, and Purity,* the appropriate pharmaceutical dosage form under *Compounded Preparations,* and the steps under *Compounding Process*).

VERIFICATION

Compounding procedures that are routinely performed, including batch compounding, shall be completed and verified according to written procedures. The act of verification of a compounding procedure involves checking to ensure that calculations, weighing and measuring, order of mixing, and compounding techniques were appropriate and accurately performed.

PATIENT COUNSELING

The patient or the patient's agent should be counseled about proper use, storage, and evidence of instability in the compounded preparation at the time of dispensing (see *Responsibility of the Pharmacist* under *Stability Considerations in Dispensing Practice* ⟨1191⟩).

▪INTRODUCTION

The purpose of this chapter is to provide compounders with guidance on applying good compounding practices for the preparation of nonsterile compounded formulations for dispensing and/or administration to humans or animals. Compounding is an integral part of pharmacy practice and is essential to the provision of health care. This chapter and applicable monographs on formulation help define what constitutes good compounding practices. Furthermore this chapter provides general information to enhance the compounder's ability in the compounding facility to extemporaneously compound preparations that are of acceptable strength, quality, and purity. It is expected that pharmacists and others engaged in the compounding of drug preparations will comply with applicable state and federal compounding laws, regulations, and guidelines.

DEFINITIONS

ACTIVE PHARMACEUTICAL INGREDIENT (API)—Any substance or mixture of substances intended to be used in the compounding of a drug preparation, thereby becoming the active ingredient in that preparation and intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or to affect the structure and function of the body.

ADDED SUBSTANCES—Ingredients that are necessary to compound a preparation but are not intended or expected to cause a pharmacologic response if administered alone in the amount or concentration contained in a single dose of the compounded preparation. The term added substances is used synonymously with the terms inactive ingredients, excipients, and pharmaceutical ingredients.

BEYOND-USE DATE (BUD)—The date after which a compounded preparation is not to be used and is determined from the date the preparation is compounded.

COMPONENT—Any ingredient used in the compounding of a drug preparation, including any active ingredient or added substance that is used in its preparation.

COMPOUNDER—A professional authorized by the appropriate jurisdiction to perform compounding pursuant to a prescription or medication order by a licensed prescriber.

COMPOUNDING—The preparation, mixing, assembling, packaging, and labeling of a drug or device in accordance with a licensed practitioner's prescription, medication order, or initiative based on the practitioner/patient/pharmacist/compounder relationship in the course of professional practice. Compounding includes the following:

- Preparation of drug dosage forms for both human and animal patients.
- Preparation of drugs or devices in anticipation of prescription drug orders based on routine, regularly observed prescribing patterns.
- Reconstitution or manipulation of commercial products that may require the addition of one or more ingredients.
- Preparation of drugs or devices for the purposes of, or as an incident to, research, teaching, or chemical analysis.
- Preparation of drugs and devices for prescriber's office use where permitted by federal and state law.

HAZARDOUS DRUG—Any drug identified by at least one of the following six criteria:

- Carcinogenicity,
- Teratogenicity or developmental toxicity,
- Reproductive toxicity in humans,
- Organ toxicity at low doses in humans or animals,
- Genotoxicity,
- New drugs that mimic existing hazardous drugs in structure or toxicity [see current National Institute for Occupational Safety and Health (NIOSH) publications].

MANUFACTURING—The production, propagation, conversion, or processing of a drug or device, either directly or indirectly, by extraction of the drug from substances of natural origin or by means of chemical or biological synthesis. Manufacturing also includes (1) any packaging or repackaging of the substance(s) or labeling or relabeling of containers for the promotion and marketing of such drugs or devices; (2) any preparation of a drug or device that is given or sold for resale by pharmacies, practitioners, or other persons; and (3) the preparation of any quantity of a drug product without a licensed prescriber/patient/licensed pharmacist/compounder relationship.

PREPARATION—For the purposes of this chapter, a compounded drug dosage form, dietary supplement, or device. This term will be used to describe compounded formulations while the term product will be used to describe manufactured pharmaceutical dosage forms. (For the definitions of official substances and official preparations, see *General Notices and Requirements*.)

STABILITY—The extent to which a preparation retains, within specified limits, and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of compounding. See the table *Criteria for Acceptable Levels of Stability* in the general information chapter *Stability Considerations in Dispensing Practice* ⟨1191⟩.

VEHICLE—A component for internal or external use that is used as a carrier or diluent in which liquids, semi-solids, or solids are dissolved or suspended. Examples include, but are not limited to, water, syrups, elixirs, oleaginous liquids, solid and semi-solid carriers, and proprietary products.

CATEGORIES OF COMPOUNDING

In the three general categories of nonsterile compounding described in this section, it is to be understood by compounders that there are different levels of experience, training, and physical facilities associated with each category.

Criteria used to determine overall classification include

- degree of difficulty of the compounding process,
- stability information and warnings,
- packaging and storage requirements,
- dosage forms,
- complexity of calculations,
- local versus systemic biological disposition,
- level of risk to the compounder, and
- potential for risk of harm to the patient.

See chapter ⟨797⟩ for risk levels associated with sterile preparations. Specialty areas such as radiopharmaceuticals require special training and are beyond the scope of this chapter. Compounders shall acquire and maintain knowledge and skills in all areas (e.g., dosage form, patient population, and medical specialty) for which they compound.

Categories

Simple—Making a preparation that has a *USP* compounding monograph or that appears in a peer-reviewed journal article that contains specific quantities of all components, compounding procedure and equipment, and stability data for that formulation with appropriate BUDs. Examples include Captopril Oral Solution, Indomethacin Topical Gel, and Potassium Bromide Oral Solution, Veterinary.

Moderate—Making a preparation, including but not limited to those with a *USP* monograph or with a peer-reviewed journal article, that requires special calculations or procedures (such as calibration of dosage unit mold cavities) to determine quantities of components per preparation or per individualized dosage units; or making a preparation for which stability data for that specific formulation is not available. Examples include Morphine Sulfate Suppositories, diphenhydramine hydrochloride troches, and mixing two or more manufactured cream products when the stability of the mixture is not known.

Complex—Making a preparation that requires special training, environment, facilities, equipment, and procedures to ensure appropriate therapeutic outcomes. Examples of possible complex preparation types include transdermal dosage forms, modified-release preparations, and some inserts and suppositories for systemic effects.

RESPONSIBILITIES OF THE COMPOUNDER

The compounder is responsible for compounding preparations of acceptable strength, quality, and purity, and in accordance with the prescription or medication order. The compounder is also responsible for dispensing the finished preparation, with appropriate packaging and labeling, and in compliance with the requirements established by the applicable State Board of Pharmacy and other regulatory agencies where appropriate. Individuals who are engaged in drug or dietary supplement compounding shall be proficient in compounding and should continually expand their compounding knowledge by participating in seminars and/or studying appropriate literature. They shall be knowledgeable of the contents of this general test chapter *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩, and should be

familiar with *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, *Pharmaceutical Dosage Forms* ⟨1151⟩, *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩, *Quality Assurance in Pharmaceutical Compounding* ⟨1163⟩, *Stability Considerations in Dispensing Practice* ⟨1191⟩, *Prescription Balances and Volumetric Apparatus* ⟨1176⟩, and all applicable compounding laws, guidelines, and standards.

To ensure the quality of compounded preparations, compounders shall adhere to the following general principles (additional information on these general principles is provided in the sections that follow.)

General Principles of Compounding

1. Personnel are appropriately trained, and are capable and qualified to perform their assigned duties.
2. Compounding ingredients of the appropriate grade and quality are purchased from reliable sources and are properly stored.
3. Bulk component containers are labeled with appropriate Occupational Safety and Health Administration (OSHA) hazard communication labels, and Material Safety Data Sheets (MSDS) are available to compounding personnel for all drugs and chemicals used in compounding.
4. All equipment used in compounding is clean, properly maintained, and used appropriately.
5. The compounding environment is suitable for its intended purpose; and procedures are implemented to prevent cross-contamination, especially when compounding with drugs (e.g., hazardous drugs and known allergens like penicillin) that require special precautions.
6. Only authorized personnel are allowed in the immediate vicinity of the drug compounding operations.
7. There is assurance that processes are always carried out as intended or specified and are under control.
8. Compounding conditions and procedures are adequate for preventing errors.
9. All aspects of compounding are appropriately documented.
10. Adequate procedures and records exist for investigating and correcting failures or problems in compounding, testing, or in the preparation itself.

COMPOUNDING PROCESS

The compounder is responsible for ensuring that each individual incidence of compounding meets the criteria given in this section (additional information on these criteria is provided in the sections that follow).

Criteria When Compounding Each Drug Preparation

1. The dose, safety, and intended use of the preparation or device has been evaluated for suitability in terms of the chemical and physical properties of the components, dosage form, and route of administration, including local and systemic biological disposition; and legal limitations, if any.
2. Ingredients used in the formulation have their expected identity, quality, and purity. If for humans or food-producing animals, the ingredients are not on the list of federally recognized drugs that have been withdrawn or removed from the market for safety reasons. Certificates of Analysis, when available, and MSDS have been consulted for all ingredients used.
3. Compounding is done in a clean area dedicated to this activity.
4. Only one preparation is compounded at one time in a specific workspace.

5. Appropriate compounding equipment has been selected and inspected for cleanliness and correct functioning, and is properly used.
6. A reliable BUD is established to ensure that the finished preparation has its expected potency, purity, quality, and characteristics, at least until the labeled BUD.
7. Personnel engaged in compounding have washed properly and wear clean clothing appropriate to the type of compounding performed, e.g., hair bonnets, coats, gowns, gloves, facemasks, shoes, aprons, or other items as needed for protection of personnel from chemical exposures and for prevention of drug contamination.
8. The preparation is made in accordance with this chapter, other official standards referenced in this chapter, and relevant scientific data and information.
9. Critical processes are verified to ensure that procedures, when used, will consistently result in the expected qualities in the finished preparation.
10. The final preparation is assessed using such factors as weight, adequacy of mixing, clarity, odor, color, consistency, and pH as appropriate, and this information is recorded on the Compounding Record.
11. The preparation is packaged as recommended in the *Packaging* section of this chapter.
12. The preparation container is labeled according to all applicable state and federal laws. The label should include the BUD and storage and handling information as well as a statement that ''this is a compounded preparation.''
13. The Master Formulation Record and the Compounding Record have been reviewed by the compounder to ensure that errors have not occurred in the compounding process.
14. The preparation is delivered to the patient or caregiver with the appropriate consultation.

COMPOUNDING FACILITIES

Compounding facilities shall have an adequate space that is specifically designated for compounding of prescriptions. This space shall provide for the orderly placement of equipment and materials to prevent mixups between ingredients, containers, labels, in-process materials, and finished preparations, and is designed, arranged, and used to prevent adventitious cross-contamination. Areas used for sterile preparations are to be separated and distinct from the nonsterile compounding area (see *Environmental Quality and Control* under *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Potable water is to be supplied for hand and equipment washing. This water meets the standards prescribed in the EPA's National Primary Drinking Water Regulations (40 CFR Part 141). Purified Water must be used for compounding nonsterile drug preparations when formulations indicate the inclusion of water. Purified Water must also be used for rinsing equipment and utensils. In those cases when a water is used to prepare a sterile preparation, follow the appropriate monographs and general chapters.

The plumbing system shall be free of defects that could contribute to contamination of any compounded preparation. Adequate hand and equipment washing facilities shall be easily accessible to the compounding areas. Such facilities shall include, but are not limited to, hot and cold water, soap or detergent, and an air-drier or single-use towels. The areas used for compounding shall be maintained in clean, orderly, and sanitary conditions, and shall be maintained in a good state of repair. Waste shall be held and disposed of in a sanitary and timely manner and in accordance with local, state, and federal guidelines.

The entire compounding and storage area is to be well-lighted. Heating, ventilation, and air conditioning systems are to be controlled to avoid decomposition and

contamination of chemicals (see *Storage Temperature and Humidity* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements* and the manufacturers' labeled storage conditions). All components, equipment, and containers shall be stored off the floor and in a manner to prevent contamination and permit inspection and cleaning of the compounding and storage area.

Hazardous drugs shall be prepared for administration only under conditions that protect the healthcare workers and other personnel in the preparation and storage areas. Hazardous drugs shall be stored separately from other inventory in a manner to prevent contamination and personnel exposure. The following are references for the safe handling of antineoplastic and hazardous drugs in health care settings:

- OSHA Technical Manual—Section VI: Chapter 2, *Controlling Occupational Exposure to Hazardous Drugs*.
- NIOSH Alert: *Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings* (DHHS (NIOSH) Publication No. 2004-165).

Disposal of all hazardous drug wastes shall comply with all applicable federal and state regulations. All personnel who perform routine custodial waste removal and cleaning activities in storage and preparation areas for hazardous drugs shall be trained in appropriate procedures to protect themselves and prevent contamination.

COMPOUNDING EQUIPMENT

The equipment and utensils used for compounding of a drug preparation shall be of appropriate design and capacity. The equipment shall be of suitable composition such that the surfaces that contact components are neither reactive, additive, nor sorptive and therefore will not affect or alter the purity of the compounded preparations. The types and sizes of equipment will depend on the dosage forms and the quantities compounded (see *Prescription Balances and Volumetric Apparatus* ⟨1176⟩, and equipment manufacturers' instruction manuals).

The equipment shall be stored in such a manner as to protect it from contamination, and shall be located in such a place as to facilitate operations for its use, maintenance, and cleaning. Automated, mechanical, electronic, and other types of equipment used in compounding or testing of compounded preparations shall be routinely inspected, calibrated as necessary, and checked to ensure proper performance.

Immediately prior to initiation of compounding operations, the equipment shall be inspected by the compounder to determine its suitability for use. After use, the equipment shall be appropriately cleaned. Extra care should be used when cleaning equipment used in compounding preparations requiring special precaution, e.g., antibiotics, cytotoxins, cancer drugs, and other hazardous materials. When possible, special equipment shall be dedicated for such use, or when the same equipment is being used for all drug products, appropriate procedures must be in place to allow meticulous cleaning of equipment prior to use with other drugs.

COMPONENT SELECTION, HANDLING, AND STORAGE

The following guidelines shall be followed when selecting, handling, and storing components for compounded preparations.

1. A *USP, NF,* or *FCC* grade substance is the preferred source of ingredients for compounding all preparations.
2. The compounder must first attempt to use components manufactured in an FDA-registered facility. When components are not obtainable from an FDA-registered facility, compounders shall use their professional judgment in selecting a source deemed acceptable and reliable and shall establish purity and

safety by reasonable means, which may include lot analysis, manufacturer reputation, or reliability of source.

3. Official compounded preparations are prepared from ingredients that meet requirements of the compendial monograph for those individual ingredients for which monographs are provided. These preparations may be labeled ''USP'' or ''NF'' as appropriate.
4. When components of compendial quality are not obtainable, components of high quality such as those that are chemically pure, analytical reagent grade or American Chemical Society-certified may be used.
5. For components in containers that have an expiration date from the manufacturer or distributor, the material may be used in compounding before that expiration date (a) when the material is stored in its original container under conditions to avoid decomposition of the chemicals (see *Storage under Nonspecific Conditions* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements* unless other conditions are noted on the label), (b) when there is minimal exposure of the remaining material each time material is withdrawn from the container, and (c) when any withdrawals from the container are by those trained in the proper handling of the material. If the component has been transferred to a different container, a new appropriate expiration date must be assigned. The new container shall be identified with the component name, the original supplier, the lot or control number, and the transfer date.
6. For components that do not have expiration dates assigned by the manufacturer or supplier, the compounder shall label the container with the date of receipt and assign a conservative expiration date, not to exceed three years after receipt, to the component based on the nature of the component and its degradation mechanism, the container in which it is packaged, and the storage conditions.
7. A manufactured drug product may be a source of active ingredient. When a manufactured drug product is used as the source of the active ingredient, the manufacturer's product container must be labeled with a batch control number and a future expiration date to be acceptable. When compounding with manufactured drug products, the compounder must consider all ingredients, including excipients, present in the drug product relative to the intended use of the compounded preparation.
8. If the preparation is intended for use as a dietary or nutritional supplement or cosmetic, then the compounder must adhere to this chapter, and must also comply with any federal and state requirements. Generally, dietary supplements are prepared from ingredients that meet *USP, FCC*, or *NF* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be acceptable food grade quality using other suitable procedures.
9. When a component is derived from ruminant animals (e.g., bovine, caprine, ovine) the supplier shall provide written assurance that these animals were certified free of bovine spongiform encephalopathy (BSE).
10. When compounding for humans or food-producing animals, the compounder shall not use components that have been withdrawn from the market for safety reasons by the FDA.
11. All components to be used in the compounding of preparations must be stored as directed by the manufacturer, or according to *USP, NF,* or *FCC* monograph requirements, in a clean, dry area, under appropriate temperature conditions (controlled room temperature, refrigerator, or freezer). All com-

ponents shall be stored off the floor, handled and stored to prevent contamination, and rotated so that the oldest stock is used first. All containers shall be properly labeled.

STABILITY CRITERIA AND BEYOND-USE DATING

The BUD is the date after which a compounded preparation is not to be used and is determined from the date the preparation is compounded. Because compounded preparations are intended for administration immediately or following short-term storage, their BUDs are assigned based on criteria different from those applied to assigning expiration dates to manufactured drug products.

Compounders are to consult and apply drug-specific and general stability documentation and literature when available, and are to consider the nature of the drug and its degradation mechanism, the container in which it is packaged, the expected storage conditions, and the intended duration of therapy when assigning a BUD (see *Expiration Date and Beyond-Use Date* under *Labeling* in the *General Notices and Requirements*). BUDs are to be assigned conservatively. When using manufactured solid dosage forms to prepare a solution or aqueous suspension, the compounder is also to consider factors such as hydrolysis, oxidation, and the freeze-thaw property of the final preparation before assigning a BUD. In assigning a BUD for a compounded drug preparation, in addition to using all available stability information, the compounder is also to use his or her pharmaceutical education and experience.

When a manufactured product is used as the source of the API for a nonsterile compounded preparation, the product expiration date cannot be used directly to assign a BUD for the compounded preparation. Instead, the compounder shall refer to the manufacturer for stability information and to the literature for applicable information on stability, compatibility, and degradation of ingredients. All stability data must be carefully interpreted in relation to the actual compounded formulation.

At all steps in the compounding, dispensing, and storage process, the compounder is to observe the compounded drug preparation for signs of instability. For more specific details of some of the common physical signs of deterioration, see *Observing Products for Evidence of Instability* under *Stability Considerations in Dispensing Practice* ⟨1191⟩. However, excessive chemical degradation and other drug concentration loss due to reactions may be invisible more often than they are visible.

General Guidelines for Assigning Beyond-Use Dates

In the absence of stability information that is applicable to a specific drug and preparation, the following maximum BUDs are recommended for nonsterile compounded drug preparations that are packaged in tight, light-resistant containers and stored at controlled room temperature unless otherwise indicated and for sterile preparations where a program of sterility testing is in place (see *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements*). Drugs or chemicals that are known to be labile to decomposition will necessitate shorter BUDs.

For Nonaqueous Formulations—The BUD is not later than 25% of the time remaining until the earliest expiration date of any API or 6 months, whichever is earlier. The BUD shall not be later than the expiration date on the container of any component.

For Water-Containing Oral and Sterile Liquid Formulations—The BUD is not later than 14 days when stored at controlled cold temperatures.

For Water-Containing External-Use Liquid and Semi-Solid Formulations—The BUD is not later than 30 days.

Water-containing preparations and other susceptible preparations should contain suitable antimicrobial agents to protect against bacteria, yeast, and mold contamination inadvertently introduced during or subsequent to the compounding process. When antimicrobial preservatives are contraindicated in such compounded preparations, storage of the preparation at controlled cold temperature is necessary; to ensure proper storage and handling of such compounded preparations by the patient or caregiver, appropriate patient instruction and consultation is essential. Antimicrobial preservatives should not be used as a substitute for good compounding practices.

For information on assigning BUDs when repackaging drug products for dispensing or administration, see *Expiration Date and Beyond-Use Date* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements*, and *Repackaging in Single-Unit Containers and Unit-Dose-Containers for Nonsterile Solid and Liquid Dosage Forms* ⟨681⟩.

Assurance of sterility in a compounded sterile preparation is mandatory. Compounding and packaging of sterile drugs (including ophthalmic preparations) requires strict adherence to guidelines presented in the general chapter *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ and in the manufacturers' labeling instructions.

PACKAGING AND DRUG PREPARATION CONTAINERS

The compounder shall ensure that the containers and container closures used in packaging compounded preparations meet USP requirements (see *Containers* under *Preservation, Packaging, Storage, and Labeling* in the *General Notices and Requirements*, *Containers—Plastics* ⟨661⟩, and *Containers—Permeation* ⟨671⟩), and when available, compounding monographs. Containers and container closures intended for compounding of sterile preparations must be handled as described in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩.

The containers and closures shall be made of suitable clean material so as not to alter the quality, strength, or purity of the compounded drug preparation. The container used depends on the physical and chemical properties of the compounded preparation. Container-drug interaction is to be considered with substances that have sorptive or leaching properties.

The containers and closures shall be stored off the floor, handled and stored to prevent contamination, and rotated so that the oldest stock is used first. The containers and container closures shall be stored in such a way as to permit inspection and cleaning of the storage area.

COMPOUNDING DOCUMENTATION

Documentation enables a compounder, whenever necessary, to systematically trace, evaluate, and replicate the steps included throughout the preparation process of a compounded preparation. All compounders who dispense prescriptions must comply with the record keeping requirements of their State Boards of Pharmacy. When the compounder compounds a preparation according to the manufacturer's labeling instructions, then further documentation is not required. All other compounded preparations require further documentation as described in this section.

These records are to be retained for the same period of time that is required for any prescription under state law. The record may be a copy of the prescription in written or machine readable form, and includes a Master Formulation Record and a Compounding Record.

Master Formulation Record

This record shall include

- name, strength, and dosage form of the preparation;
- calculations needed to determine and verify quantities of components and doses of active pharmaceutical ingredients;
- description of all ingredients and their quantities;
- compatibility and stability information including references when available;
- equipment needed to prepare the preparation, when appropriate;
- mixing instructions that should include
  1. order of mixing,
  2. mixing temperatures or other environmental controls,
  3. duration of mixing, and
  4. other factors pertinent to the replication of the preparation as compounded;
- assigned BUD;
- container used in dispensing;
- packaging and storage requirements; and
- quality control procedures.

Compounding Record

The Compounding Record shall contain

- name and strength of the compounded preparation;
- Master Formulation Record reference for the preparation;
- sources, lot numbers, and expiration dates of ingredients;
- total quantity compounded;
- name of the person who prepared the preparation and the name of the compounder who approved the preparation;
- date of preparation;
- assigned internal identification number or the prescription number;
- assigned BUD; and
- results of quality control procedures (e.g., weight range of filled capsules, pH of aqueous liquids, etc.).

Standard Operating Procedures

All significant procedures performed in the compounding area should be covered by standard operating procedures (SOPs). Procedures should be developed for the facility, equipment, personnel, preparation, packaging, and storage of compounded preparations to ensure accountability, accuracy, quality, safety, and uniformity in compounding. Implementing SOPs establishes procedural consistency and also provides a reference for orientation and training of personnel.

Material Safety Data Sheets File

Material Safety Data Sheets shall be readily accessible to all employees working with drug substances or bulk chemicals located on the compounding facility premises. Employees are to be instructed on how to retrieve and interpret needed information.

QUALITY CONTROL

The safety, quality, and performance of compounded preparations depend on correct ingredients and calculations, accurate and precise measurements, appropriate formulation conditions and procedures, and prudent pharmaceutical judgment. As a final check, the compounder shall review each procedure in the compounding process. To ensure accuracy and completeness, the compounder shall observe the finished preparation to ensure that it appears as expected and shall investigate any discrepancies and take appropriate corrective action before the prescription is dispensed to the patient.

Compounding Controls

1. The compounder shall ensure that there are written procedures for the compounding of drug preparations to ensure that the finished preparations have the identity, strength, quality, and purity that they purport to have. These procedures shall be available in either written form or electronically stored with printable documentation as described in the section *Compounding Documentation*.
2. The written procedures shall be followed in execution of the compounding process.
3. The compounder shall check and recheck each procedure at each stage of the process.
4. The compounder shall have established written procedures that will describe the tests or examinations to be conducted on the preparation compounded (e.g., the degree of weight variation among capsules) to assure uniformity and integrity of compounded drug preparations.
5. Appropriate control procedures shall be established to monitor the output and to validate the performance of those compounding processes and equipment that may be responsible for causing variability in the final compounded preparations.
6. For further guidance on recommended quality control procedures, see *Quality Assurance in Pharmaceutical Compounding* ⟨1163⟩.

PATIENT COUNSELING

At the time of dispensing, the patient or the patient's agent shall be counseled about proper use, storage, handling, and disposal of the compounded preparation. The patient or the patient's agent shall also be instructed to observe and report to the compounder any changes in the physical characteristics of the compounded preparation (see *Responsibility of the Pharmacist* under *Stability Considerations in Dispensing Practice* ⟨1191⟩). The compounder shall investigate any reported problem with a compounded preparation and take corrective action.

TRAINING

All personnel involved in the compounding, evaluation, packaging, and dispensing of compounded preparations shall be properly trained for the type of compounding conducted. It is the responsibility of the compounder to ensure that a training program has been implemented and that it is ongoing. Steps in the training procedure include the following:

- All employees involved in pharmaceutical compounding shall read and become familiar with this general chapter *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩. They should also be familiar with the contents of the USP *Pharmacists' Pharmacopeia* and other relevant publications, including how to read and interpret MSDS.
- All employees shall read and become familiar with each of the procedures related to compounding, including those involving the facility, equipment, personnel, actual compounding, evaluation, packaging, storage, and dispensing.
- All personnel who compound hazardous drugs shall be fully trained in the storage, handling, and disposal of these drugs. This training shall occur prior to preparing or handling hazardous drugs. For information on training for personnel who compound hazardous drugs, see the references in the section *Compounding Facilities* earlier in this chapter.
- All training activities shall be documented. The compounder shall meet with employees to review their work and answer any questions the employees may have concerning compounding procedures.
- The compounder shall demonstrate the procedures for the employee, and observe and guide the employee throughout the training process. The employ-

ee will then repeat the procedure without any assistance from, but under the direct supervision of, the compounder.

- When the employee has demonstrated to the compounder a verbal and functional knowledge of the procedure, then and only then, will the employee be permitted to perform the procedure without direct supervision. However, the compounder should be physically present and shall approve all ingredients and their quantities, and the final preparation.
- When the compounder is satisfied with the employee's knowledge and proficiency, the compounder will sign the documentation records to show that the employee was appropriately trained.
- The compounder shall continually monitor the work of the employee and assure that the employee's calculations and work are accurate and adequately performed.
- The compounder is solely responsible for the finished preparation.

COMPOUNDING FOR ANIMAL PATIENTS

A compounder's responsibility for providing patients with high quality compounded preparations extends beyond the human species. All portions of this chapter ⟨795⟩ apply to compounds prepared for animal patients. Intended use (e.g., companion, performance, food) of any animal patient shall be determined prior to compounding for that patient.

Because humans can consume animal patients as food, care must be taken to prevent drug residues from entering the human food chain when compounded preparations are used in animal patients. For this reason, all compounders preparing formulations for animals shall possess a functional knowledge of drug regulation and disposition in animal patients. Veterinarians are required by law to provide food-producing animal caregivers with an accurate length of time to withhold treated animal tissues (e.g. meat, milk, and eggs) from the human food supply. This length of time is referred to as a withdrawal time (WDT) and must also, by law, be included on the dispensing label of every prescription prepared for a food–producing species.

Drug use in any performance animal is strictly regulated by federal and state governments, in addition to the governing bodies of each of the specific disciplines. Penalties for violation of these rules may be severe for all contributing to the violation, including the veterinarian, pharmacist, and caregiver.

The pharmacist shall be knowledgeable of the individual species limitations in physiology and metabolic capacity that can result in toxicity when certain drugs or excipients are utilized in compounded preparations. For this reason, compounders preparing compounds for animals should utilize, when at all possible, formulations specifically developed for animal patients. If such formulations are not available, the compounder shall conduct a literature review to determine whether a specific component of the formula is toxic to the target species. Extrapolating compounding formulations intended for use in humans may not be appropriate for animal species and may contribute to negative outcomes.

Veterinarians and pharmacists preparing compounds for animal patients should be familiar with all state and federal regulations regarding drug use in animals, including but not limited to the Food, Drug, and Cosmetic Act; the Animal Drug Amendment; the Animal Medicinal Drug Use Clarification Act; FDA's Compliance Policy Guideline for Compounding of Drugs for Use in Animal Patients.

See the *Veterinary* sections of the *Pharmacists' Pharmacopoeia.*■2S (*USP33*)

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1075**⟩ **Good Compounding Practices,** *USP 32* page 523. It is proposed to delete this general information chapter. See also the briefing under *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩.

(CRX: R. Schnatz) RTS—C43023

Delete the following:

■⟨1075⟩ GOOD COMPOUNDING PRACTICES

The purpose of this chapter is to provide compounders with guidance on applying good compounding practices for the preparation of compounded formulations for dispensing and/or administration to humans or animals. This chapter is intended to provide information as a supplement to other relevant chapters. The following discussion is applicable to those engaged in compounding preparations in all pharmacies. It is expected that pharmacists or compounders engaged in the compounding of drugs will compound in conformance with applicable state and federal compounding laws, regulations, or guidelines.

APPLICABLE DEFINITIONS

Compounding (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩)—Compounding involves the preparation, mixing, assembling, packaging, and labeling of a drug or device in accordance with a licensed practitioner's prescription of medication order under an initiative based on the practitioner/patient/pharmacist/compounder relationship in the course of professional practice. Compounding includes the following:

a. Preparation of drugs or devices in anticipation of prescription drug orders based on routine, regularly observed prescribing patterns.
b. Reconstitution or manipulation of commercial products that may require the addition of one or more ingredients as a result of a licensed practitioner's prescription drug order.
c. Preparation of drugs or devices for the purposes of, or as an incident to, research, teaching, or chemical analysis.

Categories of Compounding—The categories of compounding are intended to provide an understanding among compounders when different forms of preparations are compounded. It is to be understood that there are levels of training associated with each category. In the categories of compounding described below, certain criteria were used to determine the overall classification.

Category 1	Nonsterile—Simple Generally, the mixing of two or more commercial products
Category 2	Nonsterile—Complex Generally, compounding with the bulk drug substances or when calculations are required.
Category 3	Sterile—Risk Level I (See *Low-Risk Level* in USP general chapter ⟨797⟩)
Category 4	Sterile—Risk Level II (See *Medium-Risk Level* in USP general chapter ⟨797⟩)
Category 5	Sterile—Risk Level III (See *High-Risk Level* in USP general chapter ⟨797⟩)
Category 6	Radiopharmaceuticals Preparation of radiopharmaceuticals
Category 7	Veterinary Preparation of veterinary pharmaceuticals

Manufacturing—Manufacturing involves the production, propagation, conversion, or processing of a drug or device, either directly or indirectly, by extraction of the drug from substances of natural origin or by means of chemical or biological synthesis. Manufacturing also includes (1) any packaging or repackaging of the substance(s) or labeling or relabeling of containers for the promotion and marketing of such drugs or devices; (2) any preparation of a drug or device that is given or sold for resale by pharmacies, practitioners, or other persons; (3) the distribution of inordinate amounts of compounded preparations or the copying of commercially available drug products; and (4) the preparation of any quantity of a drug product without a licensed prescriber/patient/licensed pharmacist/compounder relationship.

Component—A component is any ingredient used in the compounding of a drug product, including any that are used in its preparation, but may not appear on the labeling of such a product. (See *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩ for additional definitions.)

Compounder—A compounder is a professional authorized by the state to perform compounding pursuant to a prescription order by a licensed prescriber.

RESPONSIBILITIES OF THE COMPOUNDER

a. Compounders who are engaged in drug compounding or nutriceutical compounding shall be proficient in compounding and should continually expand their compounding knowledge by participating in seminars and/or studying appropriate literature.
b. A compounder must be familiar with all of the details of *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩, *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩, and other applicable state or federal compounding guidelines or laws. In addition, the compounder must be responsible for the following:
   • certifying all prescription orders;
   • approving or rejecting all components, drug product containers, closures, in-process materials, and labeling;
   • preparing and reviewing all compounding records to assure that errors have not occurred in the compounding process;

Chromatographic Reagents

- assuring the proper maintenance, cleanliness, and use of all equipment used in a prescription compounding practice;
- assuring that only authorized personnel shall be in the immediate vicinity of the drug compounding operations;
- assuring that the drug product and components of drug products are not on the list of federally recognized drug products that have been withdrawn or removed from the market for public health reasons.

a. The compounder must ensure that personnel engaged in compounding wear clean clothing appropriate to the type of compounding performed, e.g., coats, gowns, gloves, masks, shoes, aprons, or other items as needed for protection of personnel from chemical exposures and for prevention of drug contamination.

a. The compounder must implement procedures to prevent cross-contamination when compounding with drugs (e.g., penicillins) that require special precaution to prevent cross-contamination.

TRAINING

All personnel involved in the compounding, evaluation, packaging, and dispensing of compounded preparations shall be properly trained for the type of compounding conducted. All training activities will be covered by appropriate standard operating procedures (SOPs) and documentation.

All compounders and all personnel involved in compounding must be well trained and must participate in current, relevant training programs. It is the responsibility of the compounder to ensure that a training program has been implemented and that it is ongoing. Standards of practice require that all employees be adequately trained in their job functions and that all of the training is properly documented. Steps in the training procedure will include the following:

a. All employees involved in pharmaceutical compounding shall read and become familiar with *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩, *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, and *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩.

b. All employees shall read and become familiar with each of the procedures related to compounding, including those involving the facility, equipment, personnel, actual compounding, evaluation, packaging, storage, and dispensing.

c. The compounder shall meet with employees to review their work and answer any questions the employees may have concerning SOPs.

d. The compounder shall demonstrate the procedures for the employee, and will observe and guide the employee throughout the training process. The employee will then repeat the procedure without any assistance from, but under the direct supervision of, the compounder.

e. When the employee has demonstrated to the compounder a verbal and functional knowledge of the procedure, then and only then, will the employee be permitted to perform the procedure without direct supervision. However, the compounder should be physically present and should check off the final preparation.

f. When the compounder is satisfied with the employee's knowledge and proficiency, the compounder will sign off on the documentation records to show that the employee was appropriately trained.

g. The compounder shall continually monitor the work of the employee and assure that the employee's calculations and work are accurate and adequately performed. The compounder is completely responsible for the finished preparation. The compounder will answer any questions the employee may have concerning the SOPs.

PROCEDURES AND DOCUMENTATION

All significant procedures performed in the compounding area will be covered by SOPs and will be documented.

Procedures should be developed for the facility, equipment, personnel, preparation, packaging, and storage of compounded preparations to ensure accountability, accuracy, quality, safety (including access to Material Safety Data Sheets), and uniformity in compounding. Implementing SOPs establishes procedural consistency and also provides a reference for orientation and training of personnel.

Documentation enables a compounder, whenever necessary, to systematically trace, evaluate, and replicate the steps included throughout the preparation process of a compounded preparation.

DRUG COMPOUNDING FACILITIES

a. Compounding facilities shall have an adequate space that is specifically designated for compounding of prescriptions. This area may include a space for the storage of equipment and materials.

b. Sterile compounded preparations shall be compounded in accordance with the provisions in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, and aseptic processes shall be conducted in an area separate and distinct from the area used for the compounding of nonsterile products.

c. The areas used for compounding shall be maintained in clean, orderly, and sanitary conditions.

d. The areas for drug compounding shall be maintained in a good state of repair. The plumbing system shall be free of defects that could contribute to contamination of any compounded product. Adequate washing facilities shall be easily accessible to the compounding areas. Such facilities shall include, but not be limited to, hot and cold water, soap or detergent, and an air drier or single use towels.

e. Potable water shall be supplied under continuous positive pressure.

f. The area for compounding shall have adequate lighting and ventilation.

g. The area for compounding shall be free of infestation by insects, rodents, and other vermin. Trash shall be held and disposed of in a sanitary and timely manner.

h. Sewage and other refuse in the area of compounding shall be disposed of in a safe and sanitary manner.

i. Bulk drugs and other chemicals or materials used in the compounding of drugs must be stored as directed by the manufacturer, or according to USP monograph requirements, in a clean, dry area, under appropriate temperature conditions (controlled room temperature, refrigerator, or freezer). The bulk chemicals shall be stored in a manner such that they are protected from contamination. All containers shall be properly labeled.

j. If parenteral products are compounded, the compounder shall refer to *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, and *Injections* ⟨1⟩ for compounding technique applications.

DRUG COMPOUNDING EQUIPMENT

(See also *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩.)

a. The equipment or utensils used for compounding of a drug preparation shall be of appropriate design and capacity. The equipment should be stored in such a manner as to protect it from contamination, and shall be located in such a place as to facilitate operations for its use, maintenance, and cleaning.

b. The equipment should be of suitable composition such that the surfaces that contact components are neither reactive, additive, nor absorptive and therefore will not affect or alter the purity of the compounded preparations.

c. Automated, mechanical, electronic, and other types of equipment used in compounding or testing of compounded preparations should be routinely inspected, calibrated as necessary, and checked to ensure proper performance.

d. Immediately prior to initiation of compounding operations, the equipment shall be inspected by the compounder to determine its suitability for use.
e. After use, the equipment should be appropriately cleaned. Extra care should be used when cleaning equipment used in compounding preparations requiring special precaution, e.g., antibiotics, cytotoxins, cancer drugs, and other hazardous materials. If possible, special equipment may be dedicated for such use or if the same equipment is being used for all drug products, appropriate procedures must be in place to allow meticulous cleaning of equipment prior to use with other drugs.

COMPONENT SELECTION REQUIREMENTS

a. The compounder must first attempt to use *USP–NF* drug substances manufactured in an FDA registered facility.
b. The compounder shall also first attempt to use inactive components manufactured in an FDA registered facility.
c. If components are not obtainable from an FDA registered facility or if the FDA and/or the providing company cannot document FDA registration, compounders shall use their professional judgment in first receiving, storing, or using the components that meet official compendial requirements or are provided by another high-quality source.
d. If components of compendial quality are not obtainable, components of high quality such as those that are chemically pure, analytical reagent grade, American Chemical Society certified, or Food Chemicals Codex grade may be used.
e. When a component is not obtained from an official compendial source or is not obtainable from the sources mentioned above, the component may be obtained from a source deemed acceptable and reliable in the professional judgment of the compounder.
f. When a component is derived from ruminant animals (e.g., bovine, caprine, ovine) the supplier shall provide written assurance that these animals were born, raised, and slaughtered in countries where bovine spongiform encephalopathy (BSE) and scrapie are known not to exist.
g. The compounder shall not use components that are listed by FDA to be withdrawn from the market for public health reasons.
h. Components shall be stored off the floor, handled and stored to prevent contamination, and rotated so that the oldest stock is used first.

PACKAGING AND DRUG PREPARATION CONTAINERS

a. The compounder shall ensure that the containers and container closures used in packaging the compounded preparations meet the requirements under *Containers—Glass* ⟨660⟩, *Containers—Plastics* ⟨661⟩ and *Containers—Performance Testing* ⟨671⟩. The compounder shall obtain written records from the supplier to show that the containers meet USP requirements.
b. Containers and container closures intended for compounding of sterile preparations and nonsterile preparations must be handled, sterilized (if appropriate), and stored as described in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ and *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩. The use of commercially available presterilized containers is encouraged for sterile preparations.
c. The containers and closures shall be stored off the floor, handled and stored to prevent contamination, and rotated so that the oldest stock is used first.
d. The containers and container closures shall be stored in such a way as to permit inspection and cleaning of the work area.
e. The containers and container closures shall be made of clean materials that are neither reactive, additive, nor absorptive.
f. The containers and closures shall be of suitable material so as not to alter the quality, strength, or purity of the compounded drug.
g. The compounder shall ensure that the containers and container closures selected to dispense the finished compounded prescription, whether sterile or nonsterile or radiopharmaceutical, meet the criteria in sections (a)–(f) above.

COMPOUNDING CONTROLS

a. The compounder should ensure that there are written procedures for the compounding of drug products to assure that the finished products have the identity, strength, quality, and purity that they purport to have. These procedures should be available in either written form or electronically stored with printable documentation.
b. The compounder shall establish procedures that include a description of (1) components, their amounts, the order of component additives, and the compounding process; (2) the required equipment and utensils; and (3) the drug product container and closure system.
c. The written procedures described above shall be followed in execution of the compounding process.
d. The compounder shall accurately weigh, measure, and subdivide as appropriate.
e. The compounder shall check and recheck each procedure at each stage of the process to ensure that each weight or measure is correct as stated in the written compounding procedures.
f. If a component is transferred from the original container to another container (e.g., a powder is taken from the original container, weighed, placed in a container, and stored in that other container), the new container shall be identified with the component name, weight or measure, the lot or control number, the expiration or beyond-use date, and the transfer date.
g. The compounder should have established written procedures that will describe the tests or examinations to be conducted on the preparation compounded (e.g., the degree of weight variation among capsules) to assure uniformity and integrity of compounded drug preparations.
h. Appropriate control procedures should be established to monitor the output and to validate the performance of those compounding processes that may be responsible for causing variability in the final compounded preparations. Factors that may cause variability include (1) capsule weight variation; (2) adequacy of mixing to assure uniformity and homogeneity; and (3) clarity, completeness, or pH of solutions.
i. Appropriate written procedures should be designed to prevent microbiological contamination of compounded drug preparations purporting to be sterile, and these procedures shall be followed. Such procedures shall include validation of sterilization processes (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).
j. The compounder should establish appropriate beyond-use dates determined either from available *USP–NF* monographs, appropriate testing, or from peer-reviewed literature.
k. The compounder should adopt appropriate storage requirements as provided in *Preservation, Packaging, Storage, and Labeling* under *General Notices and Requirements*.

LABELING

1. The compounder's preparation label should contain information required by state and federal law and accepted standards of practice. [NOTES—(a) The compounder shall

~~use the established name or distinct common name (cannot use the trademarked name of a manufactured product). (b) The compounder cannot indicate that the compounded product is therapeutically equivalent to a manufactured product. (c) The label should state that this is a compounded preparation. (d) The compounder shall not use an NDC number assigned to another product.]~~

~~2. The compounder shall label any excess compounded products so as to reference them to the formula used, the assigned control number, and beyond-use date based on the compounder's appropriate testing, published data, or *USP–NF* standards.~~

~~3. Preparations compounded in anticipation of a prescription prior to receiving a valid prescription should not be prepared in an inordinate amount. A regularly used amount should be prepared on the basis of a history of prescriptions filled by the pharmacy. These preparations should be labeled or documentation referenced with the following:~~
~~a. A complete list of ingredients or preparation name and reference or established name or distinct common name~~
~~b. Dosage form~~
~~c. Strength~~
~~d. Preparation date~~
~~e. Name and address of compounder~~
~~f. Inactive ingredients~~
~~g. Batch or lot number~~
~~h. Assigned beyond-use date, based on published data, or appropriate testing, or *USP–NF* standards.~~
~~Storage conditions for these preparations should be dictated by their composition and sterility, e.g., stored in a clean, dry place under appropriate temperature conditions (controlled room temperature, refrigerator, or freezer.)~~

~~4. The compounder should examine the preparation for correct labeling after completion of the compounding process.~~

~~RECORDS AND REPORTS~~

~~a. The compounder shall maintain records, including but not limited to, the hard copy of the prescription to indicate that the prescription is compounded, and to provide formulation records and compounding records.~~
~~b. The compounder shall keep adequate records of controlled drug substances (scheduled drugs) used in compounding.~~
~~c. All records of all compounded preparations shall be kept for a period of time as set forth in the federal and state laws or regulations. Such records shall be readily available for authorized inspection.~~
~~d. The compounding records shall include the manufacturer and lot number of all ingredients.~~

~~COMPOUNDING FOR A PRESCRIBER'S OFFICE USE~~

~~a. Compounders may prepare compounded drug preparations for a prescriber's office use only where permitted by federal and state requirements.~~
~~b. An order by the prescriber indicating the formula and quantity ordered may be filled in the compounder's facility.~~
~~c. Where compounding for office use is permitted, the compounder shall compound the preparation for the sole purpose of administration by or for the prescriber.~~
~~d. A record of the compounding process shall be maintained.~~
~~e. A label must be generated and a number may be assigned.~~

~~COMPOUNDING VETERINARIAN PRODUCTS~~

~~a. Compounders shall compound prescriptions for animals on the basis of prescription orders.~~
~~b. These prescriptions shall be handled and filled according to the guideline available for compounding of veterinarian products.~~ ■2S (*USP33*)

BRIEFING

〈**1117**〉 **MICROBIOLOGICAL BEST LABORATORY PRACTICES,** *USP 32* page 616. On the basis of internal deliberations by the expert committee, (1) revisions have been proposed to several sections to enhance clarity, and (2) the new sections *Sample Handling*, *Microbiological Media Incubation Times*, and *Laboratory Resources* have been added.

(MSA: R. Tirumalai) RTS—C73547

Change to read:

INTRODUCTION

Good laboratory practices in a microbiology laboratory consist of activities that depend on several principles: aseptic technique, control of media, control of test strains,

■operation and■2S (*USP33*)
control of equipment, diligent recording and evaluation of data, and training of the laboratory staff. Because of the ~~known~~

■inherent risk of■2S (*USP33*)
variability in microbiology data, reliability and reproducibility are dependent on the use of accepted methods and adherence to good laboratory practices.

Change to read:

MEDIA PREPARATION AND QUALITY CONTROL

Media Preparation

Culture media are the basis for most microbiological tests. Safeguarding the quality of the media is therefore critical to the success of the microbiology laboratory. Media preparation, proper storage, and quality control testing can ensure a consistent supply of high-quality media.

It is important to choose the correct media or components in making media based on the use of accepted sources or references for formulas. The manufacturer's formula and instructions for preparation routinely accompany dehydrated media and ready-made media. Because different media types may have different preparation requirements (e.g., heating, additives, and pH adjustment), it is important to follow these instructions to ensure preparation of acceptable media quality. A certificate of analysis describing expiration dating and recommended storage conditions accompanies ready-made media, as well as the quality control organisms used in growth-promotion and selectivity testing of that media.

Water is the universal diluent for microbiological media. Purified Water is most often used for media preparation, but in certain cases the use of deionized or distilled water may be appropriate.

■Water of lesser quality should not be used for microbiological media preparation.■2S (*USP33*)
The volume of the water used should be recorded.

Consistent preparation of media requires accurate weighing of dehydrated media or media constituents. A calibrated balance with the appropriate weight range for the ingredients should be used

■(See *Weighing on an Analytical Balance* ⟨1251⟩).■2S (*USP33*)
Clean weighing containers and tools (such as spatulas) should be used to prevent foreign substances ~~that may alter the composition of the finished media~~

■■2S (*USP33*)
from entering the formulation. The weight of the components should be recorded.

Dehydrated media should be thoroughly dissolved in water before dispensing and sterilization. If heating is necessary to help dissolve media, care should be taken not to overheat media, becdause all culture media, to a greater or lesser extent, are heat-sensitive. Equipment used in the preparation of media should be appropriate to allow for controlled heating, constant agitation, and mixing of the media. Darkening of media (Maillard-type reaction or nonenzymatic browning) is a general indication of overheating. When adding required supplements to media, adequate mixing of the medium after adding the supplement should be performed.

Preparation of media in poorly cleaned glassware can allow inhibitory substances to enter the media. Inhibitory substances can come from detergent residue after cleaning glassware or from prior materials used in the glassware. Be sure that the cleaning process removes debris and foreign matter, and that the detergent is thoroughly rinsed out with Purified Water. See *Cleaning Glass Apparatus* ⟨1051⟩ for additional guidance.

Sterilization of media should be performed within the parameters provided by the manufacturer or validated by the user. Commercially prepared media should provide documentation of the sterilization method used. ~~Ideally the manufacturer should provide the sterility assurance level (SAL) of the media against a recognized biological indicator.~~

■■2S (*USP33*)
Autoclaving by moist heat is the preferred sterilization technique, except in instances when boiling is required in order to avoid deterioration of heat-labile components of the media. Sterilization by filtration may also be appropriate for some formulations.

The effects of the sterilization method and conditions on the media should be validated by sterility and growth-promotion testing of the media. In addition, if sterilized by moist heat, the autoclave cycle should be validated to ensure proper heat distribution for selected loads and volumes. Typically, manufacturers recommend using an autoclave cycle of 121° for 15 minutes using a validated autoclave. These conditions apply to time at temperature of the media. As

■container size and■2S (*USP33*)
the load configuration of the autoclave will influence the rate of heating, longer cycles may be required for larger loads. However, the sterilization time will be dependent on the media volume and autoclave load. Sterilization cycles in which the autoclave is slow to come up to temperature may result in overheating of the media. Therefore, care must be taken to validate a sterilization cycle, ~~to deliver the minimum SAL required~~

■■2S (*USP33*)
balancing the need for sterile media against the tendency of the media to degrade under excessive heating. Storage of the media in the autoclave after the liquid cycle is completed is not recommended after cooling, as it may damage the media. Improper heating or sterilizing conditions—for commercially prepared or internally prepared media—may result in a difference in color change, loss of clarity, altered gel strength, or pH drift from the manufacturer's recommended range,

■as well as reduced growth-promotion activity and/or selectivity.■2S (*USP33*)
The pH of each batch of medium should be confirmed after it has cooled to room temperature

■(20°–■2S (*USP33*)
25°) by aseptically withdrawing a sample for testing.

■Refrigerated purchased media should be allowed to warm up to ambient room temperature.■2S (*USP33*)
A flat pH probe is recommended for agar surfaces, and an immersion probe is recommended for liquids.

■See *pH* ⟨791⟩ for guidance with pH measurement and instrument calibration.■2S (*USP33*)
The pH of media should be in a range of ±0.2 of the value indicated by the manufacturer, unless a wider range is acceptable by the validated method.

Prepared media should be checked by appropriate inspection of plates and tubes for the following:

- Cracked containers or lids
- Unequal filling of containers
- Dehydration resulting in cracks or dimpled surfaces on solid medium
- Hemolysis
- Excessive darkening or color change
- Crystal formation from possible freezing
- Excessive number of bubbles
- Microbial contamination
- Status of redox indicators (if appropriate)
- Lot number and expiration date checked and recorded
- Sterility of the media

Media Storage

It is prudent to consider how the manufacturer or supplier transports and stores media before distribution to the end user. Manufacturers of media should use transport and storage conditions that minimize the loss of moisture, control the temperature,

■prevent microbial contamination,■2S (*USP33*)
and provide mechanical protection to the prepared media.

Media should be labeled properly with batch or lot numbers, preparation and expiration dates, and media identification. Media should be stored according to the manufacturer's instructions. Media prepared in house should be stored under validated conditions. Do not store agar at or below 0°, as freezing could damage the gel structure. Protect stored media from exposure to light and excessive temperature. Before prolonged storage, agar plates should be placed into a sealed package or container to retard moisture loss.

Remelting of an original container of solid media should be performed only once to avoid media whose quality is compromised by overheating or potential contamination. It is recommended that remelting be performed in a heated water bath or by using free-flowing steam. The use of microwave ovens and heating plates is common, but care should be taken to avoid damaging media by overheating and to avoid the potential injury to laboratory personnel from glass breakage and burns. The molten agar medium should be held in a monitored water bath at a temperature of 45° to 50° for not more than 8 hours. Caution should be taken when pouring the media from a container immersed in a water bath to prevent water from the bath commingling with the poured sterile media. Wiping the exterior of the container dry before pouring may be advisable.

Disposal of used cultured media (as well as expired media) should follow local biological hazard safety procedures.

Quality Control Testing

Although growth media can be prepared in a laboratory from individual components, many laboratories, for ease of use, use dehydrated media or purchase commercially prepared media in

■plastic■2S (*USP33*)
plates or glass containers. Manufacturers of media attempt to standardize raw materials from biological sources, but must constantly deal with unavoidable differences in raw materials obtained from natural sources, and therefore, lot-to-lot variability of media must be considered.

■In addition,■2S (*USP33*)
the performance of media prepared in a laboratory or by a manufacturer is highly dependent on preparation

■and storage conditions.■2S (*USP33*)
Improper media preparation can cause unsatisfactory conditions for microbial growth or recovery and unreliable results.

■Therefore,■2S (*USP33*)
quality control tests should be performed on all prepared media. Tests routinely performed on in-house prepared media ~~are pH, growth promotion~~

■should include pH, growth promotion, inhibition, and

indicative properties (as appropriate),■2S (*USP33*)
and periodic stability checks to confirm the expiration dating.

When in-house prepared microbiological media are properly prepared and sterilized using a validated method, the growth-promotion testing may be limited to each incoming lot of dehydrated media, unless otherwise instructed by the relevant compendial method. If the media preparation

■procedure■2S (*USP33*)
was not validated, then every batch of media ~~would~~

■should■2S (*USP33*)
be subjected to growth-promotion testing. Test organisms may be selected from the appropriate compendial test chapter.

■In addition, microorganisms used in growth-promotion

testing may be■2S (*USP33*)
based on the manufacturer's recommendation for a particular medium, or may include representative environmental isolates

■(but these latter are not to be construed as compendial

requirements).■2S (*USP33*)

Expiration dates on media should have supporting growth-promotion testing to indicate that the performance of the media still meets acceptance criteria up to and including the expiration date. The length of shelf life of a batch of media will depend on the stability of the ingredients and formulation under specified conditions, as well as the type of container and closure.

When a batch of media does not meet the requirements of growth-promotion testing, an investigation should be initiated to identify the cause. This investigation should include a corrective action plan to prevent the recurrence of the problem. ~~Any nonconforming lot should not be used if an assignable cause or corrective resolution relative to nongrowth support is undetermined.~~

■Any batch of media that fails growth-promotion testing

is unsuitable for use. [NOTE—Failed growth-promotion

test results may not be used to negate positive test re-

sults.]■2S (*USP33*)

Some reagents are used for diagnostic purposes to help support identification of microbial organisms, e.g., Gram stain and oxidase test reagents. These may have attributes that can be quality control tested similar to microbiological media. Select the correct quality control standard microorganisms, following the manufacturer's instructions, and perform the testing before unknown sample diagnostic testing.

■All relevant diagnostic reagents should be subjected to

incoming quality confirmation before use.■2S (*USP33*)

Special care should be taken with media that is used in environmental monitoring studies. Media used for environmental monitoring of critical areas should preferably be double-wrapped and terminally sterilized. If terminal sterilization is not performed, media should be subjected to ~~pre-incubation and 100% inspection~~

■100% pre-incubation and inspection■2S (*USP33*)
before use within a critical area.

■[NOTE—Growth-promotion testing for this media must

be performed after the pre-incubation stage.]■2S (*USP33*)
This will prevent extraneous contamination from being carried into controlled environments and will prevent false-positive results. A raised agar level for surface contact plates should be verified.

Change to read:

MAINTENANCE OF MICROBIOLOGICAL CULTURES

Biological specimens can be the most delicate standards to handle because their viability and characteristics are dependent on adequate handling and storage. Standardizing the handling and storage of cultures by the user laboratory should be done in a way that will minimize the opportunity for contamination or alteration of growth characteristics. The careful and consistent treatment of stock cultures is critically important to the consistency of microbiological test results. Cultures for use in compendial tests should be acquired from a national culture collection

■or a qualified secondary supplier.■2S (*USP33*)
They can be acquired frozen, freeze-dried, on slants, or in ready-to-use forms. Confirmation of the purity of the culture and the identity of the culture should be performed before its use in quality control testing. Ready-to-use cultures ~~may require confirmation of purity, identity, and inoculum size. This~~

■should be subjected to incoming testing for purity and

identity before use. The■2S (*USP33*)
confirmation of identity for commonly used laboratory strains should ideally be done at the level of ~~genotypic analysis (i.e., DNA fingerprinting, 16S rRNA gene sequencing, or PCR analysis using suitably validated probes).~~

■genus and species.■2S (*USP33*)

Preparation and resuscitation of cultures should follow the instructions of the supplier or a validated, established method. The ''Seed-Lot'' technique is recommended for storage of stock cultures.

The original sample from the national culture collection

■or a qualified secondary supplier■2S (*USP33*) is resuscitated and grown in an appropriate medium. Aliquots of this stock culture (the first transfer or passage) are suspended in a cryoprotective medium, transferred to vials, and frozen at –30° or below, until use. If stored at –70°, or in lyophilized form, strains may be kept indefinitely. These frozen stocks can then be used to inoculate monthly or weekly working cultures. Once opened, do not refreeze unused cell suspensions after culturing a working suspension. The unused portion should be discarded to minimize the risk of loss of viability and contamination of the stock.

The number of transfers of working control cultures should be tracked to prevent excessive subculturing that increases the risk of phenotypic alteration

■or mutation.■2S (*USP33*) One passage is defined as the transfer of organisms from a viable culture to a fresh medium with growth of the microorganisms. Any form of subculturing is considered to be a transfer/passage.

Change to read:

~~**MAINTENANCE OF**~~

■■2S (*USP33*)

LABORATORY EQUIPMENT

Most equipment (incubators, water baths, and autoclaves) is subject to standard validation practices of incoming qualification, operational qualification, and performance qualification. Additionally, periodic calibration (generally annually) is commonly required. New equipment, critical to the operation of the laboratory, should be qualified according to a protocol approved by the quality assurance unit (QAU).

■In addition, regular cleaning and sanitization of equipment such as incubators, refrigerators, and water baths should be performed to minimize the potential for contamination in the laboratory.■2S (*USP33*)

Instruments (pH meters and spectrophotometers) used in a microbiology laboratory should be calibrated on a regular schedule and tested to verify performance on a routine basis. The frequency of calibration and performance verification will vary based on the type of instrument and the importance of that equipment to the generation of data in the laboratory.

■Equipment that is difficult to sanitize (such as refrigerators and incubators) should be dedicated to aseptic operations (such as storage of media for testing and incubation of sterility test samples) and live culture operations to minimize the potential for inadvertent contamination of the tests.

Autoclaves are central to the operation of the laboratory and must have proper validation in place to demonstrate adequate sterilization for a variety of operations. Autoclave resources must be available (and validated) to sterilize waste media (if performed in that laboratory) as well as the media prepared in that laboratory. The choice of one or several autoclaves is not driven by a need to separate aseptic and live operations (everything in the properly maintained autoclave is sterile after the cycle) but rather driven by resource considerations (see below).■2S (*USP33*)

Change to read:

LABORATORY LAYOUT AND OPERATIONS

Laboratory layout and design should carefully consider the requirements of good microbiological practices and laboratory safety. It is essential that cross-contamination of microbial cultures be minimized to the greatest extent possible, and it is also important that microbiological samples be handled in an environment that makes contamination highly unlikely.

In general, a laboratory should be divided into clean or aseptic areas and live culture areas. Areas in which environmental or sterile product samples are handled and incubated should be maintained completely free of live cultures, if possible. If complete separation of live and clean culture zones cannot be accomplished, then other barriers and aseptic practices should be employed to reduce the likelihood of accidental contamination. These barriers include protective clothing, sanitization and disinfection procedures, and biological safety cabinets designated for clean or aseptic operations only. Procedures for handling spills or mishaps with live cultures should be in place, and all relevant technical personnel should be trained regarding these methods.

Some samples will demonstrate microbial growth and require further laboratory analysis to identify the contaminants. When growth is detected, the sample should be taken from the clean section of the laboratory to the live culture section without undue delay. Subculturing, staining, microbial identification, or other investigational operations should be undertaken in the live culture section of the laboratory. If possible, any sample found to contain growing colonies should not be opened in the clean zone of the laboratory. Careful segregation of contaminated samples and materials will reduce false-positive results.

Staff engaged in sampling activities should not enter or work in the live culture handling section of a laboratory unless special precautions are taken, including wearing protective clothing and gloves and careful sanitizing of hands upon exiting. Ideally, staff assigned to sampling activities, particularly those in support of aseptic processing, should not work in the vicinity of live culture laboratory operations. ~~Also, all microbiological samples should be taken using aseptic techniques, including those taken in support of nonsterile products. If possible, all microbiological samples should be taken under full aseptic conditions in specialized sampling areas.~~

■■2S (*USP33*)

It is important to consider that microbial contamination of samples, which leads to false-positive results, is always possible unless careful aseptic precautions are taken. Facilities should be designed so that raw material and excipient sampling can be done under controlled conditions, including proper gowning and sterilized sampling equipment. It may not always be possible to sample utility systems, such as water systems, under full aseptic conditions; however, it should be noted that when samples are not taken aseptically, their reliability is inevitably compromised.

Environmental sampling methods should require minimal aseptic handling in loading and unloading sampling instruments. Whenever possible, sampling equipment should be loaded with its microbiological recovery media in the environment that is to be sampled.

All testing in laboratories used for critical testing procedures, such as sterility testing of final dosage forms, bulk product, seed cultures for biological production, or cell cultures used in biological production, should be performed under controlled conditions. Isolator technology is also appropriate for critical, sterile microbiological testing. Isolators have been shown to have lower levels of environmental contamination than manned clean rooms, and therefore, are generally less likely to produce false-positive results. Proper validation of isolators is critical both to ensure environmental integrity and to prevent the possibility of false-negative results as a result of chemical disinfection of materials brought into or used within isolators (see *Sterility Testing—Validation of Isolator Systems* ⟨1208⟩).

Add the following:

▪SAMPLE HANDLING

Viable microorganisms in most microbiology samples, particularly water, environmental monitoring and bioburden samples, are sensitive to handling and storage conditions. Critical parameters in these conditions include product (or sample) composition, container composition, time of storage, and temperature of storage. Therefore, it is important to minimize the amount of time between the sampling event and the initiation of testing and to control, as much as possible, the conditions of storage. If the sample is to be transported to a distant location for testing, then the conditions of transport (time, temperature, etc.) should be qualified as suitable for that test and sample. Guidance for water testing in this regard can be found in *Water for Pharmaceutical Purposes* ⟨1231⟩. Product mixing before sampling may need to be evaluated and applied in order to ensure microbial dispersement and representation in the sample aliquot.

All microbiological samples should be taken using aseptic techniques, including those taken in support of nonsterile products. If possible, all microbiological samples should be taken under full aseptic conditions in specialized sampling areas. The areas should be as close to the point of use as possible to minimize contamination during transit.

Samples submitted to the microbiology laboratory should be accompanied by documentation detailing source of the sample, date the sample was taken, date of sample submission, person or department responsible for the submission, and any potentially hazardous materials associated with the sample. The testing department should acknowledge receipt of the sample and reconcile the identity and number of samples as part of this sample documentation. ■2S (*USP33*)

Add the following:

▪MICROBIOLOGICAL MEDIA INCUBATION TIMES

Incubation times for microbiological tests of less than 3 days' duration should be expressed in hours: e.g., ''Incubate at 30° to 35° for 18 to 72 hours''. Tests longer than 72 hours' duration should be expressed in days: e.g., ''Incubate at 30° to 35° for 3 to 5 days''. For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgment when exceeding the incubation time. For incubation times expressed in days, incubations started in the morning or afternoon should generally be concluded at that same time of day. ■2S (*USP33*)

Change to read:

TRAINING OF PERSONNEL

Each person engaged in each phase of pharmaceutical manufacture should have the education, training, and experience to do his or her job. The demands of microbiological testing require that the core educational background of the staff, supervisors, and managers be in microbiology or a closely related biological science. They should be assigned responsibilities in keeping with their level of skill and experience.

A coherent system of standard operating procedures (SOPs) is necessary to run the microbiology laboratory. These procedures serve two purposes in a training program. Firstly, these SOPs describe the methodology that the microbiologist will follow to obtain accurate and reproducible results, and so serve as the basis for training. Secondly, by tracking the procedures in which a particular microbiologist has demonstrated proficiency, the procedure number or title also serves to identify what training the microbiologist has received specific to his or her job function.

Training curricula should be established for each laboratory staff member specific to his or her job function. He or she should not independently conduct a microbial test until qualified to run the test. Training records should be current, documenting the microbiologist's training in the ~~proper~~

■current■2S (*USP33*)
revision to the particular SOP.

Periodic performance assessment is a wise investment in data quality. This performance testing should provide evidence of competency in core activities of the microbiology laboratory such as hygiene, plating, aseptic technique, documentation, and others as suggested by the microbiologist's job function.

Microbiologists with supervisory or managerial responsibilities should have appropriate education and in-house training in supervisory skills, laboratory safety, scheduling, ~~laboratory investigations~~

■budgeting, investigational skills,■2S (*USP33*)
technical report writing, relevant SOPs, and other critical aspects of the company's processes as suggested in their role of directing a laboratory function.

■Competency may be demonstrated by specific course work, relevant experience, and routinely engaging in relevant continuing education. Achieving certification through an accredited body is also a desirable credential. Further, it is expected that laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those they supervise. Expertise in microbiology can be achieved by a variety of routes in addition to academic course work and accreditation. Each company is expected to evaluate the credentials of those responsible for designing, implementing, and operating the microbiology program. Companies can thus ensure that those responsible for the program understand the basic principles of microbiology, can interpret guidelines and regulations based on good science, and have access to individuals with theoretical and practical knowledge in microbiology to provide assistance in areas in which the persons responsible for the program may not have adequate knowledge and understanding. It should be noted that microbiology is a scientifically based discipline that deals with biological principles substantially different from those of analytical chemistry and engineering disciplines. Many times it is difficult for individuals without specific microbiological training to make the transition.■2S (*USP33*)

Add the following:

▪LABORATORY RESOURCES

The laboratory management is responsible for ensuring that the laboratory has sufficient resources to meet the existing testing requirements. This requires some proficiency in budget management and in determining appropriate measures of laboratory performance. A measure of laboratory performance is the number of investigations performed on tests conducted by the laboratory, but this measure alone is not sufficient. In addition to tracking investigations, the period of time between sample submission and initiation of testing should be tracked, as well as the period of time between end of test and report release (or test closure). Significant delays in these measures are also indications of an underresourced laboratory staff.

The laboratory management should have sufficient budget to meet testing requirements. Particular measures of budgetary requirements will be specific to the given laboratory, but budgetary considerations related directly to the need of the laboratory for sufficient resources must be addressed to ensure reliable testing results.■2S (*USP33*)

Change to read:

DOCUMENTATION

Documentation should be sufficient to demonstrate that the testing was performed in a laboratory and by methods that were under control. This includes, but is not limited to, documentation of the following:

- Microbiologist training and verification of proficiency
- Equipment validation, calibration, and maintenance
- Equipment performance during test (e.g., 24-hour/7-day chart recorders)
- Media preparation, sterility checks, and growth-promotion and selectivity capabilities
- Media inventory and control testing
- Critical ~~components~~

■aspects■2S (*USP33*)
of test conducted as specified by a procedure

- Data and calculations ~~verified~~

■verification■2S (*USP33*)

- Reports reviewed by QAU or a qualified responsible manager
- Investigation of data deviations ~~(if needed)~~

■(when required)■2S (*USP33*)

Change to read:

MAINTENANCE OF LABORATORY RECORDS

Proper recording of data and studies is critical to the success of the microbiology laboratory. The over-riding principle is that the test should be performed as written in the SOP, the SOP should be written to reflect how the test is actually performed, and the laboratory notebook should provide a record of all critical details needed to

■reconstruct the details of the testing and■2S (*USP33*)
confirm the integrity of the data. At a minimum, the laboratory write-up should include the following:

- Date
- Material tested
- Microbiologist's name
- Procedure number
- Document test results
- Deviations (if any)
- Documented parameters (equipment used, microbial stock cultures used, media lots used)
- Management/Second review signature

Every critical piece of equipment should be noted in the write-up, and all should be on a calibration schedule documented by SOP and maintenance records. Where appropriate, logbooks or forms should be available and supportive of the laboratory notebook records. Equipment temperatures (water baths, incubators, autoclaves) should be recorded and traceable.

The governing SOP and revision should be clearly noted in the write-up. Changes in the data should be crossed off with a single line and initialed. Original data should not be erased or covered over.

Test results should include the original plate counts, allowing a reviewer to recreate the calculations used to derive the final test results. Methods for data analysis should be detailed in cited SOPs.

All laboratory records should be archived and protected against catastrophic loss. A formal record retention and retrieval program should be in place.

Change to read:

INTERPRETATION OF ASSAY RESULTS

Analytical microbiological assay results can be difficult to interpret for several important reasons: (1) Microorganisms are ubiquitous in nature, and common environmental contaminants—particularly organisms associated with humans—predominate in many types of microbiological analysis; (2) the analyst has the potential to introduce contaminating organisms during sample handling or processing in the laboratory; (3) microorganisms may not be homogeneously distributed within a sample or an environment; and (4) microbiological assays are subject to considerable variability of outcome. Therefore, ~~minor~~

■apparent■2S (*USP33*)
differences from an expected outcome may not be significant.

Because of these characteristics of microbiological analysis, laboratory studies should be conducted with the utmost care to avoid exogenous contamination as previously discussed in this chapter. Equally important, results must be interpreted from a broad microbiological perspective, considering not only the nature of the putative contaminant, but the likelihood of that organism(s) surviving in the pharmaceutical ingredient, excipient, or environment under test. In addition, the growth characteristics of the microorganism should be considered (especially in questions of the growth of filamentous fungi in liquid media).

When results are observed that do not conform to a compendial monograph or ~~another~~

■■2S (*USP33*)
other established ~~quantitative target~~

■acceptance criteria,■2S (*USP33*)
an investigation into the ~~finding~~

■microbial data deviation (MDD)■2S (*USP33*)
is required. There are generally two distinct reasons for the observation of microbial contamination that does not comply with a target or requirement: There may be either a laboratory error or laboratory environmental conditions that produced an invalid result, or the product contains a level of contamination or specific types of contaminants outside established levels or limits. In either case, laboratory management and, in most cases, ~~general management~~

■the Quality Unit■2S (*USP33*)
should be notified immediately.

A full and comprehensive evaluation of the

■laboratory■2S (*USP33*)
situation surrounding the result should be undertaken. All microbiological conditions or factors that could bring about the observed condition should be fully considered, including the magnitude of the excursion compared to established limits or levels. ~~It is critical to know if the finding is statistically significant in light of assay variability~~

■In addition, an estimate of the variability of the assay may be required in order to determine whether the finding is significant.■2S (*USP33*)

The laboratory environment, the protective conditions in place for sampling, historical findings concerning the material under test, and the nature of the material, particularly with regard to microbial survival or proliferation in contact with the material, should be considered in the investigation. In addition, interviews with the laboratory analyst(s) may provide information regarding the actual conduct of the assay that can be valuable in determining the reliability of the result and in determining an appropriate course of action. If laboratory operations are identified as the cause of the nonconforming test outcome, then a corrective action plan should be developed to address the problem(s). Following the approval and implementation of the corrective action plan, the situation should be carefully monitored and the adequacy of the corrective action determined.

If assay results are invalidated on the basis of the discovery of an attributable error, this action must be documented. Laboratories also should have approved procedures for confirmatory testing (retesting), and if necessary, resampling where specific regulatory or compendial guidance does not govern the conduct of an assay investigation.

BRIEFING

⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles, *USP 32* page 720. Based on internal deliberations of the Expert Committee, revisions have been proposed to (1) eliminate the discussion of the now defunct nonharmonized sterility tests and the references to first and second stage sterility tests and (2) eliminate the older radiation sterilization guidance and direct the reader to relevant ISO standards.

(MSA: R.Tirumalai.) RTS—C73550

⟨1211⟩ STERILIZATION AND STERILITY ASSURANCE OF COMPENDIAL ARTICLES

Change to read:

This informational chapter provides a general description of the concepts and principles involved in the quality control of articles that must be sterile. Any modifications of or variations in sterility test procedures from those described under *Sterility Tests* ⟨71⟩ should be validated in the context of the entire sterility assurance program and are not intended to be methods alternative to those described in that chapter.

Within the strictest definition of sterility, a specimen would be deemed sterile only when there is complete absence of viable microorganisms from it. However, this absolute definition cannot currently be applied to an entire lot of finished compendial articles because of limitations in testing. ~~Absolute sterility cannot be practically demonstrated without complete destruction of every finished article.~~

■■2S (*USP33*)
The sterility of a lot purported to be sterile is therefore defined in probabilistic terms, where the likelihood of a contaminated unit or article is acceptably remote. Such a state of sterility assurance can be established only through the use of ~~adequate sterilization cycles and subsequent~~

■validated sterilization processes or■2S (*USP33*)
aseptic processing, if any, under appropriate current good manufacturing practice, and not by reliance solely on sterility testing. The basic principles for validation and certification of a sterilizing process are enumerated as follows:

1. Establish that the process equipment has the capability of operating within the required parameters.
2. Demonstrate that the critical control equipment and instrumentation are capable of operating within the prescribed parameters for the process equipment.
3. Perform replicate cycles representing the required operational range of the equipment and employing actual or simulated product. Demonstrate that the processes have been carried out within the prescribed protocol limits and finally that the probability of microbial survival in the replicate processes completed is not greater than the prescribed limits.
4. Monitor the validated process during routine operation. Periodically as needed, requalify and recertify the equipment.
5. Complete the protocols, and document steps (1) through (4) above.

The principles and implementation of a program to validate an aseptic processing procedure are ~~similar to~~

■substantially more extensive than■2S (*USP33*)
the validation of a sterilization process. In aseptic processing, the components of the final dosage form are sterilized separately and the finished article is assembled in an aseptic manner.

Proper validation of the sterilization process or the aseptic process requires a high level of knowledge of the field of sterilization and clean room technology. In order to comply with currently acceptable and achievable limits in sterilization parameters, it is necessary to employ appropriate instrumentation and equipment to control the critical parameters such as temperature and time, humidity, and sterilizing gas concentration, or absorbed radiation. An important aspect of the validation program in many sterilization procedures involves the employment of biological indicators (see *Biological Indicators* ⟨1035⟩). The validated and certified process should be revalidated periodically; however, the revalidation program need not necessarily be as extensive as the original program.

A typical validation program, as outlined below, is one designed for the steam autoclave, but the principles are applicable to the other sterilization procedures discussed in this informational chapter. The program comprises several stages.

The *installation qualification* stage is intended to establish that controls and other instrumentation are properly designed and calibrated. Documentation should be on file demonstrating the quality of the required utilities such as steam, water, and air. The *operational qualification* stage is intended to confirm that the empty chamber functions within the parameters of temperature at ~~all of the~~

■■2S (*USP33*)
key chamber locations prescribed in the protocol. It is usually appropriate to develop heat profile records, i.e., simultaneous temperatures in the chamber employing multiple temperature-sensing devices. A typical acceptable range of temperature in the empty chamber is $\pm 1^\circ$ when the chamber temperature is not less than 121°. The *confirmatory* stage of the validation program is the actual sterilization of materials or articles. This determination requires the employment of temperature-sensing devices inserted into samples of the articles, as well as *either* samples of the articles to which appropriate concentrations of suitable test microorganisms have been added, *or* separate biological indicators (BIs) in operationally fully loaded autoclave configurations. The effectiveness of heat delivery or penetration into the actual articles and the time of the exposure are the two main factors that determine the lethality of the sterilization process. The *final* stage of the validation program requires the documentation of the supporting data developed in executing the program.

It is generally accepted that terminally sterilized injectable articles or critical devices purporting to be sterile, when processed in the autoclave, attain a 10^{-6} microbial survivor probability, i.e., assurance of less than 1 chance in 1 million that viable microorganisms are present in the sterilized article or dosage form. With heat-stable articles, the approach often is to ~~considerably~~

■■2S (*USP33*)
exceed the critical time necessary to achieve the 10^{-6} microbial survivor probability (overkill)

■of any presterilization bioburden.■2S (*USP33*)
However, with an article where extensive heat exposure may have a damaging effect, it ~~may not be feasible to employ this~~

■will not be feasible to employ an■2S (*USP33*)
overkill approach. In this latter instance, the development of the sterilization cycle depends heavily on knowledge of the microbial burden of the product, based on examination, over a suitable time period, of a substantial number of lots of the presterilized product.

The D value is the time (in minutes) required to reduce the microbial population by 90% or 1 log cycle (i.e., to a surviving fraction of 1/10), at a specific temperature. Therefore, where the D value of a BI preparation of, for example,

■*Geo*■2S (*USP33*)

bacillus stearothermophilus spores is 1.5 minutes under the ~~total process parameters~~

■process conditions defined,■2S (USP33) e.g., at 121°, if it is treated for 12 minutes under the same conditions, it can be stated that the lethality input is 8D. The effect of applying this input to the product would depend on the initial microbial burden. Assuming that its resistance to sterilization is equivalent to that of the BI, if the microbial burden of the product in question is 10^2 microorganisms, a lethality input of 2D yields a microbial burden of 1 (10° theoretical), and a further 6D yields a calculated microbial survivor probability of 10^{-6}. (Under the same conditions, a lethality input of 12D may be used in a typical ''overkill'' approach.) Generally, the survivor probability achieved for the article under the validated sterilization cycle is not completely correlated with what may occur with the BI. For valid use, therefore, it is essential that the resistance of the BI be greater than that of the natural microbial burden of the article sterilized. It is then appropriate to make a worst-case assumption and treat the microbial burden as though its heat resistance were equivalent to that of the BI, although it is not likely that the most resistant of a typical microbial burden isolates will demonstrate a heat resistance of the magnitude shown by this species, frequently employed as a BI for steam sterilization. In the above example, a 12-minute cycle is considered adequate for sterilization if the product had a microbial burden of 10^2 microorganisms. However, if the indicator originally had 10^6 microorganisms content, actually a 10^{-2} probability of survival could be expected; i.e., 1 in 100 BIs may yield positive results. This type of situation may be avoided by selection of the appropriate BI. Alternatively, high content indicators may be used on the basis of a predetermined acceptable count reduction.

The D value for the

■*Geo*■2S (USP33) *bacillus stearothermophilus* preparation determined or verified for these conditions should be reestablished when a specific program of validation is changed. Determination of survival curves (see *Biological Indicators* ⟨1035⟩), or what has been called the fractional cycle approach, may be employed to determine the D value of the biological indicator preferred for the specific sterilization procedure. The fractional cycle approach may also be used to evaluate the resistance of the microbial burden. Fractional cycles are studied either for microbial count-reduction or for fraction negative achievement. These numbers may be used to determine the lethality of the process under production conditions. The data can be used in qualified production equipment to establish appropriate sterilization cycles. A suitable biological indicator such as the

■*Geo*■2S (USP33) *bacillus stearothermophilus* preparation may be employed also during routine sterilization. Any microbial burden ~~method for sterility assurance~~

■based sterilization process■2S (USP33) requires adequate surveillance of the microbial resistance of the article to detect any changes, in addition to periodic surveillance of other attributes.

Change to read:

METHODS OF STERILIZATION

In this informational chapter, five methods of terminal sterilization, including removal of microorganisms by filtration and guidelines for aseptic processing, are described. Modern technological developments, however, have led to the use of additional procedures. These include blow-molding (at high temperatures), forms of moist heat other than saturated steam and UV irradiation, as well as on-line continuous filling in aseptic processing. The choice of the appropriate process for a given dosage form or component requires a high level of knowledge of sterilization techniques and information concerning any effects of the process on the material being sterilized.[1]

[1] ~~A number of guidelines dealing particularly with the development and validation of sterilization cycles and related topics have been published. These include, by the Parenteral Drug Association, Inc. (PDA), Validation of Steam Sterilization Cycles (Technical Monograph No. 1), Validation of Aseptic Filling for Solution Drug Products (Technical Monograph No. 2), and Validation of Dry Heat Processes Used for Sterilization and Depyrogenation (Technical Monograph No. 3); and by the Pharmaceutical Manufacturers Association (PMA), Validation of Sterilization of Large Volume Parenterals—Current Concepts (Science and Technology Publication No. 25). Other series of technical publications on these subjects by the Health Industry Manufacturers Association (HIMA) include Validation of Sterilization Systems (Report No. 78-4.1), Sterilization Cycle Development (Report No. 78-4.2), Industrial Sterility: Medical Device Standards and Guidelines (Document #9, Vol. 1), and Operator Training . . . for Ethylene Oxide Sterilization, for Steam Sterilization Equipment, for Dry Heat Sterilization Equipment, and for Radiation Sterilization Equipment (Report Nos. 78-4.5 through 4.8). Recommended practice guidelines published by the Association for the Advancement of Medical Instrumentation (AAMI) include Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices—Process Design, Validation, Routine Sterilization (No. OPEO-12/81) and Process Control Guidelines for the Radiation Sterilization of Medical Devices (No. RS-P 10/82). These detailed publications should be consulted for more extensive treatment of the principles and procedures described in this chapter.~~

■Documents addressing the development and validation of sterilization cycles and related topics include, by the Parenteral Drug Association, Inc. (PDA), Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control (Technical Report No. 1); Process Simulation for Aseptically Filled Products (Technical Report No. 22); Sterilizing Filtration of Liquids (Technical Report No. 26); and Validation of Dry Heat Processes Used for Sterilization and Depyrogenation (Technical Monograph No. 3); and by the Pharmaceutical Manufacturers Association (PMA), Validation of Sterilization of Large-Volume Parenterals—Current Concepts (Science and Technology Publication No. 25). Other technical publications include Health Industry Manufacturers Association (HIMA), Validation of Sterilization Systems (Report No. 78-4.1); Sterilization Cycle Development (Report No. 78-4.2); Industrial Sterility: Medical Device Standards and Guidelines (Document #9, Vol. 1); and Operator Training . . . for Ethylene Oxide Sterilization, for Steam Sterilization Equipment, for Dry Heat Sterilization Equipment, and for Radiation Sterilization Equipment (Report Nos. 78-4.5 through 4.8). Recommended practice guidelines published by the Association for the Advancement of Medical Instrumentation (AAMI) include Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices—Process Design, Validation, Routine Sterilization (No. OPEO-12/81) and Process Control Guidelines for the Radiation Sterilization of Medical Devices (No. RS-P 10/82). Additional radiation sterilization content can be found in ISO 11137—Sterilization of Health Care Products—Requirements for Validation and Routine Control—Radiation Sterilization. These more detailed publications should be consulted for more extensive treatment of the principles and procedures described in this chapter.

■2S (USP33)

Steam Sterilization

The process of thermal sterilization employing saturated steam under pressure is carried out in a chamber called an autoclave. It is probably the most widely employed sterilization process.[2]

■■2S (*USP33*)
The basic principle of operation is that the air in the sterilizing chamber is displaced by the saturated steam, achieved by employing vents or traps. In order to displace air more effectively from the chamber and from within articles, the sterilization cycle may include air and steam evacuation stages. The design or choice of a cycle for given products or components depends on a number of factors, including the heat lability of the material, knowledge of heat penetration into the articles, and other factors described under the validation program (see above). Apart from that description of sterilization cycle parameters, using a temperature of 121°, the F_0 concept may be appropriate. The F_0, at a particular temperature other than 121°, is the time (in minutes) required to provide the lethality equivalent to that provided at 121° for a stated time. Modern autoclaves generally operate with a control system that is significantly more responsive than the steam reduction valve of older units that have been in service for many years. In order for these older units to achieve the precision and level of control of the cycle discussed in this chapter, it may be necessary to upgrade or modify the control equipment and instrumentation on these units. This modification is warranted only if the chamber and steam jacket are intact for continued safe use and if deposits that interfere with heat distribution can be removed.

Dry-Heat Sterilization

■/Depyrogenation■2S (*USP33*)

The process of thermal sterilization of Pharmacopeial articles by dry heat ~~is usually carried out by a batch process in an oven designed expressly for that purpose A modern oven is supplied with heated, filtered air, distributed uniformly throughout the chamber by convection or radiation and employing a blower system with devices for sensing, monitoring, and controlling the critical parameters. The validation of a dry-heat sterilization facility is carried out in a manner similar to that for a steam sterilizer described earlier. Where the unit is employed for sterilizing components such as containers intended for intravenous solutions, care should be taken to avoid accumulation of particulate matter in the chamber.~~

■may be carried out by a batch process in an oven designed expressly for that purpose or in a dry-heat tunnel in which glass containers move on a continuous basis through the system. A dry-heat sterilization/depyrogenation system is supplied with heated, HEPA filtered air, distributed uniformly throughout the unit by convection or radiation and employing a blower system with devices for sensing, monitoring, and controlling all critical parameters.■2S (*USP33*)

A typical acceptable range in temperature in the empty chamber is ±15° when the unit is operating at not less than 250°.

In addition to the batch process described above, ~~a continuous process is frequently employed to sterilize and depyrogenate glassware as part of an integrated continuous aseptic filling and sealing system. Heat distribution may be by convection or by direct transfer of heat from an open flame. The continuous~~

■the continuous-tunnel■2S (*USP33*) system usually requires a much higher temperature than cited above for the batch process because of a much shorter dwell time. However, the total temperature input during the passage of the product should be equivalent to that achieved during the chamber process. The continuous process also usually necessitates a rapid cooling stage prior to the aseptic filling operation. In the qualification and validation program, in view of the short dwell time, parameters for uniformity of the temperature, and particularly the dwell time, should be established.

~~A microbial survival probability of 10^{-12} is considered achievable for heat-stable articles or components. An example of a biological indicator for validating and monitoring dry-heat sterilization is a preparation of *Bacillus subtilis* spores. Since dry-heat is frequently employed to render glassware or containers free from pyrogens as well as viable microbes, a pyrogen challenge, where necessary, should be an integral part of the validation program, e.g., by inoculating one or more of the articles to be treated with 1000 or more USP Units of bacterial endotoxin. The test with *Limulus* lysate could be used to demonstrate that the endotoxic substance has been inactivated to not more than 1/1000 of the original amount (3 log cycle reduction). For the test to be valid, both the original amount and, after acceptable inactivation, the remaining amount of endotoxin should be measured.~~

■Because depyrogenation is a more rigorous challenge for dry-heat processing systems than biological indicator inactivation, it is generally not necessary to include BIs when validating dry-heat processes if validation of depyrogenation is demonstrated. A 3 log cycle reduction or greater is a suitable acceptance criterion for depyrogenation and when successfully demonstrated will ensure not only adequate depyrogenation of compendial articles but also sterilization. Depyrogenation tests are typically done using articles inoculated with reference standard endotoxin. Articles are then evaluated after exposure for residual levels of endotoxin using *Limulus* lysate-based assays.■2S (*USP33*)
For additional information on the endotoxin assay, see *Bacterial Endotoxins Test* ⟨85⟩.

Gas Sterilization

The choice of gas sterilization as an alternative to heat is frequently made when the material to be sterilized cannot withstand the high temperatures obtained in the steam sterilization or dry-heat sterilization processes. ~~The active agent generally employed in gaseous sterilization is ethylene oxide of acceptable sterilizing quality. Among the disadvantages of this sterilizing agent~~

[2] ~~An autoclave cycle, where specified in the compendia for media or reagents, is a period of 15 minutes at 121°, unless otherwise indicated.~~ ■■2S (*USP33*)

■The most commonly employed method of gaseous sterilization is ethylene oxide. Among the disadvantages of ethylene oxide■2S (*USP33*) are its highly flammable nature unless mixed with suitable inert gases, its mutagenic properties, and the possibility of toxic residues in treated materials, particularly those containing chloride ions. The sterilization process is generally carried out in a pressurized chamber designed similarly to a steam autoclave but with the additional features (see below) unique to sterilizers employing this gas. Facilities employing this sterilizing agent should be designed to provide adequate post sterilization degassing, to enable microbial survivor monitoring, and to minimize exposure of operators to the potentially harmful gas.[2]

~~Qualification~~

■Validation■2S (*USP33*) of a sterilizing process employing ethylene oxide gas is accomplished along the lines discussed earlier. However, the program is more comprehensive than for the other sterilization procedures, because in addition to temperature, the humidity, vacuum/positive pressure, and ethylene oxide concentration also require ~~rigid~~

■appropriate parametric■2S (*USP33*) control. An important determination is to demonstrate that all critical process parameters in the chamber are adequate during the entire cycle. Because the sterilization parameters applied to the articles to be sterilized are critical variables, it is frequently advisable to precondition the load to achieve the required moisture content in order to minimize the time of holding at the required temperature before placement of the load in the ethylene oxide chamber. ~~The validation process is generally made~~

■Validation is generally conducted■2S (*USP33*) employing product inoculated with appropriate BIs such as spore preparations of *Bacillus ~~subtilis~~*

■*atrophaeus.*■2S (*USP33*) For validation they may be used in full chamber loads of product, or simulated product. The monitoring of moisture and gas concentration requires the utilization of sophisticated instrumentation that only knowledgeable and experienced individuals can calibrate, operate, and maintain. ~~The BI may be employed also~~

■BIs may also be employed■2S (*USP33*) in monitoring routine runs.

As is indicated elsewhere in this chapter, the BI may be employed in a fraction negative mode to establish the ultimate microbiological survivor probability in designing an ethylene oxide sterilization cycle using inoculated product or inoculated simulated product.

One of the principal ~~limitations~~

■limiting factors■2S (*USP33*) of the ethylene oxide sterilization process is the limited ability of the gas to diffuse to the innermost product areas that require sterilization. Package design and chamber loading patterns therefore must be determined ~~so that there is minimal resistance to gas diffusion.~~

■to allow for necessary gas penetration. The reader is referred to ISO 11135 for a complete description of process development, validation, and routine control of ethylene oxide sterilization processes.■2S (*USP33*)

Sterilization by Ionizing Radiation

The rapid proliferation of medical devices unable to withstand heat sterilization and the concerns about the safety of ethylene oxide have resulted in increasing applications of radiation sterilization. ~~It is applicable also to drug substances~~

■This method may also be applicable to active pharmaceutical ingredients■2S (*USP33*) and final dosage forms. The advantages of sterilization by irradiation include low chemical reactivity, low measurable residues, and the fact that there are fewer variables to control. In fact, radiation sterilization is unique in that the basis of control is essentially that of the absorbed radiation dose, which can be precisely measured. ~~Because of this characteristic, new procedures have been developed to determine the sterilizing dose. These, however, are still under review and appraisal, particularly with regard to the need, or otherwise, for additional controls and safety measures.~~

■Dose-setting and dose-substantiation procedures are used to validate the radiation dose required to achieve a sterility assurance level.■2S (*USP33*) Irradiation causes only a minimal temperature rise but can affect certain grades and types of plastics and glass.

The two types of ionizing radiation in use are radioisotope decay (gamma radiation) and electron-beam radiation. In either case the radiation dose established to yield the required degree of sterility assurance should be such that, within the range of minimum and maximum doses set, the properties of the article being sterilized are acceptable.

■The reader is referred to ISO 11137-1, - 2, and -3 for a complete description of process development, validation, and routine control of ionizing radiation processes.■2S (*USP33*)

~~For gamma irradiation, the validation of a procedure includes the establishment of article-materials compatibility, establishment of product loading pattern and completion of dose mapping in the sterilization container (including identification of the minimum and maximum dose zones), establishment of timer setting, and demonstration of the delivery of the required sterilization dose. For electron-beam irradiation, in addition, the on-line control of voltage, current, conveyor speed, and electron-beam scan dimension must be validated.~~

~~For gamma radiation sterilization, an effective sterilizing dose that is tolerated without damaging effect should be selected. Although 2.5 megarads (Mrad) of absorbed radiation was historically selected, it is desirable and acceptable in some cases to employ lower doses for devices, drug substances, and finished dosage forms. In other cases, however, higher doses are essential. In order to validate the efficacy particularly of the lower exposure levels, it is necessary to determine the magnitude (number, degree, or both) of the natural radiation resistance of the microbial population of the product. Specific product loading patterns must be established, and absorbed minimum and maximum dosage distribution must be determined by use of chemical dosimeters. (These dosimeters are usually dyed~~

[2] See *Ethylene Oxide*, Encyclopedia of Industrial Chemical Analysis, 1971, *12*, 317–340, John Wiley & Sons, Inc., and *Use of Ethylene Oxide as a Sterilant in Medical Facilities*, NIOSH Special Occupational Hazard Review with Control Recommendations, August 1977, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Criteria Documentation and Standards Development, Priorities and Research Analysis Branch, Rockville, MD.

plastic cylinders, slides, or squares that show color intensification based directly on the amount of absorbed radiation energy; they require careful calibration.)

The setting of the preferred absorbed dose has been carried out on the basis of pure cultures of resistant microorganisms and employing inoculated product, e.g., with spores of *Bacillus pumilus* as biological indicators. A fractional experimental cycle approach provides the data to be utilized to determine the D_{10} value of the biological indicator. This information is then applied in extrapolating the amount of absorbed radiation to establish an appropriate microbial survivor probability. The most recent procedures for gamma radiation sterilization base the dose upon the radiation resistance of the natural heterogeneous microbial burden contained on the product to be sterilized. Such procedures are currently being refined but may provide a more representative assessment of radiation resistance, especially where significant numbers of radiation resistant organisms are present.[4] These range from inoculation with standard resistant organisms such as *Bacillus pumilus* to subprocess (sublethal) dose exposure of finished product samples taken from production lines. Certain hypotheses are common to all these methods. Although the total microbial population present on an article generally consists of a mixture of microorganisms of differing sensitivity to radiation, the step of subjecting the article to a less than totally lethal sterilization dose eliminates the less resistant microbial fraction. This results in a residual relatively homogeneous population with respect to radiation resistance and yields consistent and reproducible results of determinations with the residual population. The amount of laboratory manipulation required is dependent upon the particular procedure used.

One such procedure requires the enumeration of the microbial population on representative samples of independently manufactured lots of the article. The resistance of the microbial population is not determined, and dose setting is based on a standard arbitrary radiation resistance assigned to the microbial population, derived from data obtained from manufacturers and from the literature. The assumption is made that the distribution of resistances chosen represents a more severe challenge than the natural microbial population on the product to be sterilized. This assumption, however, is verified by experiment. After verification, the appropriate radiation sterilization dose is read from a table.

Another and, more elaborate method does not require the enumeration of the microbial population but uses a series of incremental dose exposures to allow a dose established to be such that approximately one out of 100 samples irradiated at that dose will be nonsterile. This is not the ultimate sterilization dose, but it provides the basis on which to determine the sterilization dose by extrapolation from the dose yielding one out of 100 nonsterile samples, using an appropriate resistance factor that characterizes the remaining microorganism resistant population. A periodic audit is conducted to check that the findings continue to be operative.

More elaborate procedures, requiring more experimentation and including the isolation of microbial cultures, include one in which, after determining the substerilization dose (yielding one out of 100 nonsterile samples), the resistance of the surviving microorganisms is used to determine the sterilizing dose. Another is based on different determinations, starting with a substerilization incremental dose that results in not more than 50% of the samples being nonsterile. After irradiation of sufficient samples at this dose, a number of microbial isolates are obtained. The radiation resistance of each of these is determined. The sterilization dose is then calculated using the resistance determinations and the 50% sterilizing dose initially determined. Audit procedures are required for these methods, as for the others described.

Where the required minimum radiation dose has been determined and delivery of that dose has been confirmed (by chemical or physical dosimeters), release of the article being sterilized could be effected within the overall validation of sterility assurance (which may include such confirmation of applied dosage, the use of biological indicators, and other means).

■■2S (*USP33*)

[4] Detailed descriptions of these procedures have been published by the Association for the Advancement of Medical Instrumentation (AAMI) in the document entitled Process Control Guidelines for Radiation Sterilization of Medical Devices (No. RS-P-10/82).

Sterilization by Filtration

Filtration through microbial retentive materials is frequently employed for the sterilization of heat labile solutions by physical removal of the contained microorganisms. A filter assembly generally consists of a porous matrix sealed or clamped into an impermeable housing. The effectiveness of a filter medium or substrate depends upon the pore size of the porous material and may depend upon adsorption of bacteria on or in the filter matrix or upon a sieving mechanism. There is some evidence to indicate that sieving is the more important component of the mechanism. Fiber shedding filters, particularly those containing asbestos, are to be avoided unless no alternative filtration procedures are possible. Where a fiber shedding filter is required, it is obligatory that the process include a nonfiber shedding filter introduced downstream or subsequent to the initial filtration step.

■The sterilization of fluids by filtration is a separative process and differs from the other methods of sterilization that rely on destructive mechanisms. Filtration through microbial retentive materials is frequently employed for the sterilization of heat-labile solutions by physical removal of the contained microorganisms. A filter assembly generally consists of a porous matrix integrated with or clamped into a housing. The effectiveness of a filter medium depends upon the pore size of the porous material, the prefiltration bioburden, and may depend upon adsorption of bacteria on or in the filter matrix or upon a sieving mechanism. There is some evidence to indicate that sieving is the more important component of the mechanism. Fiber-shedding filters, particularly those containing asbestos, are to be avoided unless no alternative filtration procedures are possible. Where a fiber-shedding filter is required, it is obligatory that the process include a nonfiber-shedding filter introduced downstream or subsequent to the initial filtration step.■2S (*USP33*)

Filter Rating— The pore sizes of filter membranes are rated by a nominal rating that reflects the capability of the filter membrane to retain microorganisms of size represented by specified strains, not by determination of an average pore size and statement of distribution of sizes. Sterilizing filter membranes (those used for removing a majority of contaminating microorganisms) are membranes capable of retaining 100% of a culture of 10^7 microorganisms of a strain of *Pseudomonas diminuta* (ATCC 19146) per square centimeter of membrane surface under a pressure of not less than 30 psi (2.0 bar). Such filter membranes are nominally rated 0.22 μm or 0.2 μm, de-

~~pending on the manufacturer's practice.[5] This rating of filter membranes is also specified for reagents or media that have to be sterilized by filtration (see treatment of Isopropyl Myristate under *Oils and Oily Solutions* or *Ointments and Creams* in the chapter *Sterility Tests* ⟨71⟩). Bacterial filter membranes (also known as analytical filter membranes), which are capable of retaining only larger microorganisms, are labeled with a nominal rating of 0.45 μm. No single authoritative method for rating 0.45-μm filters has been specified, and this rating depends on conventional practice among manufacturers; 0.45-μm filters are capable of retaining particular cultures of *Serratia marcescens* (ATCC 14756) or *Ps. diminuta*. Test pressures used vary from low (5 psi, 0.33 bar for *Serratia*, or 0.5 psi, 0.34 bar for *Ps. diminuta*) to high (50 psi, 3.4 bar). They are specified for sterility testing (see *Membrane Filtration* in the section *Test for Sterility of the Product to be Examined* under *Sterility Tests* ⟨71⟩) where less exhaustive microbial retention is required. There is a small probability of testing specimens contaminated solely with small microorganisms). Filter membranes with a very low nominal rating may be tested with a culture of *Acholeplasma laidlawii* or other strain of *Mycoplasma*, at a pressure of 7 psi (0.7 bar) and be nominally rated 0.1 μm. The nominal ratings based on microbial retention properties differ when rating is done by other means, e.g., by retention of latex spheres of various diameters. It is the user's responsibility to select a filter of correct rating for the particular purpose, depending on the nature of the product to be filtered. It is generally not feasible to repeat the tests of filtration capacity in the user's establishment. Microbial challenge tests are preferably performed under a manufacturer's conditions on each lot of manufactured filter membranes.~~

■The pore sizes of filter membranes are rated by a nominal rating that reflects the capability of the filter membrane to retain microorganisms of size represented by specified strains, not by determination of an average pore size and statement of distribution of sizes. Sterilizing filters cannot be narrowly defined because, depending upon the bioburden present in the fluid stream, different filters may be considered effective for sterilization. Currently a sterilizing filter can be defined as, ''a filter that, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent''. The nominal ratings of sterilizing filters based on microbial retention properties differ when rating is done by other means, e.g., by retention of latex spheres of various diameters. It is the user's responsibility to select a filter of correct rating for the particular purpose, depending on the nature of the product (especially considering its potential bioburden) to be filtered. It is not feasible to repeat the tests of filtration capacity in the user's establishment. Microbial challenge tests are preferably performed under a manufacturer's conditions on each lot of manufactured filter membranes.■2S (*USP33*)

The user must determine whether filtration parameters employed in manufacturing will significantly influence microbial retention efficiency. Some of the other important concerns in the validation of the filtration process include product compatibility, sorption of drug, preservative or other additives, and initial effluent endotoxin content.

Because the effectiveness of the filtration process is also influenced by the microbial burden of the solution to be filtered, determining the microbiological quality of solutions prior to filtration is an important aspect of the validation of the filtration process, in addition to establishing the other parameters of the filtration procedure, such as pressures, flow rates, and filter unit characteristics. Hence, another method of describing filter-retaining capability is the use of the log reduction value (LRV). For instance, a 0.2-μm filter that can retain 10^7 microorganisms of a specified strain will have an LRV of not less than 7 under the stated conditions.

~~The process of sterilization of solutions by filtration has recently achieved new levels of proficiency, largely as a result of the development and proliferation of membrane filter technology. This class of filter media lends itself to more effective standardization and quality control and also gives the user greater opportunity to confirm the characteristics or properties of the filter assembly before and after use. The fact that membrane filters are thin polymeric films offers many advantages but also some disadvantages when compared to depth filters such as porcelain or sintered material. Since much of the membrane surface is a void or open space, the properly assembled and sterilized filter offers the advantage of a high flow rate. A disadvantage is that since the membrane is usually fragile, it is essential to determine that the assembly was properly made and that the membrane was not ruptured during assembly, sterilization, or use.~~

■■2S (*USP33*)

The housings and filter assemblies that are chosen should first be validated for compatibility and integrity by the user. While it may be possible to mix assemblies and filter membranes produced by different manufacturers, the compatibility of these hybrid assemblies should first be validated. Additionally, there are other tests to be made by the manufacturer of the membrane filter, which are not usually repeated by the user. These include microbiological challenge tests. Results of these tests on each lot of manufactured filter membranes should be obtained from the manufacturer by users for their records.

Filtration for sterilization purposes is usually carried out with assemblies having membranes of nominal pore size rating of 0.2 μm or less.~~, based on the validated challenge of not less than 10^7 *Pseudomonas diminuta* (ATCC No. 19146) suspension per square centimeter of filter surface area. Membrane filter media now available include cellulose acetate, cellulose nitrate, fluorocarbonate, acrylic polymers, polycarbonate, polyester, polyvinyl chloride, vinyl, nylon, polytef, and even metal membranes, and they may be reinforced or supported by an internal fabric. A membrane filter assembly should be tested for initial integrity prior to use, provided that such test does not impair the~~

■A membrane filter assembly must be tested for initial integrity prior to use, provided that such test does not impair the safety, integrity, and■2S (*USP33*)
validity of the system, and should be tested after the filtration process is completed to demonstrate that the filter assembly maintained its integrity throughout the entire filtration procedure. Typical use tests are the bubble point test, the diffusive airflow test, the pressure hold test, and the forward flow test. These tests should be correlated with microorganism retention.

~~[5] Consult ''Microbiological Evaluation of Filters for Sterilizing Liquids,'' Health Industry Manufacturers Association, Document No. 3, Vol. 4, 1982.~~

▪Unidirectional ■2S (USP33)

Aseptic Processing

Although there is general agreement that sterilization of the final filled container as a dosage form or final packaged device is the preferred process for ensuring the minimal risk of microbial contamination in a lot, there is a substantial class of products that are not terminally sterilized but are prepared by a series of aseptic steps. These are designed to prevent the introduction of viable microorganisms into components, where sterile, or once an intermediate process has rendered the bulk product or its components free from viable microorganisms.

▪The fundamental difference between aseptically produced sterile products and terminally sterilized products is the presence of a step that can be validated, whereby the final package is subjected to conditions shown to kill viable contaminants. Consequently, an aseptically filled product labeled as sterile must use a system of risk assessments to establish that an acceptable level of sterility assurance has been achieved. Current technology cannot provide an adequate safety assessment based on individual unit testing. In currently used methods of environmental monitoring, process simulations have not been shown to correlate directly with contaminated finished products. Finished product destructive testing (sterility tests) can only examine a very small percentage of a lot and are thus only capable of detecting grossly contaminated lots.■2S (USP33)

This section provides a review of the principles involved in producing aseptically processed products with a minimal risk of microbial contamination in the finished lot of final dosage forms.

A product defined as aseptically processed is likely to consist of components that have been sterilized by one of the processes described earlier in this chapter. For example, the bulk product, if a filterable liquid, may have been sterilized by filtration. The final empty container components would probably be sterilized by heat, dry heat being employed for glass vials and an autoclave being employed for rubber closures. The areas of critical concern are the immediate microbial environment where these presterilized components are exposed during assembly to produce the finished dosage form and the aseptic filling operation.

The requirements for a properly designed, validated, and maintained filling or other aseptic processing facility are mainly directed to (1) an air environment ~~free from viable microorganisms,~~

▪that is suitably controlled with respect to viable and nonviable particulates,■2S (USP33)

of a proper design to permit effective maintenance of air supply units, and (2) the provision of trained operating personnel who are adequately equipped and gowned. The desired environment may be achieved through the high level of air filtration technology now available, which contributes to the delivery of air of the requisite microbiological quality.[3] The facilities include both primary (in the vicinity of the exposed article) and secondary (where the aseptic processing is carried out) barrier systems.

For a properly designed aseptic processing facility or aseptic filling area, consideration should be given to such features as nonporous and smooth surfaces, including walls and ceilings that can ~~be sanitized frequently;~~

▪withstand routine decontamination;■2S (USP33)
gowning rooms with adequate space for personnel and storage of sterile garments; adequate separation of preparatory rooms for personnel from final aseptic processing rooms, with the availability, if necessary, of devices such as airlocks and air showers; proper pressure differentials between rooms, the most positive pressure being in the aseptic processing rooms or areas; the employment of ~~laminar (unidirectional)~~

▪unidirectional■2S (USP33)
airflow in the immediate vicinity of exposed product or components, and filtered air exposure thereto, with adequate air change frequency; appropriate humidity and temperature environmental controls; and a documented sanitization program. Proper training of personnel in hygienic and gowning techniques should be undertaken so that, for example, gowns, gloves, and other body coverings substantially cover exposed skin surfaces.

Certification and validation of the aseptic process and facility are achieved by establishing the efficiency of the filtration systems, by employing microbiological environmental monitoring procedures, and by processing of sterile culture medium as simulated product.

Monitoring of the aseptic facility should include periodic ~~environmental filter examination as well as routine particulate and microbiological environmental monitoring and may include periodic sterile culture medium processing.~~

▪HEPA filter evaluation and testing, as well as routine particulate and microbiological environmental monitoring. Periodic media-fill or process-simulation testing should also be performed.■2S (USP33)

Change to read:

STERILITY TESTING OF LOTS

It should be recognized that the referee sterility test might not detect microbial contamination if present in only a small percentage of the finished articles in the lot because the specified number of units to be taken imposes a significant statistical limitation on the utility of the test results. This inherent limitation, however, has to be accepted, because current knowledge offers no nondestructive alternatives for ascertaining the micro-

[3] Available published standards for such controlled work areas include the following: (1) ~~Federal Standard No. 209B, Clean Room and Work Station Requirements for a Controlled Environment, Apr. 24, 1973.~~
▪ISO 14464 1-7 Cleanrooms and Associated Controlled Environments.■2S (USP33)
(2) NASA Standard for Clean Room and Work Stations for Microbially Controlled Environment, publication NHB5340.2, Aug. 1967. (3) Contamination Control of Aerospace Facilities, U.S. Air Force, T.O. 00-25-203, 1 Dec. 1972, change 1-1, Oct. 1974.

biological quality of every finished article in the lot, and it is not a feasible option to increase the number of specimens significantly.

■For information regarding the conduct of the sterility test please see *Sterility Tests* ⟨71⟩.■2S (*USP33*)

~~The primary means of supporting the claim that a lot of finished articles purporting to be sterile meets the specifications consists of the documentation of the actual production and sterilization record of the lot and of the additional validation records that the sterilization process has the capability of totally inactivating the established product microbial burden or a more resistant challenge. Further, it should be demonstrated that any processing steps involving exposed product following the sterilization procedure are performed in an aseptic manner to prevent contamination. If data derived from the manufacturing process sterility assurance validation studies and from in-process controls are judged to provide greater assurance that the lot meets the required low probability of containing a contaminated unit (compared to sterility testing results from finished units drawn from that lot), any sterility test procedures adopted may be minimal, or dispensed with on a routine basis. However, assuming that all the above production criteria have been met, it may still be desirable to perform sterility testing on samples of the lot of finished articles. Such sterility testing is usually carried out directly after the lot is manufactured as a final product quality control test.[7] Sterility tests employed in this way in manufacturing control should not be confused with those described under *Sterility Tests* ⟨71⟩. The procedural details may be the same with regard to media, inocula and handling of specimens, but the number of units and/or incubation time(s) selected for testing may differ. The number should be chosen relative to the purpose to be served, i.e., according to whether greater or lesser reliance is placed on sterility testing in the context of all the measures for sterility assurance in manufacture. Also, longer times of incubation would make the test more sensitive to slow-growing microorganisms. In the growth promotion tests for media, such slow growers, particularly if isolated from the product microbial burden, should be included with the other test stains. Negative or satisfactory sterility test results serve only as further support of the existing evidence concerning the quality of the lot if all the pertinent production records of the lot are in order and the sterilizing or aseptic process is known to be effective. Unsatisfactory test results, however, in manufacturing quality control indicate a need for further action (see *Performance, Observation, and Interpretation*).~~

~~Definition of a Lot and Selection of Specimens for Sterility Test Purposes~~

~~Articles may be terminally sterilized either in a chamber or by a continuous process. In the chamber process, a number of articles are sterilized simultaneously under controlled conditions—for example, in a steam autoclave—so that for the purpose of sterility testing, the lot is considered to be the contents of a single chamber. In the continuous process, the articles are sterilized individually and consecutively (for example, by exposure to electron-beam radiation), so that the lot is considered to be not larger than the total number of similar items subjected to uniform sterilization for a period of not more than 24 hours.~~

~~For aseptic fills, the term "filling operation" describes a group of final containers, identical in all respects, that have been aseptically filled with the same product from the same bulk within a period not longer than 24 consecutive hours without an interruption or a change that would affect the integrity of the filling assembly. The items tested should be representative of each filling assembly and should be selected at appropriate intervals throughout the entire filling operation. If more than three filling machines, each with either single or multiple filling stations, are used for filling a single lot, a minimum of 20 filled containers (not less than 10 per medium) should be tested for each filling machine, but the total number generally need not exceed 100 containers.~~

~~For small lots, in the case of either aseptic filling or terminal sterilization, if the number of final containers in the lot is between 20 and 200, about 10% of the containers should usually be tested. If the number of final containers in the lot is 20 or less, not fewer than 2 final containers should be tested.~~

■■2S (*USP33*)

Delete the following:

■PERFORMANCE, OBSERVATION, AND INTERPRETATION

~~The facility for sterility testing should be such as to offer no greater a microbial challenge to the articles being tested than that of an aseptic processing production facility. The sterility testing procedure should be performed by individuals having a high level of aseptic technique proficiency. The test performance records of these individuals should be documented.~~

~~The extensive aseptic manipulations required to perform sterility testing may result in a probability of non-product-related contamination of the order of 10^{-3}, a level similar to the overall efficiency of an aseptic operation and comparable to the microbial survivor probability of aseptically processed articles. This level of probability is significantly greater than that usually attributed to a terminal sterilization process, namely, 1 in 1 million or 10^{-6} microbial survivor probability. Appropriate, known-to-be-sterile finished articles should be employed periodically as negative controls as a check on the reliability of the test procedure. Preferably, the technicians performing the test should be unaware that they are testing negative controls. Of these tests, a false-positive frequency not exceeding 2% is desirable.~~

~~For aseptically processed articles, these facts support the routine use of the test set forth under *Sterility Tests* ⟨71⟩ or a more elaborate one. The production and validation documentation should be acceptable and complete. For effectively terminally sterilized products, however, the lower microbial survivor probability may direct the use of a less extensive test than the compendial procedure specified under *Sterility Tests* ⟨71⟩, or even preclude altogether the necessity for performing one. This added reliability of sterility assurance of terminal sterilization depends upon a properly validated and documented sterilization process. Sterility testing alone is no substitute.~~

~~**Interpretation of Quality Control Tests**—The overall responsibility for the operation of the test unit and the interpretation of test results in relation to acceptance or rejection of a lot should be in the hands of those who have appropriate formal training in microbiology and have knowledge of industrial sterilization, aseptic processing, and the statistical concepts involved in sampling. These individuals should be knowledgeable also concerning the environmental control program in the test facility to ensure that the microbiological quality of the air and critical work surfaces are consistently acceptable.~~

~~Quality control sterility tests (either according to the official referee test or modified tests) may be carried out in two separate stages in order to rule out false positive results. *First Stage.* Regardless of the sampling plan used, if no evidence of microbial growth is found, the results of the test may be taken as indicative of absence of intrinsic contamination of the lot.~~

~~If microbial growth is found, proceed to the *Second Stage* (unless the *First Stage* test can be invalidated). Evidence for invalidating a *First Stage* test in order to repeat it as a *First Stage* test may be obtained from a review of the testing environment and the relevant records thereto. Finding of microbial growth in negative controls need not be considered the sole grounds for~~

~~[7] *Radioactive Pharmaceutical Products*—Because of rapid radioactive decay, it is not feasible to delay the release of some radioactive pharmaceutical products in order to complete sterility tests on them. In such cases, results of sterility tests provide only retrospective confirmatory evidence for sterility assurance, which therefore depends on the primary means thereto established in the manufacturing and validation/certification procedures.~~

~~invalidating a *First Stage* test. When proceeding to the *Second Stage*, particularly when depending on the results of the test for lot release, concurrently, initiate and document a complete review of all applicable production and control records. In this review, consideration should be paid to the following: (1) a check on monitoring records of the validated sterilization cycle applicable to the product, (2) sterility test history relating to the particular product for both finished and in-process samples, as well as sterilization records of supporting equipment, containers/closures, and sterile components, if any, and (3) environmental control data, including those obtained from media fills, exposure plates, filtering records, any sanitization records and microbial monitoring records of operators, gowns, gloves, and garbing practices.~~

~~Failing any lead from the above review, the current microbial profile of the product should be checked against the known historical profile for possible change. Records should be checked concomitantly for any changes in source of product components or in processing procedures that might be contributory. Depending on the findings, and in extreme cases, consideration may have to be given to revalidation of the total manufacturing process. For the *Second Stage*, it is not possible to specify a particular number of specimens to be taken for testing. It is usual to select double the number specified for the *First Stage* under *Sterility Tests* ⟨71⟩, or other reasonable number. The minimum volumes tested from each specimen, the media, and the incubation periods are the same as those indicated for the *First Stage*.~~

~~If no microbial growth is found in the *Second Stage*, and the documented review of appropriate records and the indicated product investigation does not support the possibility of intrinsic contamination, the lot may meet the requirements of a test for sterility. If growth is found, the lot fails to meet the requirements of the test. As was indicated for the *First Stage* test, the *Second Stage* test may similarly be invalidated with appropriate evidence, and, if so done, repeated as a *Second Stage* test.~~ ■*2S (USP33)*

BRIEFING

⟨1235⟩ Vaccines for Human Use—General Considerations. Because of extensive changes in the previously proposed chapter (see page 1297 of *PF* 34(5) [Sept.–Oct. 2008]), that proposal is canceled, and a new draft is proposed for this general information chapter. The proposed general information chapter *Bovine Serum* ⟨1024⟩, referred to in the section *Fermentaion and Cell Culture Media*, can be viewed on page 628 of *PF* 35(3) [May–June 2009].

(BB VV: F. Atouf) RTS—C74087

Add the following:

■⟨1235⟩ VACCINES FOR HUMAN USE—GENERAL CONSIDERATIONS

INTRODUCTION

Vaccines have been used for centuries to immunize individuals against pathogenic organisms with the goal of preventing the associated disease. Vaccines are biological products that contain antigens capable of inducing a specific and active acquired immune response in the body. Antigens present in vaccines are processed by specialized cells in the body's immune system, resulting in the development of blood proteins known as antibodies (i.e., humoral immunity) or specialized lymphocytes (i.e., cell-mediated immunity) or both. Therefore immune responses may be antibody mediated, cell mediated, or both. Thus, antigens are critical for vaccine function and generally consist of a portion of the pathogenic organism, or an attenuated form of the whole microorganism. In the case of DNA-based vaccines (currently under development), the vaccine would contain nucleotide sequences (genetic material) that encode microbial antigens.

Examples of types of licensed vaccines appear in *Appendix 1*. A current list of vaccines licensed in the United States is posted at www.fda.gov/cber/.

Vaccines can be of various types, depending on their design and processes involved in their manufacture. Vaccines for human use may contain whole killed or attenuated organisms (e.g., bacteria or viruses) or contain antigens derived from portions of a pathogen, either by partitioning and purification or derived using recombinant technology (*Table 1*). Some polysaccharide vaccines are conjugated to a carrier in order to enhance their immune response.

Table 1. Bacterial and Viral Vaccines

Live attenuated whole cell or virus[a]
Inactivated/killed[b]
Whole cell or virus[c]
Recombinant proteins[d]
Subunit[e]
Polysaccharides
Proteins
Modified toxins

[a] Live attenuated bacterial or viral vaccines are weakened (attenuated) forms of a pathogen. They contain antigens that are similar to disease-causing microbes. They may be derived from the pathogen itself, or from a different organism that contains antigens that cross-react with the virulent microbe (e.g., vaccinia and variola).

[b] Inactivated bacterial and viral vaccines are produced by growing cells of disease-causing bacteria or viruses in cell substrates and subsequently inactivating them to prevent replication in the recipient.

[c] Inactivated/killed whole-cell or virus vaccines consist of the entire microorganisms after they have been inactivated. These preparations may or may not be partially or completely purified.

[d] Recombinant protein viral and bacterial vaccines are derived from host cells that have been transformed with expression vectors that carry genes that encode antigenic material from infectious agents. The expression cells are grown in bioreactors to produce the recombinant antigenic material.

[e] Subunit vaccines are extracts from inactivated/killed viruses or bacteria. Subunit-type vaccines generally undergo some degree of purification.

In addition to antigen(s), vaccines may contain several other components, such as adjuvants that enhance the immune response to the vaccine antigen, preservatives to prevent bacterial or fungal contamination of multiple-dose vials, or other excipients needed for pharmaceutical manufacturing or vaccine stabilization. Residual components from the manufacturing process also may be present in vaccine preparations. Examples of these categories are listed in *Table 2.*

Table 2. Vaccine Components

Antigens
Whole organisms
Components/subunits
Recombinant proteins
Adjuvants
Aluminum salts
Antimicrobial preservatives
Thimerosal
2-Phenoxyethanol
Benzethonium chloride
Phenol
Stabilizers
Salts
Amino acids
Sugars
Proteins
Other
Manufacturing residuals
Cell-derived residuals
Materials of animal origin
Antibiotic residuals
Inactivating chemical agents
Other

Different vaccine antigens are often combined in one final formulation in order to elicit immunity against multiple diseases and to reduce the number of separate administrations needed to achieve immunity to the various vaccine antigens.

Despite the multiple forms vaccines may take, several common features characterize the manufacture and testing of vaccines. This chapter focuses on commonalities throughout the manufacturing process, from raw material qualifications to final release tests.

Regulations and Standards

Vaccines are regulated by FDA as biological products. The general requirements are listed in national laws and international guidances. For the U.S., national requirements are codified in 21 CFR, the 200 and 600 sections, with additional recommendations available in FDA *Points*

to Consider and Guidance documents (www.fda.gov). International guidances are available from the International Conference on Harmonization (ICH) (www.ich.org; see *Appendix 2*) and the World Health Organization. New methodologies are continually being developed and validated and will be included in *USP* as they become available. Reference standards are available from USP and FDA.

OVERALL MANUFACTURING PLAN

When considering the overall plan for manufacturing a vaccine, manufacturers need to consider the following factors:

- Physical facilities;
- Raw materials and process aids;
- Actual manufacturing process, including
  a. initial process (production of virus/bacteria and recombinant materials);
  b. downstream processes (purification or chemical modification, if applicable);
- Antigen modifications such as conjugation or toxoiding;
- Storage of process intermediates and final bulk;
- In-process and final product testing regimens and control schemes;
- Addition of adjuvants, if applicable;
- Formulation and filling;
- Container–closure system; and
- Stability program that supports the dating period of the product.

Quality systems are needed to support the following manufacturing process development: specifications for raw materials, process intermediates, and final product; change control; and failure investigations and complaints. All of these elements are important in the life cycle of the vaccine product.

The overall goal of a comprehensive manufacturing program is to consistently produce a vaccine that is safe and effective. Concurrently with clinical development of the vaccine, the manufacturing process is refined and the process and testing methods are validated for consistency. This includes systems to control changes to the process or inputs. Manufacturers should expect that changes will be required during the vaccine's manufacturing life cycle, and manufacturers necessarily will use data from development and routine manufacturing to assess the process as well as proposed changes. The manufacturers should adopt systems that continually evaluate all aspects of manufacturing to identify unanticipated changes in vaccine quality and to assess them as quickly as possible.

Manufacturing Facilities and Systems

Manufacturers should have a general layout of manufacturing facilities, including diagrams that show the following: flow of raw materials and process inputs; movement of product, intermediates, waste streams, and personnel; and air flows and pressurization levels. These diagrams assist in minimizing the risk of potential product contamination from various sources. These sources can include cross-contamination from other products, contamination from different batches of the same product, and extraneous contamination from microorganisms and personnel. Evaluation of the flow diagrams can assist with strategies for development of engineering controls, personnel procedures, and monitoring systems to enable compliance with Good Manufacturing Practices (GMPs). Analysis of potential risks may also provide insights about what information should be recorded in batch documentation to facilitate consistent manufacture and also to facilitate failure investiga-

tions. Together, physical facilities, procedures, personnel, training, and quality systems make up the GMP environment in which a vaccine will be produced.

Manufacturing Process

The manufacturing process includes process inputs such as raw materials and processing aids and unit operations comprising both the initial and downstream processing steps. A process flow map for the manufacturing process is useful and assists in validation of the manufacturing process. This map shows all unit operations, the inputs to each operation, and the outputs to subsequent manufacturing steps. Analytical testing done at relevant steps and the specifications required to proceed to the next stage of processing may be added to the map. A process map also supports a processing space to facilitate a rugged process, i.e., one based on suitable characterization studies to establish boundaries within which manufacturing can occur to promote unchanged safety and efficacy outcomes.

The process flow map should include all steps from making the seed/cell bank (described below) to formulation and filling of the final product. The validation strategy should include the steps that require validation, along with identification of the process space, associated critical process parameters (CPP), and critical quality attributes (CQA). The critical process parameters are those that directly affect core quality attributes needed to successfully manufacture a batch of product. Some manufacturers identify other processing parameters that are important for processing but do not affect critical quality attributes. These important but noncritical factors help identify the process development space, can contribute to the development of a rugged process, or can be useful when the company assesses processing deviations. The concepts of quality by design and exploration of the process space are relatively new to the biologics/vaccine industry but are becoming considerations for the overall development-planning process.

Manufacturing Surveillance

Manufacturing surveillance is the continual observation of how the process and the resulting product are performing. This section is not exhaustive; rather, the points raised here outline the types of considerations recommended for a manufacturer during development of a vaccine. Manufacturing surveillance includes the following:

- Periodic review of the performance of the manufacturing process;
- Analytical assays;
- Stability programs;
- Product complaints;
- Adverse event reports;
- Product failure investigations;
- Atypical or deviation events.

Taken as a whole, these activities allow a manufacturer to assess the state of the process and product and to evaluate which, if any, operations need to be modified. These same systems also provide a surveillance matrix to evaluate changes. In any of these programs it is also valuable to develop additional characterization assays that are not used for process intermediate or product release purposes but may be used for further evaluation when additional information is needed or desired. These additional assays for characterization are often based on different underlying analytical procedures to provide different ways to evaluate materials.

Routine surveillance processes are increasingly implemented to attempt to detect changes in processes before any critical quality attributes are adversely affected. Not all vaccine processes can be characterized to the same extent or level (e.g., a live virus vaccine vs. a recombinant

protein vaccine), and statistical tools are often used to determine alert or action levels in surveillance programs. Exceeding these levels requires the manufacturer to evaluate the situation but does not necessarily signal product failure.

GMP manufacturing entails facility design, process development, quality systems, and manufacturing surveillance. Together these systems help the manufacturer to control the production of a vaccine. As noted, many types of vaccine are marketed, and each has its unique features and therefore requires different plans for each of the steps mentioned in this section.

SEED LOT SYSTEMS

Seed lots are the stocks of specific strains of bacteria, viruses, or biotechnology-engineered cells used to express vaccine antigens. All seed lots should be documented in terms of their isolation, derivation (or construction, in the case of recombinant vector or engineered cells), and passage history. The purpose of a seed-lot system, which typically includes master and working stock seeds, and associated master and working cell banks, is to help ensure the consistency of vaccine manufacturing. The use of master and working seed lots provides a method to limit the replication of the seed and to minimize the possibility of genetic variation.

A master seed lot is a physically homogeneous preparation derived from an original seed processed at one time and passaged for a limited number of times. The master seed lot is characterized for its biological, biochemical and genetic characteristics, and to ensure its purity, its freedom from adventitious agents, and its clinical ability to produce an effective vaccine.

Cultures from the working seed lot should have the same characteristics as the master seed lot from which they are derived. For influenza vaccines, which may be reformulated with new virus antigens each year, certified seed lots can be obtained from national regulatory agencies.

A working seed lot is derived from the master seed within a limited number of passages. The working seed is tested to ensure its purity, freedom from adventitious agents, and biochemical properties. The working seed is used for production of vaccine without intervening passages.

Bacterial Vaccine Seed Lot System

In the bacterial seed lot system, a master seed is subcultured to produce a working seed one passage beyond the master seed. An aliquot of the working seed is then expanded to produce a vaccine lot. The strain(s) used for the master seed lots are identified by historical records that include information about their origin. Information about the bacterial seed lot system should include source, passage history, and raw materials to which it was exposed, with specific emphasis on raw materials of ruminant origin. Seeds should be stored at an appropriate temperature in more than one location within a facility or at a distant site in order to decrease catastrophic risk.

Identity tests may include inoculation onto suitable biochemical media, Gram stains, genotype, and serological identification with suitable specific antisera. Special tests may be added, for example, to show culture viability but also lack of virulence.

Purity of the bacterial strains used for seed lots is verified by methods of suitable sensitivity to ensure that no adventitious agents are present. These purity tests often are performed in the presence of the seed under conditions where growth is inhibited by the presence or the absence of specific nutrients. Streaking can also be used to show that the cultured seed is a pure culture.

Viral Vaccine Seed Lot System

The derivation and passage history of viral seeds should be recorded in detail. Any manipulation of the viral phenotype (e.g., cold adaptation, development of temperature sensitivity, or attenuation of virulence) or intentional genetic manipulations (e.g., reassortment or recombination) should be documented.

These viral seeds are commonly differentiated into a master viral seed and working viral seeds or working viral stock. Viral seeds should be stored at cryogenic temperatures to promote stability and in more than one location within a facility or at a distant site to decrease catastrophic risk. Manufacturers should assess the following characteristics of the viral seed stock:

- Growth characteristics on the intended production cell substrate,
- Tissue tropism;
- Genetic markers;
- Identity (for recombinant vectors);
- Viability during storage,
- Genetic stability through production;
- Attenuation properties;
- Purity;
- Absence of adventitious agents. If attenuation or derivation is achieved by passage through different species, the viral seed should be assessed for absence of adventitious agents common to those species.

The master viral seed should be extensively characterized to demonstrate the stability of genotype and phenotype for a number of passages beyond the level used in production. Generally, during assessment of genetic stability, a master seed undergoes a minimum of five passes beyond the passage that will produce the final vaccine.

Tests should be performed for identity (e.g., sequencing the entire virus or a portion of it), adventitious agents, viral phenotype, genetic stability, and, if applicable, agents that might be present in the seed as a result of its passage history). Viral phenotype can be assessed further for tissue tropism, attenuation properties, and temperature sensitivity. Not all of these tests may be necessary for every viral seed strain.

In some cases the viral seeds may have a broad host range and therefore may require neutralization of the vaccine virus before they are tested for adventitious agent(s). If possible, testing for adventitious agents should be done without neutralization in order to avoid an antiserum that may inadvertently neutralize an adventitious agent present in the seed. Sometimes it is not possible to effectively neutralize a viral seed, and in such cases alternative strategies can be used. For example, the test can be performed in a cell substrate that does not permit replication by the vaccine virus. However, such a substitution of the substrate cell may compromise the test's sensitivity for detection of other adventitious agents. Therefore, the tests may be supplemented with use of polymerase chain reaction (PCR) assays.

Assessment of neurovirulence may be appropriate if the virus is known to be neurotropic. Manufacturers should consult with regulators about appropriate animal models, methods, and scoring systems for this assessment before they initiate such studies. For viruses that are neurovirulent or may revert to neurovirulence (e.g., polioviruses), it may be necessary to assess neurovirulence beyond the master seed.

If the master viral seed is well characterized, the working viral seed may not require extensive characterization. For example, it may not be necessary to repeat testing for all the relevant viruses from the derivation history.

Systems for Biotechnology-Engineered Vaccines

For a vaccine produced via a biotechnology-engineered cell-expression system, a master seed lot or a master cell bank will be established during product development. The seed lot or cell banks should be homogenous, which is often accomplished by limiting dilu-

tions. The seed lot or cell bank system should be characterized in a manner analogous to that used for the cell substrate discussed in the next section, and additional tests can be used to demonstrate the genetic stability of the expression system.

FERMENTATION AND CELL CULTURE MEDIA

A medium is the material in which an organism is grown and amplified in quantity to produce mass material for vaccine production. Its composition is diverse and depends on the cell types that the medium supports, ranging from well-defined chemical media to chemically undefined media that contain natural components such as sera from animal origin (see *Bovine Serum* ⟨1024⟩). Culture media should be suitable for their intended purpose and should be free from adventitious agents and known undesirable components such as toxins, allergens, and similar compounds. If undefined ingredients are necessary, the amount should be kept below levels that are demonstrated to be safe for the final product.

Fermentation Media for Bacterial Growth

The nutrients consist of materials like proteins, sugars, inorganic trace elements, amino acids, and vitamins needed for bacterial growth. The protein component may be as simple as free casein (milk protein), or it can be as complex as extracts from bacterial, plant, or animal sources. Any fermentation nutrients of animal origin are sourced carefully and tested for adventitious agents. The composition of a medium is often customized to optimize product quality attributes. Medium components that are known to cause allergic reactions should be avoided.

Media for Cell Culture for Viral Vaccines

The types and composition of media used for isolation and all subsequent culture of components of viral vaccines need to be recorded in detail. Chemically defined media without materials of animal origin are preferred. The medium should be tested for sterility and suitability for the cells used in product production. If materials of animal origin are used, they are assessed for freedom from adventitious agents. If human albumin is used in a U.S.-licensed vaccine, it must be licensed by FDA. The final product should be within specified limits of residual medium components such as serum, antibiotics, selection agents or reagents added for growth enhancement.

Media for Biotechnology-Engineered Cells

The requirement for media used for the fermentation and propagation of biotechnology-engineered cells is the same as that noted above for bacterial fermentation and cell culture growth.

PROPAGATION AND HARVEST

The propagation and harvest phases follow the manufacturing process from the initiation of cell growth in the working cell bank to the separation of the crude drug substance. In addition, in these manufacturing process steps, raw materials, media, and solutions should be qualified for their intended use. Batch numbers should be clearly assigned as needed, and the relationship between component harvests and batches of individual drug substances should be recorded clearly.

Propagation and Harvest for Bacterial Vaccines

Propagation of bacteria for bacterial vaccines is performed under specified conditions for the inoculum preparation and the fermentation phases. In-process

monitoring and testing should be conducted for quality assurance. All controls and testing performed after production (e.g., purity, viability, antigen yield, and phenotypic identity) should be documented. The first step of drug-substance recovery is harvesting from the bioreactor. A variety of equipment is available, and the process equipment used depends on the nature of the process. Procedures should be established to ensure containment and prevention of contamination during harvesting and to monitor bioburden (including acceptance criteria) or sterility. The storage conditions and the stability time limit for the harvest material should be described. For most bacterial vaccines, an inactivation step is necessary. Personnel involved in bacterial inactivation should consider the following: how cell culture purity is verified after inactivation, whether culture purity should be defined before inactivation, choice of the inactivation agent, and validation of the procedure(s).

Propagation and Harvest for Viral Vaccines

The manufacturing of viral vaccines using eukaryotic cell culture includes a two-phase production process. The first is the expansion of the cell cultures used as a substrate for viral replication. The second phase includes the initial virus infection and subsequent replication and virus production.

Cell Substrate Growth Phase—The cell substrate expansion process for viral production is the phase designed to prepare the cells in a physiological state appropriate to sustain virus growth. Cell substrates often require complex animal-derived supplements such as serum. The source and testing requirements of bovine serum are subject to regulatory requirements (see *Bovine Serum* ⟨1024⟩).

Virus Production Phase—Relatively few cell types have been used as substrates in U.S.-licensed viral vaccines, but these include primary cells (e.g., certain cells derived from monkey, chick, or mouse tissue), diploid cell lines (e.g., WI38, MRC-5, or FRhL-2), and continuous cell lines (e.g., Vero). Vaccine manufacturers have optimized nutrient requirements, growth factors, and serum concentration to support robust growth and strong virus productivity for these cell lines.

PURIFICATION

The objective of the purification steps is to remove as much as possible of the impurities in the initial harvest and to maximize the purity of the final vaccine product. Process residuals may consist of materials from the culture medium and/or cellular components. Purification procedures should be optimized and validated. When applicable, viral clearance steps (viral removal or inactivation) should be included and validated using relevant model viruses. Special considerations are observed depending on the types of vaccines and production system used, as discussed below.

Bacterial Fermentation

Bacterial fermentations are typically highly productive and yield large amounts of biomass. For bacterial subunit products or recombinant components expressed by bacteria, fermentation can produce very high concentrations of the desired active ingredient. Manufacturers should initiate culture purity testing before further processing.

Live Bacterial Vaccines—Live bacterial vaccines such as Bacillus Calmette-Guérin (BCG) and *Salmonella typhi* Ty21a are relatively fragile as pharmaceutical products and therefore tolerate only fairly gentle purification approaches. If osmotic and shear forces are constrained, then the integrity of the bacteria usually can be maintained.

Inactivated Bacterial Vaccines—At present no inactivated whole-cell bacterial vaccines are licensed for use in the U.S.

Purified Bacterial Antigens—Purification of bacterial components (e.g., proteins, toxins, and polysaccharides) generally requires cell disruption. More selective purification methods can be used to remove culture media and bacterial impurities and to achieve high purity of the target bacterial component.

Biotechnology-Engineered Cells

Of special concern in the purification of recombinant-derived vaccine components is the issue of residual host cell components that could produce an adverse immunogenic response in patients. This response could be exacerbated by the presence of vaccine adjuvants.

Recombinant Virus-Like Particles (VLP)—Formation of VLPs can coincidentally result in incorporation of host cell components (e.g., DNA) into the quaternary structure of the molecular assembly, resulting in a class of impurities that has a tight association with the active pharmaceutical ingredient. As a result, modern approaches to VLP production in some cases include a disassembly step that dissociates impurities from the viral proteins. This procedure is followed by a reassembly step that reforms the VLPs in the absence of the host components. Liquid-phase extractions and chromatographic procedures can be used to provide high-purity components for use in vaccine products with no substantial risk of carrying over significant residual host components.

Viral Vaccines Derived from Cell Culture

Viral Vaccines Derived from Continuous Cell Lines—If a continuous cell line (e.g., Vero) is used for vaccine production, a validated filtration step is necessary to separate virus from intact cells. The quantity and size of any residual host cell DNA also should be determined (see general information chapter *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)* ⟨1130⟩). Currently, 10 ng of host cell DNA is permitted per dose of a parenterally administered vaccine, and regulatory agencies continue to consider on a case-by-case basis the level of risk posed by host cell DNA for vaccines that are administered by other routes (e.g., nasal or oral). Multiple purification methods to reduce the size and amount of residual host-cell DNA present in the vaccine are desirable and include steps such as treatment with DNAse, diafiltration, ultrafiltration, and column chromatography.

Viral Vaccines Derived from Human Diploid Cell Culture—FDA has licensed several vaccines made using human diploid cells. The two most commonly used diploid cell lines are MRC-5 and WI-38, both of which are derived from human embryonic cells and have the normal diploid number of human chromosomes. They are widely used to manufacture vaccines because they have been shown to have no tumorigenic or oncogenic potential and have been shown to be susceptible to a wide range of human viruses. However, unlike continuous cell lines that can be passaged indefinitely, human diploid cell lines are capable of attaining only a certain number of population doublings, after which they experience a rapid decline in their ability to proliferate. This issue is managed by freezing multiple aliquots of master and working cell banks.

Viral Vaccines Derived from Primary Cell Culture—Like diploid cells, primary cells normally are not tumorigenic or oncogenic. However, when primary cells are used to manufacture live vaccines, the donor animals from which the primary cells are obtained are extensively tested for a variety of pathogens before being used. For example, chicken flocks used to prepare chicken embryo kidney cells undergo extensive serological testing for adventitious agents before the flock can be used to prepare

the cells. Some of these tests are described in the Code of Federal Regulations (CFR, see the sections listed in *Appendix 2*) and the USP general information chapter *Virology Test Methods* ⟨1237⟩.

Viral Vaccines Derived from Chicken Eggs

The embryonated chicken egg is a highly productive growth substrate for certain viruses, such as those used to make vaccines for yellow fever and several influenza vaccines. In the case of influenza vaccines, vaccine virus is harvested from egg allantoic fluid. In the case of yellow fever vaccine, the vaccine virus is harvested from embryo tissues. Therefore, residual egg or embryo components are special considerations in vaccine purification.

Egg-based vaccine production, like all biomass expansions, requires care and quality control of the virus seed lots and egg substrates to avoid contamination with other organisms.

Live Attenuated Virus Vaccines—Viruses for live vaccines (e.g., yellow fever or live influenza) are produced using Specific Pathogen-Free (SPF) eggs. These eggs are produced by chicken flocks that are regularly screened for avian pathogens (e.g., avian leukosis virus) and are maintained using appropriate animal husbandry practices. To preserve the infectivity and antigenic integrity of the vaccine viruses while removing egg-derived components, relatively simple, mild methods (e.g., zonal sucrose gradient centrifugation and diafiltration) are used for vaccine virus concentration, purification, and buffer exchange.

Inactivated Whole Virus Vaccines—Viruses for inactivated vaccines can be produced using non-SPF eggs because of required chemical inactivation steps in the manufacturing process. Because the vaccine virus needs to be retained intact while removing egg-derived components and inactivating chemicals, relatively mild purification and concentration methods (e.g., zonal sucrose gradient centrifugation) are used. If chemical agents are used in the process, they should be minimized in the final product to below prespecified levels.

Split Virus and Purified Subunit Vaccines—Viruses for split virus and purified subunit influenza vaccines are produced in non-SPF embryonated eggs. Inactivation and purification of vaccine viruses are achieved by chemical treatment (e.g., formaldehyde or β-propiolactone) and zonal sucrose gradient centrifugation, respectively. Split virus vaccines are prepared by disruption of vaccine virus particles using a detergent (e.g., sodium deoxycholate) that preserves antigenic integrity.

INTERMEDIATES

Intermediates are defined here as the unformulated active (immunogenic) drug substances that are processed before final formulation and can be stored for long periods of time before further processing. These intermediates can be stored and should be included in a formal stability program. Examples of intermediates include bulk polysaccharides, purified recombinant proteins (concentrates), and conjugates.

Production of Intermediates

Intermediates are manufactured from starting materials by one or a combination of different processes (e.g., fermentation, cultivation, isolation, or synthesis). Subsequent steps of the procedure involve preparation, characterization, and purification, eventually resulting in the drug substance. Quality systems documents are adopted for production and all applicable information should be recorded in a controlled document (i.e., a batch record). When applicable, stability studies and release tests should be performed before proceeding to the next steps (see below).

Tests for Intermediates

The quality attributes of the intermediate are commonly tested in conjunction with further processing. Characterization beyond release testing should be considered. Characterization methods can use appropriately qualified procedures. Some tests are routinely performed before the intermediates are converted to the final bulk, depending on individual vaccines.

If intermediates need to be stored and/or subsequently shipped to a different location for further processing, the stability of these materials should be demonstrated. Stability tests can be a combination of both physicochemical analysis and biological assays.

FINAL BULK

Final bulk is the bulk drug product that contains the drug substance(s), excipients, and other ingredients at desired concentrations and is ready for filling into individual containers.

Production of Final Bulk

Appropriately controlled amounts of all ingredients are blended to uniformity to produce the final bulk. The processing may include one or more steps such as buffer exchange and addition of diluents, bulking agent, stabilizing excipients, adjuvants, and preservatives. Final bulk may be prepared aseptically or processing may include a sterilization step.

Tests for Final Bulk

The quality attributes of the final bulk should be tested. Appropriate testing should be performed with respect to identity, purity, potency, sterility (see *Sterility Tests* ⟨71⟩), and antimicrobial effectiveness (see *Antimicrobial Effectiveness Testing* ⟨51⟩). Tests demonstrating safety, if applicable, are performed. The list includes, for example, tests for the absence of adventitious agents, mycoplasma, and other microorganisms.

Testing is required for specific process-related and product-related impurities, depending on the vaccines being manufactured. In addition, tests are required for the bulking agent, stabilizing excipients, adjuvants, and/or preservatives, if used. All the testing should be done according to respective standard operating procedures (SOPs), and all tests should have specifications (or provisional specifications, where applicable).

Stability Test for Final Bulk

If final bulks are stored and/or subsequently shipped to a different location for further processing, the stability of these materials should be demonstrated. Stability tests can be a combination of both physicochemical analysis and biological assays. Implementation of a stability program is required for formal stability studies, and the studies should be executed according to a protocol that contains detailed information about types of tests, including specifications, testing intervals, and data and analysis.

FINAL CONTAINER

A final container of vaccine contains the active ingredient(s) (i.e., antigen(s)) as well as additional components, such as stabilizers, adjuvants, or antimicrobial preservatives. They also may include residual materials from the manufacturing process.

Excipients and Other Additives

In addition to specific antigens, vaccines often include excipients and other additives that are intentionally added to the vaccine by the manufacturer for a specific purpose. These include adjuvants, antimicrobial

preservatives, and stabilizers. Vaccines also contain manufacturing residuals, which are trace amounts of various components used during manufacturing. Thus, the combinations of these components comprise and define the complete vaccine product. Manufacturers must adhere to regulations governing permissible limits of such components, as indicated in the product's license.

Adjuvants—Adjuvants are agents incorporated into vaccine formulations to enhance and increase the immune responses generated by the vaccine antigens. Specifically, they can increase the amount of antibody produced, direct the immune response (Th1 or Th2), increase the duration of antibody presence (persistence), or produce a combination of these effects.

Aluminum compounds have long been the most widely used adjuvants worldwide. Two methods traditionally have been used for combining aluminum adjuvant to antigen to form aluminum-adsorbed vaccines. The first involves the addition of the antigen solution to preformed aluminum precipitate. The second involves the addition of an antigen to aluminum in solution and the addition of a compound that will coprecipitate the aluminum salt and the antigen in situ. Solutions of aluminum potassium sulfate, known as alum or aluminum chloride, have been used together with phosphate salts as precipitating agents. A number of aluminum adjuvant formulations are used in vaccines.

Tests for aluminum are based on metal detection tests described in the general test chapter *Aluminum* ⟨206⟩. Regulations limit the amount of aluminum permitted in a dose of vaccine. The Code of Federal Regulations [21 CFR 610.15(a), *Ingredients, preservatives, diluents, adjuvants*] states that ''the amount of aluminum in the recommended individual dose of a biological product shall not exceed:

1. 0.85 milligrams if determined by assay;
2. 1.14 milligrams if determined by calculation on the basis of the amount of aluminum compound added; or
3. 1.25 milligrams determined by assay provided that the data demonstrating that the amount of aluminum used is safe and necessary to produce the intended effect and are submitted to and approved by the Director, CBER [Center for Biologics Evaluation and Research at FDA].''

The third criterion above aligns U.S. regulations with World Health Organization guidance for aluminum content in a single human dose of a vaccine product.

Note that adjuvants are not licensed by themselves; they do not constitute a product. Rather, a vaccine consisting of specific antigen(s) and an adjuvant are licensed together as a drug product.

Antimicrobial Preservatives—In the case of multiple-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeated puncture of multidose vials. With certain exceptions, a preservative is required to be present in vaccines marketed in multidose containers [21 CFR 610.15(a)]. Exceptions include yellow-fever vaccine; measles, mumps, and rubella (MMR); and dried vaccines when the accompanying diluent contains a preservative.

The microbial preservatives currently used in vaccines are thimerosal, 2-phenoxyethanol, benzethonium chloride, and phenol. These agents must pass the appropriate antimicrobial effectiveness test, as described in *Antimicrobial Effectiveness Testing* ⟨51⟩. Antimicrobial test challenges should be conducted as part of the normal formal stability program, including at expiration date. Various tests for preservatives can be found in *Antimicrobial Agents—Content* ⟨341⟩.

Stabilizers—The primary purpose of stabilizers is to protect certain vaccines from adverse conditions such as heat or to serve as a cryopreservative during the lyophilization process, usually the freezing step. The particular

materials chosen for this purpose include sugars (e.g., sucrose or lactose), amino acids (e.g., glycine or glutamic acid [monosodium salt]), glycerol, and proteins (e.g., human serum albumin [HSA] or gelatin). Materials should be customized to a specific vaccine formulation and selected with patient safety in mind.

When a protein is chosen as a stabilizer, two main safety concerns arise. One stems from the source of the protein: animal or human origin raises the possibility of the presence of an adventitious agent. The second concern is the possibility of an allergic reaction in persons sensitized to that protein. This should be evaluated as part of the clinical program during vaccine development. At present two proteins are used as stabilizers for vaccines: HSA and gelatin. FDA requires that any serum-derived albumin used in manufacturing be U.S.-licensed HSA. FDA guidance further recommends that a statement indicating the source and related risks appear in the ''Warnings'' section of the labeling for HSA-containing products.

Gelatin or processed gelatin also is used as a vaccine stabilizer. The gelatin source may be either bovine or porcine. Although the conditions of manufacturing gelatin are harsh (i.e., the product is subjected to extremes of heat and pH), there remains a concern with bovine sources about the presence of the transmissible spongiform encephalopathy (TSE) agent, because this agent is known to resist such conditions. Therefore, if gelatin added to a vaccine or used in manufacturing is from a bovine source, the material should have the appropriate documentation certifying that it comes from a country or region that is in compliance with TSE guidance for industry.

Manufacturing Residuals—Vaccines may contain residual amounts of any of the materials used in the manufacturing process. These materials are termed manufacturing residuals. As a general principle, it is not possible to remove a particular substance completely, nor is it possible to conclusively demonstrate that a particular substance has been completely removed. Therefore the goal is to reduce these substances to an undetectable level, using a sensitive and validated analytical methodology. Some products are tested for pyrogenic substances as a manufacturing residual (see *Pyrogen Test* ⟨151⟩); and, if the product is freeze-dried, it should be tested for residual moisture (see *Loss on Drying* ⟨731⟩). Residual levels of manufacturing materials, including, if applicable, inactivating agents, should be justified. The release specifications of these components are required as part of the approved license.

Cell-Derived Residuals—Live attenuated bacterial vaccines are not usually subject to a high degree of post-expansion purification. But killed bacterial component vaccines typically undergo significant purification to reduce cell-derived residuals. Common cellular components to be reduced are proteins, nucleic acids, and polysaccharides. Assays for these components are routinely conducted, if appropriate, to ensure purity. A common residual in bacterial vaccines made from Gram-negative bacteria is lipopolysaccharide (LPS), commonly known as endotoxin. Endotoxin testing is performed during the manufacturing process for any Gram-negative bacterial vaccine. In the case of Gram-positive bacterial vaccines, the endotoxin testing should be conducted to ensure that no contaminants from Gram-negative bacterial growth are present. Also, there must be a release specification for this residual. Two tests are currently used to detect LPS in biological products, the *Limulus* amebocyte lysate (LAL) test (see *Bacterial Endotoxins Test* ⟨85⟩) and the rabbit pyrogen test (see *Pyrogen Test* ⟨151⟩). The *Limulus* lysate that is used to test for bacterial endotoxin in FDA-regulated products is itself a U.S.-licensed product. The rabbit pyrogenicity test requires the use of animals and is more difficult to perform; therefore, it is not employed to the extent that the LAL test is used.

Viral vaccine manufacturing requires cell substrates to produce the viruses, which are then taken through purification processes. Generally, killed viral vaccines are more highly purified than are live attenuated ones. Depending on the method used to manufacture the vaccine, manufacturers work with FDA to develop prudent specifications for the final vaccine. Animal-derived host cells have been used extensively in vaccine manufacturing, particularly viral vaccines. For example, influenza and yellow fever vaccines are produced, respectively, in egg allantoic fluid and chicken embryos. Mumps, measles, and some rabies vaccines are produced in chick embryo cells. The labels of these products must state that residual chicken proteins may be present in the final vaccine, and the label may indicate how much is present. Further, the label also urges practitioner caution when vaccinating a person with known hypersensitivity to eggs.

Two U.S.-licensed hepatitis B vaccines are based on recombinant DNA-derived proteins expressed in yeast cultures. In both cases, the labels notify health care professionals that yeast protein may be present in the vaccine and recommend that suitable precautions should be exercised. In the case of live viral vaccines, considerations may be given to the reduction of cellular residual materials (e.g., host DNA, proteins).

Materials of Animal Origin—Some raw materials and reagents, such as gelatin, calf serum (see *Bovine Serum* ⟨1024⟩), or trypsin for vaccine manufacturing raise concerns regarding the potential presence of adventitious agents. Raw materials should be sourced from countries acceptable to FDA. Additionally, manufacturers should test these materials when possible to minimize the risks of contamination with adventitious agents. Reduction of serum components (e.g. BSA) should be considered in processing.

Antibiotic Residuals—Some antibiotics (but not penicillin) can be used in minimal amounts in the manufacturing process for viral vaccines, according to 21 CFR 610.15(c). Those that have been used include gentamicin, streptomycin, neomycin, and polymyxin B. There is no requirement for tests of residual levels of these antibiotics in the final vaccine. However, according to 21 CFR 610.61(m), the calculated amount expected to remain as a residual in the final vaccine, based on the amount added and the dilution factor in the manufacturing process, must be stated on the product label.

Inactivating Chemical Agents—Several chemical agents have been used to inactivate bacteria and viruses or to detoxify toxins in vaccine production processes. Formaldehyde and β-propiolactone are the most commonly used inactivating agents. Other less often used inactivating agents include glutaraldehyde and hydrogen peroxide. As a manufacturing residual, the inactivating agent should be removed from the final product as thoroughly as possible. The upper limit for formaldehyde is generally 0.02%, equivalent to 0.1 mg per 0.5 mL vaccine dose. The limit for β-propiolactone should be below the limit of detection.

EVALUATING THE STABILITY OF VACCINES

The stability of vaccine products depends on the nature of a vaccine antigen, the product formulation, and the control of vaccine storage prior to use.

Vaccine products are evaluated with programs that include real-time long-term storage under prescribed conditions. The use of extreme temperatures to potentially accelerate degradation may help manufacturers understand the stability of the product.

Vaccine products, like all pharmaceutical products, should be evaluated to define suitable conditions for storage (21 CFR 610.50 and 610.53). General principles of stability testing for biological products are described in *Quality of Biotechnological Products: Stability Testing of Bi-*

otechnological/Biological Products ⟨1049⟩. Typically these concerns are focused on the final vaccine product, but evaluations also are needed for bulk intermediates to justify the conditions under which they are held. In both cases manufacturers define in advance the conditions to which the product will be exposed (e.g., temperature, light, and humidity) and the time range during which the product will be exposed to those conditions. Stability studies should evaluate all storage conditions to which the product or intermediate are likely to be exposed during production, handling, shipping, and storage so that appropriate time limits can be placed on the exposure to those conditions.

The primary criteria for defining the storage conditions for these intermediates and the final products are generally focused on acceptable maintenance of potency; but, as discussed below, there often are other attributes that need to be considered.

Evaluation of the stability of vaccine products has three general purposes. First, the products are shown to maintain an acceptable analytical profile throughout manufacture and use to preserve safety and effectiveness. Second, stability studies across several product batches provide an effective way to characterize the inherent properties of the product. This in turn leads to the third use, demonstrating manufacturing consistency in the product.

Stability Protocols

The overall experimental plan for evaluating the stability profile of a given set of product or intermediate batches typically includes specific definition of the conditions under which the samples will be stored and why these conditions are relevant, the length of time the samples will be stored at each condition, when samples will be tested during this time course, and the analytical measurements at each time point. Additionally, these stability protocols include itemization of the analytical procedures to be used. For stability studies that occur early in product development, the studies may be conducted to confirm the suitability of the product formulation and/or storage conditions. Later in development, stability studies are typically conducted to provide data supporting product dating period or intermediate hold time, to provide more elaborate product characterization, and to evaluate manufacturing consistency. These latter studies define product end–expiry specifications that allow definitions of acceptable and unacceptable product. Unacceptable product is defined as product that is no longer acceptable for use in clinical studies or for commercial use (e.g., because of degradation or loss of potency). Stability studies should be conducted over a duration sufficient to determine the point of loss of acceptable potency or other relevant parameters.

Analytical Measurements

Manufacturers should consider the rigor of the analytical method(s) used to evaluate the stability of complex products and improve their understanding of the parameters that are critical to immunogenicity (including stability-indicator parameters). Selection of the stability-indicator parameters varies with each vaccine's unique characteristics.

The primary parameter that reflects stability for most vaccines is the potency assay (see *Potency Tests* in *Lot Release Testing*, below). This assay can take many forms, depending on individual vaccines (e.g., an infectivity assay for a live virus vaccine or a measure of the proportion of conjugated polysaccharide for a polysaccharide–protein conjugate vaccine). The potency assay is generally the key analytical result predicting whether a vaccine remains suitable for use and whether it will produce the expected clinical response. Other analytical measurements can provide important supplemental data, particularly

those that have a clear link to the potency of the product. Examples include degradation profile, dissociation of a carrier protein from conjugated vaccines, and dissociation of an adjuvant from an antigen complex. Additionally, other common assays typically are performed as part of the stability study and may address physical or chemical changes in the product that may or may not affect its potency (e.g., general safety, degree of aggregation, pH, moisture, container, preservative, and enclosure).

Formal Evaluation of Stability Data and Product Expiry Dating

Vaccines must remain within potency specifications at the expiration date, provided that the product was stored under the normal conditions specified. Manufacturers should conduct stability studies to determine those storage conditions and that dating period to demonstrate that the product remains within the potency specifications. Manufacturers should conduct stability studies on a continuing basis. If a major manufacturing process changes, additional stability studies should be conducted to verify that there is no adverse impact on the stability profile. Under certain conditions such as process changes, accelerated stability studies could be conducted. An accelerated study involving temperatures both higher and lower than routine can evaluate the impact of temperature excursions on products. A similar evaluation should be done for product intermediates to establish how long a given intermediate can be held under defined conditions before it is processed further or discarded.

NOMENCLATURE

There are no uniform systems for naming new vaccines. 21 CFR 299 describes the cooperation of the FDA and the U.S. Adopted Names Council (USAN) in naming drugs, including vaccines. USAN is a private organization sponsored by the American Medical Association, USP, and the American Pharmacists Association. Section 262 in Title 42 of the Public Health Service Act requires that each package of the biological product be plainly marked with the proper name (name designated in the license 21 CFR 600.3) of the biological product contained in the package.

LABELING

Vaccine product labeling is regulated in compliance with 21 CFR 201 and 610. Requirements are set for container labeling and package labeling.

Container Label

Provisions are made for the following labels:

- Full label;
- Partial label; and
- No label on the container itself when the containers cannot support a label that includes all required information and should be placed in a package that does include all required information.

The label should be affixed to the container in a manner that allows visual inspection of the contents for the full length or circumference of the container. If no package exists, the container bears all of the information required for the package label.

The full container label normally contains the following:

- Proper name of the product;
- Name, address, and license number of the manufacturer;
- Lot number or other lot identification;
- Expiration date;
- Recommended individual dose, for multiple-dose containers;
- The phrase *Rx only* for prescription biologicals; and
- Any applicable cautionary statements.

Package Label

In addition to the information required on the container label, the package label should describe the following:

- Any preservative used and its concentration, or the words *no preservative* if no preservative is used and its absence is a safety factor;
- Number of containers, if more than one; or
- Amount of product in the container, expressed as number of doses, volume, units of potency, weight, and equivalent volume (for dried product to be reconstituted); or
- A combination of the above to provide an accurate description of the contents, as applicable;
- Recommended storage temperature;
- The words *shake well*, *do not freeze*, or the equivalent, as well as other instructions when indicated by the character of the product;
- Recommended individual dose, for multiple-dose containers;
- Recommended route of administration, or reference to such directions in an enclosed circular;
- Presence of known sensitizing substances;
- Type of antibiotics added during manufacture and the amount calculated to remain in the final product;
- Inactive ingredients, when they constitute a safety factor or are referenced to an enclosed circular;
- Adjuvant, if present;
- Source of the product, when this may be a factor in safe administration;
- Identity of each microorganism used in manufacture and, if applicable, the production medium and the method of inactivation or reference to an enclosed circular;
- Minimum potency in terms of official standard of potency, or the words *no U.S. standard of potency*.

Prescribing Information

Detailed information about a vaccine appears in its prescribing information, commonly called the package insert. Increasingly, vaccines are distributed with patient package inserts written in lay language. Prescribing information (21 CFR 201.56 and 201.57) includes the following:

- Highlights of prescribing information
- Product names, other required information
- Boxed warning
- Recent major changes
  a. Indications and usage
  b. Dosage and administration
  c. Dosage forms and strengths
  d. Contraindications
  e. Warnings and precautions
  f. Adverse reactions
  g. Drug interactions
  h. Use in specific populations (e.g., pregnancy, nursing mothers, pediatric, geriatric)
  i. Drug abuse and dependence
  j. Overdosage
  k. Description
  l. Clinical pharmacology
  m. Nonclinical toxicology
  n. Clinical studies
  o. References
  p. How supplied/storage and handling
  q. Patient counseling information

LOT RELEASE TESTING

General Principles

Manufacturers perform all appropriate tests for the licensed specifications for the product, according to 21 CFR 610.1 and 610.2. Samples of each licensed lot and protocols containing the manufacturers' test results are

submitted to FDA. After FDA evaluates the protocol to ensure that the product specifications are met, and after satisfactory confirmatory testing, FDA approves the release of the lot if all tests meet the standards of safety, purity, and potency established for the particular vaccine product. After approval is granted, the manufacturer distributes and markets the product.

Guidelines are available regarding alternatives to lot release and a surveillance system. All of these variations are subject to the regulations in 21 CFR 610.2 that allow FDA to require that samples of any lot of licensed product (e.g., vaccine), together with the protocols showing results of applicable tests, be sent to FDA.

Common Tests

The tests common to all lots of all products include tests for potency, general safety, sterility, purity, identity, and constituent materials. The manufacturer completes these tests for conformity with standards applicable to each product. The results of all tests are considered, except when a test has been invalidated as a result of causes unrelated to the product (21 CFR 610.1).

Potency Tests (Vaccine-Specific)—The basic definition and requirements for vaccine potency and potency assays are provided in 21 CFR 600.3 and 610.10. A vaccine potency assay should indicate the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. Potency may be expressed in terms of units by reference to a standard. Product potency tests vary with vaccine product types (e.g., viral, bacterial, live attenuated, inactivated, or polysaccharide). As a result, potency assays for vaccines span a variety of approaches to the expression of potency. In vitro potency tests for live virus may include plaque formation assays, endpoint dilution assays (e.g., the tissue culture infective dose [$TCID_{50}$], virus neutralization assays, or quantitative polymerase chain reaction [PCR] assays). Quantitative colony formation assays are used for live attenuated bacterial vaccines. Animal challenge tests for immunogenicity assays of potency, such as those for diphtheria and tetanus (U.S. Department of Health, Education, and Welfare, 1953; see *Appendix 2*), or rabies and anthrax show in vivo response. Antigenicity assays use enzyme-linked immunosorbent assays (ELISA), e.g., with hepatitis A or rate nephelometry and rocket immunoelectrophoresis (e.g., with pneumococcal polysaccharides). The potency tests for bacterial vaccines, such as the meningococcal polysaccharides, pneumococcal polysaccharides, or *Haemophilus b* protein conjugate vaccines use chemical and physical chemical assays. In the case of pure polysaccharide vaccines, the concentration or quantity of the vaccine component (polysaccharide) and its quality (e.g., size) have been shown to be indicative of human immune response.

Assay precision and reproducibility vary with the different methodologies that are used in potency assays, ranging from the high accuracy and precision of chemical tests at one end of the spectrum to bioassays at the other end. The general test chapter *Design and Analysis of Biological Assays* ⟨111⟩ provides guidance for bioassays and applies to vaccine potency assays. Other tests should be validated as described in general information chapter *Validation of Compendial Procedures* ⟨1225⟩.

Release Tests—Official release of vaccines by the vaccine regulatory authority may be based on either the bulk or the final container. It is highly desirable to perform potency tests on the final container. However, under certain circumstances this may not be practical or even possible: thus, a case-by-case approach would be required. The choice of whether to test the bulk or the final container derives from a number of considerations, such as the quantity of vaccine available for tests at the different manufacturing stages. For certain vaccines, both bulk and final container receive official release. The potency

test is generally required for the final container. If it is not feasible to perform the potency test on the final drug product, the test is performed on the bulk material.

General Safety—For biological products that are intended for administration to humans, manufacturers perform a general safety test in order to detect any extraneous toxic contaminants. Procedures and exceptions are specified in 21 CFR 610.11.

Sterility—A sterility test of each lot of each product is conducted according to procedures described in *Sterility Tests* ⟨71⟩ and 21 CFR 610.12 for both bulk and final container material.

Bacterial Endotoxins—Each lot of final containers of a vaccine intended for use by injection is tested for bacterial endotoxins, as indicated in *Bacterial Endotoxins Test* ⟨85⟩.

Purity—Vaccines need to be free of extraneous material. Approved vaccine license applications indicate extraneous materials that are unavoidable in the manufacturing process for a specific product. The application may indicate test results and allowable limits for such materials, according to procedures described in 21 CFR 610.13.

Residual Moisture—Each lot of dried product is tested for residual moisture (see 21 CFR 610.13 (a), *Loss on Drying* ⟨731⟩, and FDA's *Guideline for the Determination of Residual Moisture in Dried Biological Products* (see *Appendix 2*).

Pyrogens—Each lot of final containers of a vaccine intended for use by injection is tested for pyrogenic substances, as indicated in *Pyrogen Test* ⟨151⟩ and 21 CFR 610.13 (b).

Identity—The contents of a final container of each filling of each lot are tested for identity after labeling is completed. Identity is established by physical or chemical characteristics of the vaccine, inspection by macroscopic or microscopic methods, specific cultural tests, or in vivo or in vitro immunological tests. In large part, identity tests are performed to distinguish the subject vaccine from other materials manufactured at the same site (21 CFR 610.14).

Constituent Materials—Ingredients, preservatives, diluents, adjuvants, extraneous protein, cell culture-produced vaccines, and antibiotics are tested according to 21 CFR 610.15.

Permissible Combinations

Formulations that combine several vaccines must be licensed as combinations (21 CFR 610.17). The potency of each vaccine in the combination is individually tested and must meet the specifications in the context of the final combined product; other appropriate quality tests apply as well. For vaccines that are physically combined in clinical locations just before administration to a patient, prescribing information should describe specific procedures to follow in those settings.

Quality

In general, quality control systems for vaccine manufacture are identical to those routinely employed for production of other pharmaceuticals. These include raw material testing and release, manufacturing, process-control documentation, and aseptic processing. Manufacturers formally assign responsibility to designated staff for maintaining the continued safety, purity, and potency of the product and for ensuring compliance with applicable product and establishment standards, along with compliance with current GMPs. Analysts use reference standards and validated methods to determine active ingredients, residuals, and impurities. Manufacturers determine product safety in a variety of ways that may include the use of experimental animals, procedures to demonstrate product sterility, and tests to ensure product po-

tency. The complexity of the quality control systems for vaccines lies in the variety of methods used to produce and control production. Lot release testing proceeds according to 21 CFR 610.2 and involves evaluating lots for safety, purity, and potency before release. Manufacturers follow FDA and applicable international standards for testing and validation. The basic considerations for validation are included in *Validation of Compendial Procedures* ⟨1225⟩, in addition to guidance documents issued by FDA and the International Conference on Harmonization (ICH) (see *Appendix 2*).

Alternative Tests

Modification of test methods or manufacturing processes as licensed may be permitted if the regulatory authority can be assured that the modifications cause no reduction in safety, purity, potency, and effectiveness of the biological product. It may be necessary for the manufacturer to file the proposed changes prior to implementation (21 CFR 601.12 and 21 CFR 610.9).

APPENDIX 1. TYPES OF VACCINES CURRENTLY LICENSED IN THE U.S. (EXAMPLES)

- Bacterial, live attenuated (e.g., *Salmonella typhi*)
- Bacterial, polysaccharide (e.g., meningococcal, pneumococcal)
- Bacterial, polysaccharide-protein conjugate (e.g., meningococcal, pneumococcal)
- Bacterial, toxoid (e.g., diphtheria, tetanus)
- Bacterial, extracts (e.g., pertussis, anthrax)
- Viral, live attenuated (e.g., influenza, measles, mumps, rubella)
- Viral, whole inactivated (e.g., rabies)
- Viral, subunit (e.g., influenza, hepatitis B, human papillomavirus)

APPENDIX 2. SELECTED REGULATORY DOCUMENTS

- 21 CFR 201.
- 21 CFR 299.
- 21 CFR 600.
- 21 CFR 610.

- Section 262 in Title 42 of the Public Health Service Act
- Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA). *Guidance for Industry—Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products* (January 2002). http://www.fda.gov/cber/gdlns/cjdvcjd.pdf
- Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA). *Guideline for the Determination of Residual Moisture in Dried Biological Products* (January 1990). http://www.fda.gov/cber/gdlns/moisture.pdf
- Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA). *Guidance on Alternatives to Lot Release for Licensed Biological Products.* Federal Register 1993;58(137): 38771–38773. http://www.fda.gov/cber/gdlns/altrntvlot071493.pdf
- Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA). *Guidance For Industry—Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases* (September 2006). http://www.fda.gov/cber/gdlns/vaccsubstrates.pdf

- FDA periodically issues or updates Guidance to Industry and posts these documents at http://www.fda.gov/cber/guidelines.htm
- Department of Health, Education, and Welfare (now the National Institutes of Health). *Minimum Requirements for Immune Serum Globulin (Human).* 3rd rev. Bethesda, MD: Department of Health, Education, and Welfare, 1953.
- International Conference on Harmonization (ICH). Q2(R1). *Validation of Analytical Procedures: Text and Methodology.* Geneva, Switzerland: ICH, 2005. http://www.ich.org/LOB/media/MEDIA417.pdf

GLOSSARY

1. ***Acceptance criteria***—The product specifications and acceptance or rejection criteria, with an associated sampling plan, necessary for making a decision to accept or reject a lot or batch (or any other convenient subgroups of manufactured units).
2. ***Active ingredient***—Any component intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and may be present in the drug product in a modified form intended to furnish the specified activity or effect.
3. ***Adventitious agent***—A microorganism (e.g., bacteria, fungi, mycoplasma, spiroplasma, mycobacteria, rickettsia, viruses, protozoa, parasites, TSE agent) that is inadvertently introduced into the production of a biological product.
4. ***Batch***—A specific quantity of a drug or other material intended to have uniform character and quality, within specified limits, and produced according to a single manufacturing order during the same cycle of manufacture.
5. ***Biological product***—Any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man.
6. ***Cell bank***—Vials of cells of uniform composition (not necessarily clonal) derived from a single tissue or cell, aliquoted into appropriate storage containers, and stored under appropriate conditions.
7. ***Cell line***—Cells that have been propagated in culture since establishment of a primary culture and have survived through crisis and senescence. Such surviving cells are immortal and will not senesce. Diploid cell strains have been established from primary cultures and expanded into cell banks, but have not passed through crisis and are not immortal.
8. ***Characterization***—Determination of the properties of a substance.
9. ***Component***—Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product.
10. ***Container*** (also final container)—The immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.
11. ***Control***—Having responsibility for maintaining the continued safety, purity, and potency of the product and for compliance with applicable product and establishment standards, and for compliance with current good manufacturing practices.
12. ***Control cells***—Cells that are split off from the production culture and maintained in parallel under the same conditions and using the same reagents

(e.g., culture medium) to perform quality control tests on cells that have not been exposed to the vaccine virus (which may interfere with some tests).

13. ***Dating period***—The period beyond which the product cannot be expected beyond reasonable doubt to yield its specific results.
14. ***Diploid***—Having the expected number of chromosomes for a species, (i.e., two of each autosomal chromosome and two sex chromosomes)
15. ***Drug product***—A finished dosage form (e.g., solution, suspension) that contains an active drug ingredient generally in association with inactive ingredients.
16. ***End-of-production cells***—Cells harvested at the end of a production run or cells cultured from the master cell bank or working cell bank to a passage level or population doubling level comparable to or beyond the highest level reached in production.
17. ***End-of-production passage level***—The maximal passage level achieved during manufacturing at final vaccine harvest. Cells may be evaluated at this level or beyond.
18. ***Endogenous virus***—A virus whose genome is present in an integrated form in a cell substrate by heredity. Endogenous viral sequences may or may not encode for an intact or infectious virus.
19. ***Expiration date***—The calendar month and year, and where applicable, the day and hour, that the dating period ends.
20. ***Filling***—A group of final containers identical in all respects, which have been filled with the same product from the same bulk lot without any change that will affect the integrity of the filling assembly.
21. ***Final bulk***—The stage of vaccine production directly prior to filling of individual vials.
22. ***Free of and freedom from***—For a substance to be considered free of a contaminant, an assay must demonstrate that a defined quantity of the substance is negative for that contaminant to a defined level of sensitivity. The level of assay sensitivity is defined by the choice of assay and can be determined experimentally using standardized reagents. Alternatively, a validated process that is known to remove a contaminant to a defined level may be used to demonstrate freedom from that contaminant.
23. ***Harvest***—Collection of material at the end of vaccine virus propagation in cell culture, from which vaccine will be prepared. This material may be the culture supernatant, the cells themselves (often in disrupted form), or some combination thereof.
24. ***Inactive ingredient***—Any component other than an active ingredient.
25. ***In-process material***—Any material fabricated, compounded, blended, or derived by chemical reaction that is produced for, and used in, the preparation of the drug product.
26. ***Intermediates***—Unformulated active ingredients that are processed before final formulation and can be stored for long periods of time before further processing.
27. ***Label***—Any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.
28. ***Latent virus***—A virus that is present in a cell, without evidence of active replication, but with the potential to reactivate, is considered to be microbiologically latent.
29. ***Lot***—A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.

30. ***Lot number, control number, or batch number***—Any distinctive combination of letters, numbers, or symbols, or any combination of them, from which the complete history of the manufacture, processing, packing, holding, and distribution of a batch or lot of drug product or other material can be determined.
31. ***Manufacture***—All steps in the propagation or manufacture and preparation of products. Includes, but is not limited to, filling, testing, labeling, packaging, quality control, and storage by the manufacturer.
32. ***Manufacturer***—Any legal person or entity engaged in the manufacture of a product subject to license under the Public Health Service (PHS) Act. Manufacturer also includes any legal person or entity who is an applicant for a license where the applicant assumes responsibility for compliance with the applicable product and establishment standards.
33. ***Master cell bank***—A bank of a cell substrate from which all subsequent cell banks used for vaccine production will be derived. The master cell bank represents a characterized collection of cells derived from a single tissue or cell.
34. ***Master virus seed***—A viral seed of a selected vaccine virus from which all future vaccine production will be derived, either directly, or via working virus seeds.
35. ***Oncogenicity***—The property of certain biological agents (e.g., viruses) or materials (e.g., nucleic acids) that are capable of immortalizing cells and endowing them with the capacity to form tumors. Oncogenicity is distinct from tumorigenicity.
36. ***Package***—The immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package is used, the container shall be deemed to be the package.
37. ***Passage level***—The number of times, since establishment from a primary cell culture, a culture has been split or reseeded.
38. ***Population doubling level***—The number of times, since establishment from a primary cell culture, a culture has doubled in number of cells.
39. ***Potency***—The therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. Potency may be expressed in terms of units by reference to a standard.
40. ***Primary cells***—Cells placed into culture immediately after an embryo, tissue, or organ is removed from an animal or human and homogenized, minced, or otherwise separated into a suspension of cells. Primary cells may be maintained in medium, but are not passaged (split).
41. ***Process***—A manufacturing step that is performed on the product itself which may affect its safety, purity, or potency, in contrast to such manufacturing steps which do not affect intrinsically the safety, purity, or potency of the product.
42. ***Proper name***—The name, designated in the license, to be used on each package of the product.
43. ***Purity***—Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances.
44. ***Qualification***—Determination of the suitability of a material for manufacturing based on its characterization.
45. ***Safety***—The relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

46. ***Specification***—The quality standard (i.e., tests, analytical procedures, acceptance criteria) provided in an approved application to confirm the quality of products, intermediates, raw materials, reagents, components, in-process materials, container closure systems, and other materials used in the production of a product.
47. ***Standards***—Specifications and procedures applicable to an establishment or to the manufacture or release of products, which are prescribed in this subchapter or established in the biologics license application and designed to insure the continued safety, purity, and potency of such products.
48. ***Sterility***—Freedom from viable contaminating microorganisms, as determined by tests prescribed by the FDA.
49. ***Tumorigenic***—A property of certain cell types to form tumors when inoculated into animals (generally a syngeneic, an immunosuppressed allogeneic, or an immunosuppressed xenogeneic host). These tumors may be at the injection site or a different site and may also metastasize to other sites.
50. ***Tumorigenicity***—The process by which immortalized cells form tumors when inoculated into animals. Tumorigenicity is distinct from oncogenicity.
51. ***Unacceptable product***—Product that is no longer acceptable for use in clinical studies or for commercial use (e.g., because of degradation or loss of potency).
52. ***Validation***—The performance characteristics of an analytical procedure, based on the demonstration that the procedure is suitable for its intended purpose or use. Validation of a process is the determination of the extent to which a process meets the requirements for the various performance characteristics and the demonstration that the process uniformly performs to defined characteristics. Validation is generally performed in accordance with *Validation of Compendial Procedures* ⟨1225⟩ and the relevant ICH guidelines.
53. ***Viral clearance***—The combination of the physical removal of viral particles and the reduction of viral infectivity through inactivation.
54. ***Virus seed or viral seed***—A live viral preparation of uniform composition (not necessarily clonal) derived from a single culture process, aliquoted into appropriate storage containers, and stored under appropriate conditions.
55. ***Working cell bank***—A cell bank derived by propagation of cells from the master cell bank under defined conditions and used to initiate production cell cultures on a lot-by-lot basis.
56. ***Working virus seed***—A viral seed derived by propagation of virus from the master virus seed under defined conditions and used to initiate production cell cultures lot-by-lot.■2S (*USP33*)

DIETARY SUPPLEMENTS

General Information

BRIEFING

⟨2040⟩ Disintegration and Dissolution of Dietary Supplements, *USP 32* page 782 and page 435 of *PF* 34(2) [Mar.–Apr. 2008]. Three revisions to this dietary supplement chapter are proposed:

1. New specifications for the beaker used in *Apparatus B* in the *Disintegration* section: the new narrower specifications are based on experimental data showing that the current range of 97 to 115 mm produces significant variation in disintegration times.
2. A new test for *Delayed-Release (Enteric-Coated) Soft Shell Capsules* is included in the *Disintegration* section as a result of the increase in dietary supplements that use enteric-coated capsules.
3. In the *Dissolution* section, a new flow cell chamber for *Apparatus 4* (see *Dissolution* ⟨711⟩) is introduced for soft shell capsules with lipophilic contents.

(DS-PS: Natalia Davydova) RTS—C74668; C75300; C75302

Change to read:

DISINTEGRATION

This test is provided to determine whether dietary supplement tablets or capsules disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below. Compliance with the limits on *Disintegration* stated in the individual monographs for dietary supplements is required except where the label states that the products are intended for use as troches, are to be chewed, or are designed as extended-release dosage forms. Dietary supplements claiming to be extended-release dosage forms must comply with standards other than disintegration to verify that the release of the dietary ingredients from the dosage form is for a defined period of time. Dietary supplements claiming to be extended-release dosage forms shall not be labeled as in compliance with USP unless a USP monograph exists for such product. Determine the type of units under test from the labeling and from observation, and apply the appropriate procedure to 6 or more units.

For purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disk, if used, is a soft mass having no palpably firm core.

Apparatus

Apparatus A—Use the *Apparatus* described under *Disintegration* ⟨701⟩ for tablets or capsules that are not greater than 18 mm long. For larger tablets or capsules, use *Apparatus B.*

Apparatus B—The apparatus[1] consists of a basket-rack assembly, a 1000-mL low-form beaker for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-Rack Assembly—The basket-rack assembly

■(*Figure 1*)■2S (*USP33*)
consists of three open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 32.0 to 34.6 mm and a wall 2.0 to 3.0 mm thick; the tubes are held in a vertical position by two plastic plates, each ~~about~~

■■2S (*USP33*)
97

■ ± 2■2S (*USP33*)
mm in diameter and 7.5 to 10.5 mm in thickness, with three holes, ~~each about 33 to 34~~

■36.0 to 40.6■2S (*USP33*)
mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the undersurface of the lower plate is 10-mesh No. 23 (0.025-inch) W. and M. gauge woven stainless-steel wire cloth having a plain square weave. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device, using a point on its axis.

The design of the basket-rack assembly may be varied somewhat, provided that the specifications for the glass tubes and the screen mesh size are maintained.

[1] An apparatus and disks meeting these specifications are available from Varian Inc., 13000 Weston Parkway, Cary, NC 27513, or from laboratory supply houses.

■

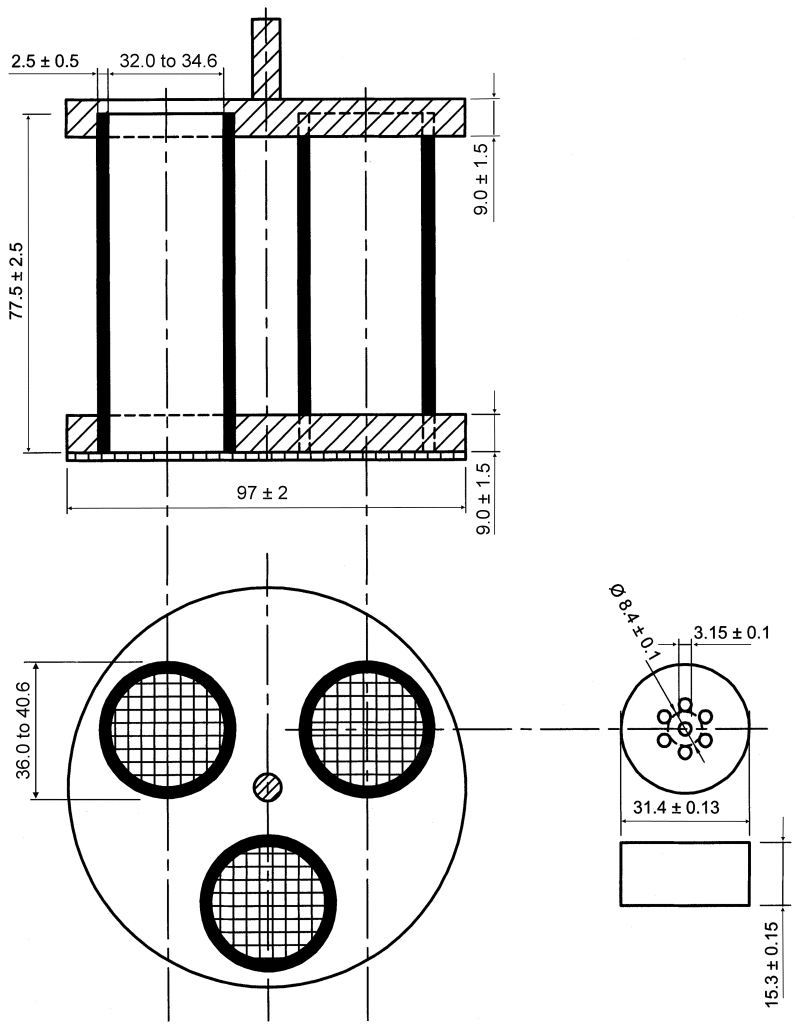


Figure 1. Basket-rack assembly, *Disintegration, Apparatus B* (dimensions in mm).

Beaker—Low form, 1000 mL; the difference between the diameter of the plastic plates, which hold the tubes in a vertical position, and the inside diameter of the beaker should not be more than 6 mm.[2] ■2S (*USP33*)

Disks—Each tube is provided with a perforated cylindrical disk 15.3 ± 0.15 mm thick and 31.4 ± 0.13 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Seven 3.15 ± 0.1-mm holes extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it and equally spaced on a 4.2 ± 0.1-mm radius from it. All surfaces of the disk are smooth.[3]

Procedure

Uncoated Tablets—Place 1 tablet in each of the tubes of the basket and, if prescribed, add a disk to each tube. Operate the apparatus, using water or the specified medium as the immersion fluid, maintained at 37 ± 2°. At the end of 30 minutes, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The requirement is met if not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Plain Coated Tablets—Place 1 tablet in each of the tubes of the basket and, if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 minutes. Then, if prescribed, add a disk to each tube, and operate the apparatus, using water or the specified medium as the immersion fluid, maintained at 37 ± 2°. At the end of 30 minutes, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The requirement is met if not fewer than 16 of the total of 18 tablets tested disintegrate completely.

[2] 1000-mL low-form beakers, designed in compliance with the current ASTM E 960 Type I or Type II or ISO 3819 specifications, are suitable. ■2S (USP33)

[3] The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimensions given in this chapter.

Delayed-Release (Enteric-Coated) Tablets—Place 1 tablet in each of the six tubes of the basket, and if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 minutes. Then operate the apparatus using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 1 hour of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the tablets: the tablets show no evidence of disintegration, cracking, or softening. Operate the apparatus, using simulated intestinal fluid TS, maintained at 37 ± 2°, as the immersion fluid for the time specified in the monograph. Lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

▪Delayed-Release (Enteric-Coated) Soft Shell Capsules—Place 1 softgel capsule in each of the six tubes of the basket. Use two baskets for a total of six tubes for *Apparatus B*. Omit the use of a disk. Operate the apparatus using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 1 hour of operation in simulated gastric fluid TS, lift the basket from the fluid and observe the softgels: the softgels show no evidence of disintegration or rupture permitting the escape of the contents. Operate the apparatus with disks, using simulated intestinal fluid TS, maintained at 37 ± 2°, as the immersion fluid. Lift the basket from the fluid, and observe the capsules. All the capsules disintegrate completely within 60 minutes. If 1 or 2 capsules fail to disintegrate completely, repeat the test on 12 additional capsules: not fewer than 16 of a total of 18 capsules tested disintegrate completely.▪2S (*USP33*)

Buccal Tablets—Apply the test for *Uncoated Tablets*. After 4 hours, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Sublingual Tablets—Apply the test for *Uncoated Tablets*. At the end of the time limit specified in the individual monograph, all the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Hard Shell Capsules—Apply the test for *Uncoated Tablets,* using as the immersion fluid, maintained at 37 ± 2°, a 0.05 M acetate buffer prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of 4.50 ± 0.05. Attach a removable wire cloth, as described under *Basket-Rack Assembly,* to the surface of the upper plate of the basket-rack assembly. At the end of 30 minutes, lift the basket from the fluid, and observe the capsules: all of the capsules disintegrate except for fragments from the capsule shell. If 1 or 2 capsules fail to disintegrate completely, repeat the test on 12 additional capsules: not fewer than 16 of the total of 18 capsules tested disintegrate completely.

Soft Shell Capsules—Proceed as directed under *Rupture Test for Soft Shell Capsules.*

Use of Disks—

VITAMIN–MINERAL DOSAGE FORMS—Add a disk to each tube unless otherwise specified in the individual monograph.

BOTANICAL DOSAGE FORMS—Omit the use of disks unless otherwise specified in the individual monograph.

DIETARY SUPPLEMENTS OTHER THAN VITAMIN–MINERAL AND BOTANICAL DOSAGE FORMS—Omit the use of disks unless otherwise specified in the individual monograph.

NOTE—The use of disks for enteric-coated tablets is not permitted.

Change to read:

RUPTURE TEST FOR SOFT SHELL CAPSULES

Medium: water; 500 mL.

Apparatus—Use *Apparatus 2* as described under *Dissolution* ⟨711⟩, operating at 50 rpm.

Time: 15 minutes.

Procedure—Place 1 capsule in each vessel, and allow the capsule to sink to the bottom of the vessel before starting rotation of the blade.

▪Use sinkers if the capsules float.▪1S (*USP32*)
Observe the capsules, and record the time taken for each capsule shell to rupture.

Tolerances—The requirements are met if all of the capsules tested rupture in not more than 15 minutes. If 1 or 2 of the capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional capsules: not more than 2 of the total of 18 capsules tested rupture in more than 15 but not more than 30 minutes.

▪For soft gelatin capsules that do not conform to the above rupture test acceptance criteria, repeat the test with the addition of purified pepsin to the *Medium* that results in an activity of 750,000 Units or less per 1000 mL.▪1S (*USP32*)

Change to read:

DISSOLUTION

This test is provided to determine compliance with the *Dissolution* requirements where stated in the individual monograph for dietary supplements, except where the label states that tablets are to be chewed.

See *Dissolution* ⟨711⟩ for a description of the apparatus used, the *Apparatus Suitability Test,* and other related information.

•Soft gelatin capsule preparations of dietary supplements meet the requirements for *Disintegration.*

Official until May 1, 2010.• (RB 1-May-2009)

▪*Figure 2* shows the schematic view of a flow-through cell specifically intended for lipid-filled soft gelatin capsules. It consists of three transparent parts that fit into each other (USP *Apparatus 4*). The lower part (1) is made up of

two adjacent chambers connected to an overflow device. The dissolution medium passes through chamber A and is subjected to an upward flow. The flow in chamber B is directed downward to a small-size bore exit that leads upward to a filter assembly. The middle part (2) of the cell has a cavity designed to collect lipophilic excipients that float on the dissolution medium. A metal grid serves as a rough filter. The upper part (3) holds a filter unit for paper, glass fiber, or cellulose filters.■2S (*USP33*)
Of the types of apparatus described in ⟨711⟩, use the one specified in the individual monograph.

■

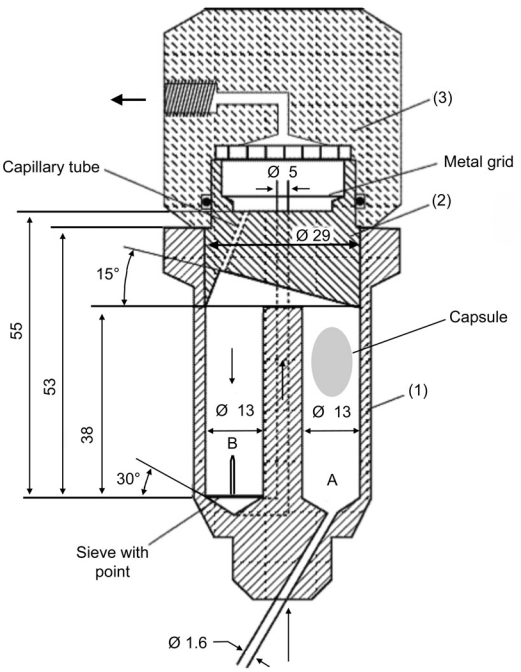


Figure 2. Flow-through cell designed for lipid-filled soft gelatin capsules (dimensions in mm).■2S (*USP33*)

For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the dissolution specification, repeat the test as follows. Where water or a medium with a pH of less than 6.8 is specified as the *Medium* in the individual monograph, the same *Medium* specified may be used with the addition of purified pepsin that results in an activity of 750,000 Units or less per 1000 mL. For media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1750 USP Units of protease activity per 1000 mL.

This nonspecific dissolution is intended to be diagnostic of known technological problems that may arise as a result of coatings, lubricants, disintegrants, and other substances inherent in the manufacturing process. For dosage forms containing botanical extracts, this dissolution measurement allows an assessment of the extent of decomposition of the extract to polymeric or other nondissoluble compounds that may have been produced by excessive drying or other manipulations involved in the manufacture of botanical extracts. The operative assumption inherent in this procedure is that if the index or marker compound(s) or the extract is demonstrated to have dissolved within the time frame and under the conditions specified, the dosage form does not suffer from any of the above formulation or manufacturing related problems.

Vitamin–Mineral Dosage Forms

All dietary supplements belonging to USP *Classes II* to *VI,* prepared as tablets or capsules, are subject to the dissolution test and criteria described in this chapter for folic acid (if present) and for index vitamins and index minerals. This test is required because of the importance of the relationship between folate deficiency and the risk of neural tube defects. The accompanying table lists the dissolution requirements for the individual USP classes of dietary supplements. *Class I* dietary supplements are combinations of oil-soluble vitamins for which dissolution standards are not established; hence, dissolution requirements do not apply to the oil-soluble vitamins contained in formulations belonging to *Class IV* or *Class V.* Vitamin–mineral combinations that may not be strictly covered by USP *Class I* to *Class VI* are subject to the dissolution test and criteria specified in the individual monographs.

Dietary Supplements—Vitamin–Mineral Dosage Forms

USP Class	Combination of Vitamins or Minerals Present	Dissolution Requirement
I	Oil-Soluble Vitamins	not applicable
II	Water-Soluble Vitamins	one index vitamin; folic acid (if present)
III	Water-Soluble Vitamins with Minerals	one index vitamin and one index element; folic acid (if present)
IV	Oil- and Water-Soluble Vitamins	one index water-soluble vitamin; folic acid (if present)
V	Oil- and Water-Soluble Vitamins with Minerals	one index water-soluble vitamin and one index element; folic acid (if present)
VI	Minerals	one index element

Unless otherwise stated in the individual monograph, test six dosage units for dissolution as directed under *Dissolution* ⟨711⟩.

DISSOLUTION CONDITIONS FOR FOLIC ACID

NOTE—Perform this test under light conditions that minimize photodegradation.

Medium: water; 900 mL. If the units tested do not meet the requirements for dissolution in water, test six additional dosage units for dissolution in a medium of 900 mL of 0.05 M pH 6.0 citrate buffer solution, prepared by mixing 9.5 mL of 0.1 M citric acid monohydrate and 40.5 mL of 0.1 M sodium citrate dihydrate in a 100-mL volumetric flask, diluting with water to volume, mixing, and adjusting to a pH of 6.0 by using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution.

Apparatus 1: 100 rpm, for capsules.

Apparatus 2: 75 rpm, for tablets.

Time: 1 hour.

NOTE—Compliance with the dissolution requirements for folic acid does not exempt the product from dissolution testing of the pertinent index vitamin or the corresponding index mineral.

DISSOLUTION CONDITIONS FOR INDEX VITAMINS AND INDEX MINERALS

Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm, for capsules.
Apparatus 2: 75 rpm, for tablets.
Time: 1 hour.

For formulations containing 25 mg or more of the index vitamin, riboflavin, use the following conditions:

Medium: 0.1 N hydrochloric acid; 1800 mL.
Apparatus 1: 100 rpm, for capsules.
Apparatus 2: 75 rpm, for tablets.
Time: 1 hour.

NOTE—Compliance with dissolution requirements for the pertinent index vitamin or index mineral does not exempt the product from dissolution testing of folic acid, if present.

SELECTION OF INDEX VITAMINS AND INDEX ELEMENTS

Compliance with the dissolution requirements for dietary supplements representing combinations of water-soluble vitamins (*Water-Soluble Vitamins Capsules* and *Water-Soluble Vitamins Tablets*) and combinations of oil- and water-soluble vitamins (*Oil- and Water-Soluble Vitamins Capsules* and *Oil- and Water-Soluble Vitamins Tablets*) is determined by measuring the dissolution of a single index vitamin from the water-soluble vitamins present. Riboflavin is the index vitamin when present in the formulation. For formulations that do not contain riboflavin, pyridoxine is the index vitamin. If neither riboflavin nor pyridoxine is present in the formulation, the index vitamin is niacinamide (or niacin), and in the absence of niacinamide (or niacin), the index vitamin is thiamine. If none of the above four water-soluble vitamins is present in the formulation, the index vitamin is ascorbic acid.

Compliance with the dissolution requirements for dietary supplements representing combinations of minerals (*Minerals Capsules* and *Minerals Tablets*) is determined by measuring the dissolution of only one index element. Iron is the index element when present in the formulation. For formulations that do not contain iron, the index element is calcium. If neither iron nor calcium is present, the index element is zinc, and in the absence of all three of these elements, magnesium is the index element.

Compliance with dissolution requirements for dietary supplements representing combinations of water-soluble vitamins and minerals (*Water-Soluble Vitamins with Minerals Capsules* and *Water-Soluble Vitamins with Minerals Tablets*) and combinations of oil- and water-soluble vitamins and minerals (*Oil- and Water-Soluble Vitamins with Minerals Capsules* and *Oil- and Water-Soluble Vitamins with Minerals Tablets*) is determined by measuring the dissolution of one index water-soluble vitamin and one index element, designated according to the respective hierarchies described above.

PROCEDURES

In the following procedures, combine equal volumes of the filtered solutions of the six individual specimens withdrawn, and determine the amount of folic acid or the index vitamin or element dissolved, based on the average of six units tested. Make any necessary modifications, including concentration of the analyte in the volume of test solution taken. Use the *Medium* for preparation of the Standard solution and dilution, if necessary, of the test solution.

Folic Acid—Determine the amount of $C_{19}H_{19}N_7O_6$ dissolved by employing the procedure set forth in the *Assay for folic acid* under *Oil- and Water-Soluble Vitamins with Minerals Tablets*, in comparison with a Standard solution having a known concentration of USP Folic Acid RS in the same *Medium*.

Niacin or Niacinamide, Pyridoxine, Riboflavin, and Thiamine—Determine the amount of the designated index vitamin dissolved by employing the procedure set forth in the *Assay for niacin or niacinamide, pyridoxine, riboflavin, and thiamine* under *Water-Soluble Vitamins Tablets.*

Ascorbic Acid—Determine the amount of $C_6H_8O_6$ dissolved by adding 10 mL of 1.0 N sulfuric acid and 3 mL of starch TS to 100.0 mL of test solution, and titrating immediately with 0.01 N iodine VS. Perform a blank determination, and make any necessary correction.

Iron, Calcium, Magnesium, and Zinc—Determine the amount of the designated index element dissolved by employing the procedure set forth in the appropriate *Assay* under *Minerals Capsules*.

TOLERANCES

The requirements are met if not less than 75% of the labeled content of folic acid and not less than 75% of the labeled content of the index vitamin or the index element from the units tested is dissolved in 1 hour.

Botanical Dosage Forms

Compliance with dissolution requirements necessitates the testing of six dosage units individually, or testing two or more dosage units in each of the six vessels of the dissolution apparatus, and measuring the dissolution of one or more index/marker compound(s) or the extract specified in the individual monograph.

PROCEDURES

Combine equal volumes of the filtered solutions of the six or more individual specimens withdrawn, and use the pooled sample as the test solution. Determine the average amount of index or marker compound(s) or the extract dissolved in the pooled sample by the *Procedure* specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the test solution taken. Use the *Medium* for preparation of the Standard solution and dilution, if necessary, of the test solution.

INTERPRETATION

Pooled Sample—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of the index or marker compound(s) or the extract dissolved from the pooled sample conform to the accompanying acceptance table. The quantity, *Q*, is the amount of dissolved index or marker compound(s) or the extract specified in the individual monograph, expressed as a percentage of the labeled content. The 5%, 15%, and 25% values in the acceptance table are percentages of the labeled content so that these values and *Q* are in the same terms.

Acceptance Table for a Pooled Sample

Stage	Number Tested	Acceptance Criteria
S_1	6	Average amount dissolved is not less than Q + 10%
S_2	6	Average amount dissolved (S_1 + S_2) is equal to or greater than Q + 5%
S_3	12	Average amount dissolved (S_1 + S_2 + S_3) is equal to or greater than Q

Dietary Supplements Other Than Vitamin–Mineral and Botanical Dosage Forms

Unless otherwise stated in the individual monographs for dietary supplement dosage forms in this category, compliance requires the testing of six individual units, measuring the dissolution of the dietary ingredient as the average of the six units tested.

PROCEDURES

Combine equal volumes of the filtered solutions of the six specimens withdrawn, and use the pooled sample as the test solution. Determine the average amount of dietary ingredient dissolved in the pooled sample by the *Procedure* specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the test solution taken. Use the *Medium* for preparation of the Standard solution and for dilution, if necessary, of the test solution.

TOLERANCES

Because of the diversity of chemical characteristics and solubilities of dietary ingredients pertaining to this category, general tolerances cannot be established. See individual monographs for *Tolerances*.

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

BRIEFING

Calcium Acetate, *USP 32* page 812. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques.) RTS—74956

Change to read:

Calcium Acetate, $Ca(C_2H_3O_2)_2 \cdot H_2O$—**176.18** ~~[62-54-4]~~ ■[*5743-26-0*]■2S (*USP33*)
—White, crystalline granules or powder. Soluble in about 3 parts of water; slightly soluble in alcohol. Use ACS reagent grade.

BRIEFING

1,3-Dicaffeoylquinic Acid. It is proposed to add this new reagent used to prepare *Standard solution 2*in the *Identification* test under *Echinacea angustifolia*.

(HDQ: M. Marques) RTS—C73258

Add the following:

■**1,3-Dicaffeoylquinic Acid** *(Cynarin; (1R,3R,4S,5R)-1,3-Bis[[3-(3,4-dihydroxyphenyl)propenoyl]oxy]-4,5-dihydroxycyclohexanecarboxylic Acid),* $C_{25}H_{24}O_{12}$—**516.45** [*30964-13-7*]—Use a suitable grade.

[NOTE— A suitable grade is available as catalog number 3991 from www.chromadex.com]■2S (*USP33*)

BRIEFING

Nitrogen Certified Standard. It is proposed to add this new reagent used in the monograph for *Medical Air.*

(HDQ: M. Marques.) RTS—C61164

Add the following:

■**Nitrogen Certified Standard**—A suitable 99.99% nitrogen certified standard is available from most suppliers of specialty gases.■2S (*USP33*)

BRIEFING

Methyl Red, *USP 32* page 833 and page 177 of *PF* 35(1) [Jan.–Feb. 2009]. It is proposed to include additional information about this reagent.

(HDQ: M. Marques.) RTS—C74960

Change to read:

Methyl Red *(2-[4-Dimethylaminophenylazo]benzoic Acid; C. I. Acid Red 2)* ~~$C_{15}H_{15}N_3O_2$— **269.30,**~~

▲ $C_{15}H_{15}N_3O_2$, free acid—**269.30** [*493-52-7*]; $C_{15}H_{14}N_3O_2Na$, sodium salt—**291.28** [*845-10-3*]▲*USP33*
—Use ACS reagent grade.

▲The free acid is recommended for nonaqueous titrations, particularly when an aprotic solvent is used. The sodium salt is recommended for titrations in aqueous media and also for nonaqueous titrations where the medium is an amphiprotic solvent.▲*USP33*

■The hydrochloride salt is recommended for titrations in aqueous media and amphiprotic solvents.■2S (*USP33*)

In-Process Revision

BRIEFING

93.0% Oxygen Certified Standard. It is proposed to add this new reagent used in the monograph for *Oxygen 93 Percent*.

(HDQ: M. Marques.) RTS—C61161

Add the following:

▪**93.0% Oxygen Certified Standard**—A suitable 93.0% oxygen certified standard is available from most suppliers of specialty gases.▪2S (*USP33*)

BRIEFING

Oxygen in Nitrogen Certified Standard. It is proposed to add this new reagent used in the monograph for *Nitrogen*.

(HDQ: M. Marques.) RTS—C73267

Add the following:

▪**Oxygen in Nitrogen Certified Standard**—A mixture of 1.0% oxygen in nitrogen. It is available from most suppliers of specialty gases.▪2S (*USP33*)

BRIEFING

3.0% Oxygen in Nitrogen Certified Standard. It is proposed to add this new reagent used in the monograph for *Nitrogen 97 Percent*.

(HDQ: M. Marques.) RTS—C61146

Add the following:

▪**3.0% Oxygen in Nitrogen Certified Standard**—A mixture of 3.0% oxygen in nitrogen. It is available from most suppliers of specialty gases.▪2S (*USP33*)

BRIEFING

21.0% Oxygen in Nitrogen Certified Standard. It is proposed to add this new reagent used in the monograph for *Medical Air.*

(HDQ: M. Marques.) RTS—C61164

Add the following:

▪**21.0% Oxygen in Nitrogen Certified Standard**—A suitable 21.0% oxygen in nitrogen certified standard is available from most suppliers of specialty gases.▪2S (*USP33*)

BRIEFING

Oxygen–Helium Certified Standard, *USP 32* page 836. It is proposed to delete this reagent because of the revisions being made to the monographs for *Nitrogen* and *Nitrogen 97 Percent*.

(HDQ: M. Marques.) RTS—C61146

Delete the following:

▪~~**Oxygen-Helium Certified Standard**—A mixture of 1.0% oxygen in industrial-grade helium. It is available from most suppliers of specialty gases.~~▪2S (*USP33*)

BRIEFING

Delta-8-tetrahydrocannabinol. It is proposed to add this new reagent used to prepare the *System suitability solution* used in the *Assay* test in the monograph for *Dronabinol Capsules*.

(HDQ: M. Marques) RTS—C73224

Add the following:

▪**Delta-8-tetrahydrocannabinol** *(Δ-8-tetrahydrocannabinol)* $C_{21}H_{30}O_2$—**314.47** [*5957-75-5*]—Use a suitable grade which may be a solid material or a solution in methanol. [NOTE—A suitable grade of a methanolic solution (1 mg/mL) is available from www.cerilliant.com, catalog number T-032.]▪2S (*USP33*)

In-Process Revision

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 32* page 881, and page 650 of *PF* 35(3) [May–June 2009].

(HDQ) RTS—C44129; C47817; C65483; C65484; C65921; C69397; C70442; C73613

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	$T_{▲USP33}$
Add the following:	
■Amlodipine Besylate Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
■Azithromycin Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Balsalazide Disodium Capsules	$T_{■2S\ (USP33)}$
Add the following:	
■Cabergoline Tablets	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Calcium Citrate Tablets	$W_{■1S\ (USP32)}$
Add the following:	
■Clonazepam Orally Disintegrating Tablets	W, $LR_{■1S\ (USP32)}$
Change to read:	
Dantrolene Sodium Capsules	T, ~~LR~~ ■$_{■2S\ (USP32)}$

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Add the following:	
■Doxycycline Hyclate Tablets, Delayed-Release	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Fluconazole Tablets	$W_{■2S\ (USP32)}$
Add the following:	
■Granisetron Hydrochloride Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
▲Guggul Tablets	W, $LR_{▲USP33}$
Add the following:	
■Ivermectin and Pyrantel Pamoate Tablets	T, $LR_{■1S\ (USP32)}$
Add the following:	
■Ketoprofen Capsules, Extended-Release	$T_{■2S\ (USP32)}$
Add the following:	
■Lamivudine and Zidovudine Tablets	W, $LR_{■1S\ (USP33)}$
Add the following:	
▲Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	$LR_{▲USP33}$
Add the following:	
▲Loratadine Orally Disintegrating Tablets	$T_{▲USP33}$
Add the following:	
■Losartan Potassium Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Metronidazole Capsules	W, $LR_{■1S\ (USP32)}$
Add the following:	
■Mycophenolate Mofetil Capsules	W, $LR_{■2S\ (USP33)}$
Add the following:	
■Mycophenolate Mofetil Tablets	W, $LR_{■2S\ (USP33)}$
Add the following:	
■Nateglinide Tablets	$T_{■1S\ (USP33)}$
Add the following:	
■Olanzapine Tablets	T, $LR_{■1S\ (USP33)}$

In-Process Revision

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Ondansetron Tablets	T, LR■2S *(USP32)*
Add the following:	
■Orbifloxacin Tablets	T■1S *(USP32)*
Add the following:	
■Orphenadrine Citrate Tablets, Extended-Release	T, LR■1S *(USP32)*
Add the following:	
▲Oseltamivir Phosphate Capsules	W▲*USP33*
Add the following:	
▲Oxcarbazepine Tablets	W▲*USP33*
Add the following:	
■Pilocarpine Hydrochloride Tablets	T ■1S *(USP32)*
Add the following:	
■Potassium Citrate Tablets	W■1S *(USP32)*
Add the following:	
■Ramipril Capsules	W■2S *(USP33)*
Add the following:	
■Ribavirin Capsules	W■1S *(USP33)*
Add the following:	
■Risedronate Sodium Tablets	W■2S *(USP32)*
Add the following:	
■Sumatriptan Tablets	W■2S *(USP33)*
Add the following:	
■Tacrolimus Capsules	T■1S *(USP33)*
Add the following:	
■Tamsulosin Hydrochloride Capsules	T■2S *(USP32)*
Add the following:	
■Telmisartan Tablets	W■1S *(USP33)*
Add the following:	
■Terazosin Capsules	W, LR■2S *(USP33)*
Add the following:	
■Terazosin Tablets	W, LR■2S *(USP33)*
Add the following:	
■Ticlopidine Hydrochloride Tablets	W■1S *(USP33)*

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Tranylcypromine Tablets	W■1S *(USP33)*
Add the following:	
■Tranylcypromine Sulfate Tablets	W■1S *(USP33)*
Add the following:	
■Valacyclovir Tablets	T■2S *(USP33)*
Add the following:	
■Valganciclovir Tablets	T■2S *(USP32)*
Add the following:	
■Zinc Citrate Tablets	W■1S *(USP32)*
Add the following:	
■Zinc Gluconate Tablets	T, LR■1S *(USP33)*
Add the following:	
■Zolpidem Tartrate Tablets	W■2S *(USP33)*
Add the following:	
■Zolpidem Tartrate Extended-Release Tablets	W■1S *(USP33)*

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 32* page 890, page 266 of *PF* 29(1) [Jan.–Feb. 2003], page 1053 of *PF* 33(5) [Sept.–Oct. 2007], page 817 of *PF* 34(3) [May–June 2008], page 1046 of *PF* 34(4) [July–Aug. 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], page 188 of *PF* 35(1) [Jan.–Feb. 2009], and page 464 of *PF* 34(2) [Mar.–Apr. 2009], and page 651 of 35(3) [May–June 2009].

(HDQ) RTS—C70442; C71283; C65791; C73593

Add the following:

■**Balsalazide Disodium:** Orange to yellow powder. Freely soluble in water and in isotonic saline; sparingly soluble in methanol and in alcohol; practically insoluble in all other organic solvents.■2S *(USP33)*

Add the following:

▪**Lactobionic Acid:** White or almost white, crystalline powder with a melting point of about 125° with decomposition. Freely soluble in water; slightly soluble in glacial acetic acid, in anhydrous ethanol and in methanol. *NF category:* Antioxidant.▪2S (*NF28*)

Add the following:

▪**Methacrylic Acid and Ethyl Acrylate Copolymer:** White powder having a faint, characteristic odor. Soluble to freely soluble in methanol, in alcohol, in isopropyl alcohol, and in acetone, each of which contains not less than 3% of water; soluble in diluted alkali, in simulated intestinal fluid TS, and in buffer solutions of pH 7 and above; insoluble in water, in diluted acids, in simulated gastric fluid TS, and in buffer solutions of up to pH 5. The solubility between pH 5.5 and pH 7 depends on the content of methacrylic acid units in the copolymer. *NF category:* Coating agent; film-forming agent.▪2S (*NF28*)

Add the following:

▪**Methacrylic Acid and Methyl Methacrylate Copolymer:** White powder having a faint, characteristic odor. Soluble to freely soluble in methanol, in alcohol, in isopropyl alcohol, and in acetone, each of which contains not less than 3% of water; soluble in diluted alkali, in simulated intestinal fluid TS, and in buffer solutions of pH 7 and above; insoluble in water, in diluted acids, in simulated gastric fluid TS, and in buffer solutions of up to pH 5. The solubility between pH 5.5 and pH 7 depends on the content of methacrylic acid units in the copolymer. *NF category:* Coating agent; film-forming agent.▪2S (*NF28*)

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* an *IRA* , or a *Revision Bulletin.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents; Indicators; and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
General Notices (entire *General Notices and Requirements* revised)	34	1	40
USP Monographs			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetaminophen and Tramadol Hydrochloride Tablets (new)	35	1	56
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Sulfate—*USP Reference standards, Assay*	34	2	242
Albuterol Tablets—*Assay*	31	3	726
Alendronate Sodium Tablets—*Dissolution*	35	1	59
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Aluminum Acetate Topical Solution—*Identification*	34	2	242
Aluminum Subacetate Topical Solution—*Identification*	34	2	242
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	35	1	62
Amodiaquine Hydrochloride—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	2	243
Amodiaquine Hydrochloride Tablets—*USP Reference standards, Identification, Assay*	34	3	558
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140
Ampicillin Sodium—*Dimethylaniline*	35	1	65
Anastrozole (new)	34	2	244
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Articaine Hydrochloride (new)	35	3	544
Aspirin—*Readily carbonizable substances*	34	5	1143
Atenolol—*Identification B*	35	3	545
Atenolol Tablets—*Dissolution*	35	1	66
Atorvastatin Calcium (new)	35	1	66
Atovaquone—*Assay*	34	2	247
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam for Injection—*Assay*	34	4	906
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Benzoin—*Botanic characteristics, Identification*	35	1	70
Betamethasone Oral Solution—*Packaging and storage, Thin-layer chromatographic identification test* (delete), *Identification A, B* (add), *Microbial limits* (add), *pH* (add), *Deliverable volume* (add), *Related compounds* (add), *Assay*	34	3	567
Bicalutamide Tablets—*Labeling, Dissolution*	34	5	1147
Bisoctrizole (new)	32	2	309
Bisoprolol Fumarate Tablets—*Dissolution*	34	3	570
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Limit of 11-ketobudesonide, Related compounds*	35	3	539
Bupropion Hydrochloride Extended-Release Tablets—*Related compounds*	35	1	70
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Cabergoline Tablets (new)	34	3	572
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Capecitabine Tablets—*Dissolution*	35	1	72
Carbidopa—*Specific rotation*	35	1	73
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Carmustine (new)	35	3	546
Carmustine for Injection (new)	35	3	548
Cefaclor Capsules—*Identification, Related compounds, Assay*	34	2	248
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Chlorhexidine Acetate (new)	34	3	582
Chlorhexidine Gluconate Oral Rinse—*Labeling, USP Reference standards*	34	2	250
Chlorhexidine Gluconate Solution—*USP Reference standards, Limit of p-chloroaniline, Assay*	34	2	250
Chlorhexidine Hydrochloride (new)	34	3	585
Chloroquine Phosphate—*USP Reference standards, Identification, Assay*	34	2	251
Chloroquine Phosphate Tablets—*USP Reference standards, Identification, Assay*	34	3	587
Cilostazol—*Loss on drying*	34	3	589
Cisapride (new)	34	2	253
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Anhydrous Citric Acid *(Harmonization)—Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)—Sulfate*	31	3	750

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49
Clarithromycin Tablets—*Dissolution*	35	1	73
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Climbazole (new)	33	5	891
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clonazepam Orally Disintegrating Tablets (new)	34	2	254
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Clozapine Tablets—*Uniformity of dosage units* (add)	34	3	589
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Human Acellular Dermal Matrix (new)	35	3	558
Microsized Human Acellular Dermal Matrix (new)	35	3	561
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclazuril (new)	35	1	73
Diclofenac Sodium Delayed-Release Tablets—*Dissolution*	35	2	271
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Dolasetron Mesylate—*Impurities*	35	2	272
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Doxycycline Hyclate Delayed-Release Tablets (new)	34	3	589
Dronabinol Capsules—*Definition, Assay, USP Reference Standards*	35	3	549
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Enalaprilat Injection (new)	34	3	593
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Epirubicin Hydrochloride (new)	35	2	273
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158
Esomeprazole Magnesium—*Identification B, Other Components—Content of Magnesium, Optical Rotation* (delete)	35	3	550
Estradiol Tablets—*USP Reference standards, Chromatographic purity* (add)	34	3	596
Estradiol Vaginal Inserts (new)	34	3	597
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Ethinyl Estradiol Tablets—*Dissolution* (add)	31	4	1067
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate—*Definition, Assay*	35	2	275
Fenoprofen Calcium—*Chromatographic purity*	34	3	601
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931
Fluconazole Injection (new)	35	3	552
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Fluticasone Propionate Cream (new)	34	3	609
Fluticasone Propionate Ointment (new)	34	3	611
Fluvestrant (new)	33	5	99
Fosinopril Sodium—*Related compounds*	34	3	613
Fosphenytoin Sodium—*Related compounds, Assay*	34	2	270
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Labeling* (add), *Dissolution*	34	6	1452
Glimepiride Tablets—*Dissolution*	33	3	411
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide Tablets—*Dissolution*	33	4	651
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163
Haloperidol Decanoate (new)	34	3	614
Heparin Sodium (entire monograph revised)	35	2	257
Heparin Sodium Injection (entire monograph revised)	35	2	266
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxyzine Pamoate—*Identification, Residue on ignition, Heavy metals, Pamoic acid content* (delete), *Assay*	34	2	271
Hydroxyzine Pamoate Capsules—*Identification, Assay*	34	2	272
Hydroxyzine Pamoate Oral Suspension—*Identification, Assay*	34	2	273
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Irbesartan—*Limit of azide*	34	5	1164
Isotretinoin Capsules—*Chromatographic purity, Assay*	34	4	942
Itraconazole (new)	34	4	947
Ivermectin and Pyrantel Pamoate Tablets (new)	34	2	277
Ketoprofen—*USP Reference standards, Chromatographic purity*	34	3	617
Ketoprofen Extended-Release Capsules (new)	34	4	951
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamivudine and Zidovudine Tablets (new)	35	2	277
Lamotrigine (new)	34	3	617
Levonorgestrel—*USP Reference standards, Chromatographic purity, Assay*	34	3	620
Levorphanol Tartrate—*Assay*	34	2	280
Levothyroxine Sodium—*Organic Impurities—Procedure 1, Procedure 2* (add), *Packaging and Storage, Labeling* (add), *USP Reference Standards*	35	3	555
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lipid Injectable Emulsion—*Definition, Limit of free fatty acids*	34	3	621
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium—*Limit of cyclohexane and isopropyl alcohol* (delete)	34	3	626
Losartan Potassium Tablets (new)	34	5	1164
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Mafenide Acetate Cream—*Identification*	34	2	280
Mafenide Acetate for Topical Solution—*Content of acetic acid*	34	3	627
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263
Meclocycline Sulfosalicylate—*Assay*	34	3	627
Meclocycline Sulfosalicylate Cream—*Assay*	34	3	628
Mefenamic Acid—*Heavy metals*	34	2	281
Megestrol Acetate Oral Suspension—*Dissolution*	35	1	75
Meloxicam—*Impurities, Procedure 1*	35	2	278
Mesna (new)	34	5	1168
Metformin Hydrochloride Extended-Release Tablets—*Dissolution*	35	1	76
Methacholine Chloride—*Identification, Melting range* (delete)	34	3	629
Methotrexate—*USP Reference standards, Chromatographic purity*	34	3	630
Methylcellulose (new)—*Stage 6 Harmonization*	35	3	683
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Metronidazole—*Packaging and storage, USP Reference standards, Identification, Melting range* (delete), *Non-basic substances* (delete), *Chromatographic purity* (delete), *Related compounds* (add), *Assay*	34	3	631
Metronidazole Capsules (new)	34	3	633
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Misoprostol (new)	35	3	564
Mometasone Furoate Cream—*Packaging and storage, Related compounds* (add), *Assay*	35	1	82
Mometasone Furoate Ointment—*Packaging and storage, Related compounds* (add), *Assay*	35	1	84
Mometasone Furoate Topical Solution—*Packaging and storage, Related compounds* (add), *Assay*	35	1	87
Morantel Tartrate—*pH*	32	6	1735
Morphine Sulfate Extended-Release Capsules—*Assay, Organic Impurities—Procedure, USP Reference Standards*	35	3	565
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Cream—*Related compounds, Assay*	34	2	281
Mupirocin Nasal Ointment (new)	34	4	966
Mycophenolate Mofetil—*Identification, Melting range* (delete), *Related compounds, Assay*	35	1	89
Naltrexone Hydrochloride—*Related compounds*	34	2	283
Naratriptan Hydrochloride Oral Suspension (new)	35	1	90
Nefazodone Hydrochloride—*Identification*	35	3	540
Nateglinide (new)	34	6	1463
Nateglinide Tablets (new)	35	2	281
Niacinamide—*Readily carbonizable substances*	34	5	1176
Nitrofurantoin—*Packaging and storage*	35	1	92
Nitrofurantoin Capsules—*Packaging and storage*	35	1	92
Nitrofurantoin Oral Suspension—*Packaging and storage*	35	1	92
Nitrofurantoin Tablets—*Packaging and storage*	35	1	92
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethynodrel (delete entire monograph)	35	1	92
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine (new)	34	3	641
Olanzapine Tablets (new)	35	2	282
Olopatadine Hydrochloride (new)	35	3	567
Olopatadine Hydrochloride Ophthalmic Solution (new)	35	3	568
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Orbifloxacin (new)	34	2	283
Orbifloxacin Tablets (new)	34	2	286
Orlistat Capsules (new)	32	6	1739
Orphenadrine Citrate Extended-Release Tablets (new)	34	3	643
Oseltamivir Phosphate (new)	34	6	1468
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin Injection (new)	35	2	284
Oxaliplatin for Injection (new)	34	6	1473
Oxazepam Capsules—*Dissolution*	35	2	286
Oxcarbazepine (new)	34	5	1177
Oxcarbazepine Tablets (new)	34	6	1478
Oxybutynin Chloride Tablets—*Dissolution*	35	1	93
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxycodone Hydrochloride Extended-Release Tablets—*Related compounds*	31	4	1104
Oxymetazoline Hydrochloride Nasal Solution—*pH*	33	5	932
Oxytocin—*Definition, USP Reference standards, Identification, Vasopressor activity* (delete), *Acetic acid content* (add)	34	3	647
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Paricalcitol—*Identification, Assay*	33	2	252
Pectin—*Chemical information; Definition; Identification—A, B, C, D* (delete), *Procedure* (add); *Assay—Methoxy Groups* (name change), *Galacturonic Acid, Methoxy Groups* (add); *Impurities—Lead, Procedure 1, Procedure 2* (add), *Procedure 3* (add); *Microbial Enumeration Tests; Packaging and Storage; Labeling; USP Reference Standards* (add)	35	2	287
Penicillamine Capsules—*Dissolution*	31	2	436
Pentamidine Isethionate (new)	35	3	570
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Pergolide Oral Suspension, Veterinary (new)	34	2	289
Permethrin (new)	32	4	1100
Permethrin Cream (new)	34	1	103
Petrolatum (new)—*Harmonization*	28	2	569
White Petrolatum (new)—*Harmonization*	28	2	570
Liquefied Phenol—*Identification* (add), *Other requirements*	35	1	93
Phenylephrine Hydrochloride—*Assay*	34	2	291
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride Tablets (new)	34	2	291
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Pravastatin Sodium—*Chromatographic purity, Assay*	34	2	294
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Praziquantel Tablets—*Dissolution*	35	2	291
Primidone—*Identification B, C* (delete), *Assay, Organic Impurities—Procedure, Melting Range or Temperature* (delete), *USP Reference Standards*	35	3	571
Primidone Tablets—*Assay, Organic Impurities—Procedure* (add), *USP Reference Standards*	35	3	573
Proguanil Hydrochloride (new)	34	2	296
Promethazine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	292
Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution (new)	35	2	295
Promethazine and Phenylephrine Hydrochloride Oral Solution (new)	35	2	298
Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	301
Propafenone Hydrochloride—*USP Reference standards, Chromatographic purity* (delete), *Related compounds* (add)	35	1	94
Propoxyphene Hydrochloride Capsules—*Identification B* (delete), *Identification C*	35	3	574
Pseudoephedrine Hydrochloride—*Definition, USP Reference standards, Ordinary impurities* (delete), *Chromatographic purity* (add), *Assay*	34	2	298
Psyllium Husk—*Impurities—Heavy Metals* (add), *Procedure 3* (add)	35	2	304
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ramipril—*Definition, Assay*	31	3	787
Ranitidine Hydrochloride—*Chromatograpic purity, Assay*	34	2	299
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Repaglinide Tablets—*Loss on Drying* (delete)	35	2	306
Ribavirin Capsules (new)	35	3	576
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Ritonavir—*Identification*	35	1	95
Rocuronium Bromide (new)	34	3	648
Salmeterol Xinafoate (new)	35	2	307
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sennosides—*Content of Sennosides A and B* (add), *USP Reference Standards* (add)	35	2	308
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Simethicone Emulsion—*Assay*	34	3	652
Simethicone Tablets—*Disintegration*	34	3	652
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Fluoride—*Assay*	34	3	653
Sodium Sulfate—*Assay*	34	5	1192
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Stavudine—*Specific rotation*	34	3	653
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadiazine Tablets—*Dissolution*	35	3	577
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfadoxine and Pyrimethamine Tablets—*Assay*	34	2	301
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfasalazine—*Identification*	34	3	653
Sulfazalazine Tablets—*Identification*	34	3	653
Sulfinpyrazone—*Identification A, B* (add), *Melting Range or Temperature* (delete), *Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	35	3	577

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Tacrolimus (new)	35	2	310
Tacrolimus Capsules (new)	35	2	312
Tamsulosin Hydrochloride (new)	35	3	578
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Tazobactam—*Identification, Specific rotation, Related compounds, Organic volatile impurities* (delete), *Assay*	34	2	302
Telmisartan (new)	35	3	580
Telmisartan Tablets (new)	35	3	581
Terbinafine Oral Suspension (new)	35	1	96
Terbutaline Oral Suspension (new)	35	1	97
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride—*Chromatographic purity*	34	2	306
Tiagabine Hydrochloride Oral Suspension (new)	35	1	98
Ticlopidine Hydrochloride (new)	35	3	582
Ticlopidine Hydrochloride Tablets (new)	35	3	584
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798
Tizanidine Tablets—*Dissolution*	35	3	585
Tobramycin Inhalation Solution—*Identification* (add), *Osmolarity, Chromatographic purity, Other requirements* (delete), *Assay*	34	2	307
Topiramate Tablets (new)	34	5	1197
Tramadol Hydrochloride (new)	34	5	1200
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Trandolapril (new)	34	2	310
Tranylcypromine Sulfate (new)	35	2	314
Tranylcypromine Tablets (new)	35	3	587
Travoprost (new)	32	4	1115
Travoprost Ophthalmic Solution (new)	32	4	1118
Trenbolone Acetate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Limit of trenbolone acetate 17α-isomer* (delete), *Related compounds* (add), *Assay*	35	1	100
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800
Triamterine Capsules—*USP Reference standards, Related compounds* (add), *Assay*	34	3	654
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tromethamine—*Melting Range or Temperature*	35	2	316
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Valacyclovir Hydrochloride (new)	35	3	589
Valganciclovir Tablets (new)	33	1	89
Valproic Acid Capsules—*Disintegration* (delete)	35	3	591
Valrubicin—*Definition, USP Reference standards, Identification, Loss on drying* (delete), *Water* (add), *Limit of residual solvents* (delete), *Related compounds, Assay*	35	1	103
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486
Vancomycin Hydrochloride—*Labeling* (add), *USP Reference standards, Chromatographic purity, Other requirements* (add)	34	1	111
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Labeling* (add), *Identification* (add), *Water* (add), *pH* (add), *Uniformity of dosage units* (add), *Chromatographic purity, Assay*	34	4	992
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Vincristine Sulfate Injection—*Identification*	35	1	106
Vincristine Sulfate for Injection—*Identification*	35	1	106
Pure Steam (new)	31	2	467
Water for Hemodialysis—*Bacterial endotoxins*	31	2	468
Water for Injection—*Definition, Bacterial Endotoxins Test, Water Conductivity, Sterility Tests* (add), *Packaging and Storage* (add), *Labeling* (add)	35	2	316
Purified Water—*Definition, Packaging and Storage* (add), *Labeling* (add)	35	2	317
Sterile Water for Inhalation—*pH* (delete), *Ammonia* (delete), *Calcium* (delete), *Carbon dioxide* (delete), *Chloride* (delete), *Sulfate* (delete), *Conductivity* (add), *Oxidizable substances*	31	3	802
Sterile Water for Injection—*Oxidizable substances*	31	3	803
Sterile Water for Irrigation—*Oxidizable substances*	31	3	804
Sterile Purified Water—*Oxidizable substances*	31	3	804
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Zidovudine—*Assay*	34	3	656
Zidovudine Capsules—*Related compounds, Assay*	34	3	657
Zidovudine Injection—*Related compounds, Assay*	34	3	658
Ziprasidone Hydrochloride (new)	35	3	592
Zolpidem Tartrate (new)	34	6	1487
Zolpidem Tartrate Extended-Release Tablets (new)	35	3	595
Zonisamide (new)	34	6	1489
Dietary Supplements Monographs			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
N-Acetyltyrosine (new)	35	1	107
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Calcium Citrate Tablets (new)	34	2	312
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Glucosamine Hydrochloride—*Assay*	33	4	691
Glucosamine Sulfate Potassium Chloride—*Assay*	33	4	692
Glucosamine Sulfate Sodium Chloride—*Assay*	33	4	692
Glutamic Acid (new)	34	4	997
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004
Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Powdered Hawthorn Leaf with Flower—*Labeling*	34	5	1209
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Omega-3 Acid Triglycerides (new)	34	3	662
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Potassium Citrate Tablets (new)	34	2	313
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Vitamin A Oral Liquid Preparation (new)	35	3	596
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Citrate (new)	34	2	315
Zinc Citrate Tablets (new)	34	2	316
Zinc Gluconate Tablets (new)	35	3	597
Zinc and Vitamin C Lozenges (new)	34	2	317
USP General Test Chapters			
⟨1⟩ Injections—*Ingredients, Foreign and Particulate Matter*	35	3	601
⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests (new)	35	3	602
⟨11⟩ USP Reference Standards	29	6	2022
	30	5	1674
	31	2	507
	31	4	1154
	31	6	1680
	32	4	1161
	33	1	95
	33	5	981
	34	2	332
	34	3	680

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
	34	4	1021
	34	5	1230
	34	6	1531
	35	1	144
	35	2	330
	35	3	612
⟨41⟩ Weights and Balances—*Introduction, Repeatability, Verification of Accuracy, Calibration Check*	35	2	331
⟨63⟩ Mycoplasma Tests (new)	35	1	146
⟨85⟩ Bacterial Endotoxins Test—*Stage 6 Harmonization*	35	3	695
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨191⟩ Identification Tests—General—*Introduction*	34	2	333
⟨197⟩ Spectrophotometric Identification Tests (entire chapter revised)	35	1	153
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨223⟩ Dimethylaniline—*Chromatographic System, Procedure*	35	1	156
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨271⟩ Readily Carbonizable Substances Test—*Introduction*	33	6	1258
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨381⟩ Elastomeric Closures for Injections—*Introduction*	35	3	614
⟨401⟩ Fats and Fixed Oils—*Ester Value, Hydroxyl Value, Iodine Value, Peroxide Value, Saponification Value, Polyunsaturated Fatty Acids Determination and Profile* (add), *Trace Metals* (add), *Sterol Composition* (add)	34	3	736
⟨429⟩ Light Diffraction Measurement of Particle Size (new)—*Stage 6 Harmonization*	35	3	707
⟨467⟩ Organic Volatile Impurities—*Identification, Control, and Quantification of Residual Solvents*	34	3	747
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	35	2	334
⟨525⟩ Sulfur Dioxide—*Method IV* (add), *Method V* (add)	35	2	341
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density (new)—*Stage 6 Harmonization*	35	3	715
⟨621⟩ Chromatography—*System Suitability*	34	5	1238
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨661⟩ Containers—Plastics—*Introduction, Polyethylene Containers, Polypropylene Containers*	34	2	335
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533
⟨671⟩ Containers—Performance Testing—*Introduction, Moisture Permeation, Light Transmission Test*	34	2	337
⟨696⟩ Characterization of Crystallinity Determination by Solution Calorimetry—*Stage 4 Harmonization*	35	3	675
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨711⟩ Dissolution—*Stage 6 Harmonization*	35	3	719
⟨725⟩ Topical and Transdermal Drug Products—Product Performance Tests (new)	35	3	615
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	35	3	626
⟨731⟩ Loss on Drying—*Introduction*	34	3	760
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨788⟩ Particulate Matter in Injections—*Introduction*	35	3	628
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨851⟩ Spectrophotometry and Light-Scattering—(entire chapter revised)	35	1	157
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨905⟩ Uniformity of Dosage Units—*Stage 6 Harmonization*	35	3	724
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	35	2	346
⟨941⟩ X-Ray Diffraction (new)—*Stage 6 Harmonization*	35	3	731
General Information Chapters			
⟨1010⟩ Analytical Data—Interpretation and Treatment—*Prerequisite Laboratory Practices and Principles, Measurement Principles and Variation, Comparison of Analytical Methods, Appendixes B, C, D, E, F*	34	3	764
⟨1024⟩ Bovine Serum (new)	35	3	628
⟨1033⟩ Biological Assay Validation (new)	35	2	349
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients (entire chapter revised)	34	2	343
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1097⟩ Bulk Powder Sampling Procedures (new)	35	2	367
⟨1113⟩ Microbial Identification (new)	35	1	167
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1180⟩ Human Plasma (new)	35	2	388
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients (new)	34	2	375
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation*	30	5	1729
⟨1225⟩ Validation of Compendial Procedures—*Validation*	35	2	444
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1237⟩ Virology Test Methods (new)	34	2	391
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	35	2	448
⟨1788⟩ Particulate Matter Determination in Parenteral and Ophthalmic Products (new)	34	2	421
Reagents, Indicators, and Solutions			
Reagents, Indicators, and Solutions—*Introduction*	35	1	176
Acetylactone	34	3	808
Alcohol	35	1	177
Alcohol, Denatured (new)	34	3	808
p-Aminophenol	34	2	442
Ammonium Molybdate	35	1	177
Barium Chloride	34	2	442
Beclomethasone (new)	34	3	808
1-Butanesulfonic Acid Sodium Salt (new)	33	4	766
t-Butylthiol (new)	35	3	648
Calcium Chloride	34	3	808
Chloramine T	34	2	442
Chromotropic Acid	35	1	177
Chromotropic Acid Disodium Salt	35	1	177
Cobalt Nitrate	35	3	648
Diatomaceous Earth (new)	34	3	809
Diaveridine	35	3	648
2,7-Dihydroxynaphthalene (new)	34	3	809
N,N-Dimethyldecylamine (new)	34	4	1041
Dimethyltin Dibromide (new)	34	2	442
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Ethylenediamine (new)	34	2	442
Ferric Chloride	34	2	443
Heptyl *p*-Hydroxybenzoate (new)	35	2	460
Hydrogen Peroxide, 30 Percent	34	2	443

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Hydrogen Peroxide, 30 Percent, Unstabilized (new)	34	3	809
Hydrogen Peroxide, 50 Percent in Water (new)	34	3	809
Lead Acetate	34	2	443
Maltotriose (new)	34	3	809
7-Methoxycoumarin (new)	34	2	443
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Methyl Red	35	1	177
Morin (new)	34	2	443
p-Naphtholbenzein	35	3	648
1-Octanol (new)	32	6	1804
Pectate Lysate (new)	35	2	460
Phosphatase Enzyme, Alkaline	34	3	809
Phosphorous Acid (new)	35	1	178
Potassium Metabisulfite (new)	35	1	178
Potassium Sodium Tartrate	35	1	178
Silver Nitrate	34	3	810
Sodium Acetate	35	2	461
Sodium Biphenyl	35	3	648
Sodium Cholate Hydrate (new)	34	3	810
Sodium 1-Decanesulfonate	34	5	1319
Sorbitol (new)	34	3	810
Stannous Chloride	35	3	649
Sulfuric Acid, Nitrogen Free (new)	35	3	649
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Tetrabutylammonium Hydroxide 30-Hydrate (new)	34	3	810
Tetrabutylammonium Hydroxide, 40 Percent in Water (delete)	34	3	810
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled	34	3	810
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled	34	3	811
Triethylenediamine (new)	34	2	443
Trimethyltin Bromide (new)	34	2	444
Test Solutions			
Acetic Acid, Glacial, TS	35	1	179
Alcoholic TS (new)	34	3	811
Denatured Alcoholic TS (new)	35	1	179
Ammonia TS 2 (new)	34	2	444
Cupric Citrate TS 2, Alkaline	35	1	179
Iodine and Potassium Iodide TS 3 (new)	34	2	444
Lanthanum Nitrate TS (new)	34	2	444
Methyl Red TS 2 (new)	34	2	445
Potasssium Pyroantimonate TS	34	3	812
Dibasic Sodium Phosphate TS	35	3	649
Volumetric Solutions			
Hydrochloric Acid, Normal (1 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N) in Methanol	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid	35	1	181
Potassium Iodate, Twentieth-Molar (0.05 M)	34	3	813
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Sodium Tetraphenylboron, Fiftieth-Molar (0.02 M)	34	2	447
Chromatographc Reagents			
Chromatographic Reagents—*Title, Packings*	35	1	182
Reference Tables			
Container Specifications for Capsules and Tablets	35	3	650
Description and Solubility	29	1	266
	31	2	591
	31	4	1193
	33	5	1053
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
	35	1	188
	35	2	464
	35	3	651
Atomic Weights—*Standard Atomic Weights of the Elements*	35	1	189

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Excipients			
USP and *NF* Excipients, Listed by Category	35	2	318
NF General Notices and Requirements—*Title* (delete), *''Official'' and ''Official Articles''* (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
NF Monographs			
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
Alpha-Lactalbumin (new)	34	3	670
Amino Methacrylate Copolymer—*Definition, Packaging and storage, Viscosity, Limit of monomers*	34	2	326
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Benzyl Alcohol—*Stage 6 Harmonization*	35	3	685
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Harmonization*	33	3	537
Silicified Microcrystalline Cellulose (new)	34	5	1218
Chitosan (new)	35	1	115
Hydrogenated Coconut Oil (new)	34	2	327
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
High Fructose Corn Syrup—*Total solids, Assay*	34	2	329
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone (new)—*Stage 4 Harmonization*	35	3	671
Cystine (new)	35	1	122
Desoxycholic Acid (new)	34	6	1523
Egg Phospholipids (new)	33	4	703
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity, Coagulum content*	35	1	123
Ethyl Maltol (new)	34	5	1224
Ethylene Glycol and Vinyl Alcohol Graft Copolymer (new)	35	2	324
Ethylparaben—*Harmonization*	34	6	1594
Fumaric Acid—*Identification*	35	3	598
Hydrogenated Palm Oil (new)	34	2	330
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Hydroxypropyl Cellulose—*Identification*	35	1	124
Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	672
Low-Substituted Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	673
Anhydrous Lactose—*Harmonization*	32	6	1847
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Viscosity, Limit of monomers, Coagulum content*	35	1	124
Methyl Alcohol—*Readily carbonizable substances*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Definition, Packaging and storage, Assay*	31	4	1145
Nitrogen 97 Percent—*Definition, Packaging and storage, Assay*	31	4	1146
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Olive Oil—*CAS number* (add), *Definition, Packaging and storage, Identification* (add), *Fatty acid composition* (add), *Specific gravity* (delete), *Cottonseed oil* (delete), *Peanut oil* (delete), *Sesame oil* (delete), *Teaseed oil* (delete), *Absence of sesame oil* (add), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Specific absorbance* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add)	35	1	126
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete), *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227
Polyethylene Glycol—*Harmonization*	31	3	897
Polyoxyl 15 Hydroxystearate (new)	35	1	128
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polysorbate 80—*Harmonization*	33	5	1075
Polyvinyl Acetate (new)	34	6	1526
Polyvinyl Acetate Dispersion (new)	35	1	134
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylene Glycol Dilaurate—*Chemical information, Identification A, Assay*	35	3	599
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Corn Starch—*Stage 6 Harmonization*	35	3	687
Hydrogenated Starch Hydrolysate (new)	35	1	136
Pea Starch (new)	35	1	140
Potato Starch (new)—*Stage 6 Harmonization*	35	3	689
Rice Starch (new)—*Stage 6 Harmonization*	35	3	690
Wheat Starch (new)—*Stage 6 Harmonization*	35	3	692
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Sucrose Palmitate (new)	35	2	326
Sucrose Stearate (new)	35	2	328
Tagatose (new)	30	5	1672
Tetrafluoroethane (new)	31	6	1672
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	***PF Volume, Issue, and Page Numbers of Canceled Proposals***		
	Vol.	***No.***	***Page(s)***
USP Monographs			
Carvedilol Tablets—*Title* (add), *Definition* (add), *Packaging and storage* (add), *USP Reference standards* (add), *Identification* (add), *Uniformity of dosage units* (add), *Related compounds* (add), *Assay* (add)	33	5	888
Conjugated Estrogens—*Definition*	30	3	840
Desogestrel and Ethinyl Estradiol Tablets—*Related compounds*	30	5	1604
Estradiol Vaginal Inserts—*Dissolution*	31	6	1617
Flavoxate Hydrochloride Tablets—*Dissolution* (add)	33	6	1174
Hydrocodone Bitartrate and Homatropine Methylbromide Tablets—*Dissolution*	30	3	853
†Isotretinoin Capsules—*Labeling (add), Dissolution*	34	4	942
Ketoprofen Extended-Release Capsules—*Drug release*	31	5	1378
Leflunomide Tablets—*Dissolution*	31	5	1383
Mirtazapine Orally Disintegrating Tablets—*Water, Method Ia* (add)	33	6	1189
Norethindrone Tablets—*Dissolution* (add)	32	6	1736
Norethindrone Tablets—*Dissolution* (add)	33	6	1193
†Piperacillin and Tazobactam for Injection—*Definition* (add), *USP Reference standards* (add), *pH* (add), *Particulate matter* (add), *Assay* (add)	31	2	439
Promethazine Hydrochloride—*USP Reference standards, Related substances*	32	2	365
Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add), *Assay*	32	2	367
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add)	32	4	1107
†Terbinafine Hydrochloride—*Melting range*	34	5	1197
Dietary Supplements			
Asian Ginseng Capsules (entire submission)	30	2	571
USP General Test Chapters			
⟨191⟩ Identification Tests—General—*Acetate, Ammonium*	33	4	719
USP General Information Chapters			
⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire submission)	31	2	524
⟨1024⟩ Bovine Serum (entire submission)	34	3	776
†⟨1235⟩ Vaccines for Human Use—General Considerations (entire submission)	34	5	1297
NF Monographs			
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
†Methacrylic Acid Copolymer (entire submission)	33	6	1251
Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
Sucralose—*Related compounds*	33	6	1255

† New cancellation in *PF* 35(4).

STAGE 4 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF,* this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

MONOGRAPHS (NF)

BRIEFING

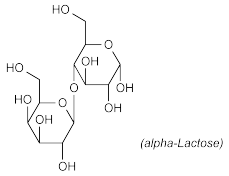
Anhydrous Lactose. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Anhydrous Lactose monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the revised **OFFICIAL INQUIRY STAGE 4** document, is based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia. Differences between this draft and the current monograph for Anhydrous Lactose include the following:

1. Clarification of the *Clarity and Color of Solution* test to allow the solution to cool before examination.
2. An updated procedure for the *Content of Alpha and Beta Anomers* is included.
3. The acceptance criteria for total combined molds and yeasts is updated at the request of EP.
4. A test for *Salmonella* is added at the request of JP.

(EM1: K. Moore.) RTS—C72359

Add the following:

Anhydrous Lactose



(alpha-Lactose)

DEFINITION

Anhydrous Lactose is *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (β-lactose), or a mixture of *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose and *O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (α-lactose).

ASSAY

• **CONTENT OF ALPHA AND BETA ANOMERS**

Silylation reagent: Dimethyl sulfoxide, pyridine, and trimethylsilylimidazole (19.5:58.5:22)

Standard solution: Prepare a mixture of alpha lactose monohydrate and beta lactose having an anomeric ratio of about 1:1, based on the labeled anomeric contents of the alpha lactose monohydrate and beta lactose. Introduce 10 mg of this mixture into a vial with a screw cap. Add 4 mL of *Silylation reagent*. Sonicate for 20 min at room temperature. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial and mix well.

Sample solution: Transfer 10 mg of Anhydrous Lactose into a vial with a screw cap. Add 4 mL of *Silylation reagent*. Sonicate for 20 min at room temperature. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial and mix well.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC
Detector: Flame ionization
Precolumn[1]: 0.53-mm × 2-m intermediate polarity deactivated fused-silica.
Column[2]: 0.25-mm × 15-m G27 on fused silica, film thickness 0.25 µm
Temperature: See temperature table below.

	Time (min)	Temperature (°)
Detector		325
Injection port		275 or use cold on-column injection
Column	0	80
	1	80
	3	150
	15.5	300
	17.5	300

Column pressure: 70 kPa
Carrier gas: Helium
Flow rate: 40 mL/min
Injection size: 0.5-µL splitless or by cold on-column injection
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between the peaks due to alpha lactose and beta lactose
Analysis
Sample: *Sample solution*
[NOTE—The relative retention times for beta lactose and alpha lactose are 12 and 0.9, respectively. The retention time for beta lactose is about 12 min.]
Calculate the percentage content of alpha lactose:

$$\text{Result} = (S_A/S_A + S_B) \times 100$$

S_A = area of the peak due to alpha lactose
S_B = area of the peak due to beta lactose

Calculate the percentage content of beta lactose:

$$\text{Result} = (S_B/S_A + S_B) \times 100$$

S_B = area of the peak due to beta lactose
S_A = area of the peak due to alpha lactose

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%, determined on a specimen ignited at a temperature of 600 ± 50°

Organic Impurities

• **PROCEDURE: CLARITY AND COLOR OF SOLUTION**

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water and dilute to 100.0 mL. Allow to stand for 4–6 h.

Hexamethylenetetramine solution: In a 100-mL ground-glass-stoppered flask, dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water.

Primary opalescent suspension: To the *Hexamethylenetetramine solution* in the flask add 25.0 mL of the *Hydrazine sulfate solution*. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Standard of opalescence: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of *Standard of opalescence* add 95.0 mL of water. Mix and shake before use.

[1] A Restek guard column is suitable.
[2] A Varian CP-Sil 8 CB guard column is suitable.

Brown-yellow stock solution: Mix 2.4 mL of ferric chloride CS, 1.0 mL of cobaltous chloride CS, 0.4 mL of cupric sulfate CS, and 6.2 mL of hydrochloric acid (10 g/L HCl).
Brown-yellow reference solution: Mix 2.5 mL of *Brown-yellow stock solution* and 97.5 mL of hydrochloric acid (10 g/L HCl).
Sample solution: 1 g in 10 mL of boiling water is clear and nearly colorless.
Spectrometric conditions
Mode: UV-Vis
Analytical wavelength: 400 nm
Acceptance criteria: Its clarity is the same as that of water or its opalescence is not more pronounced than that of the *Reference suspension*, and it is not more colored than the *Brown-yellow reference solution*. The absorbance divided by the path length in cm is NMT 0.04.

SPECIFIC TESTS

- **Loss on Drying** ⟨**731**⟩**:** Dry a sample at 80° for 2 h: it loses NMT 0.5% of its weight.
- **Water Determination,** *Method II* ⟨**921**⟩**:** NMT 1.0%, determined on a preparation containing anhydrous lactose in a mixture of methanol and formamide (2:1)
- **Microbial Enumeration Tests** ⟨**61**⟩ and **Tests for Specified Microorganisms** ⟨**62**⟩**:** Total aerobic microbial count is NMT 100 cfu/g; total combined molds and yeasts count is NMT 10 cfu/g; and it meets the requirements of the test for absence of *Escherichia coli* and *Salmonella* species.
- **Protein and Light-Absorbing Impurities** ⟨**851**⟩
Sample solution: 1% solution
Spectrometric conditions
Mode: UV
Wavelength range: 210–300 nm
Acceptance criteria: The absorbance divided by the path length in cm at 210–220 nm: NMT 0.25; and at 270–300 nm: NMT 0.07.
- **Acidity or Alkalinity**
Sample solution: Dissolve 6 g by heating in 25 mL of carbon dioxide-free water, cool, and add 0.3 mL of phenolphthalein TS.
Acceptance criteria: The solution is colorless, and NMT 0.4 mL of 0.1 N sodium hydroxide is required to produce a pink or red color.
- **Optical Rotation,** *Specific Rotation* ⟨**781S**⟩
Sample solution: Dissolve 10 g by heating in 80 mL of water to 50°. Allow to cool, and add 0.2 mL of 6 N ammonium hydroxide. Allow to stand for 30 min, and dilute with water to 100 mL.
Acceptance criteria: +54.4° and +55.9°, calculated on the anhydrous basis, at 20°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where the labeling indicates the relative quantities of alpha and beta lactose, determine compliance using *Content of Alpha and Beta Anomers*. Where the labeling states the particle size distribution, it also indicates the d_{10}, d_{50}, and d_{90} values and the range for each.

STAGE 6 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). This section includes Stage 6 adopted text which is provided for information. USP cannot incorporate public comments at Stage 6 without consulting PDG partners. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 27* page 1143 and page 318 of *PF* 35(2) [Mar.–Apr. 2009]. It is proposed to add *Carmellose* to the *Suspending and/or Viscosity-Increasing Agent* category; *Lactobionic Acid* to the *Antioxidant* category; and *Methacrylic Acid and Ethyl Acrylate Copolymer* and *Methacrylic Acid and Methyl Methacrylate Copolymer* to the *Coating Agent* and *Film-Forming Agent* categories, to complement the proposed new monographs for *Carmellose, Lactobionic Acid, Methacrylic Acid and Ethyl Acrylate Copolymer***, and** *Methacrylic Acid and Methyl Methacrylate Copolymer,* **which appear elsewhere in this issue of** *PF.*

(EM1; EM2) RTS—C65791; C71283; C73593

In the following reference table, the grouping of excipients by functional category is intended to summarize the most typically identified purpose that these excipients serve in drug product formulations. The list of substances included in each category is not comprehensive. The statement of category is intended neither to limit in any way the choice or use of the substance nor to indicate that it has no other utility.

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

▲Carmellose▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

▲Chitosan▲*NF28*

▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

▲Alpha-Lactalbumin▲*NF28*

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin
Polyethylene Oxide
Polyvinyl Alcohol
Povidone
Propylene Glycol Alginate
Pullulan
Hydrophobic Colloidal Silica
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca
Starch, Wheat

■Sucrose Palmitate■1S (*NF28*)

Tragacanth
Xanthan Gum

MONOGRAPHS (NF)

BRIEFING

Carmellose. The Japanese Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Carmellose monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the Official Inquiry Stage 4 document which appeared in PF 33(3). Because there is no existing monograph for this excipient, a new monograph based on the **ADOPTION STAGE 6** document is presented.

(EM2: K. Moore.) RTS—C71283

Add the following:

▲Carmellose

Attributes	EP	JP	USP
Definition	+	+	+
Identification A*	+	+	+
Identification B	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+

*EP and USP will adopt Carmellose Reference Standard; JP will adopt a Reference spectrum.

Legend: + will adopt and implement; – will not stipulate
Nonharmonized attributes: *Heavy Metals* and *Packaging and Storage* (USP)
Reagents and reference materials: Each pharmacopeia will adapt the text to take account of local reference substances and spectra and reagent specifications.

Carboxymethylcellulose [9000-11-7].

DEFINITION
Carmellose is a carboxymethyl ether of cellulose.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B. pH ⟨791⟩**
  Sample solution: 10 mg/mL of Carmellose suspension
  Acceptance criteria: 3.5–5.0

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 1.5% on 1 g, calculated on the dried basis
- **CHLORIDES**
  Sample solution: Shake well 0.8 g of Carmellose with 50 mL of water, dissolve in 10 mL of sodium hydroxide TS, and add water to make 100 mL. Heat 20 mL of this solution with 10 mL of nitric acid, diluted on a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Take 25 mL of this solution, add 6 mL of nitric acid, and dilute with water to make 50 mL.
  Control solution: 0.40 mL of 0.01 N hydrochloric acid VS and 6 mL of dilute nitric acid. Add water to make 50 mL.
  Analysis: Add 1 mL of silver nitrate TS to the *Sample solution* and to the *Control solution*, mix well, and allow to stand for 5 min protected from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.
  Acceptance criteria: The turbidity produced in the *Sample solution* is NMT that in the *Control solution* (NMT 0.36%).
- **SULFATES**
  Sample solution: Shake well 0.40 g of Carmellose with 25 mL of water, dissolve in 5 mL of sodium hydroxide TS, and add 20 mL of water. Heat this solution with 2.5 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Filter this solution, discard 5 mL of the first filtrate, take 25 mL of the subsequent filtrate, add 1 mL of hydrochloric acid, and dilute with water to make 50 mL.
  Control solution: 1.5 mL of 0.01 N sulfuric acid VS and 1 mL of dilute hydrochloric acid. Add water to make 50 mL.
  Analysis: Add 2 mL of barium chloride TS to the *Sample solution* and to the *Control solution*, mix well, and allow to stand for 10 min. Compare the turbidity developed in both solutions against a black background by viewing downward or transversely.
  Acceptance criteria: The turbidity produced in the *Sample solution* is NMT that of the *Control solution* (NMT 0.72%).
- **✦HEAVY METALS,** *Method II* **⟨231⟩:** Proceed with 1.0 g of Carmellose and perform the test. Prepare the control solution with 2.0 mL of *Standard Lead Solution*.
  Acceptance criteria NMT 20 ppm✦

SPECIFIC TESTS
- **LOSS ON DRYING ⟨731⟩:** Dry 1 g at 105° for 4 h: it loses NMT 8.0% of its weight.

ADDITIONAL REQUIREMENTS
- **✦PACKAGING AND STORAGE:** Preserve in tight containers.✦
- **✦USP REFERENCE STANDARDS ⟨11⟩**
  USP Carmellose RS✦▲*NF28*

BRIEFING

Polysorbate 80. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Polysorbate 80 monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Polysorbate 80 that was prepared by the European Pharmacopoeia and published in *PF* 33(5) [Sept.–Oct. 2007]. The European Pharmacopoeia draft was based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia in response to the Provisional Harmonized Text Stage 5A and 5B drafts prepared by the European Pharmacopoeia. No significant differences were made from the draft published in *PF* 33(5) [Sept.–Oct. 2007].

(EM2: K. Moore.) RTS—C65925

Add the following:

▲Polysorbate 80

Attributes	EP	JP	USP
Definition	+	+	+
Characters (Description and Solubility, Specific Gravity, Viscosity)	+	+	+
Identification (Composition of Fatty Acids)	+	+	+
Acid Value	+	+	+
Hydroxyl Value	+	+	+
Peroxide Value	+	+	+
Saponification Value	+	+	+
Composition of Fatty Acids	+	+	+
Ethylene Oxide and Dioxane	+	+	+
Water	+	+	+
Residue on Ignition	+	+	+
Storage	+	+	+

Legend: + will adopt and implement; – will not stipulate
Nonharmonized attributes: *Identification* by IR (EP), *Heavy Metals* (USP)
Each pharmacopeia will adapt the text to take account of local reference substances and spectra and reagent specifications.

Sorbitan, mono-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs., (*Z*)-;
Polyoxyethylene 20 sorbitan monooleate [9005-65-6].

DEFINITION

Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

IDENTIFICATION

• **PROCEDURE**
Acceptance criteria: It complies with the test for *Composition of Fatty Acids*.

ASSAY

• **COMPOSITION OF FATTY ACIDS**
Diluent: 20 g/L sodium hydroxide in methanol
Saturated sodium chloride solution: Sodium chloride and water (1:2). Before use, decant the solution from any undissolved substance and filter, if necessary.
Reference solution A: Prepare 0.50 g of the mixture of calibrating substances with the composition described in *Table 1*. Dissolve in heptane, and dilute with heptane to 50.0 mL.
Reference solution B: *Reference solution A* in heptane (1 in 10)
Reference solution C: Prepare 0.50 g of a mixture of fatty acid methyl esters, which corresponds to the composition of the substance to be examined. Dissolve in heptane, and dilute with heptane to 50.0 mL. [NOTE—Commercially available mixtures of fatty acid methyl esters may also be used.]
Sample solution: Dissolve 0.10 g of Polysorbate 80 in 2 mL of *Diluent* in a 25-mL conical flask, and boil under a reflux condenser for 30 min. Add 2.0 mL of 14% boron trifluoride–methanol through the condenser, and boil for 30 min. Add 4 mL of heptane through the condenser, and boil for 5 min. Cool and add 10.0 mL of *Saturated sodium chloride solution*, shake for about 15 s, and add a quantity of *Saturated sodium chloride solution* such that the upper phase is brought into the neck of the flask. Collect 2 mL of the upper phase, wash with three quantities, each of 2 mL, of water and dry over anhydrous sodium sulfate.

Table 1

Mixture of the Following Substances	Composition (%)
Methyl myristate	5
Methyl palmitate	10
Methyl stearate	15
Methyl arachidate	20
Methyl oleate	20
Methyl eicosenoate	10
Methyl behenate	10
Methyl lignocerate	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m G16 on fused silica, film thickness 0.5 µm
Column temperature: See the temperature program table below.

	Time (min)	Temperature (°)
Detector temperature		250
Injection port		250
Column	0	80
	14	220
	54	220

Carrier gas: Helium
Linear velocity: 50 cm/s
Injection size: 1 µL
System suitability
Samples: *Reference solution A* and *Reference solution B*
Suitability requirements
Resolution: NLT 1.8 between the peaks due to methyl oleate and methyl stearate, *Reference solution A*
Signal-to-noise ratio: NLT 5 for the peak of methyl myristate, *Reference solution B*
Theoretical plates: NLT 30,000 calculated for the peak of methyl stearate, *Reference solution A*

Analysis
Sample: *Sample solution*
Identify the peaks from *Reference solution C*. Calculate the percentage of each component in the *Sample solution*:

$$\text{Result} = A_c/A_T \times 100$$

A_c = peak area for the component of interest
A_T = total area of all peaks related to fatty acids

Acceptance criteria: Myristic acid, NMT 5.0%; Palmitic acid, NMT 16.0%; Palmitoleic acid, NMT 8.0%; Stearic acid, NMT 6.0%; Oleic acid, NLT 58.0%; Linoleic acid, NMT 18.0%; Linolenic acid, NMT 4.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Evenly distribute 2.00 g of the substance to be examined in the crucible. Dry at 100° to 105° for 1 h and ignite to constant mass in a muffle furnace at 600° ± 25°, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up with hot water, filter through an ashless filter paper and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant mass.
  Acceptance criteria: NMT 0.25%
- **‡HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm‡

Organic Impurities

- **PROCEDURE: ETHYLENE OXIDE AND DIOXANE**
  Ethylene oxide standard solution: Dilute 0.5 mL of a commercially available solution of ethylene oxide in methylene chloride (50 mg/mL) with water to 50.0 mL. [NOTE—The solution is stable for 3 months, if stored in vials with a teflon-coated, silicon membrane crimped caps at –20°.] Allow to reach room temperature. Dilute 1.0 mL of this solution with water to 250.0 mL.
  Dioxane standard solution: Dioxane in water (v/v) 1 in 20,000
  Acetaldehyde standard solution: 0.01 mg/mL acetaldehyde in water
  Standard solution: Dilute 6.0 mL of *Ethylene oxide standard solution* and 2.5 mL of *Dioxane standard solution* with water to 25.0 mL.
  Sample solution A: 1.0 g of Polysorbate 80 into a 10-mL headspace vial. Add 2.0 mL of water, and seal the vial immediately with a teflon-coated, silicon membrane and an aluminum cap.
  Sample solution B: 1.0 g of Polysorbate 80 into a 10-mL headspace vial. Add 2.0 mL of *Standard solution*, and seal the vial immediately with a teflon-coated, silicon membrane and an aluminum cap.
  Reference solution: Introduce 2.0 mL of *Acetaldehyde standard solution* and 2.0 mL of *Ethylene oxide standard solution* to a 10-mL headspace vial, and seal the vial immediately with a teflon-coated, silicon membrane and an aluminum cap.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: Headspace GC
  Detector: Flame ionization
  Column[1]**:** 0.53-mm × 50-m G27 on fused silica, film thickness 5 µm
  Column temperature: See the temperature program table below.

	Time (min)	Temperature (°)
Detector temperature		250
Injection port		85
Column	0	70
	18	250
	23	250

  Split ratio: 3.5
  Carrier gas: Helium
  Flow rate: 4.0 mL/min
  Injection size: 1 mL
  [NOTE—The relative retention times for ethylene oxide, acetaldehyde, and dioxane are 1.0, 0.9, and 1.9, respectively. The retention time for ethylene oxide is about 6.5 min.]
  System suitability
  Sample: *Reference solution*
  Suitability requirements
  Resolution: NLT 2.0 between the peaks due to acetaldehyde and ethylene oxide
  Analysis
  Samples: *Sample solution A* and *Sample solution B*
  Calculate the content of ethylene oxide:

$$\text{Result} = (2 \times C_{EO} \times A_a)/(A_b - A_a)$$

C_{EO} = concentration of ethylene oxide in *Sample solution A* (µg/mL)
A_a = peak area of ethylene oxide from *Sample solution A*
A_b = peak area of ethylene oxide from *Sample solution B*

  Calculate the content of dioxane:

$$\text{Result} = (2 \times 1.03 \times C_D \times A_{a'})/(A_{b'} - A_{a'})$$

1.03 = density of dioxane (g/mL)
C_D = concentration of dioxane in *Sample solution A* (µg/mL)
$A_{a'}$ = peak area of dioxane from *Sample solution A*
$A_{b'}$ = peak area of dioxane from *Sample solution B*

  Acceptance criteria: NMT 1 ppm for ethylene oxide; NMT 10 ppm for dioxane

SPECIFIC TESTS

- **SPECIFIC GRAVITY ⟨841⟩:** about 1.10 at 20°
- **VISCOSITY ⟨911⟩:** about 400 mPa · s at 25°
- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩
  Sample solution: Dissolve 5.0 g in 50 mL of a mixture of equal volumes of alcohol and hexane (previously neutralized with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide), using 0.5 mL of phenolphthalein solution as indicator. If necessary, heat to about 90° to dissolve the substance to be examined.
  Analysis: Titrate the *Sample solution* with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide until the pink color persists for at least 15 s. When heating has been applied to aid dissolution, maintain the temperature at about 90° during the titration.
  Acceptance criteria: NMT 2.0
- **FATS AND FIXED OILS,** *Hydroxyl Value* ⟨**401**⟩
  Sample: 2.0 g
  Analysis: Introduce the *Sample* into a 150-mL acetylation flask fitted with an air condenser. Add 5.0 mL of *Pyridine–Acetic Anhydride Reagent*, and attach the air condenser. Heat the flask in a water bath for 1 h keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask, and allow to cool. Add 5 mL of water through the upper end of the condenser. If a cloudiness appears, add sufficient pyridine to clear it, noting the volume added. Shake the flask, and replace in the water bath for 10 min. Withdraw the flask, and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of alcohol, previously neutralized with

[1] CP-Sil 8 CB is suitable.

phenolphthalein solution. Titrate with 0.5 N alcoholic potassium hydroxide using 0.2 mL of phenolphthalein solution as indicator. Carry out a blank test under the same conditions.
Acceptance criteria: 65–80

- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩
Sample: 10.0 g
Saturated potassium iodide solution: Prepare a saturated solution of potassium iodide in carbon dioxide-free water. Make sure the solution remains saturated as indicated by the presence of undissolved crystals.
Analysis: Introduce the *Sample* into a 100-mL beaker, and dissolve with 20 mL of glacial acetic acid. Add 1 mL of *Saturated potassium iodide solution*, and allow to stand for 1 min. Add 50 mL of carbon dioxide-free water and a magnetic stirring bar. Titrate with 0.01 M sodium thiosulfate, determining the endpoint potentiometrically. Carry out a blank titration.
Acceptance criteria: NMT 10
- **FATS AND FIXED OILS,** *Saponification Value* ⟨**401**⟩
Sample: 4.0 g
Analysis: Introduce the *Sample* into a 250-mL borosilicate glass flask fitted with a reflux condenser. Add 30.0 mL of 0.5 N alcoholic potassium hydroxide and a few glass beads. Attach the condenser, and heat under reflux for 60 min. Add 1 mL of phenolphthalein solution and 50 mL of dehydrated alcohol, and titrate immediately with 0.5 N hydrochloric acid. Carry out a blank test under the same conditions.
Acceptance criteria: 45–55
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 3.0%, determined on 1.0 g

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in an airtight container, protected from light.▲*NF28*

Stage 6 Harmonization

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

〈**11**〉 **USP Reference Standards,** *USP 32* page 35 and page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 1161 of *PF* 32(4) [July–Aug. 2006], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], page 144 of *PF* 35(1) [Jan.–Feb. 2009], page 330 of *PF* 35(2) [Mar–Apr. 2009], and page 612 of *PF* 35(3) [May–June 2009].

(HDQ) RTS—C44494; C47817; C51559; C56006; C65791; C70442; C71283; C73290; C73593; C73680; C73681

Add the following:

▲USP Carmellose RS.▲*USP33*

REFERENCE TABLES

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 32* page 890, page 266 of *PF* 29(1) [Jan.–Feb. 2003], page 1053 of *PF* 33(5) [Sept.–Oct. 2007], page 817 of *PF* 34(3) [May–June 2008], page 1046 of *PF* 34(4) [July–Aug. 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], page 188 of *PF* 35(1) [Jan.–Feb. 2009], and page 464 of *PF* 34(2) [Mar.–Apr. 2009], and page 651 of 35(3) [May–June 2009].

(HDQ) RTS—C70442; C71283; C65791; C73593

Add the following:

▲Carmellose: White powder. Practically insoluble in ethanol (99.5%). Swells with water to form a suspension. Becomes viscous in sodium hydroxide TS. Is hygroscopic. *NF category:* Suspending and/or viscosity increasing agent.▲*NF28*

STIMULI TO THE REVISION PROCESS

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- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

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Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication.

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Co-processed Excipients

Lawrence H. Block,[a] Richard C. Moreton,[a] Shireesh P. Apte,[a] Richard H. Wendt,[a] Eric J. Munson,[a] Joseph R. Creekmore,[a] Indira V. Persaud,[a] Catherine Sheehan,[b] and Hong Wang[c]

ABSTRACT This *Stimuli* article summarizes the thinking of the USP Excipient Monographs 2 Expert Committee and USP staff regarding a class of excipients—collectively labeled *co-processed excipients*—that have been and continue to be introduced into the *National Formulary* (*NF*). This article presents some suggested criteria for acceptance of such monograph proposals into *NF* and solicits public input.

INTRODUCTION

In recent years drug formulation scientists have recognized that single-component excipients do not always provide the requisite performance to allow certain active pharmaceutical ingredients to be formulated or manufactured adequately. In response to these deficiencies, drug formulation scientists have relied on increasing numbers of combination excipients introduced by excipient manufacturers into the commercial market. Combination excipients fall into two broad categories: physical mixtures and co-processed excipients.

Physical mixtures, as the name suggests, are simple admixtures of two or more excipients typically produced by short duration low-shear processing. They may be either liquids or solids and are generally used for convenience rather than for facilitating the manufacturing process or improving the resultant pharmaceutical product. Examples of such physical mixtures include immediate-release film coating powders for dispersion that reduce the time required to prepare film coating suspensions and to minimize color variation of the final product. Such physical mixtures are not appropriate for consideration for *National Formulary* (*NF*) monographs because the individual components are isolated (distinct and intact) before mixing; i.e., the manufacturing process of each of the individual components has been taken to completion, and consequently these components can be adequately controlled before mixing.

Co-processed excipients are combinations of two or more excipients that possess performance advantages that cannot be achieved using a physical admixture of the same combination of excipients. Typically they are produced using some form of specialized manufacturing process. The performance benefits relate to the manufacture or performance of the finished pharmaceutical product. This improvement in performance has been a primary driver for the introduction of co-processed excipients into the marketplace. Co-processed excipients are appropriate for consideration as new monographs because one or more of the components may be formed in situ, or the component may not be isolated prior to co-processing. That is, the manufacturing process for one component may not have been taken to completion before the addition of the other components, and/or the co-processed excipient combination cannot be adequately controlled using the monograph tests for the individual component excipients.

Because many co-processed excipients contain a macromolecular excipient as one of the constituents, responsibility for reviewing these monographs and recommending them for inclusion in *NF* falls within the purview of the EM2 Expert Committee, one of three Expert Committees that set excipient standards for *NF* in USP's Council of Experts.[d] Recently there has been increased interest in *NF* monographs for co-processed excipients. The Expert Committee is therefore addressing the more general question of compendial acceptance of these types of excipients. To this end the EM2 Expert Committee believes that guidelines for the acceptance of monograph proposals for co-processed excipients would be useful.

CURRENT STATUS OF CO-PROCESSED EXCIPIENT MONOGRAPHS IN *NF*

During the past 20 years, several monographs, each of which presents a co-processed combination of existing excipients, have appeared in *NF*. These excipients may be either liquids or solids. *Table 1* lists several examples of co-processed excipient combinations included in *NF 27* (2009). The co-processed excipient monographs listed in *Table 1* meet current *NF* submission requirements as defined by the following: Each of them is either included in an approved drug application (in the FDA inactive ingredient database) or has a Generally Recognized as Safe (GRAS) designation.

[a] Excipient Monographs 2 Expert Committee. Lawrence H. Block is the chair, and Richard C. Moreton is the vice chair.

[b] Director, Excipients, USP.

[c] Correspondence should be addressed to: Hong Wang, PhD, Scientific Liaison to Excipient Monographs 2 Expert Committee, Documentary Standards Division, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8351; hw@usp.org.

[d] The other two Expert Committees that set excipient standards for *NF* are: Excipient Monographs 1 Expert Committee, which works with small-molecule excipients, and Excipient General Chapter Expert Committee, which prepares General Chapters for excipients.

Table 1. Co-processed Excipients

Name	Form
Ammonio Methacrylate Copolymer Dispersion	Liquid
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion	
Ethylcellulose Aqueous Dispersion	
Methacrylic Acid Copolymer Dispersion	
Microcrystalline Cellulose and Carboxymethylcellulose	Solid

The excipients shown in *Table 1* typically are manufactured using some type of specialized manufacturing process such as high-shear dispersion, granulation, spray drying, or melt extrusion. Such combination excipients produced using these specialized manufacturing processes are commonly called co-processed excipients (*1–4*). Researchers have reported that these co-processed excipients, when incorporated into the finished product, offer one or more improvements in performance in comparison with the corresponding physical admixtures of the constituent excipients or individual components (*5*).

RECOMMENDATIONS FROM USP EM2 EXPERT COMMITTEE

The EM2 Expert Committee together with involved USP staff has considered the issues regarding co-processed excipients, especially for solid forms, during several years of monograph development and in collaboration and discussion with monograph sponsors. The Expert Committee has formulated the following guidelines that may help determine whether or not such combination excipients are co-processed and whether they will be eligible to be considered for a *NF* monograph. The Expert Committee welcomes comments regarding these guidelines.

Due to the complex nature of co-processed excipients and the diverse composition of their individual constituents, the Expert Committee is unable to propose a single test or other criterion that can be applied to distinguish these co-processed excipients from their corresponding simple physical admixtures. The committee will therefore evaluate *NF* submissions for co-processed excipients on a case-by-case basis using the following criteria:

1. A co-processed excipient is a combination of existing pharmacopeial excipients, and it must be distinguishable—in at least one non–performance-related property—from the admixture obtained by physically mixing the corresponding constituent excipients. A co-processed excipient typically is produced by some specialized manufacturing process such as high-shear dispersion, granulation, spray drying, or melt extrusion. When it is submitted as a potential *NF* monograph, information relating to its quality must meet current *NF* submission requirements: The claimed co-processed excipient is either included in an FDA-approved drug application or has a GRAS designation or is under special consideration by the Council of Experts.
2. A physical mixture of the various excipient components—which have not been individually modified in order to change their inherent thermodynamic state prior to being physically mixed—will not quantitatively exhibit one or more characteristics of the co-processed excipient. Co-processed excipients demonstrate one or more different properties regardless of whether a comparative analysis is performed using the physical mixture as is or using a sample of the physical mixture whose particle size distribution is very similar to that of the co-processed excipient. This or other characteristics of the co-processed excipient can be determined by a suitable test method.
3. The physical or chemical characteristic(s) of the co-processed excipient that differ from those of the physical mixture may cause or may be correlated with improvements in the performance of the finished product. However, these unique characteristic(s) must be inherent, demonstrably analyzable, and quantitatively different in the co-processed excipient itself *before* incorporation into the finished product. Thus, if the proposed co-processed excipient does not exhibit any analytical differences from the physical mixture, then it may not be considered a co-processed excipient even if it alters the performance of the finished product.
4. At least one of the components of the co-processed excipient is capable of being analyzed qualitatively and quantitatively in the co-processed state, i.e., without the use of any specific physical or chemical methods to separate the components of the co-processed excipient before analysis of the individual component(s).
5. No unintended covalently bonded chemical entity is formed when the individual ingredients are mixed to form the co-processed excipient. The absence of any chemical reaction(s) between individual ingredients in the co-processed excipient must be analytically demonstrated initially and over the proposed storage period of the co-processed excipient. However, intentional in situ salt formation, or formation of a known excipient by in situ polymerization or covalent cross-linking would be allowed.
6. The individual ingredients used in a co-processed admixture must have *USP–NF* monographs, or at least monograph proposals published in *Pharmacopeial Forum* as part of in-process revision. This does not necessarily imply that those individual ingredients must demonstrably meet monograph specifications in *USP–NF* before being incorporated or processed into the co-processed excipient. Indeed, this may not be possible because one or more individual component(s) of the co-processed excipient may not be capable of being isolated before co-processing.

However, the proposed co-processed excipient cannot be considered for inclusion as a monograph in *NF* if its production or manufacture involves incorporation of a noncompendial ingredient. In such cases, the co-processed excipient is excluded from *NF* regardless of whether or not the noncompendial ingredient is isolated before co-processing. Thus, if a sponsor wishes to propose a monograph for a co-processed excipient that contains a noncompendial excipient, the sponsor would first be required to secure an approved *NF* monograph for the noncompendial excipient.

CASE STUDIES

Since 2005 the EM2 Expert Committee has been working with a monograph sponsor to develop a new monograph titled Silicified Microcrystalline Cellulose (*6*). This excipient is recorded in the FDA inactive ingredient database as Prosolv, Prosolv 50, Prosolv 90, Prosolv HD 90, Prosolv SMCC 50, or Prosolv SMCC 90. It is categorized as a co-processed excipient (*3*). The monograph for this excipient underscores the importance of identification tests for this type of material. In the proposed Silicified Microcrystalline Cellulose monograph, four individual tests are used to identify this co-processed excipient. They are Fourier-transform infrared spectroscopy (FTIR), wet chemical tests for cellulose and silicon, and a *Silica dispersion uniformity test*, which is used to distinguish the co-processed excipient from the equivalent physical admixture of microcrystalline cellulose and silica. FTIR is useful for providing a confirmation of molecular structure. The FTIR measurement ensures that the material is predominantly a microcrystalline cellulose–like material. Additional identification tests in combination provide differentiation among microcrystalline cellulose, silicified microcrystalline cellulose, and a physical admixture of microcrystalline cellulose and colloidal silicon dioxide.

The EM2 Expert Committee believes that Silicifed Microcrystalline Cellulose meets all the criteria for inclusion as a separate monograph in *NF* based on the criteria listed above under Recommendations.

The EM2 expert committee also published a new monograph: Polyvinyl Acetate Dispersion (*7*). This material is a liquid co-processed excipient manufactured by an in situ polymerization process. The polymerization occurs in the presence of emulsifiers and stabilizers in water to form the dispersion, which is different from a physical admixture because the monomer, vinyl acetate, is polymerized during the manufacturing process. This excipient is used in an FDA-approved drug product, although it is not yet recorded in the FDA inactive ingredient database. Its application was confirmed by a US drug company. In addition to the major component, polyvinyl acetate, some minor components in the dispersion are povidone and sodium lauryl sulfate. *NF* includes monographs for Povidone and Sodium Lauryl Sulfate, as well as Polyvinyl Acetate. In 2008 the EM2 Expert Committee published a new monograph proposal for Polyvinyl Acetate (*8*) to replace a previous proposal (*9*). In the new monograph proposal for Polyvinyl Acetate Dispersion, the Expert Committee recommended strengthening the *Labeling* section: Any added surface active agent or stabilizer should be named, and its quantity should be provided. The Expert Committee further recommended that a test for Povidone should be included under a section called Stabilizers or Surface Active Agents and a limit test should be used.

The EM2 Expert Committee believes that Polyvinyl Acetate Dispersion also meets the criteria for inclusion as a separate monograph in *NF* based on the Recommendations listed above.

SUMMARY

The EM2 Expert Committee continues to work on developing/updating monographs that satisfy current regulatory requirements and industrial practices and that appear to be scientifically sound. The development of guidelines to facilitate the incorporation of co-processed excipients in *NF* and to differentiate them from physical mixtures promises to be a challenging and interesting topic for the EM2 Expert Committee for the remainder of the current cycle and for new Expert Committees and/or Expert Panels in coming cycles. With staff, the USP EM2 Expert Committee welcomes public comments on these proposals (please send comments to hw@usp.org).

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Liquid-filled Gelatin Capsules

Margareth R.C. Marques,[ab] Ewart Cole,[c] Dale Kruep,[d] Vivian Gray,[e] Dennis Murachanian,[f] William E. Brown,[a] Gabriel I. Giancaspro[a]

ABSTRACT This *Stimuli* article provides an overview of the manufacturing, characteristics, and in vitro performance evaluation of liquid-filled gelatin capsules. The intent of the article is to initiate discussion, to solicit public comments, and to invite participation of interested parties in the efforts of the USP Biopharmaceutics Expert Committee in either updating USP General Chapter *Dissolution* ⟨711⟩ or creating a new General Chapter that will address the particularities and special approaches required to develop and carry out in vitro performance evaluations of liquid-filled gelatin capsules.

HISTORY OF LIQUID-FILLED GELATIN CAPSULES

Initially capsules were used because it was simple to formulate them as unit dosage forms for drugs in powdered or granular form and because they provided an easy-to-swallow container that effectively masked the bitter taste of drugs. With the advent of pellet technology that enabled modified drug release, capsules provided a useful vehicle into which multiparticulates could be filled without risk of modifying the release characteristics associated with other processing methods such as compression of multiparticulates into tablets (*1*). Since the early 1980s technology has been available to permit accurate dosing and sealing of liquids into hard gelatin capsules (*2–4*).

Advantages of liquid- and semisolid-filled hard gelatin capsules include:

- Improved bioavailability
- Improved content uniformity of low-dose active substances
- Reduced dust for handling potent compounds
- Process simplification for low-melting-point active substances
- Enhanced stability
- Sustained release.

Unlike soft gelatin capsules, in which the fill and the shell are manufactured in one operation, hard capsules are manufactured and supplied to the pharmaceutical manufacturer empty. The capsule first needs to be filled and then sealed. Most modern capsule-filling machines can be modified to allow hard gelatin capsules to be filled with hot or cold liquids. Equipment requirements that allow industrial manufacture of liquid-filled capsules are reported by Cole (*5*), and examples of some available models are given in *Table 1*.

Table 1. Capsule-filling Machines for Liquid Filling of Hard Gelatin Capsules up to Production Scale

Machine Type	Filling Action	Approximate Filling Rate (Capsules/h)
Robert Bosch GmbH	Intermittent motion	
GKF 1400 L		60,000
GKF 701 L		36,000
Harro Hoefliger GmbH	Intermittent motion	
KFM III-C		25,000
IMA Zanasi Division	All intermittent motion	
Zanasi 6/12		6,000–12,000
Zanasi 25/40		25,000–40,000
Zanasi Lab 8/16		8,000–16,000
Zanasi Plus 2E/48E/70E/85E		32,000–85,000

[a] Documentary Standards Division, USP.

[b] Correspondence should be addressed to Margareth R.C. Marques, MSc, PhD, Senior Scientist, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790, tel. 301.816.8106; e-mail mrm@usp.org.

[c] Consultant, Capsugel, Division of Pfizer, Switzerland.

[d] Banner Pharmacaps, Inc.

[e] Member USP Biopharmaceutics Expert Committee.

[f] Capsugel, Division of Pfizer, USA.

Table 1. Capsule-filling Machines for Liquid Filling of Hard Gelatin Capsules up to Production Scale
(Continued)

Machine Type	Filling Action	Approximate Filling Rate (Capsules/h)
MG2	All continuous motion	
MG Compact		6,000–96,000
MG Futura		6,000–96,000
Planeta 100		100,000
Qualicaps	All continuous motion	
F-5		4,000
F-40		30,000
F-80		60,000
F-120		90,000
F-150		120,000
Schaefer Technologies Inc.	Semi-automatic	
LF-10		10,000–25,000
Bonapace	Intermittent motion	
IN-CAP		3,000

An essential part of a liquid-filling operation is the ability to effectively seal the capsule. Various methods are available to seal hard gelatin capsules, and these have been reviewed by Wittwer (*6*). The two most studied methods are banding using a gelatin band as described by Bowtle (*7*) and sealing using a hydroalcoholic solution as described by Cole (*8, 9*). Both methods are described in USP General Information Chapter *Pharmaceutical Dosage Forms, Capsules* ⟨1151⟩ (*10*).

NOMENCLATURE

Liquid-filled capsules can be classified into two main types based on the physical characteristics of the shell: soft gelatin capsules or softgels, and hard gelatin capsules or hardgels. Softgels have a thicker shell and typically exhibit a higher degree of elasticity because of a greater amount of added plasticizer relative to gelatin. Hardgel shells are thinner and more rigid than softgels. Both soft and hard gelatin capsules basically are composed of gelatin, a plasticizer, and water. For hardgels water acts as the plasticizer, whereas softgels use high-boiling-point polyols such as glycerol and sorbitol. Although many parameters affect the physical and chemical performance of the shell, the ratio of gelatin to plasticizer primarily determines the rigidity, brittleness, and dissolution performance of the shell.

Liquid-filled capsules also can be characterized by the chemical properties of the fill material (hydrophobic-based vs hydrophilic-based fill materials) or by the physical properties of the fill material (solution vs suspension). Hydrophobic solutions include neat oils, combinations of miscible oils, or drugs dissolved in oil vehicles. Hydrophobic suspensions include drugs suspended in oils or in oil–wax mixtures, often referred to as semisolids. Hydrophilic solutions may be neat liquids or syrups, combinations of water-soluble liquids or syrups, or drugs dissolved in water-soluble vehicles. Hydrophilic suspensions include drugs suspended in hydrophilic vehicles such as polyethylene glycol.

GEL COMPOSITION AND MATERIAL PROPERTIES

Composition and Properties of Gelatin Raw Material

Considerable interest lies in replacements for gelatin as a suitable capsule shell matrix, especially in the dietary supplement industry that serves a large number of consumers who are strict vegetarians and do not want to consume articles derived from animal origin. In addition, some consumers avoid consumption of products of porcine origin. Alternative suitable materials include nonanimal sources such as potato starch and carrageenans from seaweeds, as well as synthetic hydrogels, and ongoing research aims to improve the quality and reduce the cost of these replacements. However, as previously noted, gelatin is the material used to produce the shell matrix for most of the liquid-filled capsule pharmaceutical products currently on the market. Consequently, this *Stimuli* article focuses on products for which the fill material is encapsulated in a gelatin-based shell.

Gelatin is obtained from the partial hydrolysis of collagen, which is the most abundant animal protein in nature. Collagen is an insoluble, highly ordered fibrous protein and is the primary fibrous component of bone, skin, and connective tissue. The majority of pharmaceutical gelatin is produced from collagen from bovine bone, bovine hide, and porcine skin, although some reports describe the use of the skin from poultry and fish. Collagen is a highly structured protein. It consists of individual polypeptide chains containing approximately 1000 amino acid units of which glycine, proline, hydroxyproline, and alanine constitute ~60%. The primary structure is dominated by regions of repeating amino

acid sequences rich in glycine, proline, hydroxyproline, and alanine. These regions readily form left-handed helices that make up most of collagen's secondary structure. Although collagen contains minimal tertiary structure, significant quaternary structure is formed when the helical regions of three different protein chains wind around each other in a right-handed superhelix, forming a ropelike structure. This superhelix, also called tropocollagen, is held together by hydrogen bonding and creates a three-dimensional protein matrix. The regions of the primary structure not involved in the helices contain most of the amino acids with ionic side chains such as lysine, arginine, glutamic acid, and aspartic acid. These regions contribute to the stabilization of the three-dimensional structure by entering into ionic and covalent linkages between protein chains and forming the characteristic collagen fiber (*11*).

As noted above and in *USP–NF* (*12*), gelatin is a product obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue, and bones of animals. The hydrolysis may be catalyzed by the addition of strong acid or base. Gelatin derived from acid-catalyzed hydrolysis is referred to as Type A, and gelatin derived from base-catalyzed hydrolysis is referred to as Type B. The main difference between gelatins derived from these two processes is that the gelatin derived from the acid-catalyzed process typically exhibits an isoelectric point (pI) of approximately 7–9, whereas the pI of gelatin obtained from the base-catalyzed process is typically 4.7–5.4. The lower pI resulting from the treatment with base is due to the hydrolysis of the amide groups of glutamine and asparagine, creating glutamic acid and aspartic acid. Because of the manufacturing process used, gelatin molecules exhibit significant polydispersity: The molecular weight of individual molecules typically ranges from 15,000 to 250,000. Gelatin maintains many of the chemical and physical properties of collagen, the most important of which is its ability to form superhelices and to associate into a three-dimensional matrix. For gelatin this is a thermally reversible process at relatively low temperatures and is an important reason why gelatin has been the material of choice for providing the shell matrix of liquid-filled capsules.

Gelatin is graded primarily on the strength of the gel it forms, and, depending on the process used and the tissue source, noticeable differences in strength are apparent among suppliers and even between lots from the same supplier. Consequently, controlling the strength of the gel from batch to batch (measured as *bloom strength*) is a key to obtaining a consistently performing product. Bloom strength is a measure of the ability of a given weight of gelatin to set up in water under controlled conditions and is a function of the molecular weight of the gelatin molecules, the concentration of the gelatin in the gel, and the pH of the gel. It is a measure of the resultant gel's resistance to compression and is reported in bloom-grams or simply grams. Bloom strength increases when the gelatin concentration in the gel increases, when the average molecular weight of the gelatin increases, and when the pH of the gel approaches neutrality (from either direction). In addition, as bloom strength increases the cost of gelatin increases and gel dissolution rate decreases. Bloom strength also can have an effect on the clarity and color of liquid-filled capsules. For gelatin with higher bloom strength, less gelatin is needed to produce a suitable shell, which results in a clearer shell and a reduced need for colorants and dyes in order to produce the desired hue. Although gelatin with bloom strengths ranging from 50 to 300 is available, most gelatins used in the manufacture of liquid-filled capsules have a bloom strength of approximately 150–200 for softgels and 220–280 for hardgels. Gelatin manufacturers commonly blend different sublots of gelatin to meet bloom requirements.

Composition and Properties of the Gel Mass and Finished Shell

In its simplest form the shell of a liquid-filled capsule is prepared from a molten gel mass that is composed of gelatin and a plasticizer dissolved in an aqueous vehicle. For hardgels water acts as both the plasticizer and the vehicle. For softgels small polyhydroxy compounds such as glycerol, sorbitol, and maltitol typically are used as plasticizers. Normally, during the preparation of the gel mass gelatin is first hydrated in a measured amount of excess water before plasticizer is added and the mass is heated to complete the solution. Less frequently, gelatin may be added directly into hot water and dissolved immediately. The gel mass for hardgels normally contains about 30–35 parts gelatin and 65–70 parts water by weight. The gel mass for softgels commonly starts out at approximately 40–45 parts gelatin, 30–35 parts plasticizer, and 20–30 parts water. However, depending on the desired performance traits of the shell, the size of the shell, and the composition of the fill material, the gelatin:plasticizer ratio for softgels may vary from 1.0 to 3.0. Other minor components added to the gel mass may include colorants, flavors, stabilizers, buffers, and opacifiers.

Before the encapsulation process the gel mass is kept warm (about 60 °C) and at a high water content so that it remains pliable and free flowing, in some cases as long as 4 days. During this time the gelatin exists as single protein molecules in a random configuration and sheathed by water molecules. The proteins slowly hydrolyze over time at high temperatures, and this occurs more rapidly the further the pH of the gel mass departs from neutrality. This phenomenon may be detected by a decrease in the viscosity of the gel mass.

For softgels the capsule shells are manufactured and filled in one continuous operation. The warm gel mass is allowed to flow onto a chilled rotating casting drum where gelation begins to occur and a gel ribbon is formed. As the temperature of the gel decreases below 40 °C and lower, the gelatin molecules begin to re-form the secondary structural characteristics that were exhibited by the parent collagen fibers and were held in place by hydrogen and ionic bonding. The degree to which the superhelices form depends on how fast the gel cools and to what extent the plasticizer associates with the gelatin. The plasticizer breaks up the ordered structure, thereby increasing elasticity. At this time the gel also begins to evaporate water, the rate of which also affects the final association and alignment of the gelatin molecules.

Within minutes a relatively wet, elastic gel ribbon is formed and is routed through lubricating rollers on its way to the dies and wedge on the encapsulation machine. At the wedge two opposing gel ribbons are brought together on the dies, and a sandwich is created as the fill material is injected between the ribbons. As the fill material is injected between the ribbons the latter are pressed into the die cavities, thereby creating the capsule. As the dies come together the two halves of the capsule shell are sealed. This process is assisted by the wedge, which is held at slightly elevated temperature (37–40 °C) to soften the ribbon. The dies finish the encapsulation process by cutting the capsules from the ribbon. Next, the capsules begin the drying process and are carefully dried to their final moisture content. Drying is performed under conditions of highly controlled temperature, relative humidity, and airflow. Along with gelatin concentration and gel strength, the rate and extent of water loss determine the hardness and brittleness of the capsules, their ability to undergo dissolution, and their tendency to stick to each other. In the completed product hardgel shells contain approximately 13%–16% water, and softgels contain approximately 6.5%–8.0%.

Gelatin is a hygroscopic material, and the relationships among relative humidity, gelatin moisture content, and hard gelatin capsule properties are shown in *Figure 1* (*13*). Kontny and Mulski (*14*) also have studied the relationship between relative humidity and brittleness of hard gelatin capsules. Because certain solvents are known hydrophilic agents, it is particularly important to monitor the mechanical properties of liquid-filled capsules stored under various conditions of temperature and relative humidity. Methodology to determine the compatibility of fill materials has been described (*15*).

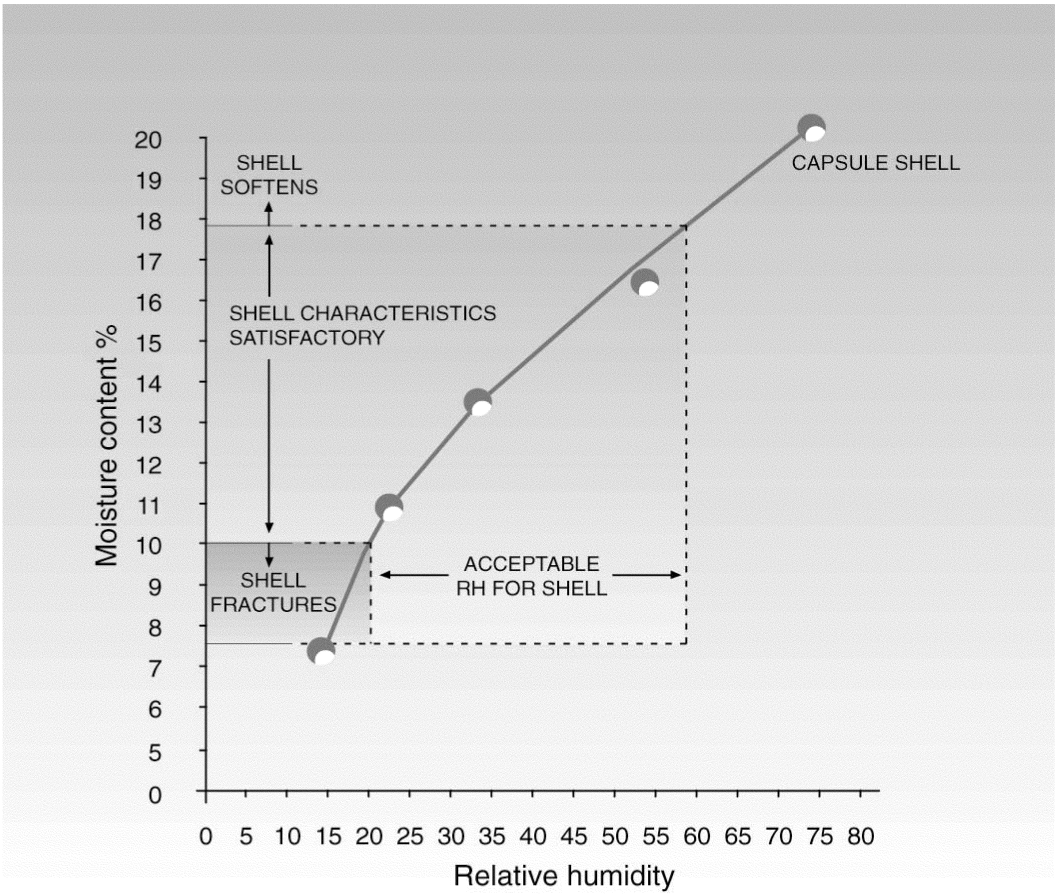

Figure 1. Relative Humidity (RH), Gelatin Moisture Content, and Hard Gelatin Capsule Properties.

Stability and Reactivity of Gelatin

At room temperature the shells of fully dried capsules are relatively effective at protecting the fill from oxygen and its effects, and when an opacifier such as titanium dioxide is added to the shell it can prevent photodegradation of the fill. In addition, the low water content of the shell does not promote microbial growth. Bacteria, yeasts, and molds require high water content of at least 0.80% (w/w), and the water content of capsule shells typically is less that half of that. However, it must be stressed that capsule shells are not unreactive, physically or chemically. As storage conditions change in temperature and humidity, so too does the gelatin matrix in the shell. As temperature and humidity increase the shell absorbs moisture, and the gelatin molecules assume additional freedom of movement. The sol–gel transition temperature for many gel formulas occurs just above 40 °C. Stress from temperature and relative humidity can result in weakened seams, leaking, and their capsules

sticking together. Recommended storage conditions of the final product are typically 15–30 °C and 30%–60% relative humidity, or standard room temperature conditions. Brief excursions outside of these conditions are rarely cause for concern.

Another phenomenon seen in capsules, especially softgels, is extensive cross-linking of the gelatin, which can occur during storage and can result in the formation during dissolution testing of swollen, rubbery water-insoluble membranes known as *pellicles* that may act as a barrier to drug release. A pellicle is a thin clear membrane of cross-linked protein surrounding the fill or the capsule and preventing the fill from being released. This cross-linking involves strong chemical linkages beyond simple hydrogen and ionic bonding between gelatin chains, and the linkages formed can affect the thermal reversibility of the gelatin shell. One of the strongest and most common types of cross-linking involves the covalent bonding of the amine group of a lysine side chain of one gelatin molecule to a similar amine group on another molecule. This reaction generally is catalyzed by trace amounts of reactive aldehydes (*16, 17*). Formaldehyde, glutaraldehyde, glyoxal, and reducing sugars are the most common catalysts. The covalent bonding produced with this type of cross-linking is, for all practical purposes, irreversible, and dissolution of the shell must involve the breaking of other bonds, e.g., by enzyme-mediated breaking of peptide bonds in protein chains. It has been proposed that chemically modifying gelatin, perhaps by adding succininc acid groups to the lysine side chains, may prevent or at least diminish aldehyde-mediated cross-linking. Another, weaker, type of cross-linking is complexation of free carboxylic acid groups on two different gelatin molecules with trivalent metal ions, such as Fe^{3+} and Al^{3+}. These cations may be found in some of the dyes used as colorants or as low levels of contaminants in excipients. Higher bloom gelatin, which normally is associated with higher quality, facilitates efficient cross-linking because fewer links are needed to join greater lengths of gelatin chains.

Common causes of cross-linking include:

- Aldehydes present in active pharmaceutical ingredients (APIs), excipients, packaging materials, or degradants formed in situ during storage
- High humidity
- Indirect catalysis in cross-linking reactions
- Decomposition of a stabilizer in corn starch (hexamethylenetetramine), which forms ammonia and formaldehyde, which in turn promote cross-linking reactions
- Rayon coilers that contain an aldeydic functional group (*18*)
- Polyethylene glycols that may auto-oxidize to form aldehydes
- UV light, especially in the presence of high heat and humidity (*19, 20*)
- Heat, which can catalyze aldehyde formation.

Dissolution testing of capsules that are affected by cross-linking can yield results that suggest an apparently slower drug-release profile. However, in vivo disintegration of cross-linked capsules in healthy volunteers was found to be rapid and to be equivalent to drug release from fresh nonstressed capsules (*21*). Bioequivalence between stressed and unstressed acetaminophen hard and soft gelatin capsules also has been established (*22*). These findings have led to the acceptance of a two-tier dissolution test using enzymes (*23*). Techniques to monitor any potential incompatibility between the fill material and the capsule have been described (*15*).

APPLICATIONS AND FORMULATION STRATEGIES FOR HARD GELATIN CAPSULES

Table 2 summarizes drug characteristics for which liquid-filling technology is applicable. The important factors during a liquid-filling operation are temperature and viscosity of fill material and, in the case of a suspension, the particle size of the suspended drug. In principle, any excipient found to be compatible with the gelatin shell can be used, but in a manufacturing environment the viscosity of the fill material is important. If the viscosity is too low, splashing of the bushings may contaminate the area of overlap between the capsule body and cap, preventing the capsule from being effectively sealed. Absence of a clean break during dosing (stringing) can have the same effect. Recommended guidelines for dosing liquids or semisolids into hard gelatin capsules are given in *Table 3*. Excipients that are solid at room temperature but melt at temperatures up to 70 °C are well suited for formulating APIs, provided they yield the desired pharmacokinetic and stability profiles. Bowtle (*7*) has described a process for selecting bases for thermosetting formulations.

Table 2. Drug Characteristics and Liquid-filling Technologies

Characteristic	References	Examples of Marketed Products
Poorly soluble	24–30	Nifedipine (Apricel) Ibuprofen (Solufen)
Short half-life requiring frequent dosing	31–37	Captopril (Captoril)
Low melting point	38	Oils of avocado and soya (Piascledine) Danthron (Co-danthramer)
Low dose/high potency	8, 31, 38, 39	Cytotoxic products in development
Critical stability	40–43	Vancomycin hydrochloride (Vancocin)

Other process-related factors include:

- Excipient/drug stability over time
- Temperature-dependent solubility of drug in lipid
- Aging/polymorphic characteristics of the lipid

- Adequate characterization of the saturation/supersaturation status of the drug in the lipid formulation in order to avoid drug ''crashing out.'' Optimally saturation status should be determined when the formulation is at equilibrium with the shell
- Stability in solution under stress
- The potential for aldehyde formation and drug degradation if hot melts are held for prolonged times at elevated temperatures
- The influence, if any, of the rate of cooling on the structure of certain excipients that may modify drug-release characteristics from the matrix (*43*).

Table 3. Recommended Guidelines for Dosing Liquids and Semisolids into Hard Gelatin Capsules

Parameter	Recommendation
Temperature of fill material	Maximum approximately 70 °C
Viscosity at the temperature of dosing	0.1–1.0 Pa · s
Dosing characteristics	Clean break from dosing nozzle and absence of ''stringing''
Particle size of suspended drug	$\leq$ 50 μm

As increasing numbers of poorly water soluble drugs enter the pipeline, so do the challenges of finding innovative ways of developing bioavailable and stable dosage forms. In the dietary supplement industry, formulators also are finding an increased interest in new liquid-encapsulated presentations of fat-soluble vitamins and lipophylic products such as Co-enzyme Q10, lycopene, or lutein that are prepared for improved bioavailability. Excipient suppliers, encouraged by potential opportunities, are developing new materials comprising mixtures of functional excipients.

A range of excipients have been tested and found to be compatible with hard gelatin capsules. They can be classified into three arbitrary groups:

- Lipophilic liquid vehicles
- Semisolid lipophilic vehicles/viscosity modifiers for lipophilic liquid vehicles
- Solubilizing agents, surfactants, emulsifying agents, and adsorption enhancers.

These excipients are listed below:

- Lipophilic liquid vehicles compatible with hard gelatin capsules*
- Refined specialty oils:
  - Arachis oil
  - Castor oil
  - Cottonseed oil
  - Maize (corn) oil
  - Olive oil
  - Sesame oil
  - Soybean oil
  - Sunflower oil.
- Medium-chain triglycerides and related esters:
  - Caprylic/capric triglycerides (Akomed E, Akomed R, Miglyol 810, and Captex 355)
  - Medium-chain triglyceride (Labrafac CC)
  - Propylene glycol diester of caprylic/capric acid (Labrafac PG)
  - Propylene glycol monolaurate (Lauroglycol FCC)
  - Fractionated coconut oil (Miglyol 812)
  - Caprylic/capric/diglyceryl succinate (Miglyol 829)
  - Medium-chain diesters of propylene glycols (Miglyol 840)
  - Partial ester of diglycerides with natural fatty acids (Softisan 645).
- Solubilizing agents, surfactants, emulsifying agents, and adsorption enhancers compatible with hard gelatin capsules:†
  - Propylene glycol monocaprylate (Capryol 90)
  - Polyglycolized glycerides (Gelucire 44/14 and 50/13)
  - Polyoxyl-40 hydrogenated castor oil (Cremophor RH 40)
  - Glycerol monostearate/di-triglycerides + glycerin (Imwitor 191)
  - Glyceryl monocaprylate (Imwitor 308*)
  - Glyceryl cocoate/citrate/lactate (Imwitor 380)
  - Glyceryl mono-di-caprylate/caprate (Imwitor 742)
  - Isosteryl diglyceryl succinate (Imwitor 780 K)
  - Glyceryl cocoate (Imwitor 928)
  - Glyceryl caprylate (Imwitor 988)
  - Oleoyl macrogol-8 glycerides (Labrafil M 1944 CS)
  - Linoleoyl macrogolglycerides (Labrafil M 2125 CS)
  - PEG-8 caprylic/capric glycerides (Labrasol)
  - Lauric acid
  - Propylene glycol laurate (Lauroglycol 90)
  - Oleic acid
  - PEG MW > 4000
  - Polyglycerol dioleate (Plurol Oleique CC 497)
  - Polyoxyethylene-polyoxypropylene copolymer (Poloxamer 124 and 188)
  - Partial glycerides of hydroxylated unsaturated fatty acids (Softigen 701)
  - PEG-6 caprylic/capric glycerides (Softigen 767)
  - Polyoxyethylene glyceryl trioleate (Tagat TO)
  - Polyoxyethylene(20)sorbitan monooleate (Tween 80).

The excipients shown below are generally incompatible with hard gelatin capsules and should be avoided in high concentrations. They may, however, be used in mixed systems (i.e., systems that involve nonreactive, acceptable excipients for hard gelatin capsules). In such cases the critical concentration that would lead to incompatibility must be determined experimentally. The com-

* Quality may vary among suppliers and also from batch to batch and should be routinely checked. The thermal history of excipients should be recorded during manufacturing.

† Quality may vary among suppliers and also from batch to batch and should be routinely checked. The thermal history of excipients should be recorded during manufacturing.

patibility of the final formulation, including the API, must be monitored as part of the routine development process.

Excipients that are incompatible with hard gelatin capsules in unmixed systems[‡]:

Ethanol
Hydrogenated polyoxyl castor oil (Cremophor EL)
Glycerin (with a content > 5%)
Glycofurol 75
Medium-chain mono- and diglycerides (Akoline MCM and Capmul MCM)
Glyceryl monocaprylate (Imwitor 308*)
PEGs of MW < 4000
N-methyl-2-pyrrollidone (Pharmasolve)
Propylene glycol
Sorbitan monooleate (Span 80)
Diethylene glycol monoethylether (Transcutol P).

APPLICATIONS AND FORMULATION STRATEGIES FOR LIQUID-FILLED CAPSULES

Hard Gelatin Capsules

Liquid-filled capsules often are used to deliver APIs such as oily or waxy substances that are not easily formulated in solid delivery systems. Substances that are easily formulated in solid dosage forms also may be formulated in liquid-filled capsules for product line extensions and to improve product performance or bioavailability. Reformulation may include coating the capsule or adding ingredients into the shell to produce delayed- or extended-release properties. Chewable products, which often require additional texture and flavor components, are being developed.

With some limitations and exceptions, most of the excipients used to develop pharmaceutical solutions or semisolids can be used for developing fill materials for liquid-filled capsules, including emulsifiers and liquid polymers. Hydrophilic substances can be formulated into a capsule filling material, but water or low molecular weight alcohols should be kept at or below 10%. Higher levels may initiate erosion of the shell or cause appearance problems because of diffusion and evaporation from the fill material and/or the shell. Levels of glycerol and other plasticizers should be kept to a minimum or should be accounted for in the shell formula because they can diffuse into the shell and produce unacceptably soft capsules. Conversely, glycerol in the shell may migrate into very hydrophilic fill material, and it may be necessary to add some glycerol to the fill material to establish equilibrium.

Soft Gelatin Capsules

The fill material cannot be so thin that it leaks around the pump and wedge assembly, but it must not be so viscous that it cannot flow through the lines to the encapsulation pump or be accurately pumped into the capsule. Thicker fill materials may be warmed to reduce viscosity, but the temperature must be kept below 35 °C so that integrity of the gelatin shell and the sealing operation are not compromised. Both shell and fill excipients should be controlled for levels of known cross-linking agents such as formaldehyde and reducing sugars. Shell and fill excipients should be controlled for particle size (normally $\leq$ 170 μm or 80 mesh) and the presence of fibers. Large particles in the shell and seams can create weak points where leaking can occur, and a fiber that crosses a seam can act as a wick that facilitates leaks.

A few processing variables and shell formulations should be controlled with a high degree of precision because certain fill materials behave unpredictably if processing steps are not well controlled. Die speeds that are too slow or too fast may create poor seam quality and result in leaking capsules. Gel ribbon thickness must be precisely controlled to ensure consistent product appearance and dissolution performance within and between lots of product. The rate and extent of capsule drying probably is the most important processing parameter. Drying parameters must be individually determined for each combination of shell and fill material, and they must be consistently maintained within and between lots of product. Removal of too much water may result in hard, brittle capsules that have a higher propensity to develop cracked shells and/or require a longer time for dissolution. Not enough drying results in capsules that are too soft and that may weep, become tacky, and/or tightly stick to each other. If the drying conditions remove water too rapidly—caused by high drying temperatures, very low relative humidity, and/or high airflow—*case hardening* may occur. Case hardening takes place when the exterior surface of the capsule dries very rapidly and forms a temporarily seal that prevents further egress of moisture from the fill and shell. During case hardening capsule hardness is temporarily increased, but when the capsule is removed from the rapid drying conditions excess moisture within the fill and shell diffuse into the hardened surface. Thus the capsule emerges as a too-soft, under-dried product.

DISSOLUTION PROCEDURE DEVELOPMENT

Purpose of Test

Among the reasons for measuring the dissolution performance of liquid-filled capsules is to ensure the quality of the product. A well-designed dissolution test indicates when significant batch-to-batch variations occur and is a surrogate for demonstrating manufacturing consistency or significant changes in the dissolution performance of a single batch during the product's shelf life (e.g., gelatin cross-linking) (*44–47*). Dissolution performance also can be used as a research tool. For example, during the development of a generic drug product the dissolution performance of various formulations may be compared to that of the reference listed drug to ensure that product development is proceeding correctly. Dissolution tests also are used to evaluate formulation design and storage conditions (*48*). More recently, dissolution testing has been

[‡] Mixed systems may permit use of some of these excipients in low concentrations, but the limit of the excipients listed here must be determined experimentally.

used to make in vitro/in vivo correlations (IVIVC) between different products or between variations of the same product.

Although conceptually designed for tablets, dissolution testing has been extended to encapsulated drug-delivery formulations. In these systems, the drug is presented, often solubilized, in a liquid or semisolid formulation, and the relationship between solid drug dissolution and ultimate bioavailability has been disconnected. When a dissolution test is conducted on this type of formulation, the result is either a dissolution of the contents or the formation of an emulsion and is complicated by the sensitivity of these emulsions to their physical environment. Further, many of these formulations undergo digestive processes in vivo resulting in a breakdown of the formulation that is not mimicked in a dissolution test. Thus, the translation of the concept of dissolution testing to bioavailability of lipid-based drug delivery formulations is confusing. Nevertheless, attempts to design dissolution tests for lipid-based systems have been reported and discussed (*49–52*).

The ''dissolution'' of a encapsulated liquid formulation depends on its design. The gelatin capsule must rupture to release its contents. Rupture is a process more relevant than complete disintegration, which usually measures the dissolution of the shell. Therefore, for dietary supplements, a general rupture test was recently incorporated in USP General Chapter *Disintegration and Dissolution of Dietary Supplements* ⟨2040⟩ (*53*), where disintegration for soft shell capsules is required. What happens next depends on the composition of the formulation. For water-based formulations containing concentrated solutions, the rupture of the shell may be the only relevant step for bioavailability and IVIVC. For this reason, several monographs in *USP* contain rupture tests as the only performance standard for these dosage forms. For lipid-based formulations, which often are less dense than the aqueous medium, the release from the capsule is buoyancy driven.

If the formulation has been designed to be self-emulsifying, the formulation will efficiently disperse into most aqueous media. Formulations such as those consisting simply of a triglyceride with no additional cosolvent or emulsifier will rapidly float to the top of the vessel. The assay results from a dissolution test in this situation provide the rate of extraction of drug from this floating layer. As is the case with dissolution tests for traditional tablets, the variables to consider are rotation speed, surfactant type and concentration, medium pH, and apparatus.

Because ''drug release'' and its connection with bioavailability are difficult to define, alternative tests may be useful to better guide the dosage form design. These tests include dispersibility, measurement of globule size for emulsions, micelle formation, lipolysis, precipitation on dilution in biorelevant media, phase behavior studies, and burst tests to detect gelatin crosslinking (*52, 54, 55*).

Design of the Test

Developing a suitable dissolution test procedure for a liquid-filled capsule depends on the physical and chemical properties of the drug substance and the product. Developing a dissolution method for most liquid-filled capsules that contain a hydrophilic fill material typically follows the approach used for solid oral dosage forms. It may be possible to use disintegration as the performance test if the drug is already dissolved in the hydrophilic matrix because rupture of the shell to release the capsule content is the limiting step (*49, 56*). Dissolution testing should be required if the drug is dispersed as a suspension or emulsion in the hydrophilic matrix. FDA's *Guidance for Industry—Dissolution Testing of Immediate-release Solid Oral Dosage Forms* is a general resource that lists the types of media and equipment settings that are acceptable from a regulatory standpoint (*57*). The first step involves identifying a dissolution medium or several media candidates that provide sink conditions, preferably using physiologically relevant media, for the drug product. At minimum, the medium selected must provide sink conditions for the drug, must not interfere with the activity of enzymes used to overcome crosslinking of gelatin in Tier 2 tests, and must not show negative interactions with the formulation. Next, during preliminary trials the apparatus, media, media volume, agitation rate, and other test parameters are varied to determine what effect each has on the dissolution performance of the product. A statistical matrix design can assist in identifying critical parameters for a procedure that will consistently provide a percentage dissolved of approximately 75%–80% of label claim within 30–45 min. Testing aged and/or stressed capsules typically provides valuable insight into a product's dissolution performance.

Developing a suitable dissolution procedure for liquid-filled capsules containing a hydrophobic matrix often poses considerable challenges and requires a creative approach. Typically problems are encountered when one tries to identify sink and agitation conditions for dispersing the fill material and solubilizing the drug. This is especially the case for suspensions and waxy paste-filled products. The ''release'' profile from hydrophobic matrices is sensitive to the solubility of both the drug and the matrix in the medium, as well as the relative partitioning of the drug between the matrix and the medium. Before choosing the apparatus and agitation intensity, formulators must determine which media will disperse the fill and dissolve the drug. Profiles that relate drug solubility to media pH and ionic strength may be constructed. Surfactants available for testing include the following:

- Polysorbates (Tween)
- Sodium dodecyl sulfate (sodium lauryl sulfate)
- Lauryl dimethyl amine oxide
- Cetyltrimethylammonium bromide
- Polyethoxylated alcohols
- Polyoxyethylene sorbitan
- Octoxynol (Triton X100)
- *N,N*–dimethyldodecylamine–*N*–oxide
- Hexadecyltrimethylammonium bromide
- Polyoxyl 10 lauryl ether
- Brij 721
- Bile salts (sodium deoxycholate and sodium cholate)
- Polyoxyl castor oil (Cremophor)
- Nonylphenol ethoxylate (Tergitol)
- Cyclodextrins

- Lecithin
- Methylbenzethonium chloride (Hyamine) (*58, 59*).

Although it is frequently used to improve the solubility of poorly soluble drugs in dissolution tests of tablets, sodium dodecyl sulfate should be considered only in the absence of other alternatives because it readily denatures enzymes that are used to overcome crosslinking in Tier 2 dissolution tests (*60, 61*). Use of pancreatin, which has lipase activity, may help to increase the dissolution rate not only because it hydrolyzes triglyceride matrices but also because of the additional surfactant effect derived from the resulting fatty acid salts.

Initial efforts attempt to identify suitable surfactants, concentrations, and pH conditions that provide sink conditions in an environment that ensures suitable stability. Then formulators can evaluatie the effect of these conditions on the formulation. During a dissolution test of a lipid-filled capsule, the gelatin capsule opens and the lipid-based formulation is released. The resulting fluid jet breaks up into droplets, and if the formulation is not solubilized by the medium it often floats to the top of the vessel. The fluid jet develops due to interfacial forces, and droplet break-up occurs due to Rayleigh–Taylor instability and the shear of mixing. Important physical properties include the density of the fluid, viscosity, and interfacial tension between the fill and the medium. However, upon dilution the formulation may undergo phase transitions that may affect the viscosity, density, and interfacial tension.

Simple visual evaluation can be used to screen the interactions between surfactant solutions and formulations by preparing blends of varying composition that span the range from the neat formulation to a final dilution in the surfactant medium that represents the expected dilution in a dissolution test (e.g., 1 part in 250 parts aqueous). Complete solubilization of the formulation will provide a more robust dissolution test. When the formulation is not solubilized by the medium, the dissolution profile is a measure of the extraction of the drug from the matrix and is influenced directly by the surfactant concentration and the rotation speed of the apparatus. Any surfactant–formulation combinations that form viscous phases at intermediate dilutions should be used with caution because they may negatively influence extraction of the compound or the rate of solubilization of the formulation and extend the ''release'' profile. Cationic surfactants should be avoided in formulations that contain fatty acids because of their propensity to form insoluble precipitates.

The dissolution profile obtained for a lipid-based formulation in a given medium is designed for that particular formulation. This specific dissolution procedure probably cannot be used to compare drug product release from several different formulations to predict in vivo performance. Such a comparison should be made only with a complete understating of the dispersion behavior of the formulations and their interactions with the medium. Often other measurements such as rate of dispersion, particle size, and lipolysis provide appropriate predictors of physico-chemical phenomena for correlation with in vivo performance. Depending on the nature of the drug and its mechanisms of absorption, it may be helpful to use dissolution conditions that mimic the in vivo environment. Such conditions include the use of specific enzymes to transform the drug into the entity that will be absorbed in vivo (*62*) or to affect excipients in a manner that modifies the in vivo release of the drug from the matrix.

Regulatory bodies discourage the use of organic co-solvents to improve sink conditions, and thus co-solvents should be employed as a last resort.

Procedures for sampling and analysis of aliquots are similar to those for traditional dissolution tests. Some precautions must be taken to avoid disturbance of partitioned formulations. Disruption or sampling of the floating layer will result in anomalously high release measurements and significant variability. Further, floating material or emulsion droplets can accumulate around the shaft of the paddle or basket, which can result in radial concentration gradients. Such gradients can lead to variability if sample position is not controlled.

Delayed-release and enteric-coated capsule products are developed as line extensions, therapeutic improvements, and protection for acid-labile drugs. Because of the special claims and requirements of these products the dissolution test conditions in *USP* General Chapters *Dissolution* ⟨711⟩ and *Drug Release* ⟨724⟩ are used (*63*). Deviations from ⟨711⟩ or ⟨724⟩ are rare, but the addition of surfactants, bile salts, or other solubilizing aids may be justified for drugs that present poor water solubility.

Selection of Apparatus

As is the case with solid dosage forms, baskets (USP Apparatus 1) and paddles (USP Apparatus 2) are most often chosen as the dissolution apparatus for gelatin capsules, and both have been used successfully. However, with liquid-filled capsules baskets may not be suitable in certain instances. As a capsule breaks down the gelatin from the shell may clog the basket's mesh, and, with very hydrophobic fill materials, oil phase released from the capsule may not disperse into fine enough droplets in the basket to efficiently pass through the mesh. Occasionally, the capsule and its contents may float in the dissolution media. In these instances wire coils can be wound around the capsules, or commercially available sinkers can be used to encase the capsules to hold them on the bottom of the vessel and to allow the fill to become exposed to more of the medium. In rare instances the capsule shell may stick to the basket or the bottom of the vessel. The reciprocating cylinder (USP Apparatus 3) may overcome this problem because of its different mechanism of agitation. Also, USP Apparatus 3 may be a more appropriate system for generating IVIVC data than the basket or paddle when one is testing liquid-filled capsules (*64*). However, USP Apparatus 3 has a tendency to generate foam when surfactants such as sodium lauryl sulfate are added to the media. The rotating bottle apparatus may be an alternative that overcomes this problem. For drugs formulated in soft gelatin capsules, an IVIVC was established using the rotating dialysis cell method (*65*). This cell is placed into a normal USP dissolution vessel. The cell encloses a small volume (maximum 30 mL) of inner fluid by means

of a dialysis membrane, and it can simulate the transfer of drugs from the intestinal lumen (inside the cell) to tissues (outside the cell) (*66–68*).

New dosage forms may require new approaches to using compendial apparatus. For example, floating dosage forms have been investigated as a means of prolonging retention in the stomach in order to maintain sustained release. Such dosage forms may use a delivery system that consists of a biphasic gelatin capsule that contains a rapid-release component such as a lipophilic drug dissolved in an appropriate lipophilic solvent and a sustained-release solid erodible matrix that contains an emulsifier. To demonstrate the biphasic release characteristics of this delivery system, USP Apparatus 2 (paddles) was modified in such a way that the top of the paddle blade was just breaking the surface film of the dissolution medium (*69–71*).

The use of USP Apparatus 4 (flow cell) is another suitable alternative for the dissolution test of liquid-filled capsules, mainly for drugs with poor solubility (*72, 73*). USP Apparatus 2 and Apparatus 4 were compared for dissolution of soft gelatin capsule formulations of a poorly water-soluble amine drug. During testing with 0.01 N hydrochloric acid containing 0.25% polysorbate 80, both apparatus gave similar dissolution profiles. Apparatus 2 tended to give a faster rate of dissolution, but Apparatus 4 was better able to distinguish between different formulations (*74*).

Table 4 gives a summary of the dissolution conditions that formulators should consider when developing a dissolution test for liquid-filled capsules.

Table 4. Dissolution Conditions That Should Be Considered for Liquid-filled Capsules

Apparatus Type and Agitation	Use of Sinkers
Medium	Aqueous
	Sink conditions
	Stability of the active ingredient
	Buffer strength
	Counter-ion effect
Surfactant	Sink conditions
	Dispersion of the lipophilic fill
	Compatibility
Medium pH	Biorelevant
Enzymes	Maximum activity at the medium pH

UNIQUE ISSUES ASSOCIATED WITH LIQUID-FILLED CAPSULES: SUMMARY AND CONCLUSIONS

The chemical and physical properties of liquid-filled capsules pose unique challenges when formulators attempt to develop dissolution methods, and this *Stimuli* article has reviewed some of these. One of the more common problems is pellicle formation and cross-linking of the gel. To address this issue formulators can use proteolytic preparations that contain pepsin or pancreatin and certain enzymes as additives to dissolution media when cross-linking occurs in gelatin shells (*23, 75*). Evaluation of the amount of enzymes is necessary in such cases and there may be circumstances in which the amount of enzyme added currently recommended in *USP 31* (*23*) may be insufficient. Occasionally, liquid-filled capsules may float in the dissolution medium, but this can be overcome if the capsule is held loosely in a sinker or if a wire coil is wound around the capsule. The use of commercially available sinkers is preferred and is strongly recommended to ensure consistent results between laboratories and between tests. Technology transfer studies have identified differences that were not related to sample performance but rather to the techniques used in different laboratories to prevent samples from floating.

Liquid-filled capsules are useful dosage forms for delivering oily or waxy pharmaceutical preparations, but, as discussed previously, obtaining sink conditions with universally accepted dissolution media may be a challenge. This challenge is compounded if cross-linking occurs because surfactants normally used to disperse the fill material and solubilize the drug may tend to diminish the protease activity of pepsin and pancreatin. This problem has recently been solved with a two-stage dissolution approach: The dissolution was performed according to a procedure involving 800 mL of 0.1 N HCl with the addition of pepsin to initiate hydrolysis of the pellicle. After 25 min, 100 mL of a sodium lauryl sulfate solution in 0.1 N HCl was added to complete the dispersion of the fill and solubilize the active ingredient. Samples were taken for analysis 5 min later. This approach worked very well for single-point quality control testing but would not be suitable for generating dissolution profiles. In many instances involving hydrophobic drugs and fill materials the only recourse may be to increase the dissolution time. Some procedures for hydrophobic drugs have shown a linear rate of dissolution that continues for up to 5 h (*58*).

Dissolution vs Disintegration

Historically, liquid-filled capsules have often employed disintegration testing—or, more accurately, rupture testing—instead of dissolution testing. *USP* monographs for calcifediol, chloral hydrate, cyclosporine, dronabinol, and docusate sodium are a few examples. For solution-filled capsules, especially those with a highly soluble drug in a hydrophilic fill material, this may be a reasonable approach (*49*). The active drug is already in solution, and the shell needs only to rupture to release the API. The point at which the disintegration of a liquid-filled capsule is considered complete is different from that of a solid dosage form. In order to disintegrate a tablet must fully break apart and collapse into a loose pile of drug and excipients, whereas a liquid-filled capsule needs only to rupture to meet the definition of disintegration. However, for capsules filled with a thick suspension or a waxy paste a simple rupture of the shell may not adequately demonstrate that the finished dosage form is delivering the drug in a suitable manner. Measures of the dispersion

and subsequent solubilization of the drug by the dissolution test also are required in order to evaluate the performance of the dosage form.

A risk-based approach (*56*) can be applied to the application of disintegration for testing the in vitro performance of liquid-filled capsules. The suitability of the disintegration test for this type of dosage form can be justified on the basis of the ICH Q6A decision tree #7 (*76*). For some products Q6A supports the use of the disintegration test instead of dissolution if the following conditions are met: a) the drug is highly soluble between pH 1.2 and 6.8; b) the drug releases rapidly, i.e., more than 80% in 15 min at pH 1.2, 4.0, and 6.8; and c) a relationship is established between dissolution and disintegration.

Regulatory Aspects

To date FDA has not released any guidances specifically related to the design of dissolution and/or disintegration tests for liquid-filled capsules. The FDA guidance for dissolution testing of immediate-release solid oral dosage forms (*77*) indicates the possible need for enzymes to dissolve pellicles, if any are formed, to permit drug dissolution, but it makes no other recommendations about this type of dosage form.

In *USP XXII* (1990), the sentence "Requirements for Dissolution do not apply to soft gelatin capsules unless otherwise specified in the monographs" was added to *Dissolution* ⟨711⟩. *Pharmacopeial Forum* 19(3) (1993) included a proposal to delete that exemption, and the deletion became official in *USP 23* (1995). *Pharmacopeial Forum* 23(4) contained a proposal to add a rupture test to all monographs for soft gelatin capsules. The monographs affected by this proposal were: Calcifediol Capsules, Chloral Hydrate Capsules, Chlorotrianisene Capsules, Clofazimine Capsules, Docusate Sodium Capsules, Dronabinol Capsules, and Ethchlorovynol Capsules. At that time the USP Subcommittee on Dissolution and Bioavailability wanted to include a dissolution test in all monographs for soft gelatin capsules, but, because of the inherent challenges associated with the development of dissolution testing for this type of dosage form, the Subcommittee proposed an interim Dissolution rupture test that requires observation of the rupture time for the capsule shell. The Subcommittee received no suggestions regarding dissolution conditions for these monographs and thus made the rupture test official. This test can still be found in the following *USP* monographs: Calcifediol Capsules, Chloral Hydrate Capsules, Clofazimine Capsules, Cyclosporine Capsules, Docusate Calcium Capsules, Docusate Sodium Capsules, Docusate Potassium Capsules, Dronabinol Capsules, Ergoloid Mesylate Capsules, Ethchlorovynol Capsules, Cod Liver Oil Capsules, and Saw Palmetto Capsules. The rupture test has been included in the *USP* General Information Chapter *Disintegration and Dissolution of Dietary Supplements* ⟨2040⟩ as a replacement of disintegration for liquid-filled soft shell capsules that contain dietary supplements (*52, 54, 55*).

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NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–218; Vol. 35 No. 2, pp. 219–498; Vol. 35 No. 3, pp.499–784; and Vol. 35 No. 4, pp. 785–1050.*]

MONOGRAPHS

Index

EXCIPIENTS

GENERAL CHAPTERS

Index

Index

CHROMATOGRAPHIC COLUMNS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Columns Used in *USP–NF* and *Pharmacopeial Forum*
July–Aug. 2009

ABACAVIR SULFATE (DSD Mgh #2654)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Hypersil BDS C-18	Assay	4.6 mm × 5 cm, 5 µm. Manufacturer: Thermo Scientific
0(0)	L51	Chiralpak AD	Enantiomeric Purity	4.6 mm × 25 cm, 10 µm. Manufacturer: Chiral Technologies, Inc.
0(0)	L1	Symmetry C-18	Related Compounds	3.9 mm × 15 cm, 5 µm. Manufacturer: Waters Corp.

BALSALAZIDE DISODIUM (DSD Mgh #6988)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Eclipse XDB C18	Organic Impurities	4.6 mm × 25 cm, 5 µm. Manufacturer: Agilent

BALSALAZIDE DISODIUM CAPSULES (DSD Mgh #6990)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Hypersil BDS C-18	Assay	4.6 mm × 25 cm, 5 µm. Manufacturer: Thermo Scientific
35(4)	L1	Eclipse XDB C18	Organic Impurities	4.6 mm × 25 cm, 5 µm. Manufacturer: Agilent

BEMOTRIZINOL (DSD Mgh #3022)

PF	LGS#	Reagent Brand	Type of Test	Comments
32(4)	L1	Hypersil BDS C-18	Assay and Related Compounds	3.0 mm × 12.5 cm. Manufacturer: Thermo Scientific

BRINZOLAMIDE (DSD Mgh #10206)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Spherisorb ODS2	Assay and Related Compounds	Related compounds test 2. 4.6 mm × 25 cm, 5 µm. Manufacturer: Waters Corp.

CARVEDILOL (DSD Mgh #2730)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(5)	L7	Spherisorb C8	Limit of	Limit of carvedilol related compound F. 4.6 mm × 30 mm, 3 µm. Manufacturer: Waters Corp.

CARVEDILOL TABLETS (DSD Mgh #2701)

PF	LGS#	Reagent Brand	Type of Test	Comments
33(5)	L7	IB-SIL C8	Assay and Related Compounds	4.6 mm × 5 cm, 5 µm. Alternative column: Hypersil MOS C8, 2.5 µm, manufactured by Thermo-Electron. Manufacturer: Phenomenex

CEFDITOREN PIVOXIL (DSD Mgh #13988)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	YMC-Pack ODS-AM	Assay and Related Compounds	4.6 mm × 25 cm, 5 µm. Manufacturer: YMC Co. Ltd.

CEFTIOFUR SODIUM (DSD Mgh #14135)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(4)	G43	None Cited	Limit of	Limit of acetone and tetrahydrofuran. 0.32 mm × 30 m, 1.8 µm. Manufacturer: n/a

CLIMBAZOLE (DSD Mgh #1371)

PF	LGS#	Reagent Brand	Type of Test	Comments
33(5)	L7	LiChrospher 60 RP-Select B	Assay	4 mm × 25 cm, 5 μm. Manufacturer: Merck KGaA

DEFEROXAMINE MESYLATE (DSD Mgh #22400)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	ZORBAX ODS	Assay and Related Compounds	4.6 mm × 7.5 cm, 3.5 μm. Alternative column: Zorbax Eclipse XDB C18. Manufacturer: Agilent Technologies

FLUCONAZOLE INJECTION (DSD Mgh #33243)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(3)	L1	Symmetry C-18	Organic Impurities	Procedure 3. 4.6 mm × 15 cm, 3.5 μm. Manufacturer: Waters Corp.

METHACRYLIC ACID COPOLYMER (DSD Mgh #49850)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Nucleosil 100 C18	Limit of	Limit of monomers. 4 mm × 12.5 cm, 7 μm. Manufacturer: Macherey-Nagel

METHACRYLIC ACID AND ETHYL ACRYLATE COPOLYMER (DSD Mgh #4368)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Nucleosil 100 C18	Limit of	Limit of methacrylic acid and ethyl acrylate. 4 mm × 12.5 cm, 7 μm. Manufacturer: Macherey-Nagel

METHACRYLIC ACID AND METHYL METHACRYLATE COPOLYMER (DSD Mgh #4367)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Nucleosil 100 C18	Limit of	Limit of methacrylic acid and methyl methacrylate. 4 mm × 12.5 cm, 7 μm. Manufacturer: Macherey-Nagel

MYCOPHENOLATE MOFETIL CAPSULES (DSD Mgh #55075)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L7	Luna C8(2)	Assay and Limit of	Limit of degradation products. 4.6 mm × 25 cm, 5 μm. Manufacturer: Phenomenex
35(4)	L7	ZORBAX SB C8	Limit of	Limit of *z*-mycophenolate mofetil. 4.6 mm × 15 cm, 3.5 μm. Manufacturer: Agilent Technologies

MYCOPHENOLATE MOFETIL TABLETS (DSD Mgh #55077)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L7	ZORBAX SB C8	Limit of	Limit of *z*-mycophenolate mofetil. 4.6 mm × 15 cm, 3.5 μm. Manufacturer: Agilent Technologies
35(4)	L7	Luna C8(2)	Assay and Limit of	Limit of degradation products. 4.6 mm × 25 cm, 5 μm. Manufacturer: Phenomenex

PROPOXYPHENE HYDROCHLORIDE (DSD Mgh #70590)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	ZORBAX Eclipse XDB-C18	Assay and Related Compounds	4.6 mm × 15 cm, 3.5 μm. Manufacturer: Agilent Technologies

RAMIPRIL CAPSULES (DSD Mgh #73034)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Zorbax SB-C18	Assay, Dissolution, and Related Compounds	4.6 mm × 15 cm, 5 μm. Manufacturer: Agilent

SERTRALINE HYDROCHLORIDE (DSD Mgh #75100)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(5)	L45	Chiradex	Assay and Related Compounds	4.0 mm × 25 cm, 5 µm. Manufacturer: Merck KGaA
34(5)	L40	Chiralcel OD-H	Limit of	Limit of (*R,R*) sertraline hydrochloride. 4.6 mm × 25 cm, 5 µm. Manufacturer: Daicel Chemical Industries

SUMATRIPTAN SUCCINATE (DSD Mgh #80104)

PF	LGS#	Reagent Brand	Type of Test	Comments
33(3)	L3	Spherisorb Silica	Limit of	Limit of sumatriptan succinate related compound A. 4.6 mm × 25 cm, 5 µm. Manufacturer: Waters Corp.

SUMATRIPTAN TABLETS (DSD Mgh #80108)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Spherisorb ODS1	Assay and Organic Impurities	4.6 mm × 25 cm, 5 µm. Manufacturer: Waters Corp.

TERAZOSIN CAPSULES (DSD Mgh #80838)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Burdick & Jackson C18	Assay	4.6 × 15 cm, 5 µm. Manufacturer: B & J
35(4)	L1	SymmetryShield RP18	Related Compounds	4.6 × 25 cm, 5 µm. Manufacturer: Waters Corp.

TERAZOSIN TABLETS (DSD Mgh #80842)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	SymmetryShield RP18	Related Compounds	4.6 mm × 25 cm, 5 µm. Manufacturer: Waters Corp.
35(4)	L1	Burdick & Jackson C18	Assay	4.6 mm × 15 cm, 5 µm. Manufacturer: B & J

VALACYCLOVIR TABLETS (DSD Mgh #87565)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Hypersil BDS C-18	Dissolution	4.6 mm × 5 cm, 5 µm. Manufacturer: Thermo Scientific
0(0)	L66	Crownpak CR (+)	Assay and Related Compounds	4 mm × 15 cm, 5 µm. Manufacturer: Daicel

Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 5 **SEPT.–OCT. 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *Pharmacopeial Forum* content to
USP Executive Secretariat
12601 Twinbrook Parkway,
Rockville, MD 20852
execsec@usp.org

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

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

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STANDARDS DEVELOPMENT

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

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

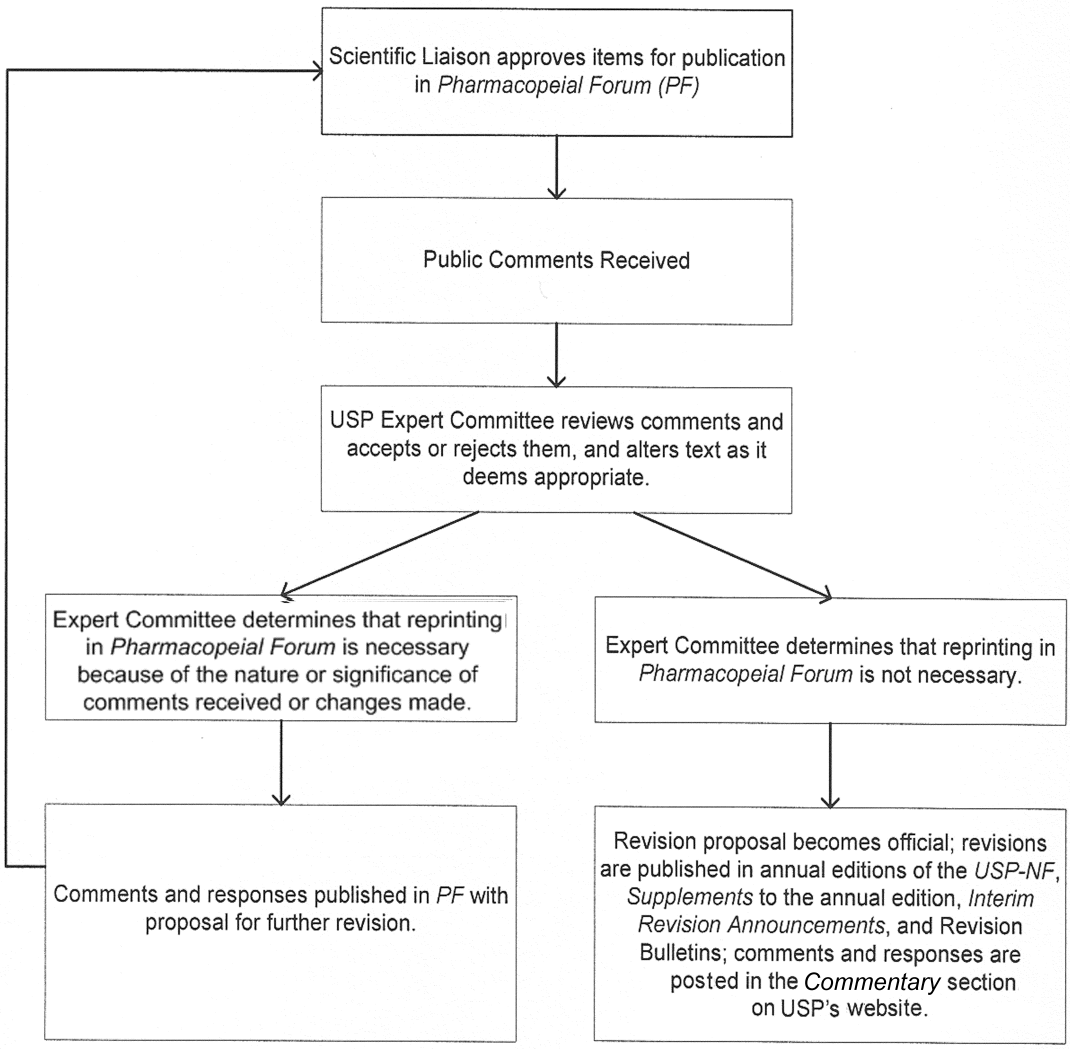
PF includes the following:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's typical Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that reprinting in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Accelerated Revisions**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP website) (e.g., *Interim Revision Announcements*, *Revision Bulletins*, and *Errata*). Accelerated Revisions allow for a revision to become official prior to the next *USP–NF* or *Supplement* and do not always require notice and comment. *Interim Revision Announcements* are first presented for a 60-day public comment period in the *Proposed Interim Revision Announcement* section before becoming official in a later *PF* in the *Interim Revision Announcements* section of the *PF*. Note that *Revision Bulletins* appear only on the USP website.

USP welcomes comments and data on proposed revisions. Comments, along with USP's responses, will be published in the *Commentary* section of the USP website (www.usp.org).

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

''How to Use PF'' describes the various parts of *Pharmacopeial Forum*, lists the *Committee Designations*, and includes the *Staff Directory*.

The contents of the various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
Proposed Interim Revision Announcement Accelerated Revision targeted to become official in an upcoming *PF.*	Proposals for *Interim Revision Announcements (IRAs)* that will be published as official in a future *Pharmacopeial Forum.* BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Scientific Liaison who handled the issue.	Review material and send comments within 60 days of the *PF* publication where the standard was proposed (or per the Briefing). Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing.
Interim Revision Announcement Official Accelerated Revision (on the first day of the second month, unless otherwise indicated).	Standards that have been adopted and will become official on the date that is specified in the section's introductory page or within parentheses following a particular item. New or revised text is marked by the symbols •.	Review material to see whether affected by any of the changes. Note date when standards become official, and ensure compliance.
Errata Accelerated Revision.	Corrections to official standards that will be printed in *USP–NF.*	Review material to see whether affected by any changes.
In-Process Revision Revisions for public review and comment.	Proposals for standards that will be published as official in a future *USP–NF* book or *Supplement*. BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the appropriate USP Scientific Liaison.	Review material and send comments. Comment deadlines are found at the end of the *Policies and Announcements* section. Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839.
Previous PF Proposals	Proposals from previous *PF*s that did not advance to official status in an official USP publication. This section is cumulative.	Review material to track pending proposals.
Canceled Proposals	Items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption in *USP–NF.*	Review material to track canceled proposals.

Proposed and Adopted Revisions to the *USP–NF* *(Continued)*

Section	Content	How Readers Can Respond
Stage 4 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize.	BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. Stage 4 is available for comment.	Review material and provide comments to the appropriate Scientific Liaison cited in the Briefing. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP, for a given article. The addresses for the European (PhEur) and Japanese (JP) pharmacopoeias are as follows: PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp
Stage 6 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize.	Stage 6 is the final official harmonized standard and is not available for comment. This information is published for informational purposes only. New or revised text to Stage 6 documents is marked with symbols that indicate the publication in which the book or *Supplement* becomes official.	Review material to see whether affected by any changes.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including Scientific Liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Columns Used in USP–NF and Pharmacopeial Forum
Update of chromatographic columns based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* *(Continued)*

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, M.S., Senior Scientific Associate	kxb@usp.org	(301) 816-8356	Food Ingredients (FI)
Colleen Brennan, R.Ph., Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	*USP–NF* Spanish Edition
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D., Scientist	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards (DS-PS)
Anthony DeStefano, Ph.D., Vice President, General Chapters	ajd@usp.org	(301) 230-6303	
Susan S. de Mars, J.D., Chief Documentary Standards Officer and General Counsel	sdm@usp.org	(301) 816-8296	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE); Radiopharmaceuticals and Medical Imaging Agents (RMI); Veterinary Drugs (VET)
James Griffiths, Ph.D., Vice President, Food, Dietary Supplement, and Excipient Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, M.Sc., Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	*USP–NF* Spanish Edition; General Chapters (GC); Pharmaceutical Waters (PW)
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products—Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexey Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B Proteins and Polysaccharides (BB PP)
Randy Kiser, M.S., M.B.A., Program Manager	rwk@usp.org	(301) 816-8324	USP Reference Standards Acquisition
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Ph.D., Vice President, Reference Standards Operations	psl@usp.org	(301) 816-8337	
Markus Lipp, Ph.D., Director, Foods Standards	mxl@usp.org	(301) 816-6366	Food Ingredients (FI)
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, M.S., Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. C. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Kate Meringolo, M.S., M.B.A., Manager, Publication Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Scientist	jm@usp.org	(301) 816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Senior Scientist	ktm@usp.org	(301) 816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Vice President, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements—Non-Botanicals (DSN)
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Ph.D., Senior Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D., Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D., Manager, Pharmacy Compounding	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, M.S., Director, Excipients	cxs@usp.org	(301) 816-8262	Excipients
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB VV); Proteins and Polysaccharides (BB PP) (small peptides)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Ph.D., Senior Scientist	dwv@usp.org	(301) 998-6828	Monograph Development—Pulmonary and Steroids (MD-PS)
Mary ''Jeanie'' Waddell, Scientist	msw@usp.org	(301) 816-8124	Monograph Development—Pulmonary and Steroids (MD-PS)
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	lw@usp.org	(301) 816-8129	USP Reference Standards Evaluation
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, M.S., Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section provides general information resources for *USP–NF* standards and processes. Information resources include announcements on scientific and policy issues currently under consideration, schedules for USP publications, and schedules for comment periods for proposed standards.

USP ANNOUNCES REVISION TO BUDESONIDE MONOGRAPH. The Monograph Development—Pulmonary and Steroids Expert Committee has approved two revisions to *Limit of 11-ketobudesonide*. Resolution cannot be established due to the absence of a signal for 14,15-dehydrobudesonide and 21-dehydrobudesonide; therefore, the Expert Committee removed the resolution requirements as part of the system suitability. In addition, the Expert Committee added a requirement that the *Mobile phase* be pre-heated in order to stabilize the retention times. These revisions were previously proposed in *Pharmacopeial Forum* 35(3) [May–June 2009]. The approved *Interim Revision Announcement* supersedes the monograph printed in *USP 32–NF 27* until it is printed in *USP 33–NF 28*.

Please contact Domenick Vicchio, Ph.D, at 301-998-6828 or dwv@usp with any questions.

USP ANNOUNCES REVISION TO NEFAZODONE HYDROCHLORIDE MONOGRAPH. The Monograph Development—Psychiatrics and Pyschoactives Expert Committee has approved the revision of *Identification* test *B* for counter ion. The revision utilizes methanol as a solvent rather than water because the drug substance has better solubility in methanol. This revision was previously proposed in *Pharmacopeial Forum* 35(3) [May–June 2009]. The approved *Interim Revision Announcement* supersedes the monograph printed in USP 32–NF 27 until it is printed in USP 33–NF 28.

Please contact Ravi Ravichandran, Ph.D., at 301-816-8330 or rr@usp.org with any questions about the Nefazadone Hydrochloride monograph.

USP ANNOUNCES CHROMATOGRAPHIC REAGENTS IS NOW CALLED CHROMATOGRAPHIC COLUMNS. Starting with *PF* 35(5), USP is changing the *Pharmacopeial Forum* section title *Chromatographic Reagents* to *Chromatographic Columns* in order to reflect the current standard practice of using prepared columns in the laboratory. References to *Chromatographic Reagents* have also been revised throughout *Pharmacopeial Forum*. The column information that is contained within this renamed section is the same information that has been presented under the previous *Chromatographic Reagents* title.

Please contact Margareth Marques, Ph.D., at 301-816-8106 or mrm@usp.org with any questions.

USP ISSUES CALL FOR CANDIDATES FOR 2010–2015 COUNCIL OF EXPERTS, ITS EXPERT COMMITTEES, AND ITS EXPERT PANELS. In accordance with the Bylaws of the USP Convention, USP is issuing a Call for Candidates for the 2010–2015 Council of Experts (COE). The 2010–2015 COE includes Expert Committees in the areas of Nomenclature, Small Molecules, Biologics and Biotechnology, Excipients, General Chapters, Reference Standards, Compounding, Food Ingredients, and Dietary Supplements. In the 2010–2015 cycle, USP is expanding the number of Expert Panels that report to Expert Committees.

These Expert Committees and Panels align with the new USP Strategic Plan, which focuses on expanding and enhancing USP's core compendial and standards-setting activities. The ability to add Expert Panels according to USP's needs introduces flexibility and scalability into USP's activities. USP plans to continue to attract a global base of experts and therefore encourages any qualified individual to apply. Importantly, this approach also enables USP to closely align its documentary and reference standards activities for a more efficient standards-setting process.

Specific Expert Committees and Expert Panels for which USP is seeking candidates are listed at USP's nominations Web site (www.usp.org/goto/nominate). The deadline for applications for the COE (Expert Committee Chairs) is **December 31, 2009**. The deadline for applications for Expert Committee members is **May 15, 2010**. Recruitment for Expert Panel members will begin in July 2010 and will be continuous.

For further information, please contact Nelufar Mohajeri, Director, Volunteer Affairs and Compendial Initiatives (nym@usp.org or nominate@usp.org).

***PHARMACOPEIAL FORUM* PUBLIC REVIEW AND COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section and incorporated in the upcoming *USP–NF* or *Supplement*. They may also be published as *Revision Bulletins* on www.usp.org in the ''New Official Text'' section. The official publication in which an *Interim Revision Announcement* (*IRA*) is incorporated will depend upon publication deadlines. See the table below. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and, therefore, contains all official text up to and including the contents of the latest *Supplement*. The table below outlines the publications and their release and official dates, and the *USP–NF* or *Supplement* that supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
USP 32–NF 27	November 1, 2008	May 1, 2009	*1st Supplement to USP 32–NF 27*
IRA [*PF* 35(1)]	January 1, 2009	February 1, 2009	*2nd Supplement to USP 32–NF 27*
1st Supplement to USP 32–NF 27	February 1, 2009	August 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*

PRIORITY NEW MONOGRAPH ITEMS. USP is seeking monographs for the following drug substances and drug products that are off patent, are approaching off patent status, and thus are of the highest priority. USP also is seeking monographs for the excipients listed below. Monographs are marked ''Received'' upon receipt of a monograph proposal. Received monographs are removed from this list upon publication in *Pharmacopeial Forum* or when posted in the *USP Pending Monographs* section of the USP website (http://www.usp.org/standards/pending/). This list has been updated as of June 15, 2009; monographs received since the last update to the list are noted in bold.

Monograph sponsors should consult USP's Guideline for Submitting Requests for Revision to the *USP–NF* at http://www.usp.org/USPNF/submitMonograph/subGuide.html.

For additional information, contact Randy Kiser, MS, MBA, rwk@usp.org.

Small Molecules (Drug Substances)—As of June 15, 2009

1. Allopurinol Sodium	2. Aminopropazine Fumarate	3. Aminopterin Sodium
4. Anagrelide Hydrochloride ***(Received)***	5. Arsenic Trioxide	6. Auranfoin
7. Azelaic Acid ***(Received)***	8. Bentoquatam	9. Benzphetamine Hydrochloride
10. Bivalirudin ***(Received)***	11. Calcipotriene	12. Calcium Trisodium Pentetate
13. Calfactant	14. Candesartan Cilexetil ***(Received)***	15. Ceftibuten
16. Cetrorelix	17. Cevimeline Hydrochloride ***(Received)***	18. Chloroxine
19. Choline Salicylate	20. Cysteamine Bitartrate	21. Dalfopristin
22. Dapirazole Hydrochloride	23. Desirudin	24. Desonide ***(Received)***
25. Dexrazoxane	26. Difenoxin Hydrochloride	27. Entacapone ***(Received)***
28. Epoprostenol Sodium ***(Received)***	29. Erythromycin Phosphate	30. Erythromycin Thiocyanate
31. Esmolol Hydrochloride ***(Received)***	32. Estazolam ***(Received)***	33. Estramustine Phosphate Sodium
34. Ethanolamine Oleate	35. Etomidate ***(Received)***	36. Etoposide Phosphate
37. Exemestane	38. Famciclovir ***(Received)***)	39. Felbamate ***(Received)***
40. Fluoromethane F 18	41. Fosfomycin Tromethamine ***(Received)***	42. Gadobenate Dimeglumine
43. Gadopentetic Acid	44. Gallium Nitrate	45. Ganirelix
46. Guanidine Hydrochloride	47. Halobetasol Propionate ***(Received)***	48. Haloperidol Decanoate ***(Received)***
49. Hydrocodone Polistirex	50. Ibandronate Sodium	51. Imipramine Pamoate
52. Imiquimod	53. Isosulfan Blue	54. Latanoprost ***(Received)***
55. Lomustine ***(Received)***	56. Metipranolol Hydrochloride	57. Miglitol
58. Milrinone Lactate	59. Moexipril Hydrochloride	60. Nalbuphine Hydrochloride
61. Nedocromil Sodium	62. Nicardipine Hydrochloride ***(Received)***	63. Nilutamide
64. Nisoldipine	65. Olsalazine Sodium ***(Received)***	66. Oxiconazole Nitrate
67. Pemirolast Potassium	68. Pioglitazone Hydrochloride ***(Received)***	69. Piperonyl Butoxide
70. Pirbuterol Acetate ***(Received)***	71. Poractant Alpha	72. Porfimer Sodium
73. Pramiprexole Dihydrochloride ***(Received)***	74. Quetiapine Fumarate ***(Received)***	75. Ranitidine
76. Rivastigmine Tartrate ***(Received)***	77. Ropinirole Hydrochloride ***(Received)***	78. Rose Bengal Disodium
79. Rosiglitazone Maleate	80. Sodium Phenylbutyrate	81. Sodium Phosphates
82. Spectinomycin Sulfate	83. Streptozocin	84. Tenofovir Disoproxil Fumarate ***(Received)***
85. Tiludronate Disodium	86. Tiopronin	87. Trimetrexate Glucuronate

Small Molecules (Drug Substances)—As of June 15, 2009 *(Continued)*

88. Venlafaxine Hydrochloride ***(Received)***	89. Voriconazole ***(Received)***	90. Zaleplon ***(Received)***
91. Zinc Tridosium Pentetate	92. Zoledronic Acid	

Small Molecules (Drug Products)—As of June 15, 2009

1. Abacavir Sulfate, Lamivudine, and Zidovudine Tablets	2. Acarbose Tablets	3. Acetaminophen, Butalbital, Caffeine, and Codeine Phosphate Capsules
4. Acetaminophen, Clemastine Fumarate and Pseudoephedrine Hydrochloride Tablets	5. Acetazolamide Extended-Release Capsules	6. Albuterol and Ipratropium Bromide Inhalation Aerosol
7. Albuterol and Ipratropium Bromide Inhalation Solution	8. Albuterol Extended-Release Tablets	9. Albuterol Inhalation Aerosol
10. Albuterol Sulfate Inhalation Solution	11. Albuterol Sulfate Oral Solution	12. Alendronate Sodium Oral Solution
13. Alfuzosin Extended-Release Tablets	14. Allopurinol for Injection	15. Alprazolam Extended-Release Tablets
16. Alprostadil Urethral Suppository	17. Aminopropazine Fumarate and Neomycin Sulfate Tablets	18. Aminopropazine Fumarate Injection
19. Aminopropazine Fumarate Tablets	20. Aminopterin Sodium Tablets	21. Amiodarone Hydrochloride Injection
22. Amlodipine and Benazepril Hydrochloride Capsules ***(Received)***	23. Amphotericin B Injection	24. Anagrelide Hydrochloride Capsules ***(Received)***
25. Arsenic Trioxide Injection	26. Atovaquone and Proguanil Hydrochloride Tablets	27. Atovaquone Tablets
28. Auranofin Capsules	29. Azatadine Maleate and Pseudoephedrine Sulfate Extended-Release Tablets	30. Azelaic Acid Cream
31. Azithromycin for Injection ***(Received)***	32. Azithromycin Tablets ***(Received)***	33. Baclofen Injection
34. Beclomethasone Dipropionate Inhalation Aerosol	35. Beclomethasone Dipropionate Nasal Suspension	36. Benazepril Hydrochloride and Hydrochlorothiazide Tablets
37. Bentoquatam Topical Suspension	38. Benzocaine and Cetylpyridinium Chloride Lozenges	39. Benzocaine and Menthol Lotion
40. Benzphetamine Hydrochloride Tablets	41. Bivalirudin for Injection ***(Received)***	42. Brompheniramine Maleate, Dextromethorphan Hydrobromide, and Pseudoephedrine Hydrochloride Oral Solution
43. Budesonide Inhalation Aerosol	44. Bupivacaine and Lidocaine Hydrochlorides Injection	45. Buprenorphine Hydrochloride Injection
46. Butalbital and Acetaminophen Capsules	47. Butalbital and Acetaminophen Tablets	48. Calcipotriene Cream
49. Calcipotriene Ointment	50. Calcipotriene Topical Solution	51. Calcitriol Capsules
52. Calcitriol Oral Solution	53. Calcium Acetate Capsules	54. Calcium Trisodium Pentetate Injection
55. Calfactant Intratracheal Suspension	56. Carbidopa and Levodopa Tablets For Oral Suspension ***(Received)***	57. Carbidopa, Levidopa, and Entacapone Tablets
58. Carmustine Implant	59. Cefdinir Tablets	60. Cefditoren Pivoxil Tablets
61. Ceftibuten Capsules	62. Ceftibuten for Oral Suspension	63. Ceftiofur Hydrochloride Oral Suspension
64. Cetirizine Hydrochloride Tablets ***(Received)***	65. Cetrorelix Injection	66. Cevimeline Hydrochloride Capsules
67. Chloroxine Cream	68. Chlorpromazine Hydrochloride Extended-Release Capsules	69. Choline and Magnesium Salicylates Oral Solution
70. Choline and Magnesium Salicylates Tablets	71. Choline Salicylate Oral Solution ***(Received)***	72. Ciclopirox Shampoo
73. Ciclopirox Topical Gel	74. Ciclopirox Topical Solution ***(Received)***	75. Cimetidine Oral Solution
76. Ciprofloxacin Extended-Release Tablets	77. Ciprofloxacin Hydrochloride and Hydrocortisone Otic Suspension	78. Ciprofloxacin Otic Solution
79. Cisplatin Injection	80. Citalopram Hydrobromide Oral Solution	81. Citric Acid, Gluconolactone, and Magnesium Carbonate Irrigation
82. Cladribine Injection	83. Clemastine Fumarate Syrup	84. Clobetasol Propionate Gel
85. Clorazepate Dipotassium Capsules	86. Clorazepate Dipotassium Extended-Release Tablets	87. Clotrimazole and Betamethasone Dipropionate Lotion

Small Molecules (Drug Products)—As of June 15, 2009 *(Continued)*

88. Compound Undecylenic Acid Cream	89. Compound Undecylenic Acid Topical Powder	90. Conjugated Estrogens and Medroxyprogesterone Acetate Tablets
91. Cyclosporine Modified Capsules	92. Cyclosporine Modified Oral Solution	93. Cyclosporine Ointment
94. Cyclosporine Topical Solution	95. Cysteamine Bitartrate Capsules	96. Cytarabine Liposome Injection
97. Dalfopristin and Quinupristin Injection	98. Dantrolene Sodium Oral Suspension	99. Dapiprazole for Ophthalmic Solution
100. Desirudin for Injection	101. Desonide Cream	102. Dexrazoxane for Injection
103. Dextroamphetamine Sulfate Extended-Release Capsules	104. Dextromethorphan Polistirex Extended-Release Oral Suspension	105. Diazepam Injectable Emulsion
106. Diclofenac Sodium Ophthalmic Solution	107. Diethylpropion Hydrochloride Extended-Release Tablets	108. Difenoxin Hydrochloride and Atropine Sulfate Tablets
109. Difloxacin Hydrochloride Tablets	110. Dihydroergotamine Mesylate Metered Spray	111. Diltiazem Hydrochloride Injection
112. Dinoprostone Vaginal Suppositories	113. Diphenhydramine Hydrochloride and Acetaminophen Tablets	114. Divalproex Sodium Delayed-Release Capsules ***(Received)***
115. Dorzolamide and Timolol Ophthalmic Solution	116. Dorzolamide Ophthalmic Solution	117. Doxepin Hydrochloride Cream
118. Doxycycline Oral Gel	119. Econazole Nitrate Cream	120. Edrophonium Chloride and Atropine Sulfate Injection
121. Enalapril Maleate and Felodipine Extended-Release Tablets	122. Entacapone Tablets ***(Received)***	123. Ephedrine Sulfate and Guaifenesin Tablets
124. Epirubicin Hydrochloride for Injection	125. Epirubicin Hydrochloride Injection	126. Epoprostenol for Injection
127. Epoprostenol Injection	128. Esmolol Hydrochloride Injection	129. Esomeprazole Magnesium Capsules
130. Estazolam Tablets ***(Received)***	131. Estramustine Phosphate Sodium Capsules	132. Ethanolamine Oleate Injection
133. Etidronate Disodium Injection Concentrate	134. Etomidate Injection ***(Received)***	135. Exemestane Tablets
136. Famotidine Orally Disintegrating Tablets	137. Felbamate Oral Suspension	138. Felbamate Tablets
139. Fentanyl Lozenges	140. **Famciclovir Tablets** ***(Received)***	141. Fentanyl Transdermal System ***(Received)***
142. Ferrous Fumarate and Docusate Sodium Extended-Release Capsules	143. Fluconazole Oral Suspension	144. Flunisolide Inhalation Aerosol
145. Flunisolide Nasal Spray	146. Fluocinolone Acetonide Shampoo	147. Fluorescein Sodium Ophthalmic Solution
148. Fluorometholone Ointment	149. Fluticasone Propionate Inhalation Powder ***(Received)***	150. Fluticasone Propionate Pressurized Inhaler
151. Foscarnet Sodium Injection	152. Fosfomycin for Oral Solution	153. Gabapentin Oral Solution
154. Gadobenate Dimeglumine Injection	155. Gallium Nitrate Injection	156. Ganciclovir Capsules
157. Ganirelix Acetate Injection	158. Gatifloxacin Injection	159. Gatifloxacin Tablets
160. Gentamicin Sulfate Oral Solution	161. Gentamicin Sulfate Soluble Powder	162. Glipizide Extended-Release Tablets
163. Guaifenesin and Pseudoephedrine Hydrochloride Extended-Release Tablets	164. Guaifenesin and Salts of Dextromethorphan and Pseudoephedrine Oral Solution	165. Guanidine Hydrochloride Tablets
166. Halobetasol Propionate Cream	167. Halobetasol Propionate Ointment	168. Haloperidol Decanoate Injection
169. Haloperidol Lactate Injection	170. Haloperidol Lactate Oral Concentrate	171. Hydralazine Hydrochloride and Hydrochlorothiazide Capsules
172. Hydrochlorothiazide Capsules ***(Received)***	173. Hydrochlorothiazide Oral Solution	174. Hydrocodone Bitartrate and Acetaminophen Capsules
175. Hydrocodone Bitartrate and Acetaminophen Oral Solution	176. Hydrocodone Bitartrate and Aspirin Tablets	177. Hydrocodone Bitartrate and Guaifenesin Oral Solution
178. Hydrocodone Bitartrate and Homatropine Methylbromide Syrup	179. Hydrocortisone Acetate Dental Paste	180. Hydrocortisone Acetate Rectal Foam Aerosol
181. Hydrocortisone Butyrate Lotion	182. Hydroflumethiazide and Reserpine Tablets	183. Hydroquinone Lotion
184. Ibandronate Sodium Tablets	185. Ibuprofen Capsules	186. Idarubicin Hydrochloride Injection
187. Imipramine Pamoate Capsules	188. Imiquimod Topical Cream	189. Ipratropium Bromide Inhalation Aerosol
190. Ipratropium Bromide Inhalation Solution	191. Irinotecan Hydrochloride Injection	192. Isosulfan Blue Injection

Small Molecules (Drug Products)—As of June 15, 2009 *(Continued)*

193. Isradipine Extended-Release Tablets	194. Itraconazole Injection	195. Itraconazole Oral Solution
196. Ketoconazole Cream	197. Ketoconazole Shampoo	198. Ketoprofen Capsules ***(Received)***
199. Ketoprofen Extended-Release Capsules	200. Ketoprofen Tablets	201. Ketotifen Fumarate Ophthalmic Solution
202. Lactic Acid Lotion	203. Lamotrigine Tablets ***(Received)***	204. Latanoprost Ophthalmic Solution
205. Leucovorin Calcium for Injection	206. Levetiracetam Tablets ***(Received)***	207. Levocabastine Ophthalmic Suspension
208. Levofloxacin Solution	209. Lincomycin Hydrochloride and Spectinomycin Sulfate Soluble Powder	210. Liothyronine Injection
211. Lomustine Capsules ***(Received)***	212. Lopinavir and Ritonavir Solution	213. Lopinavir Capsules
214. Lopinavir Solution	215. Melphalan for Injection	216. Mesalamine Suppositories
217. Mesoridazine Besylate Concentrate	218. Metaraminol Bitartrate Injection	219. Methacholine Chloride for Inhalation Solution
220. Methadone Hydrochloride Oral Concentrate	221. Methocarbamol and Aspirin Tablets	222. Methoxsalen Softgels
223. Methyclothiazide and Deserpidine Tablets	224. Methylphenidate Hydochloride Chewable Tablets	225. Metipranolol Ophthalmic Solution
226. Metronidazole Cream	227. Metronidazole Extended-Release Tablets	228. Metronidazole Hydrochloride for Injection
229. Metronidazole Lotion	230. Miconazole Nitrate Topical Aerosol	231. Mifepristone Tablets
232. Miglitol Tablets	233. Milrinone Injection	234. Misoprostol Tablets ***(Received)***
235. Moexipril Hydrochloride and Hydrochlorothiazide Tablets	236. Moexipril Hydrochloride Tablets	237. Molindone Hydrochloride Oral Solution
238. Morphine Sulfate for Injection Concentrate	239. Morphine Sulfate Oral Solution	240. Morphine Sulfate Oral Solution Concentrate
241. Morphine Sulfate Tablets	242. Nalbuphine Hydrochloride Injection	243. **Mycophenolate Mofetil Oral Solution** ***(Received)***
244. Nalmefene Hydrochloride Injection	245. Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution	246. Naproxen Sodium Extended-Release Tablets
247. Nedocromil Sodium Inhalation Aerosol	248. Neomycin Sulfate Oral Powder	249. Nicardipine Hydrochloride Capsules
250. Nilutamide Tablets	251. Nimodipine Capsules	252. Nisoldipine Extended-Release Tablets
253. Nitroglycerin Solution In Acrylic Adhesive	254. Nitroglycerin Transdermal System	255. Nizatidine Tablets
256. Ofloxacin In Dextrose Injection	257. Ofloxacin Injection	258. Olsalazine Sodium Capsules
259. Orphenadrine Citrate Extended-Release Tablets ***(Received)***	260. Orphenadrine Citrate, Aspirin, and Caffeine Tablets	261. Oxcarbazepine Suspension
262. Oxiconazole Cream	263. Pamidronate Disodium Injection	264. Pantoprazole Sodium for Injection
265. Pantoprazole Sodium Tablets	266. Paroxetine Hydrochloride Extended-Release Tablets	267. Paroxetine Oral Suspension
268. Pemirolast Potassium Ophthalmic Solution	269. Penicillin G Potassium Tablets for Oral Solution	270. Pentamidine Isethionate for Inhalation
271. Pentamidine Isethionate Injection ***(Received)***	272. Pentazocine Hydrochloride and Acetaminophen Tablets	273. Phendimetrazine Tartrate Extended-Release Capsules
274. Phenobarbital Capsules	275. Phentermine Resin Complex Capsules	276. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Capsules
277. Phenylephrine Hydrochloride, Chlorpheniramine Maleate, and Acetaminophen Extended-Release Tablets	278. Pilocarpine Hydrochloride Ophthalmic Gel	279. Pilocarpine Hydrochloride Ophthalmic Ointment
280. Pioglitazone Hydrochloride Tablets ***(Received)***	281. Piperonyl Butoxide and Pyrethrins Aerosol Foam	282. Pirbuterol Acetate Inhalation Aerosol
283. Poractant Alpha Suspension	284. Porfimer Sodium for Injection	285. Povacrylate Solution
286. Povacrylate–Iodine Topical Solution	287. Povidone–Iodine Gauze	288. Povidone–Iodine Swabsticks

Small Molecules (Drug Products)—As of June 15, 2009 *(Continued)*

289. Povidone–Iodine Topical Aerosol Foam	290. Povidone–Iodine Vaginal Suppositories	291. Pramipexole Dihydrochloride Tablets
292. Prednisolone Sodium Phosphate Oral Solution	293. Prochlorperazine Maleate Extended-Release Capsules	294. Progesterone Capsules
295. Propafenone Hydrochloride Tablets	296. Pseudoephedrine Hydrochloride and Brompheniramine Maleate Extended-Release Tablets	297. Pseudoephedrine Hydrochloride and Naproxen Sodium Extended-Release Tablets
298. Pseudoephedrine Hydrochloride, Chlorpheniramine Maleate, and Codeine Phosphate Oral Solution	299. Pseudoephedrine Hydrochloride, Guaifenesin, and Codeine Phosphate Oral Solution	300. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Extended-Release Tablets
301. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Oral Solution	302. Pseudoephedrine Sulfate, Dexbrompheniramine Maleate, and Acetaminophen Extended-Release Tablets	303. Pyrilamine Maleate Injection
304. Quinapril Hydrochloride and Hydrochlorothiazide Tablets	305. Quinidine Sulfate Injection	306. **Ranitidine Capsules** ***(Received)***
307. Rauwolfia Serpentina and Endroflumethiazide Tablets	308. Reserpine and Polythiazide Tablets	309. Rimantadine Hydrochloride Oral Solution
310. Rivastigmine Tartrate Capsules ***(Received)***	311. Rivastigmine Tartrate Oral Solution ***(Received)***	312. Rocuronium Bromide Injection
313. Ropinirole Hydrochloride Tablets	314. Rosiglitazone Maleate Tablets	315. Salicylic Acid and Sulfur Cleansing Lotion
316. Salicylic Acid and Sulfur Lotion	317. Salicylic Acid and Sulfur Shampoo	318. Salicylic Acid Cream
319. Salicylic Acid Ointment	320. Salmeterol Inhalation Aerosol	321. Salmeterol Xinafoate Inhalation Powder
322. Scopolamine Transdermal System	323. Selegiline Hydrochloride Capsules	324. Sertraline Hydrochloride Oral Solution
325. Sibutramine Hydrochloride Capsules	326. Sodium Bicarbonate and Sodium Citrate for Oral Solution	327. Sodium Bicarbonate, Sodium Citrate, and Sodium Tartrate for Oral Suspension
328. Sodium Iodide Injection	329. Sodium Phenylbutyrate Oral Powder	330. Sodium Phenylbutyrate Tablets
331. Sodium Phosphates for Oral Suspension	332. Sodium Phosphates Tablets	333. Sodium Salicylate and Sulfur Shampoo
334. Sterile Talc Aerosol	335. Streptozocin for Injection	336. Sucralfate Oral Suspension
337. Sulconazole Nitrate Cream	338. Sulfacetamide Sodium and Fluorometholone Ophthalmic Suspension	339. Sulfacetamide Sodium and Prednisolone Sodium Phosphate Ophthalmic Solution
340. Sulfasalazine Oral Suspension	341. Sulisobenzone Lotion	342. Sumatriptan Injection
343. Tacrolimus Injection	344. Tacrolimus Ointment	345. Technetium Tc 99m Teboroxime Injection
346. Tenofovir Disoproxil Fumarate Tablets ***(Received)***	347. Terbinafine Hydrochloride Cream	348. Terbinafine Tablets ***(Received)***
349. Terbinafine Topical Solution	350. Terconazole Vaginal Cream	351. Terconazole Vaginal Suppositories
352. Testosterone Transdermal Gel	353. Testosterone Transdermal System	354. Tetracycline Hydrochloride Periodontal Fiber
355. Theophylline Extended-Release Tablets	356. Tioconazole Vaginal Ointment	357. Tiopronin Tablets
358. Tolnaftate Topical Aerosol Solution	359. Topiramate Capsules ***(Received)***	360. Torsemide Injection
361. Torsemide Tablets ***(Received)***	362. Trandolapril and Verapamil Hydrochloride Extended-Release Tablets	363. **Trandolapril Tablets** ***(Received)***
364. Tranexamic Acid Injection	365. Tretinoin Capsules	366. Tretinoin Microsphere Gel
367. Triamcinolone Acetonide Nasal Suspension	368. Trifluridine Ophthalmic Solution	369. Trimetrexate for Injection
370. Trimipramine Maleate Capsules	371. Triprolidine and Pseudoephedrine Hydrochlorides and Codeine Phosphate Syrup	372. Trolamine Salicylate Cream
373. Trolamine Salicylate Gel	374. Trolamine Salicylate Topical Emulsion	375. Undecylenic Acid Topical Foam Aerosol
376. Urea Cream	377. Vecuronium Bromide for Injection	378. Venlafaxine Extended-Release Capsules ***(Received)***
379. Venlafaxine Tablets ***(Received)***	380. Verapamil Hydrochloride Capsules	381. Verapamil Hydrochloride Extended-Release Capsules

Small Molecules (Drug Products)—As of June 15, 2009 *(Continued)*

382. Voriconazole Injection	383. Voriconazole Oral Suspension	384. Voriconazole Tablets
385. Yttrium Y-90 Chloride Solution	386. Yttrium Y-90 Glass Microspheres	387. Yttrium Y-90 Microspheres Injection
388. Zaleplon Capsules ***(Received)***	389. Zidovudine and Lamivudine Tablets ***(Received)***	390. Zinc Acetate Capsules
391. Zinc Tridosium Pentetate Injection	392. Ziprasidone Hydrochloride Capsules	393. Zoledronic Acid for Injection
394. Zonisamide Capsules ***(Received)***		

Excipients—As of June 15, 2009

1. Acetone Sodium Bisulfite	2. Acetylated Monoglycerides	3. Aconitic Acid (Achilleic Acid)
4. Acrylic Acid–Octyl Acrylate Copolymer	5. Albumin Colloidal	6. Aliphatic Polyesters
7. Allantoin–Sodium Pyrrolidone Carboxylate	8. Aluminum Ammonium Sulfate	9. Aluminum Lactate
10. Aluminum Oxide	11. Aluminum Potassium Sulfate	12. Aluminum Silicate
13. Aluminum Sodium Sulfate	14. Aluminum Stearate	15. Ammonium Bicarbonate
16. Ammonium Calcium Alginate	17. Ammonium Phosphate	18. Batylalcohol Monostearate
19. Beeswax, Synthetic	20. Benzododecinium Bromide	21. Benzyl Chloride
22. Benzyl Nicotinate	23. Beta Naphthol	24. Brominated Vegetable Oil
25. Butadiene–Styrene Rubber	26. Butyl Stearate ***(Received)***	27. Butylated Hydromethylphenol
28. Butylene Glycol	29. Butylphthalyl Butylglycolate	30. Calcium Acid Pyrophosphate
31. Calcium Alginate	32. Calcium Alginate and Ammonium Alginate	33. Calcium Bromide
34. Calcium Chloride Solution	35. Calcium Phosphate Monobasic	36. Calcium Propionate
37. Calcium Pyrophosphate	38. Calcium Sorbate	39. Calcium Stearoyl Lactylate
40. Caldiamide Sodium	41. Calteridol Calcium	42. Capric Acid
43. Caprylic/Capric Diglyceryl Succinate	44. Carbon	45. Carboxymethyl Starch
46. Carboxymethylamylopectin Sodium	47. Carboxymethylcellulose Potassium	48. Cetostearyl Isononanoate
49. Chlorodifluoroethane	50. Cholic Acid	51. Cinnamaldehyde
52. Cocamide Diethanolamine	53. Cocamide Oxide	54. Cocoyl Caprylocaprate
55. Crystal Gum	56. Cutina	57. Cystine
58. Dammar Gum	59. Decanoic Acid	60. Decyl Oleate
61. Dextrin Palmitate	62. Dextrins Modified	63. Diacetyl Tartaric Acid Esters of Mono- and Digoycerides
64. Dicetyl Phosphate	65. Dichlorofluoromethane	66. Diethyl Sebacate ***(Received)***
67. Difluoroethane	68. Diglycol Stearate	69. Diisobutyl Adipate
70. Diisopropyl Adipate	71. Diisopropylbenzothiazyl-2-Sulfenamide	72. Dilauryl Thiodipropionate
73. Dimethyl Dicarbonate	74. Dimyristoyl Lecithin	75. Dimyristoyl Phosphatidylglycerol
76. Dipropylene Glycol	77. Disodium Edisylate	78. Disodium Guanylate
79. Disodium Inosinate	80. Disodium Monooleamide Sulfasuccinate	81. D-Mannose
82. Docusate Sodium/Sodium Benzoate	83. Erythrosine	84. Ethoxylated Mono- and Diglycerides
85. Ethoxyquin	86. Ethyl Hexanediol	87. Ethyl Linoleate
88. Ethyl Maltol ***(Received)***	89. Ethylene Dichloride	90. Ethylurea
91. Ferric Ammonium Citrate	92. Ferric Citrate	93. Ferric Oxide, Brown
94. Ferric Phosphate	95. Ferric Pyrophosphate	96. Ferrous Citrate
97. Ferrous Glycinate	98. Ferrous Lactate	99. Fluorochlorohydrocarbons
100. Formic Acid	101. Furcelleran	102. Gentistic Acid
103. Geraniol	104. Gluten	105. Glycerol Ester of Gum Rosin (Ester Gum)
106. Glyceryl Laurate	107. Glyceryl Palmitate	108. Glyceryl Ricinoleate
109. Glyceryl Tristearate	110. Glycine Hydrochloride	111. Glycofurol
112. Glycol Stearate	113. Heptafluoropropane	114. Heptylparaben
115. Hexadecyl Isostearate	116. Hexane	117. Hexanetriol(-1,2,6-)

Excipients—As of June 15, 2009 *(Continued)*

118. Hydrocarbon Gel	119. Hydroxyethylmethylcellulose	120. Hydroxylated Lecithin
121. Indigotine	122. Iron Carbonyl	123. Iron Subcarbonate
124. Isobutylated-Isoprene Copolymer	125. Isooctylacrylate	126. Isopropyl Isostearate
127. Isopropyl Stearate	128. Isostearic Acid	129. Isostearyl Alcohol
130. Lactose Ferrin, Bovine	131. Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol	132. Lactylic Esters of Fatty Acids
133. Lanolin (Wool Fat), Hydrogenated	134. Lanolin Alcohols, Acetylated	135. Lanolin Hydrous
136. L-Ascorbyl Stearate	137. Lauramine Oxide	138. Lauric Myristic Diethanolamide
139. Lauric Acid	140. Lauric Diethanolamide	141. Lavender Oil
142. L-Cysteine Monohydrochloride	143. Lecithin, Hydroxylated	144. L-Glutamic Acid ***(Received)***
145. Linoleic Acid ***(Received)***	146. L-Leucine	147. Macrogol Sorbitan Tristearate
148. Macrogolglycerol Cocoates	149. Macrogolglycerol Triisostearate	150. Magnesium Aluminum Silicate Hydrate
151. Magnesium Aspartame Dihydrate	152. Magnesium Aspartate	153. Magnesium Phosphate Tribasic
154. Magnesium Phosphate, Diabasic, Trihydrate	155. Magnesium Tartrate	156. Malt Syrup
157. Maltitol Syrup	158. Maltol Isobutyrate	159. Manganese Chloride
160. Manganese Citrate	161. Manganese Glycerophosphate	162. Manganese Hypophosphite
163. Medical Antifoam Emulsion C	164. Medronate Disodium	165. Medronic Acid
166. Methyl Chloride	167. Methylchloroisothiazolinone	168. Methylisothiazolinone
169. Microcrystalline Cellulose, Silicified ***(Received)***	170. Mineral Spirits	171. Monoisostearyl Glyceryl Ester
172. Monopotassium Glutamate Monohydrate	173. Monosodium Citrate	174. Mullein Leaf
175. Myristyl Gamma-Picolinium Chloride	176. Myristyl Lactate	177. *N,N*-Bis(2-Hydroxyethyl)Stearamide
178. *N*-Acetyl-L-Methionine	179. Naphtha	180. *N*-Methylpyrrolidone ***(Received)***
181. Non-Pareil Seeds	182. Nutmeg Oil	183. Octanoic Acid
184. Oxystearin	185. Pentasodium Triphosphate	186. Pentetate Calcium Trisodium
187. Pentetate Pentasodium	188. Phenprobamate	189. Phenylmercuric Acetate
190. Phenylmercuric Nitrate	191. Pine Oil	192. Polacrilin
193. Polyglycerol Esters of Fatty Acids	194. Polyglycerol Polyricinoleic Acid	195. Polyoxyethylene Castor Oil (USP has 35)
196. Polyoxyl Stearate (USP has 40)	197. Polypropylene Oleate	198. Polypropylene Stearyl Ether
199. Polysorbate 65	200. Polyvinylacetal Diethylanoacetate	201. Polyvinylpolypyrrolidone
202. Polyvinylpyrrolidone Ethylcellulose	203. Potassium Acid Tartrate	204. Potassium Bromate
205. Potassium Carbonate Solution	206. Potassium Dichloroisocyanurate	207. Potassium Gibberellate
208. Potassium Glycerophospate	209. Potassium Iodate	210. Potassium Nitrite
211. Potassium Phosphate	212. Potassium Phosphate Tribasic	213. Potassium Polymetaphosphate
214. Potassium Pyrophosphate	215. Potassium Stearate	216. Potassium Sulfate
217. Potassium Sulfite	218. Potassium Tripolyphosphate	219. Propyl Propionate
220. Propylene Glycol Diacetate	221. Propylene Glycol Mono- and Diesters	222. Rice Bran Wax
223. Rosin	224. Silicone	225. Sodium Acid Pyrophosphate
226. Sodium Aluminosilicate ***(Received)***	227. Sodium Aluminum Phosphate Acidic	228. Sodium Aluminum Phosphate Basic
229. Sodium Aspartate	230. Sodium Bisulfate	231. Sodium Bisulfite
232. Sodium Carbonate Hydrate	233. Sodium Carboxymethyl Betaglucan	234. Sodium Caseinate
235. Sodium Chlorate	236. Sodium Citrate, Dibasic	237. Sodium Citrate, Monobasic
238. Sodium Dehydroacetate	239. Sodium Diacetate	240. Sodium Erythorbate
241. Sodium Ferric Pyrophosphate	242. Sodium Ferrocyanide	243. Sodium Hypophosphite ***(Received)***
244. Sodium Laureth Sulfate	245. Sodium Lauroyl Sarcosinate	246. Sodium Lauryl Sulfoacetate
247. Sodium Magnesium Aluminosilicate	248. Sodium Magnesium Silicate	249. Sodium Malate
250. Sodium Metaphosphate, Insoluble	251. Sodium Metasilicate	252. Sodium Methylate

Excipients—As of June 15, 2009 *(Continued)*

253. Sodium Polyphosphates Glassy	254. Sodium Potassium Tripolyphosphate	255. Sodium Pyrophosphate
256. Sodium Pyrrolidone Carboxylate	257. Sodium Sesquicarbonate	258. Sodium Sesquinoleate
259. Sodium Stearoyl Lactylate	260. Sodium Thiomalate	261. Sodium Trimetaphosphate
262. Sodium Trioleate	263. Sodium Tripolyphosphate	264. Soy Polysaccharides
265. Stannous Tartrate	266. Starch, Pregelatinized Corn	267. Starch, Pregelatinized Tapioca
268. Stearalkonium Chloride	269. Stearyl Citrate	270. Stearyl Monoglyceridyl Citrate
271. Succinylated Monoglycerides	272. Sucrose Acetate Isobutyrate	273. Sucrose Fatty Acid Esters
274. Sucrose Stearate ***(Received)***	275. Sugar Fruit Fine	276. Sulfobutyl Ether Beta Cyclodextrin ***(Received)***
277. Tallow	278. Tallow Glycerides	279. Tallow Oil
280. Tetrafluoroethane	281. Thioglycerol	282. Thyme Oil
283. Tribehenin	284. Tricetеareth-4 Phosphate	285. Trichloroethylene
286. Trimyristin	287. Trisodium Citrate	288. Trolamine Lauryl Sulfate
289. Vegetable Oil	290. Wheat Flour	291. Wheat Germ Oil
292. Wheat Gluten ***(Received)***	293. Whey	

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:

- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become official before the official date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The official date for these revisions is stated on the next page.)
- Errata

Readers should review this section to determine if they are affected by any of the changes.

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

Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the issue of a given *PF* volume.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements,* that does not accurately reflect the intended official requirements of the Council of Experts. Beginning with *PF* 35(2), Errata will be published both in the *Pharmacopeial Forum* and on the usp.org website. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to *USP 32–NF 27*. The page number indicates where the item is found in *USP–NF.* Errata are updated as necessary in each *Pharmacopeial Forum* issue and monthly on the usp.org website. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF.* The list of Errata has been relocated to www.usp.org, where updates will be posted monthly.

INTERIM REVISION ANNOUNCEMENT to *USP 32* and to *NF 27*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

Duane M. Kirking, Pharm.D., Ph.D.,
Chair, USP Board of Trustees,
USP Trustee At-Large

Roger L. Williams, M.D., *Chief Executive Officer*
and *Chairman, USP Council of Experts*

Susan de Mars, J.D., *Chief Documentary Standards Officer and General Counsel*
William F. Koch, Ph.D., FACB, *Chief Standards Acquisitions and Metrology Officer*

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All inquiries and comments regarding *USP 32* text and *NF 27* text should be addressed to the Executive Secretariat, *USP–NF*, 12601 Twinbrook Parkway, Rockville, MD 20852 (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 32* or *NF 27* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard. Note that the official date is six months after publishing in this PF.

USP *S*-Adenosyl-L-homocysteine RS (March 1, 2010)
USP Alpha Lipoic Acid RS (March 1, 2010)
USP Lypressin RS (March 1, 2010)
USP Propylene Glycol Dilaurate RS (March 1, 2010)
USP Valrubicin RS (March 1, 2010)
USP Vasopressin RS (March 1, 2010)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 32* or *NF 27* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of June 1, 2009. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at http://www.uspcatalog.com.

USP Acarbose
USP Acarbose System Suitability Mixture
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Fludeoxyglucose Related Compound B RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Propylene Glycol Dilaurate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin Related Compound A RS

Budesonide

Change to read:

Limit of 11-ketobudesonide—

Buffer solution—Proceed as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and isopropanol (65 : 26 : 9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*

Test solution—Proceed as directed for the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 3.5-µm packing L1. The flow rate is about 1.5 mL per minute. •Pre-heat the *Mobile phase* to 50° and•$_{5}$ maintain the column at 50°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.73 and 0.78, respectively, for the two epimers of 11-ketobudesonide, about 0.68 for 21-dehydrobudesonide, about 0.84 for 14,15-dehydrobudesonide, and 1.0 for the first eluted epimer of budesonide (epimer B). •$_{5}$ The column efficiency is not less than 5500 theoretical plates, determined from the budesonide epimer B peak.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of 11-ketobudesonide in the portion of Budesonide taken by the formula:

$$100(r_i / r_U)$$

in which r_i is the sum of the areas of the two ketobudesonide peaks; and r_U is the sum of the areas of the two budesonide peaks; not more than 0.2% of 11-ketobudesonide is found.

Change to read:

Heparin Sodium

•DEFINITION

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process should be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* ⟨1050⟩ for general guidance on viral safety evaluation. It is composed of polymers of alternating derivatives of α-D-glucosamido (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and *O*-sulfated uronic acid (α-L-iduronic acid or β-D-glucuronic acid). The component activities of the mixture are in ratios corresponding to those shown by the USP Heparin Sodium for Assays RS. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin and heparin cofactor II to potentiate the inactivation of thrombin (factor IIa). Other coagulation proteases in the clotting sequence, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity to anti-factor IIa potency is between 0.9 and 1.1. The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

IDENTIFICATION

• A. ^{1}H NMR SPECTRUM

(See *Nuclear Magnetic Resonance* ⟨761⟩.)

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in deuterium oxide with 0.02% (w/v) deuterated trimethylsilylpropionic (TSP) acid sodium salt

System suitability solution: Prepare 1% (w/w) USP Oversulfated Chondroitin Sulfate RS in *Standard solution.*

Sample solution: NLT 20 mg/mL of Heparin Sodium in deuterium oxide with 0.02% (w/v) deuterated TSP

Analysis

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

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for ^{1}H, acquire a free induction decay (FID) using NLT 16 scans using a 90° pulse and 20 s delay. Record the ^{1}H NMR spectra of the *Standard solution* and *System suitability solution* at 25°. Collect the ^{1}H NMR spectrum with a spectral window of at least 10 to –2 ppm and without spinning. The number of transients should be adjusted until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm. The *Standard solution* shall be run at least daily when *Sample solutions* are being run. For all samples, the TSP methyl signal should be set to 0.00 ppm. The chemical shift for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution* should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the ^{1}H NMR spectrum of the *Sample solution* at 25°. Draw a baseline from 8.00 ppm to 0.10 ppm. The ppm values for H1 of GlcNAc/GlcNS, 6S (signal 1), H1 of IdoA2S (signal 2), the H2 of GlcNS (signal 3), and the methyl of GlcNAc (signal 4) of heparin are present at 5.42, 5.21, 3.28 (doublet centered at 3.28 ppm), and 2.05 ppm, respectively.[1] The ppm values of these signals do not differ by more than ±0.03 ppm. Measure the signal heights above the baseline of signal 1 and signal 2, and calculate the mean of these signal heights. Other signals of variable heights and ppm values, attributable to heparin and HOD, may be seen between signal 2 and 4.55 ppm. Residual solvent signals may be observed in the 0.10–3.00 range. Heparin Sodium must meet the requirements stated in *Residual Solvents* ⟨467⟩.

Acceptance criteria: No unidentified signals greater than 4% of the mean of signal height of 1 and 2 are present in the following ranges: 0.10–2.00, 2.10–3.20, and 5.70–8.00 ppm. No signals greater than 200% signal height of the mean of the signal height of 1 and 2 are present in the 3.35–4.55 ppm for porcine heparin.

• B. CHROMATOGRAPHIC IDENTITY

Solution A: Dissolve 0.8 g of monobasic sodium phosphate dihydrate in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Filter the solution through a 0.45-µm membrane and degas prior to use.

Solution B: Dissolve 0.8 g of monobasic sodium phosphate dihydrate and 280 g of sodium perchlorate monohydrate in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Filter the solution through a 0.45-µm membrane and degas prior to use.

[1] GlcNAc, *N*-acetylated glucosamine; GlcNS, *N*-sulfated glucosamine; S, Sulfate; IdoA, iduronic acid; GlcN, glucosamine; GalN, galactosamine.

Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)	Elution
0	80	20	Equilibration
60	10	90	Linear gradient
61	80	20	Linear gradient
75	80	20	Re-equilibration

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in water

System suitability solution: Prepare 1% (w/w) USP Oversulfated Chondroitin Sulfate RS and 1% (w/w) USP Dermatan Sulfate RS in *Standard solution*.

Sample solution: NLT 20 mg/mL of Heparin Sodium in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 202 nm

Column: 2-mm × 25-cm; packing L61

Guard column: 2-mm × 50-mm; packing L61

Column temperature: Maintain columns at 40°

Flow rate: 0.22 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

NOTE—The retention times for dermatan sulfate, heparin, and oversulfated chondroitin sulfate are about 20, 30, and 50 min, respectively.

Suitability requirements

Resolution: NLT 1.0 between the dermatan sulfate and the heparin peaks, and NLT 1.5 between the heparin and the oversulfated chondroitin sulfate

Relative standard deviation: NMT 2% for the heparin peak determined from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms, and measure the retention times for the major peaks.

Acceptance criteria: The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*.

• **C. Anti-Factor Xa to Anti-Factor IIa Ratio**

Anti-factor Xa activity

pH 8.4 buffer: Dissolve amounts of tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% of polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust, if necessary, with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) as directed by the manufacturer, and further dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 1.0 Antithrombin IU/mL.

Factor Xa solution: Reconstitute bovine factor Xa as directed by the manufacturer (see *Factor Xa* in *Reagents, Indicators, and Solutions—Reagent Specifications*), and further dilute in *pH 8.4 buffer* to obtain a solution that gives an absorbance value between 0.65 and 1.25 at 405 nm when assayed as described below but using 30 µL of *pH 8.4 buffer* instead of 30 µL of the *Standard solutions* or the *Sample solutions*. [NOTE—*Factor Xa solution* contains about 3 nanokatalytic units/mL, but can vary depending upon the manufacturer of factor Xa or the substrate used.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) specific for factor Xa in water to obtain a concentration of 1 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least 5 dilutions in the concentration range between 0.03 and 0.375 USP Heparin Units/mL.

Sample solutions: Dissolve or dilute an accurately measured quantity of Heparin Sodium in *pH 8.4 buffer*, and dilute with the same buffer to obtain solutions having activities approximately equal to those of the *Standard solutions*.

Analysis

NOTE—The procedure can also be performed using alternative platforms. Perform the test with each *Standard solution* and *Sample solution* in duplicate.

To each of a series of suitable plastic tubes placed in a water bath set at 37°, transfer 120 µL of *pH 8.4 buffer*. Then separately transfer 30 µL of the different dilutions of the *Standard solutions* or the *Sample solutions* to the tubes. Add 150 µL of *Antithrombin solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Factor Xa solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Chromogenic substrate solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for exactly 2 min. Add 150 µL of *Stopping solution* to each tube, and mix. Prepare a blank for zeroing the spectrophotometer by adding the reagents in reverse order, starting with the *Stopping solution* and ending with the addition of 150 µL of *pH 8.4 buffer*, and excluding the *Standard solutions* or the *Sample solutions*. Record the absorbance at 405 nm against the blank.

Calculations: Plot the log of the absorbance values of the *Standard solutions* and the *Sample solutions* versus heparin concentrations in USP Units. Calculate the activity of Heparin Sodium in USP Units/mg using statistical methods for slope ratio assays. Calculate the anti-factor Xa activity of Heparin Sodium by the formula:

$$A \times (S_T/S_S)$$

A = the potency of USP Heparin Sodium for Assays RS

S_T = slope of the line for the *Sample solutions*

S_S = slope of the line for the *Standard solutions*

Express the anti-factor Xa activity of the *Sample solution* as USP Heparin Units/mg, calculated on the dried basis. Calculate the ratio of anti-factor Xa activity against anti-factor IIa potency (see *Assay* below) by the formula:

anti-factor Xa activity/anti-factor IIa potency

Acceptance criteria: NLT 0.9 and NMT 1.1

• **D. Identification Tests— General**, *Sodium* ⟨191⟩**:** It meets the requirements of the flame test for sodium.

ASSAY

• **Anti-Factor IIa Potency**

pH 8.4 buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Interim Revision Announcement

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates to be tested: for example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just prior to use.

Two different types of measurements can be recorded:

1. Endpoint Measurement: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). The RSD over the blank readings is less than 10%.
2. Kinetic Measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values as they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

Acceptance criteria: The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

INORGANIC IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** Between 28.0% and 41.0%

• **NITROGEN DETERMINATION**, *Method I, Nitrates and Nitrites Absent* ⟨461⟩*:* Between 1.3% and 2.5%, calculated on the dried basis

• **HEAVY METALS**, *Method II* ⟨231⟩*:* NMT 30 ppm

ORGANIC IMPURITIES

• **LIMIT OF GALACTOSAMINE IN TOTAL HEXOSAMINE** (a measure of dermatan sulfate and other galactosamine containing impurities)

Mobile phase: 14 mM potassium hydroxide

Glucosamine standard solution: 1.6 mg/mL of USP Glucosamine Hydrochloride RS in 5 N hydrochloric acid

Galactosamine standard solution: 16 µg/mL of USP Galactosamine Hydrochloride RS in 5 N hydrochloric acid

Standard solution: Mix equal volumes of *Glucosamine standard solution* and *Galactosamine standard solution*.

Hydrolyzed standard solution: Transfer 5 mL of the *Standard solution* to a 7-mL screw-cap test tube, cap, and heat for 6 h at 100°. Cool to room temperature, quantitatively transfer the solution to a 500-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 12 mg of Heparin Sodium to a 7-mL screw-cap test tube, dissolve in 5 mL of 5 N hydrochloric acid, and cap.

Hydrolyzed sample solution: Heat the *Sample solution* for 6 h at 100°. Cool to room temperature and dilute with water (1 in 100).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*)

Mode: HPIC

Detector: Pulsed amperometric detector, set to the following waveform:

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.1	—
2	0.20	+0.1	Begins
3	0.40	+0.1	Ends
4	0.41	–2.0	—
5	0.42	–2.0	—
6	0.43	+0.6	—
7	0.44	–0.1	—
8	0.50	–0.1	—

Column: 3-mm × 30-mm amino acid trap column in series with a 3- × 30-mm guard column and a 3-mm × 15-cm column that contains packing L69

Column temperature: Maintain columns at 30°

Flow rate: 0.5 mL/min

Pre-equilibration: At least 60 min with *Mobile phase*

Injection size: 10 µL

Elution: 10 min with *Mobile phase*

Column cleaning: At least 10 min with 100 mM potassium hydroxide

Equilibration: At least 10 min with *Mobile phase* before each injection

System suitability

Sample: *Hydrolyzed standard solution*

Suitability requirements

Resolution: NLT 2 between the galactosamine and glucosamine peaks

Column efficiency: NLT 2000 theoretical plates for glucosamine

Tailing factor: Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

Analysis

Samples: *Hydrolyzed standard solution* and *Hydrolyzed sample solution*

Record the chromatograms, and measure the responses for the peaks at the retention time of galactosamine and glucosamine. Calculate the response ratio of galactosamine to glucosamine ($GalN_R$) in the *Hydrolyzed standard solution*:

$$(GalN_B/GalN_W) \times (GlcN_W/GlcN_B)$$

$GalN_B$ = galactosamine peak area from the *Hydrolyzed standard solution*
$GalN_W$ = weight of galactosamine for the *Standard solution*
$GlcN_B$ = glucosamine peak area from the *Hydrolyzed standard solution*
$GluN_W$ = weight of glucosamine for the *Standard solution*
Calculate the percentage of galactosamine in the portion of total hexosamine taken:

$$\{[(GalN_U/GalN_R)]/[(GalN_U/GalN_R) + GlcN_U]\} \times 100$$

$GalN_U$ = galactosamine peak area from the *Hydrolyzed sample solution*
$GalN_R$ = galactosamine response ratio
$GlcN_U$ = glucosamine peak area from the *Hydrolyzed sample solution*

Acceptance criteria: The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

• **NUCLEOTIDIC IMPURITIES:** *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩*, Method I* with the following modifications

Analysis: Dissolve 40 mg of Heparin Sodium in 10 mL of water. Measure the absorbance of this solution at 260 nm using the light scattering correction procedure of *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩*, Method I*.

Acceptance criteria: The absorbance of this solution at 260 nm is NMT 0.20.

• **ABSENCE OF OVERSULFATED CHONDROITIN SULFATE**

A: Proceed as directed in *Identification* test *A*. No features associated with oversulfated chondroitin sulfate are found between 2.12 and 3.00 ppm.

B: Proceed as directed in *Identification* test *B*. No peaks corresponding to oversulfated chondroitin sulfate should be detected eluting after the heparin peak.

• **PROTEIN IMPURITIES**

Standard stock solution: 0.100 mg/mL of bovine serum albumin in water

Standard solutions: Dilute portions of the *Standard stock solution* with water to obtain NLT 5 standard solutions having concentrations between 0.005 and 0.100 mg/mL of bovine serum albumin, the concentrations being evenly spaced.

Sample solution: 5 mg/mL of Heparin Sodium in water. Prepare in triplicate.

Blank: Water

Lowry reagent A: Prepare a solution of 10 g/L of sodium hydroxide in water and a solution of 50 g/L of sodium carbonate in water. Mix equal volumes (2V:2V) of each solution, and dilute with water to 5V.

Lowry reagent B: Prepare a solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate in water. Mix equal volumes of both solutions (2V:2V), and dilute with water to 5V.

Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*.

Diluted Folin-Ciocalteu's phenol reagent: Dilute Folin-Ciocalteu's phenol reagent 2–4 times with water. The dilution should be chosen such that the pH of the samples (i.e., *Standard solution* and *Sample solution* after addition of *Lowry reagent C* and the *Diluted Folin-Ciocalteu's phenol reagent*) is 10.25 ± 0.25.

Analysis

Samples: *Standard solutions, Sample solution,* and *Blank*

To 1 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 5 mL of *Lowry reagent C*. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin-Ciocalteu's phenol reagent* to each solution, mix immediately, and allow to stand at room temperature for 30 min. Determine the absorbance as directed in *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩*, Method 2*.

Calculations: See *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩*, Method 2*.

Acceptance criteria: NMT 1.0% (w/w) is found.

• **RESIDUAL SOLVENTS** ⟨467⟩**:** It meets the requirements.

SPECIFIC TESTS

• **pH** ⟨791⟩*:* 5.0–7.5, (1 in 100) solution

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩*:* NMT 0.03 USP Endotoxin Unit/USP Heparin Unit

• **STERILITY TESTS** ⟨71⟩*:* Where it is labeled as sterile, it meets the requirements.

• **LOSS ON DRYING** ⟨731⟩*:* Dry under vacuum at 60° for 3 h. It loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE***:* Preserve in tight containers, and store below 40°, preferably at room temperature.

• **LABELING***:* Label it to indicate the tissue and the animal species from which it is derived.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Oversulfated Chondroitin Sulfate RS
USP Dermatan Sulfate RS
USP Endotoxin RS
USP Galactosamine Hydrochloride RS
USP Glucosamine Hydrochloride RS
USP Heparin Sodium for Assays RS
USP Heparin Sodium Identification RS•5

Change to read:

Heparin Sodium Injection

•DEFINITION

Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits a potency NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

ASSAY

• **ANTI-FACTOR IIa POTENCY**

NOTE—Allow alternative platforms.

pH 8.4 Buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 Buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagents Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 Buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Interim Revision Announcement

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water and dilute with *pH 8.4 Buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. To each tube add twice the volume (100–200 µL), of *Antithrombin solution* and one volume (50–100 µL), of either the *pH 8.4 Buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just prior to use.

Two different types of measurements can be recorded:

1. ENDPOINT MEASUREMENT: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). The RSD over the blank readings is less than 10%.
2. KINETIC MEASUREMENT: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values as they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Acceptance criteria: NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.03 USP Endotoxin Unit/USP Heparin Unit.

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

• **pH** ⟨791⟩*:* 5.0–7.5

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.

ADDITONAL REQUIREMENTS

• **LABELING:** Label it to indicate the volume of the total contents and the potency in terms of USP Heparin Units only per mL, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, and store at a temperature below 40°, preferably at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Endotoxin RS

USP Heparin Sodium for Assays RS•5

Nefazodone Hydrochloride

Change to read:

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: A solution of 10 mg per mL •in methanol•5 meets the requirements of the test for *Chloride* ⟨191⟩.

Interim Revision Announcement

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

USP 32–NF 27 **Page**	**Title**	**Section**	**Description**
307	⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*	*Table 1*	Column 5 heading: Change ''Recommended USP Sieves (mesh)'' to: Recommended USP Sieves (microns)
817	*Reagent Specifications*	*Decanol*	Line 1: Change ''[25339-17-7]'' to: [112-30-1]
924	*Description and Solubility*	*Polydextrose*	Line 3: Change ''insoluble in alcohol.'' to: soluble in alcohol.
1175	*Betadex*	*Reducing sugars*	Line 3 under *Standard solution*: Change ''1.0 g of anhydrous Betadex.'' to: 1 mL of 10 mg/mL Betadex solution.
1296	*Paraffin*	*Alkalinity*	Line 2: Change ''methyl red TS:'' to: methyl red TS 2:
2466	*Gadodiamide*	*Content of gadolinium*	Line 3 under *Procedure*: Change ''342 nm'' to: 342.3 nm
2963	*Metoprolol Succinate*	*Assay*	Line 6 under *Chromatographic system*: Change ''between metoprolol related compound A and metoprolol related compound B is not less than 1.5; and the resolution, *R,* between metoprolol related compound B and metoprolol related compound C is not less than 2.5.'' to: between metoprolol related compound A and metoprolol related compound B is not less than 2.5; and the resolution, *R,* between metoprolol related compound B and metoprolol related compound C is not less than 1.5.
3001	*Monensin*	*Assay*	Line 1 under *Derivatizing reagent*: Change ''3 g of vanillin in a mixture of methanol and sulfuric acid (95:2).'' to: 3 g of vanillin in a mixture of 95 mL of methanol and 2 mL of sulfuric acid.

USP 32–NF 27			
Page	**Title**	**Section**	**Description**
3113	*Norgestimate*	*Chromatographic purity*	Line 2 under *Test 1*: Change ''*Diluent, Mobile phase, Sensitivity solution,* and *Chromatographic system*—'' to: *Diluent, Mobile phase,* and *Sensitivity solution*— Line 4 under *Chromatographic system*: Change ''1.2 mL per minute. Chromatograph the *Resolution solution,*'' to: 1.2 mL per minute. The column temperature is maintained at about 40°. Chromatograph the *Resolution solution,* Line 8 under *Chromatographic system*: Change ''related compound A, and 1.0 for (*E*)-norgestimate;'' to: related compound A, 0.86 for (*Z*)-norgestimate, and 1.0 for (*E*)-norgestimate; Line 11 under *Chromatographic system*: Change ''is not less than 1.5.'' to: is not less than 1.5; the tailing factor for (*E*)-norgestimate and for (*Z*)-norgestimate is not more than 1.5; and the relative standard deviation for replicate injections, determined from the peak area ratio of (*E*)-norgestimate to (*Z*)-norgestimate, is not more than 2.0%. Chromatograph the *Sensitivity solution*, and record the peak areas as directed for *Procedure*: the signal-to-noise ratio for (*Z*)-norgestimate is not less than 3.0.
3220	*Penicillamine*	*Limit of penicillin activity*	Line 1 under *Standard preparation*: Change ''*Table 2* under *Antibiotics—Microbial Assays* ⟨81⟩'' to: *Table 1* under *Antibiotics—Microbial Assays* ⟨81⟩
First Supplement to USP 32–NF 27			
4066	*Isotretinoin Capsules*	*Chromatographic purity*	Line 4 under *Chromatographic system*: Change ''Chromatograph the *System suitability solution*,'' to: Chromatograph the *System suitability solution* [NOTE—The injection volume is about 20 µL.],
Online	*Estradiol Vaginal Inserts*	*Loss on Drying*	Delete the test for *Loss on drying*, which was incorrectly published in the online version of the *First Supplement to USP 32–NF 27*, and carried forward to the *Second Supplement to USP 32–NF 27*.

Interim Revision Announcement

PROPOSED INTERIM REVISION ANNOUNCEMENTS

This section includes proposals for *Interim Revision Announcements* (*IRAs*) that will be published as official *USP* or *NF* standards. There is a 60-day comment period for these proposals, beginning on the 15th of the first month of this *Pharmacopeial Forum*. The approved official text will be published in a future *Pharmacopeial Forum* and additionally in the ''New Official Text'' section of USP's web site (www.usp.org). Readers should review material in this section and provide comments to the Scientific Liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Expert Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

Acitretin Capsules, *USP 32* page 1426. On the basis of comments received, in the *Assay* it is proposed to revise the *Assay preparation* to state that only the contents of the Capsules will be used, rather than the entire Capsule including the shells. The acitretin concentration of the *Assay preparation* will also be specified. In the absence of any significant adverse comment, it is proposed to implement this revision via an *Interim Revision Announcement* pertaining to *USP 32–NF 27* in *PF* 36(1) [Jan.–Feb. 2010], with an official date of February 1, 2010.

(MD-OOD: F. Mao) RTS—C75260

Change to read:

Assay—

Diluent—Prepare a suitable mixture of methanol and tetrahydrofuran (13 : 10).

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, alcohol, and glacial acetic acid (74 : 21 : 5 : 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—In a 100-mL volumetric flask, dissolve about 10 mg of USP Acitretin RS, accurately weighed, in 80 mL of *Diluent,* and sonicate for 5 minutes. Add 8 mL of water, and quantitatively dilute with *Diluent* to obtain a solution having a concentration of about 0.1 mg per mL.

System suitability solution—Transfer 2 mL of the *Standard preparation* to a clear 4-mL glass vial. After sealing the vial with a teflon-lined silicone septum and cap, place the vial on its side in a light chamber, expose it to 400 foot-candles of fluorescent light for 5 minutes, and then completely wrap the vial with aluminum foil. [NOTE—Exposure to the fluorescent light allows for the formation of two degradation products: acitretin related compound A and the 9-*cis* isomer [*(E,E,Z,E)*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid].]

Assay preparation—~~Carefully separate and place both halves of 10 Capsules into a 100 mL volumetric flask. Stopper and shake the flask to remove the fill. Add 8 mL of water while rinsing any fill from the neck of the flask. Place the flask in a water bath set at 45° for 10 minutes, shaking initially and at 5-minute intervals up to 10 minutes. Place the resulting suspension in an ultrasonic bath for 15 minutes. Dilute with *Diluent* to volume, and sonicate for 5 additional minutes. Cool to room temperature and, if necessary, dilute with *Diluent* to volume. Filter the suspension, and use the clear filtrate.~~

•Open not fewer than 20 Capsules, composite the Capsule fill, and mix well. Transfer the Capsule fill, equivalent to 10 mg of acitretin, into a 100-mL volumetric flask. Add 8 mL of water to wet the sample, and sonicate for 5 minutes. Dilute with *Diluent* to volume, and sonicate for 5 minutes. Cool to room temperature. Pass the suspension through a suitable filter having a porosity of 0.5 μm, and use the clear filtrate. [NOTE—Inject the *Sample solution* within an hour of preparation.]•1

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm × 15-cm column that contains 5-μm L1 packing. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between acitretin related compound A (relative retention time of about 0.84) and acitretin is not less than 3.0; the resolution, *R,* between the 9-*cis* isomer (relative retention time of about 1.09) and acitretin is not less than 1.8. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections of acitretin is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of acitretin ($C_{21}H_{26}O_3$) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acitretin RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

BRIEFING

Docusate Sodium, *USP 32* page 2195. Comments were received indicating that the ignition temperature of 600 ± 50° specified in the harmonized general test chapter *Residue on Ignition* ⟨281⟩ is not sufficient to effectively ash the material. It is proposed to specify that the test for *Residue on ignition* should be performed at 800 ± 25°, which is also consistent with the specifications in the *Food Chemicals Codex*.

Subject to consideration of comments received, it is proposed to implement this change via an *Interim Revision Announcement* pertaining to *USP 33–NF 28* in *PF* 36(1) [Jan.–Feb. 2010], with an official date of February 1, 2010. Comments regarding this proposal should be received by Nov. 15, 2009.

(MD-GRE: E. Gonikberg) RTS—C77132

Change to read:

Residue on ignition ⟨281⟩: between 15.5% and 16.5%, calculated on the anhydrous basis.

•*Procedure:* Transfer about 1 g, accurately weighed, to a tared crucible, ignite until thoroughly charred, and cool. Moisten the ash with 1 mL of sulfuric acid, and complete the ignition by heating at 800 ± 25° for 15-minute periods to constant weight.•1

BRIEFING

Propranolol Hydrochloride Extended-Release Capsules, *USP 32* page 3426. It is proposed to clarify how to measure time when running *Dissolution Test 1* and *Test 2*. It is proposed to add a *Dissolution Test 3* for a new generic product. In the absence of negative comments, it is proposed to implement these revisions through an *Interim Revision Announcement* with an official date of February 1, 2010.

(BPC: M. Marques.) RTS—C55653

Change to read:

Dissolution ⟨711⟩**—**

TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

pH 1.2 Buffer solution—Dissolve 2.0 g of sodium chloride in water, add 7.0 mL of hydrochloric acid, dilute with water to 1 L, and mix.

pH 6.8 Buffer solution—Dissolve 21.72 g of anhydrous dibasic sodium phosphate and 4.94 g of citric acid monohydrate in water, dilute with water to 1 L, and mix.

Media—Proceed as directed under *Method B* for *Delayed-Release Dosage Forms,* using 900 mL of *pH 1.2 Buffer solution* during the *Acid stage,* ~~run for 1.5 hours, and use the acceptance criteria given under *Acceptance Table 3*~~

•and conduct the test for 1.5 hours.•1
For the *Buffer stage*, use 900 mL of *pH 6.8 Buffer solution*, ~~run for the time specified~~

•conduct the test for 2.5 hours (this is the 4-hour time point: 1.5 hours in *Acid stage* plus 2.5 hours in *Buffer stage*), conduct the test for the additional time points, always considering T_1 = 1.5 hours,•1
and use the acceptance criteria given under *Tolerances*.

Apparatus 1: 100 rpm.

Times: 1.5, 4, 8, 14, and 24 hours.

Procedure—Using filtered portions of the solution under test, diluted if necessary, determine the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved, using UV absorbances at the wavelength of maximum absorbance at about 320 nm, with respect to a baseline drawn from 355 nm through 340 nm, by comparison with a Standard solution in water having a known concentration of USP Propranolol Hydrochloride RS.

Tolerances—The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.5	not more than 30%
4	between 35% and 60%
8	between 55% and 80%
14	between 70% and 95%
24	between 81% and 110%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

pH 1.2 Buffer solution—Dissolve 2.0 g of sodium chloride in water, add 7.0 mL of hydrochloric acid, dilute with water to 1 L, and mix.

pH 7.5 Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.6 g of sodium hydroxide in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.5, dilute with water to 1 L, and mix.

Media—Proceed as directed under *Method B* for *Delayed-Release Dosage Forms,* using 900 mL of *pH 1.2 Buffer solution* during the *Acid stage,* ~~run for 1 hour, and use the acceptance criteria given under *Acceptance Table 3*~~

•and conduct the test for 1 hour.•1
For the *Buffer stage*, use 900 mL of *pH 7.5 Buffer solution*, ~~run for the time specified~~

•conduct the test for 2 hours (this is the 3-hour time point: 1 hour in *Acid stage* plus 2 hours in *Buffer stage*), conduct the test for the additional time points, always considering T_1 = 1 hour,•1
and use the acceptance criteria given under *Tolerances.*

Apparatus 1: 50 rpm.

Times: 1, 3, 6, and 12 hours.

Procedure—Using filtered portions of the solution under test, diluted if necessary, determine the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved, using UV absorbances at the wavelength of maximum absorbance at about 320 nm, with respect to a baseline drawn from 355 nm through 340 nm, by comparison with a Standard solution in water having a known concentration of USP Propranolol Hydrochloride RS.

Tolerances—The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	not more than 20%
3	between 20% and 45%
6	between 45% and 80%
12	not less than 80%

•TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Acid stage medium: pH 1.2 buffer solution (prepared by dissolving 2.0 g of sodium chloride in water, adding 7.0 mL of hydrochloric acid, and diluting with water to 1000 mL); 900 mL.

Buffer stage medium: pH 6.8 phosphate buffer; 900 mL.

Apparatus 1: 100 rpm.

Standard stock solution: Transfer 50 mg, accurately weighed, of USP Propranolol Hydrochloride RS to a 50-mL volumetric flask, and dilute with water to volume.

Working standard solution—Quantitatively dilute the *Standard stock solution* with water to obtain a final concentration of about L/1000 mg per mL, where L is the capsule label claim in mg.

Procedure—Conduct the test in *Acid stage medium* for 1.5 hours, sample, and pass through a suitable 0.45-μm filter. Replace the *Acid stage medium* with the *Buffer stage*

Proposed IRA

medium, and conduct the test for 2.5 hours (this is the 4-hour time point: 1.5 hours in *Acid stage medium* plus 2.5 hours in *Buffer stage medium*), conduct the test for the additional time points, always considering T_1 = 1.5 hours, and use the acceptance criteria given under *Tolerances*.

Determine the percentage of propanolol hydrochloride dissolved using the spectrophotometric procedure as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.5	not more than 15%
4	not more than 30%
8	between 25% and 60%
14	between 55% and 85%
24	not less than 75%

●1

Proposed IRA

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision,* or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

Name of Item, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:

(DSI: D. Sarma.) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. Because of the redesign of monographs, any proposed new text with revisions for *USP 33–NF 28* and beyond will be set off from the current official text by shading where the symbols surround the text changes. Standards that become official as *Interim Revision Announcements (IRAs)* in *Pharmacopeial Forum* will continue to identify changed text in a larger font (print edition only). All *USP–NF* revisions use the following symbols that indicate the final destination of the official text: •new text• if slated for an *IRA*; ▲new text▲ if slated for *USP–NF*; and ■new text■ if slated for a *Supplement* to *USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as ••, or ■■, or ▲▲, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (*USP 32*) indicates that the proposed revision is slated for the *Second Supplement to USP 32*, and ▲*USP33* and ▲*NF28* indicates that the revisions are proposed for *USP 33* and *NF 28*, respectively.

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

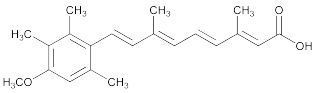
USP MONOGRAPHS

BRIEFING

Acitretin, *USP 32* page 1425. On the basis of comments received, it is proposed to revise the *System suitability* criteria in the *Assay*. The relative standard deviation is calculated on the basis of the acitretin peak from the *Standard solution* to make it consistent with the system suitability requirement in the monograph submission. It is also proposed to revise the test for *Heavy Metals* by replacing chapter ⟨231⟩, *Method I*, with *Method II*, because acitretin yields a yellow solution under the test condition.

(MD-OOD: F. Mao.) RTS—C71343

Acitretin



$C_{21}H_{26}O_3$ 326.43

2,4,6,8-Nonatetraenoic acid, 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-, (*all-E*)-;
(*all-E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid [55079-83-9].

DEFINITION

Acitretin contains NLT 98.0% and NMT 102.0% of $C_{21}H_{26}O_3$, calculated on the dried basis.

[**CAUTION—**Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

[NOTE—Use low-actinic glassware, and perform all tests under yellow and subdued light.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Alcohol, glacial acetic acid, and water (92:0.3:8)

System suitability solution: In a 200-mL volumetric flask dissolve 2.0 mg each of USP Acitretin RS and USP Tretinoin RS in tetrahydrofuran. Dilute with alcohol to volume. Pipet 5.0 mL of this solution into a 200-mL volumetric flask, and dilute quantitatively with alcohol. [NOTE—Store the solution at 4° before injection.]

Standard solution: 0.1 mg/mL of USP Acitretin RS in alcohol. [NOTE—Use tetrahydrofuran to dissolve USP Acitretin RS before diluting with alcohol. The final concentration of tetrahydrofuran in the preparation will be 2%. Store the solution at 4° before injection.]

Sample stock solution: 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

Sample solution: 0.1 mg/mL of Acitretin from *Sample stock solution* in alcohol. [NOTE—Store the solution at 4° before injection.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 0.6 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* ■and *Standard solution*■2S (USP33)

[NOTE—The relative retention times for tretinoin and acitretin are 0.84 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between tretinoin and acitretin, ■*System suitability solution*■2S (USP33)

Relative standard deviation: NMT 1.0% of acitretin, ■*Standard solution*■2S (USP33)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{26}O_3$ in the portion of Acitretin taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)
C_U = concentration of Acitretin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method ~~I~~*■*II*■2S (USP33) ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.8 µg/mL each of USP Acitretin RS, USP Acitretin Related Compound A RS, and USP Acitretin Related Compound B RS in tetrahydrofuran. Dilute quantitatively with alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol. Store the solution at 4° before injection.]

Sample solution: 0.25 mg/mL of Acitretin in tetrahydrofuran, and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol. Store the solution at 4° before injection.]

System suitability

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Sample: *Standard solution*

[NOTE—The relative retention times for acitretin related compound A, acitretin, and acitretin related compound B are 0.78, 1.0, and 1.61, respectively.]

Suitability requirements

Resolution: NLT 1.5 between acitretin related compound A and acitretin; NLT 1.5 between acitretin related compound B and acitretin

Relative standard deviation: NMT 10.0% for acitretin related compound A and NMT 10.0% for acitretin related compound B

In-Process Revision

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acitretin related compound A and acitretin related compound B in the portion of Acitretin taken:

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

r_U = peak response of the relevant impurity from the *Sample solution*
r_S = peak response of the relevant impurity from the *Standard solution*
C_S = concentration of USP Acitretin Related Compound A RS or USP Acitretin Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Acitretin in the *Sample solution* (µg/mL)

Calculate the percentage of impurities other than acitretin related compounds A and B in the portion of Acitretin taken:

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

r_U = peak response of each individual unspecified impurity in the *Sample solution*
r_S = peak response of USP Acitretin RS in the *Standard solution*
C_S = concentration of USP Acitretin RS in the *Standard solution* (µg/mL)
C_U = concentration of Acitretin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.3% of acitretin related compound A; NMT 0.3% of acitretin related compound B; NMT 0.1% of any individual unspecified impurity; and NMT 0.4% of total unspecified impurities

Total impurities: NMT 1.0%

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry a sample in a vacuum at a pressure not exceeding 19 mm of mercury at 100° for 4 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Acitretin RS
  USP Acitretin Related Compound A RS
  USP Acitretin Related Compound B RS
  USP Tretinoin RS

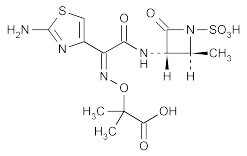
BRIEFING

Aztreonam, *USP 32* page 1616. On the basis of comments received, the following revisions are proposed:

- The *Definition* is revised to provide one set of acceptance criteria for both polymorphic forms.
- The *Assay* is revised to update the *Mobile phase*, the *System suitability solution*, the analytical wavelength, column dimensions, and system suitability based on a validated method. The format of the relative retention times is updated to follow current chromatographic conventions. Solution storage conditions are added to prevent isomerization of aztreonam *Z*-isomer to aztreonam *E*-isomer.
- A test for *Organic Impurites* is added to the monograph based on a validated method. The liquid chromatographic procedure in this test and in the *Assay* is based on analyses performed using a Waters µBondapak C18 brand of L1 column. The typical retention time for aztreonam under the conditions specified is 6 min.
- A test for *Limit of Alcohol* is added to the monograph based on a validated method. This is a test for residual alcohol. The gas chromatographic procedure in this test is based on analyses performed using a Supelco OVI-G43 brand of G43 column. The typical retention time for alcohol under the conditions specified is 3.5 min.

(MD-ANT: A. Wise.) RTS—C60576

Aztreonam



$C_{13}H_{17}N_5O_8S_2$ 435.43

Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methyl-, [*2S*-[2α,3*β*(*Z*)]]-;
(*Z*)-2-[[[(2-Amino-4-thiazolyl)[[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid [78110-38-0].

DEFINITION

Change to read:

~~Aztreonam is anhydrous or hydrated. The anhydrous form contains NLT 90.0% and NMT 105.0% of $C_{13}H_{17}N_5O_8S_2$, calculated on the as-is basis. The hydrated form contains NLT 92.0% and NMT 105.0% of $C_{13}H_{17}N_5O_8S_2$, calculated on the anhydrous basis.~~

■Aztreonam, which may be anhydrous or hydrated, contains NLT 92.0% and NMT 105.0% of $C_{13}H_{17}N_5O_8S_2$, calculated on the anhydrous and solvent-free basis.■2S (*USP33*)

IDENTIFICATION

- **INFRARED ABSORPTION ⟨197K⟩:** If a difference appears in the IR spectra of the analyte and the standard, dissolve an equal portion of the test specimen and the Reference Standard in equal volumes of methanol. [NOTE—To achieve a complete dissolution, it is suggested to use about 25 mL of methanol for each 50 mg of material, and stir the mixture for 40 min at room temperature.]
  Evaporate the solutions to dryness under vacuum. Dry under vacuum at 40° for 4 h. Perform the test on the residues.

In-Process Revision

ASSAY

Change to read:

• **PROCEDURE**

■[NOTE—Store the *Standard solution*, *System suitability solution*, and the *Sample solution* at 5° and protect from light to prevent isomerization of aztreonam *Z*-isomer to aztreonam *E*-isomer.]■2S *(USP33)*

Buffer: 6.8 mg/mL of monobasic potassium phosphate in water; adjust with 1 M phosphoric acid to a pH of ~~3.0 ± 0.1~~■ 3.0■2S *(USP33)*

Mobile phase: Methanol and *Buffer* (1:4)

Standard solution: 1 mg/mL of USP Aztreonam RS in *Mobile phase*

System suitability solution: ~~0.2 mg/mL~~■1 mg/mL■2S *(USP33)* of USP Aztreonam RS and ~~0.2 mg/mL~~■1 mg/mL■2S *(USP33)* of USP Aztreonam *E*-Isomer RS in *Mobile phase*

Sample solution: 1 mg/mL of Aztreonam in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV ~~270 nm~~■254 nm■2S *(USP33)*

Column

Guard: 2-mm × 10-cm; packing L2

Analytical: ~~4.6-mm~~ ■3.9-mm■2S *(USP33)* × 30-cm; ■10-µm■2S *(USP33)* packing L1

Flow rate: 1.5 mL/min

Injection size: ~~20 µL~~■10 µL■2S *(USP33)*

System suitability

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

[NOTE—The relative retention times for aztreonam and aztreonam *E*-isomer are ~~0.6 and 1.0~~■1.0 and 1.8,■2S *(USP33)* respectively.]

Suitability requirements

Resolution: NLT 2.0 between aztreonam and aztreonam *E*-isomer, *System suitability solution*

Tailing factor: NMT ~~2.0~~■2■2S *(USP33)* for aztreonam, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{13}H_{17}N_5O_8S_2$ in the portion of Aztreonam taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
P = potency of USP Aztreonam RS (µg/mg)
F = unit conversion factor (0.001 mg/µg)

Acceptance criteria: ~~90.0%–105.0%, anhydrous form, on the as-is basis; 92.0%–105.0%, hydrated form, on the anhydrous basis~~■92.0%–105.0% on the anhydrous and solvent-free basis■2S *(USP33)*

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm

Add the following:

■**Organic impurities**

• **PROCEDURE**

[NOTE—Store the *Standard solution*, *System suitability solution*, and the *Sample solution* at 5° and protect from light to prevent isomerization of aztreonam *Z*-isomer to aztreonam *E*-isomer.]

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Aztreonam taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of aztreonam from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
P = potency of USP Aztreonam RS (µg/mg)
F = unit conversion factor (0.001 mg/µg)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 3.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Open-ring aztreonam[a] and open-ring desulfated aztreonam[b,c]	0.55	1.0
Aztreonam (*Z*-isomer)	1.0	—
Desulfated aztreonam[d]	1.6	1.5
Aztreonam *E*-isomer[e]	1.8	0.5
Aztreonam ethyl ester[f]	3.9	1.5
Any individual unspecified impurity	—	0.1

[a] (2*S*,3*S*)-2-{(*Z*)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido}-3-(sulfoamino)butanoic acid.

[b] (2*S*,3*S*)-3-Amino-2-{(*Z*)-2-[2-aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido}butanoic acid.

[c] Open-ring aztreonam and open-ring desulfated aztreonam coelute. The limit is for the sum of these two impurities.

[d] (*Z*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionic acid.

[e] (*E*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionic acid.

[f] Ethyl (*Z*)-2-({[(2-amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionate.

■2S *(USP33)*

In-Process Revision

SPECIFIC TESTS

- **STERILITY TESTS ⟨71⟩:** Where the label states that Aztreonam is sterile, it meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration,* using *Fluid A,* to which 23.4 g of sterile arginine has been added to each 1000 mL.
- **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 2.0%; if labeled as the hydrated form: 12.0%–18.0%
  [NOTE—The term *hydrated form* refers to the α-form of Aztreonam, which is not a stoichiometric hydrate.]
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** Where the label states that aztreonam is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.

Add the following:

■• **LIMIT OF ALCOHOL**
[NOTE—This test is to be performed if alcohol is used while manufacturing Aztreonam.]
Standard solution: 0.004 mL/mL of alcohol from USP Alcohol Determination—Alcohol RS and 0.004 mL/mL of acetonitrile from USP Alcohol Determination—Acetonitrile RS in dimethylformamide. [NOTE—The *Standard solution* contains 0.4% v/v alcohol and 0.4% v/v acetonitrile.]
Sample solution: 80 mg/mL of Aztreonam and 0.004 mL/mL of acetonitrile in dimethylformamide. [NOTE—Dissolve Aztreonam in dimethylformamide using 20% of the final volume, add a suitable aliquot of USP Alcohol Determination—Acetonitrile RS and dilute with dimethylformamide to volume. The concentration of acetonitrile in the *Sample solution* is 0.4% v/v.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; phase G43
Film thickness: 3.0-µm
Temperature
Injector: 210°
Detector: 280°
Column: See the temperature program table.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	200	4

Carrier gas: He
Linear velocity: 35 cm/s
Injection mode: Split
Split ratio: 5:1
Injection size: 0.5 µL
System suitability
[NOTE—The relative retention times for alcohol and acetonitrile are 1.0 and 1.3, respectively.]
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between alcohol and acetonitrile
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Aztreonam taken:

$$\text{Result} = (R_U/R_S) \times (C_S \times D/C_U) \times F \times 100$$

R_U = peak response ratio of alcohol to acetonitrile from the *Sample solution*
R_S = peak response ratio of alcohol to acetonitrile from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mL/mL)
D = density of alcohol (g/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
F = unit conversion factor (1000 mg/g)
Acceptance criteria: NMT 4%■2S (*USP33*)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is the hydrated form, the label so indicates.

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
■USP Alcohol Determination—Acetonitrile RS
USP Alcohol Determination—Alcohol RS■2S (*USP33*)
USP Aztreonam RS
USP Aztreonam *E*-Isomer RS
USP Endotoxin RS

BRIEFING

Soluble Bacitracin Methylene Disalicylate, *USP 32* page 1622. On the basis of comments received, it is proposed to change the title of this monograph in order to provide the chemically correct name of the methylenedisalicylate derivative.

(NOM: A. Wilk.) RTS—C72264

Change to read:

Soluble Bacitracin ~~Methylene Disalicylate~~ ■ Methylenedisalicylate■2S (*USP33*)

DEFINITION

Change to read:

Soluble Bacitracin ~~Methylene Disalicylate~~■Methylenedisalicylate■2S (*USP33*) is a mixture of ~~Bacitracin Methylene Disalicylate~~ ■Bacitracin Methylenedisalicylate■2S (*USP33*) and Sodium Bicarbonate. It has a potency of NLT 8 Bacitracin Units/mg, calculated on the dried basis.

ASSAY

- **ANTIBIOTICS—MICROBIAL ASSAYS ⟨81⟩**

Sample stock solution: Transfer a sample to a high-speed glass blender jar, add 99.0 mL of a 20 mg/mL sodium bicarbonate solution and 1.0 mL of polysorbate 80, and blend for 3 min. Add a sufficient volume of 0.01 N hydrochloric acid so that the amount of hydrochloric acid will be the same as in the median dose level of the Standard.

Sample solution: Dilute the *Sample stock solution* with *Buffer No. 1* (see *Phosphate Buffers and Other Solutions*) to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard.

Analysis: Proceed as directed for *Bacitracin* in *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: NLT 8 Bacitracin Units/mg on the dried basis

SPECIFIC TESTS

- **pH ⟨791⟩:** 8.0–9.5, in a solution of 25 mg/mL
- **LOSS ON DRYING ⟨731⟩:** Dry 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Bacitracin Zinc RS

BRIEFING

Bacitracin Methylene Disalicylate Soluble Powder, *USP 32* page 1622. On the basis of the comments received, the title of the monograph is being changed in order to provide the chemically correct name of the methylenedisalicylate derivative.

(NOM: A. Wilk.) RTS—C72264

Change to read:

~~Bacitracin Methylene Disalicylate Soluble Powder~~■Bacitracin Methylenedisalicylate Soluble Powder■2S (*USP33*)

DEFINITION

Change to read:

~~Bacitracin Methylene Disalicylate Soluble Powder~~■Bacitracin Methylenedisalicylate Soluble Powder■2S (*USP33*) contains NLT 90.0% and NMT 120.0% of the labeled amount of bacitracin.

ASSAY

- **ANTIBIOTICS—MICROBIAL ASSAYS ⟨81⟩**

Sample stock solution: Transfer a sample to a high-speed glass blender jar. Add 99.0 mL of sodium bicarbonate solution (1 in 50) and 1.0 mL of polysorbate 80, and blend for about 3 min. Add a sufficient volume of 0.01 N hydrochloric acid to a measured volume of *Sample solution* so that the amount of hydrochloric acid will be the same as in the median dose level of the Standard.

Sample solution: Dilute the *Sample stock solution* with *Buffer No. 1* (see *Phosphate Buffers and Other Solutions*) to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard.

SPECIFIC TESTS

- **pH ⟨791⟩:** 8.0–9.5, 50 mg/mL
- **LOSS ON DRYING ⟨731⟩:** Dry 100 mg in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of g/lb, each g of bacitracin being equivalent to 42,000 Bacitracin Units.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Bacitracin Zinc RS

BRIEFING

Bendroflumethiazide Tablets, *USP 32* page 1637. Because of the low solubility of bendroflumethiazide in aqueous solvents, instructions are being added in the *Dissolution* test on how to prepare the *Standard solution*.

(BPC: M. Marques..) RTS—75670

Bendroflumethiazide Tablets

DEFINITION

Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

- **PROCEDURE**[NOTE—Use low-actinic glassware for the *Sample solution* and the *Standard solution*.]

Mobile phase: Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

Standard solution: 50 µg/mL of USP Bendroflumethiazide RS in methanol

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to about 5 mg of bendroflumethiazide, to a 100-mL volumetric flask. Add about 70 mL of methanol, and sonicate for 15 min, with occasional shaking. Dilute with methanol to volume, mix, and centrifuge a portion of the solution for 15 min.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 30-cm; packing L11
Temperature: 35 ± 5°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0% for five replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{14}F_3N_3O_4S_2$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bendroflumethiazide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of bendroflumethiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩
[NOTE—Protect solutions from light throughout this test.]

Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV 271 nm
Sample solution: Sample per *Dissolution* ⟨711⟩.
Standard solution: ~~USP Bendroflumethiazide RS in *Medium*~~ ■Prepare a stock solution of USP Bendroflumethiazide RS in an appropriate organic solvent, and dilute this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.■2S *(USP33)*
Analysis: Determine the amount of $C_{15}H_{14}F_3N_3O_4S_2$ dissolved by using UV absorption on filtered portions of the *Sample solution*, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Bendroflumethiazide RS.
Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{14}F_3N_3O_4S_2$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

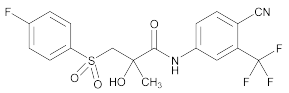
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Bendroflumethiazide RS

BRIEFING

Bicalutamide, *USP 32* page 1673. On the basis of comments received, it is proposed to delete the acceptance criteria for *Total unspecified impurities* in the test for *Organic Impurities* to be consistent with the ICH guideline. It is also proposed to add the relative retention times for bicalutamide and bicalutamide related compound B, and the run time in the test for *Organic Impurities* for clarification.

(MD-OOD: F. Mao.) RTS—C70497

Bicalutamide



$C_{18}H_{14}F_4N_2O_4S$ 430.37
Propanamide, *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-,(±)-;
(±)-4′-Cyano-α,α,α-trifluoro-3-[(*p*-fluorophenyl)sulfonyl]-2-methyl-*m*-lactotoluidide [90357-06-5].

DEFINITION
Bicalutamide contains NLT 98.0% and NMT 102.0% of $C_{18}H_{14}F_4N_2O_4S$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197M**⟩
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (6:3:11)
Standard solution: 0.05 mg/mL of USP Bicalutamide RS in a minimum amount of tetrahydrofuran, and diluted with *Mobile phase*
Sample solution: 0.05 mg/mL of Bicalutamide in a minimum amount of tetrahydrofuran, and diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 5-mm × 25-cm; 5-µm packing L1
Temperature: 35°–40°
Flow rate: 1.8 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{14}F_4N_2O_4S$ in the portion of Bicalutamide taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

In-Process Revision

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

Change to read:

Organic Impurities

- **PROCEDURE**

Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 0.05 mg/mL of USP Bicalutamide RS and 0.02 mg/mL of USP Bicalutamide Related Compound B RS in a minimum amount of tetrahydrofuran, and dilute with *Mobile phase*

Standard solution: 0.02 mg/mL of USP Bicalutamide RS in a minimum amount of tetrahydrofuran, and dilute with *Mobile phase*

Sample solution: 4.0 mg/mL of Bicalutamide in a minimum amount of tetrahydrofuran, and dilute with *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 5-mm × 25-cm; 5-µm packing L1

Temperature: 35°–40°

Flow rate: 1.8 mL/min

Injection size: 10 µL

■**Run time:** NLT 3 times the retention time for bicalutamide■2S (*USP33*)

System suitability

Sample: *System suitability solution*

■[NOTE—The relative retention times for bicalutamide and bicalutamide related compound B are 1.00 and 1.12, respectively.]■2S (*USP33*)

Suitability requirements

Resolution: NLT 2.0 between bicalutamide and bicalutamide related compound B

Tailing factor: Less than 1.3 for bicalutamide

Relative standard deviation: NMT 4% for bicalutamide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bicalutamide taken:

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

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for bicalutamide from the *Standard solution*

C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)

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

Acceptance criteria

Individual impurities: See *Impurity Table*.

Total impurities: NMT 0.5%

Impurity Table

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Des-fluoro analog[1]	0.74	0.2
2-Fluoro isomer[2]	0.78	0.2
Des hydroxy analog[3]	1.19	0.2
Individual unspecified impurity	—	0.1
~~Total unspecified impurities~~	~~—~~	~~0.3~~■2S (*USP33*)

[1](*RS*)-4′-cyano-3-phenylsulfonyl-2-hydroxy-2-methyl-3′-(trifluoromethyl)-propionanilide.

[2](*RS*)-4′-cyano-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methyl-3′-(trifluoromethyl)-propionanilide.

[3](*RS*)-4′-cyano-3-(4-fluorophenylsulfonyl)-2-methyl-3′-(trifluoromethyl)propionanilide.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Bicalutamide RS

USP Bicalutamide Related Compound B RS

BRIEFING

Biperiden Hydrochloride Tablets, *USP 32* page 1680. It is proposed to correct the volume of the *Standard solution*, *Sample solution*, and *Blank* to be used in the *Dissolution* test.

(BPC: M. Marques.) RTS—75629

Biperiden Hydrochloride Tablets

DEFINITION

Biperiden Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of $C_{21}H_{29}NO \cdot HCl$.

IDENTIFICATION

- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Standard solution: Proceed as directed for the *Sample solution* using 10 mg of USP Biperiden Hydrochloride RS in place of the powdered Tablets.

Sample solution: To a quantity of finely powdered Tablets, equivalent to 10 mg of biperiden hydrochloride, add 5 mL of water, mix, and sonicate to disperse the powder. Add 5 mL of methanol to the flask, mix, and sonicate for 15 min. Filter the solution into a separator, add 2 mL of 1 N sodium hydroxide and 10 mL of chloroform, and shake for 3 min. Filter the chloroform layer into a stoppered flask, and use the chloroform filtrate as the *Sample solution.*

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture. Condition by heating the plate at 105° for 1 h and allowing to cool.

Application volume: 20 µL

Developing solvent system: Methanol and ammonium hydroxide (100:1.5)

Visualization: Iodine vapor, 10 min

Analysis: Separately apply the *Sample solution* and the *Standard solution* to the chromatographic plate. Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by exposing the plate for 10 min to iodine vapors in a pre-equilibrated closed chamber, on the bottom of which there are iodine crystals.

In-Process Revision

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

- **PROCEDURE**

Solution A: 38 mg/mL of monobasic sodium phosphate and 2 mg/mL of anhydrous dibasic sodium phosphate in water. Adjust to a pH of 5.3 ± 0.1, if necessary.

Solution B: Dissolve 400 mg of bromocresol purple in 30 mL of water, add 6.3 mL of 0.1 N sodium hydroxide, and dilute with water to 500 mL.

Phosphate buffer–bromocresol purple solution: Mix equal volumes of *Solution A*, *Solution B*, and chloroform, shake in a separator, and discard the chloroform. If appreciable color is extracted, repeat with additional portions of chloroform until no color is extracted.

Standard stock solution: 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

Standard solution: 40 µg/mL of USP Biperiden Hydrochloride RS from *Standard stock solution*. Transfer 5.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of water, and dilute with methanol to volume.

Sample solution: Transfer a portion of finely powdered Tablets, equivalent to 2 mg of biperiden hydrochloride, from NLT 20 Tablets, to a 50-mL volumetric flask, add 12.5 mL of water, and heat on a steam bath for 15 min. Cool, and dilute with methanol to volume.

Blank: Methanol and water (3:1)

Analysis

Samples: *Standard solution, Sample solution*, and *Blank*

Transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 20.0 mL of chloroform for 2 min. After the layers have separated, pass each chloroform extract through filter paper (Whatman No. 31 or equivalent) into separate glass-stoppered, 50-mL volumetric flasks. In the same manner, extract the solution in each separator with another 20.0-mL portion of chloroform, filter, and wash each filter with 8 mL of chloroform, collecting each combined filtrate and washing, respectively, in the 50-mL volumetric flask containing the first extract. Dilute each with chloroform to volume. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 408 nm, with a suitable spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of $C_{21}H_{29}NO \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Biperiden Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)
F = conversion factor, 0.001 (µg to mg)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

[NOTE—Determine the amount of $C_{21}H_{29}NO \cdot HCl$ dissolved by using the following method.]

Phosphate buffer–bromocresol purple solution: Prepare as directed for *Assay*.

Standard stock solution: 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

Standard solution: 2 µg/mL of USP Biperiden Hydrochloride RS, prepared as follows: Pipet 5 mL of *Standard stock solution* into a 500-mL volumetric flask, and add 0.01 N hydrochloric acid to volume. Pipet 25 mL of this solution into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

Sample solution: Sample per *Dissolution* ⟨711⟩. Filter 75 mL of the solution under test, pipet 50 mL of the clear filtrate into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

Blank: Water

Analysis

Samples: *Standard solution, Sample solution*, and *Blank*

Pipet ~~20~~■20.0■*2S (USP33)* mL each of the *Standard solution*, the *Sample solution*, and the *Blank* into individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 40.0 mL of chloroform for 10 min. After the layers have separated, pass each chloroform extract through filter paper into separate, glass-stoppered containers, discarding the first 10 mL of each filtrate. Determine the amount of $C_{21}H_{29}NO \cdot HCl$ dissolved from absorbances at the wavelength of maximum absorbance at about 408 nm (10-cm cells) of the extract from the *Sample solution* in comparison with that of the extract from the *Standard solution*, using the *Blank* to set the instrument.

Tolerances: NLT 75% (Q) of $C_{21}H_{29}NO \cdot HCl$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Biperiden Hydrochloride RS

BRIEFING

Bupropion Hydrochloride Extended-Release Tablets, *USP 32* page 1723, page 4033 of the *First Supplement*, page 70 of *PF* 35(1) [Jan.–Feb. 2009], and the *Interim Revision Announcement* on page 531 of *PF* 35(3) [May–June 2009]. It is proposed to add the option of using sinkers, if necessary, in *Dissolution Test 3*.

(BPC: M. Marques.) RTS—C75800

Bupropion Hydrochloride Extended-Release Tablets

DEFINITION

Bupropion Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩

Sample: Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

Acceptance criteria: The *Sample* shows strong bands at about 1690, 1560, and 1240 cm^{-1} and a weaker band at about 740 cm^{-1}, similar to the reference preparation.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

In-Process Revision

ASSAY

• **PROCEDURE**

Diluent: Methanol and 0.001 N hydrochloric acid (2:8)

Solution A: Acetonitrile, trifluoroacetic acid, and water (10:0.04:90)

Solution B: Acetonitrile, trifluoroacetic acid, and water (95:0.03:5)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

System suitability solution A: Separate solutions of 0.20 mg/mL each of USP Bupropion Hydrochloride Related Compound C RS and USP Bupropion Hydrochloride Related Compound F RS in methanol. Transfer suitable volumes of each solution to a single volumetric flask to obtain 0.0018 mg/mL of USP Bupropion Hydrochloride Related Compound C RS and 0.018 mg/mL of USP Bupropion Hydrochloride Related Compound F RS. Dilute with *Diluent* to volume.

System suitability stock solution B: 0.09 mg/mL of *m*-chlorobenzoic acid in water

System suitability solution B: 0.0018 mg/mL of *m*-chlorobenzoic acid from *System suitability stock solution B* and *Diluent*

Standard solution: 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent*

Sample solution: Transfer a number of Tablets to a suitable homogenizer vessel containing sufficient methanol to obtain a concentration of 3.0 mg of bupropion hydrochloride/mL. Immediately homogenize the sample for 30 s at 20,000 rpm. Allow extraction for 3 min, and follow by two additional 10-s pulses, each at 20,000 rpm, pausing 3 min between these pulses to ensure complete extraction. Pass a portion of the solution through a nylon filter having a 0.45-µm porosity, discarding the first 2–4 mL of the filtrate. Pipet 10.0 mL of the filtrate into a 50-mL volumetric flask, and add about 25 mL of 0.001 N hydrochloric acid. Allow to cool to room temperature, and dilute with 0.001 N hydrochloric acid to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 10-cm; 3.5-µm packing L1

Temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 5 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5, between bupropion hydrochloride related compound C and bupropion hydrochloride related compound F, *System suitability solution A*

Tailing factor: NMT 1.9, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Relative response factor: Between 0.22 and 0.26 for *m*-chlorobenzoic acid. [NOTE—Use the responses from *System suitability solution B* and the *Standard solution*.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{13}H_{18}ClNO \cdot HCl$ in the portion of Tablets taken:

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

r_U = peak response for bupropion hydrochloride from the *Sample solution*

r_S = peak response for bupropion hydrochloride from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of bupropion hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩

For products labeled for dosing every 12 h

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 1, 4, and 8 h

Standard solution: USP Bupropion Hydrochloride RS at a known concentration in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter, dilute with *Medium* if necessary.

Spectrometric conditions

Mode: UV-Vis

Analytical wavelength: 298 nm

Cell: 1.0 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2*.

Time (h)	Amount Dissolved (%)
1	25–45
4	60–85
8	NLT 80

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5 ± 0.05); 900 mL

Apparatus 1: 50 rpm

Times: 1, 2, 4, and 6 h

Determine the percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved by using the following method.

Buffer: 3.45 g of monobasic sodium phosphate monohydrate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.80 ± 0.05.

Mobile phase: Methanol and *Buffer* (7:13)

Standard solution: USP Bupropion Hydrochloride RS in *Medium* at a known concentration similar to the one expected in the *Sample solution*

Sample solution: Use portions of the solution under test, and pass through a 0.45-µm nylon filter.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
1	25–50
2	40–65
4	65–90
6	NLT 80

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium, Apparatus, Standard solution, Sample solution, Spectometric conditions, and **Analysis:** Proceed as directed for *Test 1*, except the wavelength is about 250 nm ■and with wire coil sinkers, if necessary.■2S *(USP33)*
Times: 1, 2, 4, and 6 h
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2*.

Time (h)	Amount Dissolved (%)
1	30–55
2	50–75
4	70–90
6	NLT 80

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium and **Analysis:** Proceed as directed for *Test 1.*
Spectrometric conditions: Proceed as directed for *Test 1*, except to use a 0.5-cm cell.
Times: 1, 3, and 6 h
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
1	35–55
3	65–85
6	NLT 80

•**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium, Apparatus 1, and **Times:** Proceed as directed for *Test 2*, including the quantitative chromatographic method, but using as the *Mobile phase* a mixture of *Buffer* with methanol (55:45).
Tolerances: The percentages of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
1	between 25 and 50
2	between 45 and 70
4	NLT 70
6	NLT 80

•5
For products labeled for dosing every 24 h
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 16 h
Standard solution: USP Bupropion Hydrochloride RS at a known concentration in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, dilute with *Medium*, if necessary.
Spectrometric conditions
Mode: UV-Vis
Analytical wavelength: 252 nm
Cell: 1.0 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
2	NMT 20
4	20–45
8	65–90
16	NLT 80

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium and Apparaus: Proceed as directed for *Test 4*.
Times: 1, 2, 4, 8, and 12 h
Standard solution: USP Bupropion Hydrochloride RS at a known concentration in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, dilute with *Medium*, if necessary.
Spectrometric conditions
Mode: UV-Vis
Analytical wavelength: 298 nm
Cell: 1.0 cm
Blank: *Medium*
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
1	between 15 and 35
2	between 25 and 50
4	between 40 and 65

Time (h)	Amount Dissolved (%)
8	between 65 and 90
12	NLT 80

•**Test 8:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 16 h
Standard solution
For Tablets labeled to contain 150 mg: USP Bupropion Hydrochloride RS dissolved in *Medium* (about 0.167 mg/mL)
For Tablets labeled to contain 300 mg: USP Bupropion Hydrochloride RS dissolved in *Medium* (about 0.333 mg/mL)
Sample solution: Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm.
Spectrometric conditions
Mode: UV-Vis
Analytical wavelength: 298 nm
Blank: *Medium*
Tolerances: The percentage of the labeled amount of $C_{13}H_{18}ClNO \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (h)	Amount Dissolved (%)
2	NMT 10
4	between 10 and 35
8	between 45 and 75
16	NLT 80

•3

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements
Procedure for content uniformity
Standard solution: 0.33 mg/mL of USP Bupropion Hydrochloride RS in water
Sample solution: Transfer 1 Tablet to a suitable homogenizer vessel containing a volume of water to obtain a concentration of about 0.33 mg of bupropion hydrochloride/mL. Immediately homogenize the sample using single 30-s pulses each at 5,000, 10,000, and 15,000 rpm, and follow by two pulses each at 20,000 rpm. After the homogenate has settled, mix at 5000 rpm for an additional 30 s. Pass a portion of the solution through a nylon filter having a 0.45-µm porosity, discarding the first 4 mL of the filtrate.
Analysis: Proceed as directed for the appropriate *Dissolution* prodecure, using a 0.5-cm cell, and correct for dilution.

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**
Solution A, Solution B, Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Analysis: Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for bupropion hydrochloride from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bupropion hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Impurity Table 1* for values)
Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)	
			100 mg or less	150 mg or greater
2-Amino-1-(3-chlorophenyl)-1-propanone	0.38	0.80	0.3	0.3
(3*S*,5*S*,6*S*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	0.56	0.86	1.0	1.5
(3*S*,5*R*,6*R*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	0.78	0.88	0.5	0.4
Bupropion	1.0	—	—	—
Bupropion related compound F	1.71	0.55	1.2	2.3
Bupropion related compound C	1.75	0.59	0.3	0.3
m-Chlorobenzoic acid	1.80	0.24	0.3	0.3
■1-(3-Chlorophenyl)-1,2-propanedione■1S *(USP32)*	2.25	1.00	0.4	0.4
Any unspecified impurity	—	1.00	0.2	0.2
Total impurities	—	—	3.2	3.3

In-Process Revision

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Bupropion Hydrochloride RS
  USP Bupropion Hydrochloride Related Compound C RS
  USP Bupropion Hydrochloride Related Compound F RS

BRIEFING

Capecitabine Tablets, *USP 32* page 1775 and page 835 of *PF* 35(4) [July–Aug. 2009]. It is proposed to modify the wavelength used in the quantitation step in the *Dissolution* test.

(BPC: M. Marques.) RTS—C75630

Capecitabine Tablets

DEFINITION
Capecitabine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of capecitabine ($C_{15}H_{22}FN_3O_6$).

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**
  Wavelength range: 1500–1760 cm^{-1}
  Sample: Grind one Tablet to a fine powder with a mortar and pestle. Mix 1 mg of this sample with 300 mg of potassium bromide.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

Change to read:

- **PROCEDURE**
  Diluent: Methanol, acetonitrile, and water (7:1:12)
  Solution A: 0.1% mixture of ■glacial■*2S (USP33)* acetic acid in water
  Solution B: Methanol, acetonitrile, and *Solution A* (7:1:12)
  Solution C: Methanol, acetonitrile, and *Solution A* (16:1:3)
  Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

  System suitability solution: Includes 0.6 µg/mL of USP Capecitabine RS, 0.6 µg/mL of USP Capecitabine Related Compound A RS, 0.6 µg/mL of USP Capecitabine Related Compound B RS, and 0.6 µg/mL of USP Capecitabine Related Compound C RS in *Diluent.* [NOTE—Sonicate if necessary.]
  Standard solution: 0.6 mg/mL of USP Capecitabine RS in *Diluent.* [NOTE—Sonicate if necessary.]
  Sample solution: Equivalent to 0.6 mg/mL of Capecitabine, from powdered Tablets (NLT 20), in *Diluent.* Pass through a PVDF 0.45-µm membrane filter, and use the filtrate. [NOTE—Sonicate if necessary.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)
  Mode: LC
  Detector: UV 250 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Autosampler temperature: 5°
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1.* The relative retention times are measured with respect to capecitabine.]
  Suitability requirements
  Resolution: NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*
  Tailing factor: NMT 1.5, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{15}H_{22}FN_3O_6$ in the portion of Tablets taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)
  Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION ⟨711⟩**
  Medium: Water; 900 mL, degassed
  Apparatus 2: 50 rpm
  Time: 30 min
  Sample solution: *Sample* per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution.* Pass a portion of the solution under test through a 0.45-µm fiberglass filter.
  Standard solutions
  For Tablets labeled to contain 150 mg: 17 mg of USP Capecitabine RS in 100 mL of *Medium*
  For Tablets labeled to contain 500 mg: 28 mg of USP Capecitabine RS in 50 mL of *Medium*
  Analysis: Determine the amount of $C_{15}H_{22}FN_3O_6$ dissolved ~~by employing UV absorption at the wavelength of maximum absorbance at 304 nm~~■by selecting a wavelength with appropriate sensitivity between 300 and 330 nm■*2S (USP33)* ~~(for Tablets labeled to contain 150 mg) and at 325 nm (for Tablets labeled to contain 500 mg)~~▲*USP33* on portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with the appropriate *Standard solution*, using a 1-mm quartz cell. Calculate the percentage of $C_{15}H_{22}FN_3O_6$ dissolved in each Tablet:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  C_S = concentration of capecitabine in the *Standard solution* (mg/mL)
  V = volume of medium, 900 mL
  L = label claim (mg/Tablet)
  Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{22}FN_3O_6$ is dissolved.

In-Process Revision

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**

Diluent, Solution A, Solution B, System suitability solution, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for capecitabine from the *Standard solution*

C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)

F = relative response factor for each impurity, from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total ~~impurities~~ degradation products: ■2S (*USP33*) NMT 2.0%

~~Impurity Table~~

~~Name~~	~~Relative Retention Time~~	~~Relative Response Factor~~	~~Acceptance Criteria, NMT (%)~~
~~Capecitabine related compound A~~	~~0.18~~	~~1.05~~	~~1.0~~
~~Capecitabine related compound B~~	~~0.19~~	~~0.81~~	~~1.0~~
~~Capecitabine~~	~~1.00~~	~~1.00~~	~~—~~
~~Capecitabine related compound C~~	~~1.11~~	~~0.91~~	~~0.5~~
~~Individual unspecified impurity~~	~~—~~	~~1.00~~	~~0.1~~
~~Total unspecified impurities~~	~~—~~	~~—~~	~~0.5~~

■**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	1.0
Capecitabine related compound B	0.19	0.81	1.0
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine*	0.36	0.89	—

The impurities marked with an "*" are process impurities and are not included in the total degradation products.

Impurity Table 1 *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5′-Deoxy-5-fluoro-*N*4-(2-methyl-1-butyloxycarbonyl)cytidine + 5′-Deoxy-5-fluoro-*N*4-(3-methyl-1-butyloxycarbonyl)cytidine*	0.95	1.01	—
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.06	1.00	—
[1-[5-Deoxy-2-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.09	1.00	—
Capecitabine related compound C	1.11	0.91	0.5
[1-[5-Deoxy-3-*O*-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.20	1.00	—
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-*N*4-(pentyloxycarbonyl)cytidine*	1.37	0.85	—
Individual unspecified degradation product	—	1.00	0.1

The impurities marked with an "*" are process impurities and are not included in the total degradation products.

■2S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Capecitabine RS
USP Capecitabine Related Compound A RS
USP Capecitabine Related Compound B RS
USP Capecitabine Related Compound C RS

In-Process Revision

BRIEFING

Cefdinir Capsules, *USP 32* page 1827. On the basis of comments received, it is proposed to revise the column and autosampler temperatures in the test for *Organic Impurities*. Robustness studies do not demonstrate the need for a narrow temperature range. In the test for *Organic Impurities*, *Impurity Table 1* is updated to provide chemical names for the related compounds and to update the impurity names in the footnotes to match those listed in the table. The 'Limit of Quantification' column header was revised to 'Reporting Threshold' as this more accurately represents the values in the table.

(MD-ANT: A. Wise.) RTS—C69056

Cefdinir Capsules

DEFINITION

Cefdinir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
  Buffer: Prepare as directed in the *Assay*.
  Blank: Use the *Buffer*.
  Standard solution: 10 µg/mL of USP Cefdinir RS in *Buffer*
  Sample solution: Equivalent to 10 µg/mL of cefdinir in *Buffer*. Filter before use.
  Cell size: 1 cm
  Acceptance criteria: *Sample solution* maxima and minima occur at the same wavelengths as those in the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 10.65 mg/mL of dibasic sodium phosphate and 3.40 mg/mL of monobasic potassium phosphate. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.
  Solution A: 7.0 mg/mL of citric acid monohydrate. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.
  Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)
  Standard solution: 50 µg/mL of USP Cefdinir RS in *Buffer*
  System suitability solution: 50 µg/mL of cefdinir and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*
  Sample solution: Equivalent to 50 µg/mL of Cefdinir, from Capsule contents (NLT 20) in the *Buffer*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 15-cm; 4-µm packing L1
  Flow rate: 1.4 mL/min
  Injection size: 15 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: Greater than 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*
  Tailing factor: NMT 2.0 for cefdinir, *System suitability solution*
  Relative standard deviation: NMT 1.0% for cefdinir, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{13}N_5O_5S_2$ in the portion of Capsules taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of the *Standard solution* (µg/mL)
  C_U = nominal concentration of cefdinir in the *Sample solution* (µg/mL)
  Acceptance criteria: 90.0%–100.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**
  Medium: 50 mM phosphate buffer pH 6.8; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Detector: UV 290 nm
  Cell length: 1 cm
  Standard solution: 0.33 mg/mL of USP Cefdinir RS in *Medium*
  Sample solution: Sample per *Dissolution* ⟨711⟩. Pass each sample through a suitable 0.45-µm filter. Dilute with *Medium* to a concentration of about 0.33 mg/mL of cefdinir.
  Blank: Dissolve one empty capsule in 100 mL of *Medium*, and dilute to 900 mL; filter if necessary.
  Analysis: Determine the percentage of $C_{14}H_{13}N_5O_5S_2$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  D = dilution factor of the *Sample solution* (mL/mL)
  V = volume of *Medium*, 900 mL
  L = label claim (mg)
  Tolerances: NLT 80% (Q) of the labeled amount of $C_{14}H_{13}N_5O_5S_2$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

Organic Impurities

- **PROCEDURE**
  Solution A: 14.2 mg/mL of anhydrous dibasic sodium phosphate
  Solution B: 13.6 mg/mL of monobasic potassium phosphate
  Buffer: Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of 7.0 ± 0.1.
  Solution C: Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of 5.5 ± 0.1.
  Solution D: 37.2 mg/mL of edetate disodium
  Solution E: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.
  Solution F: Acetonitrile, methanol, *Solution C*, and *Solution D* (150:100:250:0.2)
  Mobile phase: See gradient table below.

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50

In-Process Revision

Time (min)	Solution E (%)	Solution F (%)
28	95	5
58	95	5

Standard stock solution: 750 µg/mL of USP Cefdinir RS, *Buffer*

Standard solution: 15 µg/mL of USP Cefdinir RS, from the *Standard stock solution* in *Solution C*

System suitability stock solution 1: 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*

System suitability stock solution 2: 40 µg/mL of USP Cefdinir Related Compound B RS in *Solution C*

System suitability solution: Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL of each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

Sample solution: Transfer an equivalent to 300 mg of Cefdinir, from Capsule contents (NLT 20), into a 200-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume to obtain a solution having a ~~known~~ ■nominal■ *(2S USP33)* concentration of about 1.5 mg/mL of cefdinir.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40° ~~± 0.5°~~
~~**Sample solution temperature:** 4° ± 3°~~
■**Autosampler temperature:** 4°■ *(2S USP33)*
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between cefdinir and the third peak of the USP Cefdinir Related Compound A RS, *System suitability solution*
Tailing factor: NMT 1.5 for cefdinir related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0% for the cefdinir peak response, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 5.0%

~~**Impurity Table 1**~~

~~Name~~	~~Relative Retention Time~~	~~Relative Response Factor~~	~~Limit of Quantification (% Cefdinir)~~	~~19 Criteria, NMT (%)~~
~~Impurity VIII~~	~~0.10~~	~~1.1~~	~~0.1~~	~~0.5~~
~~Impurity IV~~	~~0.13~~	~~1.1~~	~~0.1~~	~~0.5~~
~~Impurity XIV~~	~~0.36~~	~~1.0~~	~~0.05~~	~~0.2~~
~~Impurity V~~	~~0.46~~	~~1.5~~	~~0.05~~	~~0.7~~
~~Impurity B~~	~~0.77~~	~~1.0~~	~~0.05~~	~~0.3~~
~~Impurity XI~~	~~0.75~~	~~1.0~~	~~0.05~~	~~0.7~~
~~Cefdinir Related Compound A (lactam ring cleavage lactones-a)~~	~~0.85~~	~~1.5~~	~~0.1~~	~~2.5~~
~~Cefdinir Related Compound A (lactam ring cleavage lactones-b)~~	~~0.94~~	~~1.5~~	~~0.1~~	~~—~~
~~Cefdinir Related Compound A (lactam ring cleavage lactones-c)~~	~~1.11~~	~~1.5~~	~~0.1~~	~~—~~
~~Cefdinir Related Compound A (lactam ring cleavage lactones-d)~~	~~1.14~~	~~1.5~~	~~0.1~~	~~—~~
~~Impurity VI~~	~~1.18~~	~~1.1~~	~~0.05~~	~~0.2~~
~~Impurity I~~	~~1.23~~	~~1.2~~	~~0.05~~	~~1.0~~
~~Cefdinir Related Compound B~~	~~1.28~~	~~1.1~~	~~0.05~~	~~0.2~~
~~Impurity XIII~~	~~1.37~~	~~1.4~~	~~0.05~~	~~0.5~~
~~Impurity E~~	~~1.44~~	~~1.0~~	~~0.05~~	~~0.5~~
~~Impurity XV~~	~~1.49~~	~~1.0~~	~~0.05~~	~~0.2~~
~~Impurity VII~~	~~1.51~~	~~1.1~~	~~0.05~~	~~0.7~~
~~Impurity IIIa~~	~~1.62~~	~~1.3~~	~~0.05~~	~~1.0~~
~~Impurity IIIb~~	~~1.64~~	~~1.3~~	~~0.05~~	~~—~~
~~Impurity D~~	~~1.82~~	~~1.0~~	~~0.05~~	~~0.2~~
~~Individual unidentified impurities~~	~~—~~	~~1.0~~	~~—~~	~~0.2~~
~~Total unspecified impurities~~	~~—~~	~~—~~	~~—~~	~~1.0~~

~~[a]RS II is a mixture of 4 isomers designated as RS IIa, RS IIb, RS IIc, and RS IId. The sum of all values is reported and the total limit for all 4 isomers combined is 2.5%.~~
~~[b]RS III is a mixture of 2 isomers designated as RS IIIa and RS IIIb. The sum of both values is reported and the total limit for both isomers combined is 1.0%.~~
~~[c]Impurity B, Impurity D, and Impurity E are unidentified impurities.~~
~~[d]The total unidentified impurities limit includes the % total of unidentified impurities B, D, and E and any other unidentified impurities.~~

In-Process Revision

■Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Reporting Threshold (% Cefdinir)	Acceptance Criteria, NMT (%)
Impurity VIII[a]	0.10	1.1	0.1	0.5
Impurity IV[b]	0.13	1.1	0.1	0.5
Impurity XIV[c]	0.36	1.0	0.05	0.2
Impurity V[d]	0.46	1.5	0.05	0.7
Impurity B[e]	0.77	1.0	0.05	0.3
Impurity XI[f]	0.75	1.0	0.05	0.7
Cefdinir related compound A (lactam ring cleavage lactones-a)[g,h]	0.85	1.5	0.1	2.5
Cefdinir related compound A (lactam ring cleavage lactones-b)[g,h]	0.94	1.5	0.1	—
Cefdinir related compound A (lactam ring cleavage lactones-c)[g,h]	1.11	1.5	0.1	—
Cefdinir related compound A (lactam ring cleavage lactones-d)[g,h]	1.14	1.5	0.1	—
Impurity VI[i]	1.18	1.1	0.05	0.2
Impurity I[j]	1.23	1.2	0.05	1.0
Cefdinir related compound B[k]	1.28	1.1	0.05	0.2
Impurity XIII[f]	1.37	1.4	0.05	0.5
Impurity E[e]	1.44	1.0	0.05	0.5
Impurity XV[l]	1.49	1.0	0.05	0.2
Impurity VII[m]	1.51	1.1	0.05	0.7
Impurity IIIa[n,o]	1.62	1.3	0.05	1.0
Impurity IIIb[n,o]	1.64	1.3	0.05	—
Impurity D[e]	1.82	1.0	0.05	0.2
Individual unidentified impurities	—	1.0	—	0.2
Total unspecified impurities[p]	—	—	—	1.0

[a] *N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.
[b] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.
[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] (*R*,*Z*)-2-{(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxy)methyl}-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[e] Impurity B, Impurity D, and Impurity E are unidentified impurities.
[f] This information cannot be disclosed.
[g] Cefdinir related compound A is a mixture of 4 isomers designated as lactam ring cleavage lactones a, b, c, and d. The sum of all values is reported and the total limit for all 4 isomers combined is 2.5%.
[h] 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.
[i] (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[j] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl)acetamide.
[k] (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] (6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[m] (6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] Impurity III is a mixture of 2 isomers designated as Impurity IIIa and Impurity IIIb. The sum of both values is reported and the total limit for both isomers combined is 1.0%.
[o] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-(((2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3]thiazin-2-yl)methyl)acetamide.
[p] The total unidentified impurities limit includes the percent total of unidentified impurities B, D, and E and any other unidentified impurities.

■2S (*USP33*)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Cefdinir RS
  USP Cefdinir Related Compound A RS
  USP Cefdinir Related Compound B RS

BRIEFING

Cefdinir for Oral Suspension, *USP 32* page 1830. On the basis of comments received, it is proposed to revise the column and autosampler temperatures in the test for *Organic Impurities*. Robustness studies do not demonstrate the need for a narrow temperature range. *Impurity Table 1* in the test for *Organic Impurities* is updated to provide chemical names for the related compounds. The 'Limit of Quantification' column header was revised to 'Reporting Threshold' as this more accurately represents the values in the table.

(MD-ANT: A. Wise.) RTS—C69057

Cefdinir for Oral Suspension

DEFINITION

Cefdinir for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_{14}H_{13}N_5O_5S_2$. It may contain one or more suitable buffers, flavors, preservatives, stabilizing agents, sweeteners, and suspending agents.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩
  Adsorbent: 0.25-mm layer of chromatographic silica gel, preconditioned with *n*-hexane and tetradecane (95:5)
  Buffer: Prepare as directed in the *Assay*.
  Standard solution: 600 µg/mL of USP Cefdinir RS in methanol and *Buffer* (3:1)
  Sample solution: Transfer an equivalent to 125 mg of cefdinir from reconstituted Suspension to a 100-mL volumetric flask, add 50 mL of *Buffer*, and dilute with methanol to volume. Pass a portion through a suitable 0.45-µm filter, transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, and dilute with methanol to volume.
  Application volume: 10 µL
  Developing solvent system: Methanol and water (4:1)
  Visualization: Short-wavelength UV
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop the chromatogram until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, and allow the solvent to evaporate.
  Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 10.65 mg/mL of anhydrous dibasic sodium phosphate and 3.40 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.
  Solution A: 7.0 mg/mL of citric acid monohydrate. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.
  Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)
  Standard solution: 50 µg/mL of USP Cefdinir RS in *Buffer*
  System suitability solution: 50 µg/mL of cefdinir and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*
  Sample solution: Equivalent to 50 µg/mL of cefdinir, from constituted Suspension in *Buffer*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 15-cm; 4-µm packing L1
  Flow rate: 1.4 mL/min
  Injection size: 15 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*
  Tailing factor: NMT 2.0 for cefdinir, *System suitability solution*
  Relative standard deviation: NMT 1.0% for cefdinir, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{13}N_5O_5S_2$ in the portion of Cefdinir for Oral Suspension taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of the *Standard solution* (µg/mL)
  C_U = nominal concentration of cefdinir in the *Sample solution* (µg/mL)
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩
  Medium: 50 mM phosphate buffer pH 6.8; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Detector: UV 290 nm
  Standard solution: 0.14 mg/mL of USP Cefdinir RS in *Medium*
  Sample solution: Dilute a portion of each filtered sample with *Medium* as necessary to obtain a solution having a concentration of about 0.14 mg/mL of cefdinir.
  Blank: *Medium*
  Analysis: Transfer 5 mL, by weight, of the reconstituted Oral Suspension into the vessel. After the appropriate time, withdraw a portion of the solution under test and pass through a suitable 0.45-µm filter.
  Determine the percentage of $C_{14}H_{13}N_5O_5S_2$ dissolved:

$$\text{Result} = (A_U/A_S) \times ([C_S \times d \times D \times V]/W \times L) \times 100$$

  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  C_S = concentration of the *Standard solution* (mg/mL)
  d = density of the Oral Suspension (mg/mL)
  D = dilution factor of the *Sample solution* (mL/mL)
  V = volume of *Medium*, 900 mL
  W = weight of Oral Suspension taken (mg)
  L = label claim (mg)
  Tolerances: NLT 80% (Q) of the labeled amount of $C_{14}H_{13}N_5O_5S_2$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩ (for solid packaged in single-unit containers)**:** Meets the requirements
- **DELIVERABLE VOLUME** ⟨**698**⟩ (for solid packaged in multiple-unit containers)**:** Meets the requirements

In-Process Revision

IMPURITIES

Change to read:

Organic Impurities

• **PROCEDURE**

Solution A: 14.2 mg/mL of anhydrous dibasic sodium phosphate

Solution B: 13.6 mg/mL of monobasic potassium phosphate

Buffer: Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a pH 7.0 ± 0.1 solution.

Solution C: Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of 5.5 ± 0.1.

Solution D: 37.2 mg/mL of edetate disodium

Solution E: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

Solution F: Acetonitrile, methanol, *Solution C*, and *Solution D* (150:100:250:0.2)

Mobile phase: See the gradient table below.

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

Standard stock solution: 750 µg/mL of USP Cefdinir RS in *Buffer*

Standard solution: 15 µg/mL of USP Cefdinir RS, from the *Standard stock solution* in *Solution C*

System suitability stock solution 1: 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*

System suitability stock solution 2: 40 µg/mL of USP Cefdinir Related Compound B RS in *Buffer*

System suitability solution: Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL of each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

Sample solution: Transfer an equivalent to 150 mg of cefdinir, from constituted Oral Suspension, into a 100-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40° ~~± 0.5°~~

~~**Sample solution temperature:** 4° ± 3°~~

■**Autosampler temperature:** 4°■2S *(USP33)*

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between cefdinir and the third peak of the USP Cefdinir Related Compound A RS, *System suitability solution*

Tailing factor: NMT 1.5 for cefdinir related compound B, *System suitability solution*

Relative standard deviation: NMT 2.0% for the cefdinir peak response, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefdinir for Oral Suspension taken:

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

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 6.2%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Limit of Quantification (% Cefdinir)	Acceptance Criteria, NMT (%)
Impurity VIII	0.10	1.1	0.1	0.5
Impurity IV	0.13	1.1	0.1	0.6
Impurity XIV	0.36	1.0	0.05	0.2
Impurity V	0.46	1.5	0.05	0.3
Impurity B[c]	0.77	1.0	0.05	0.2
Impurity XI	0.75	1.0	0.05	0.7
Cefdinir Related Compound A (lactam ring cleavage lactones-a)[a]	0.85	1.5	0.1	3.3
Cefdinir Related Compound A (lactam ring cleavage lactones-b)[a]	0.94	1.5	0.1	—
Cefdinir Related Compound A (lactam ring cleavage lactones-c)[a]	1.11	1.5	0.1	—
Cefdinir Related Compound A (lactam ring cleavage lactones-d)[a]	1.14	1.5	0.1	—
Impurity VI	1.18	1.1	0.05	0.2
Impurity I	1.23	1.2	0.05	0.8
Cefdinir Related Compound B	1.28	1.1	0.05	0.2
Impurity XIII	1.37	1.4	0.05	0.5
Impurity E[c]	1.44	1.0	0.05	0.2
Impurity XV	1.49	1.0	0.05	0.2
Impurity VII	1.51	1.1	0.05	1.2
Impurity IIIa[b]	1.62	1.3	0.05	1.1
Impurity IIIb[b]	1.64	1.3	0.05	—
Impurity D[c]	1.82	1.0	0.05	0.2
Individual unidentified impurities	—	1.0	—	0.2
Total unidentified impurities[d]	—	—	—	0.9

[a] Cefdinir related compound A is a mixture of 4 isomers designated as lactam ring cleavage lactones a, b, c, and d. The sum of all values is reported and the total limit for all 4 isomers combined is 3.3%.

[b] Impurity III is a mixture of 2 isomers designated as Impurity IIIa and Impurity IIIb. The sum of both values is reported, and the total limit for both isomers combined is 1.5%.

[c] Impurity B, Impurity D, and Impurity E are unidentified impurities.

[d] The total unidentified impurities limit includes the % total of unidentified impurities B, D, and E and any other unidentified impurities detected.

In-Process Revision

▪Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Reporting Threshold (% Cefdinir)	Acceptance Criteria, NMT (%)
Impurity VIII[a]	0.10	1.1	0.1	0.5
Impurity IV[b]	0.13	1.1	0.1	0.6
Impurity XIV[c]	0.36	1.0	0.05	0.2
Impurity V[d]	0.46	1.5	0.05	0.3
Impurity B[e]	0.77	1.0	0.05	0.2
Impurity XI[f]	0.75	1.0	0.05	0.7
Cefdinir related compound A (lactam ring cleavage lactones-a)[g,h]	0.85	1.5	0.1	3.3
Cefdinir related compound A (lactam ring cleavage lactones-b)[g,h]	0.94	1.5	0.1	—
Cefdinir related compound A (lactam ring cleavage lactones-c)[g,h]	1.11	1.5	0.1	—
Cefdinir related compound A (lactam ring cleavage lactones-d)[g,h]	1.14	1.5	0.1	—
Impurity VI[i]	1.18	1.1	0.05	0.2
Impurity I[j]	1.23	1.2	0.05	0.8
Cefdinir related compound B[k]	1.28	1.1	0.05	0.2
Impurity XIII[f]	1.37	1.4	0.05	0.5
Impurity E[e]	1.44	1.0	0.05	0.2
Impurity XV[l]	1.49	1.0	0.05	0.2
Impurity VII[m]	1.51	1.1	0.05	1.2
Impurity IIIa[n,o]	1.62	1.3	0.05	1.1
Impurity IIIb[n,o]	1.64	1.3	0.05	—
Impurity D[e]	1.82	1.0	0.05	0.2
Individual unidentified impurities	—	1.0	—	0.2
Total unidentified impurities[p]	—	—	—	0.9

[a] *N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.
[b] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.
[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] (*R*,*Z*)-2-{(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxy)methyl}-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[e] Impurity B, Impurity D, and Impurity E are unidentified impurities.
[f] This information cannot be disclosed.
[g] Cefdinir related compound A is a mixture of 4 isomers designated as lactam ring cleavage lactones a, b, c, and d. The sum of all values is reported and the total limit for all 4 isomers combined is 3.3%.
[h] 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.
[i] (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[j] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl)acetamide.
[k] (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] (6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[m] (6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] Impurity III is a mixture of 2 isomers designated as Impurity IIIa and Impurity IIIb. The sum of both values is reported and the total limit for both isomers combined is 1.1%.
[o] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-(((2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3]thiazin-2-yl)methyl)acetamide.
[p] The total unidentified impurities limit includes the percent total of unidentified impurities B, D, and E and any other unidentified impurities.

■2S (*USP33*)

SPECIFIC TESTS
- **pH** ⟨**791**⟩**:** 3.5–4.5
- **Loss on Drying** ⟨**731**⟩**:** Dry about 1 g over phosphorous pentoxide in a vacuum not exceeding 5 mm of mercury at 70° for 4–4.5 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **Labeling:** The label specifies the directions for the constitution of the powder and states the equivalent amount of $C_{14}H_{13}N_5O_5S_2$ in a given volume of Oral Suspension after constitution.
- **USP Reference Standards** ⟨**11**⟩
  USP Cefdinir RS
  USP Cefdinir Related Compound A RS
  USP Cefdinir Related Compound B RS

BRIEFING

Cefotetan for Injection, *USP 32* page 1847. On the basis of comments received, in the *Other Requirements* section it is proposed to delete the cross-reference to the *Identification* test under *Cefotetan Disodium* and to replace it with a new expanded *Identification* section that is specific for this monograph.

(MD-ANT: A. Wise.) RTS—C69120

Cefotetan for Injection

DEFINITION
Cefotetan for Injection contains an amount of Cefotetan Disodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

IDENTIFICATION

Add the following:

■• **A:** The retention time of the major peak of the appropriate *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*■2S *(USP33)*

Add the following:

■• **B. Identification tests—General,** *Sodium* ⟨**191**⟩**:** Meets the requirements■2S *(USP33)*

ASSAY
- **Procedure**
  [Note—Protect the *Standard solution*, the *System suitability solution*, and the *Sample solutions* from light, and use within 2 h.]
  Solution A: Methanol, acetonitrile, and water (1:1:18)
  Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)
  Standard solution: 20 mg of USP Cefotetan RS in a 100-mL volumetric flask. Add 5 mL of methanol, swirl for several min, add 5 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume.
  System suitability solution: 10 mL of *Standard solution* in a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-µm or finer porosity. Use the clear filtrate.
  Sample solution A (where the package is represented as being in a single-dose container)**:** Constitute Cefotetan for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and quantitatively dilute with *Solution A* to obtain a solution containing the equivalent of 200 µg of cefotetan/mL.
  Sample solution B (where the label states the quantity of cefotetan in a given volume of constituted solution)**:** Constitute Cefotetan for Injection as directed in the labeling. Dilute a volume of the constituted solution quantitatively with *Solution A* to obtain a solution containing the equivalent of 200 µg of cefotetan/mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 2 mL/min
  Injection size: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [Note—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively, *System suitability solution*.]
  Suitability requirements
  Resolution: NLT 2.0 between the cefotetan peak and the cefotetan tautomer peak, *System suitability solution*
  Column efficiency: NLT 1500 theoretical plates, *Standard solution*
  Tailing factor: NMT 1.5, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{17}H_{17}N_7O_8S_4$ withdrawn from the container, or in the portion of solution taken:

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

  r_U = peak response of the *Sample solution*
  r_S = peak response of the *Standard solution*
  C_S = concentration of USP Cefotetan RS in the *Standard solution* (µg/mL)
  C_U = nominal concentration of cefotetan in *Sample solution A* or *Sample solution B* (µg/mL)
  Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **Uniformity of Dosage Units** ⟨**905**⟩**:** Meets the requirements

SPECIFIC TESTS
- **Injections,** *Constituted Solutions* ⟨**1**⟩**:** Meets the requirements
- **Bacterial Endotoxins Test** ⟨**85**⟩**:** NMT 0.17 USP Endotoxin Unit/mg of cefotetan
- **Sterility Tests** ⟨**71**⟩**:** Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **Particulate Matter in Injections** ⟨**788**⟩**:** Meets the requirements for small-volume injections
- **pH** ⟨**791**⟩**:** 4.0–6.5, in a solution (1 in 10)
- **Water Determination,** *Method Ic* ⟨**921**⟩**:** NMT 2.8%

Change to read:

• **Other Requirements:** It ~~meets the requirements of the *Identification* tests under *Cefotetan Disodium*~~ and ■■2S *(USP33)* meets the requirements under *Injections* ⟨1⟩*, Labels and Labeling*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve as described under *Injections* ⟨1⟩*, Containers for Sterile Solids*.
- **USP Reference Standards** ⟨**11**⟩
  USP Cefotetan RS
  USP Endotoxin RS

In-Process Revision

BRIEFING

Cefuroxime Axetil for Oral Suspension, *USP 32* page 1864. On the basis of comments received, it is proposed to revise the test for *pH* to indicate how the solution should be prepared.

(MD-ANT: A. Wise.) RTS—C72175

Cefuroxime Axetil for Oral Suspension

DEFINITION

Cefuroxime Axetil for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

IDENTIFICATION

- The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 23.0 mg/mL of monobasic ammonium phosphate in water

Mobile phase: Methanol and *Solution A* (19:31)

System suitability stock solution A: 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol

System suitability stock solution B: 0.16 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

System suitability solution: Transfer 10.0 mL of *System suitability stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of methanol and 3.8 mL of *System suitability stock solution B*. Dilute with *Solution A* to volume.

Standard stock solution: 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol. [NOTE—Use this solution promptly.]

Standard solution: Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 8.8 mL of methanol, and dilute with *Solution A* to volume. [NOTE—Use this *Standard solution* promptly, or refrigerate and use on the day prepared.]

Sample stock solution: Equivalent to 2.5 mg/mL of cefuroxime, from constituted Oral Suspension, in methanol. Filter before further use. [NOTE—The Oral Suspension to be used should be constituted as directed on the label, and is freshly prepared and free of bubbles. After the addition of a portion of methanol, the solution should be shaken by mechanical means for about 10 min. Protect from light and use promptly.]

Sample solution: Transfer 5.0 mL of the filtered *Sample stock solution* to a 50-mL volumetric flask. Add 13.8 mL of methanol, and dilute with *Solution A* to volume. [NOTE—Protect this *Sample solution* from light and use promptly, or refrigerate and use on the day prepared.]

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L13
Flow rate: 1.5 mL/min
Injection size: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for acetanilide, cefuroxime axetil diastereoisomer B, cefuroxime axetil diastereoisomer A, and cefuroxime axetil delta-3 isomers are 0.4, 0.8, 0.9, and 1.0, respectively.]

Suitability requirements
Resolution: NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*
Column efficiency: NLT 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{16}N_4O_8S$ in each mL taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P_S \times F \times (100 - K)$$

R_U = sum of the peak responses of the cefuroxime axetil diastereoisomers A and B from the *Sample solution*
R_S = sum of the peak responses of the cefuroxime axetil diastereoisomers A and B from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of cefuroxime axetil in the *Sample solution* (mg/mL)
P_S = designated cefuroxime content of anhydrous USP Cefuroxime Axetil RS (µg/mg)
F = unit conversion factor (0.001 mg/µg)
K = percentage of water content of USP Cefuroxime Axetil RS

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

Medium: 0.07 M pH 7.0 phosphate buffer, prepared by dissolving 3.7 mg/mL of monobasic sodium phosphate and 5.7 mg/mL of anhydrous dibasic sodium phosphate in water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Analysis: Test 5.0 mL of constituted Cefuroxime Axetil for Oral Suspension equivalent to 125 or 250 mg of cefuroxime. Determine the amount of cefuroxime equivalent dissolved by using UV absorption at the wavelength of maximum absorbance at 280 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefuroxime Axetil RS in the same *Medium*.
Tolerances: NLT 60% (Q) of the labeled amount of $C_{16}H_{16}N_4O_8S$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩

For solid packaged in single-unit containers: Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. Mix, and allow the contents of the container to drain into a beaker for 5 s. Withdraw and assay 5.0 mL of Cefuroxime Axetil for Oral Suspension from the beaker, or the total amount if it is less than 5 mL. It meets the requirements.

- **DELIVERABLE VOLUME** ⟨**698**⟩

For solid packaged in multiple-unit containers: Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. It meets the requirements.

SPECIFIC TESTS

Change to read:

- **pH** ⟨**791**⟩**:** 3.5–7.0, ■in the solution constituted as directed in the labeling■*2S (USP33)*
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 6.0%

In-Process Revision

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefuroxime Axetil RS
  USP Cefuroxime Axetil Delta-3 Isomers RS

BRIEFING

Clarithromycin Tablets, *USP 32* page 1958, and page 73 of *PF* 35(1) [Jan.–Feb. 2009]. It is proposed to make some modifications in the preparation of the *Buffer solution* used in the *Dissolution* test.

(BPC: M. Marques.) RTS—C75373

Clarithromycin Tablets

DEFINITION
Clarithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{38}H_{69}NO_{13}$.

IDENTIFICATION
- The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Methanol and 0.067 M monobasic potassium phosphate (13:7). Adjust with phosphoric acid to a pH of 4.0, and then pass through a filter having a 0.5-µm or finer porosity.

Standard stock solution: 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in methanol.
[NOTE—Shake and sonicate to facilitate dissolution.]

Standard solution: 125 µg/mL of clarithromycin from *Standard stock solution*. Dilute with *Mobile phase*, and pass through a 0.5-µm porosity filter.

System suitability solution: Prepare 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol. Transfer 10 mL of this solution and 10 mL of *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Finely powder a counted number of Tablets, nominally equivalent to 2000 mg of clarithromycin, and with the aid of methanol, transfer the powder to a 500-mL volumetric flask, add 350 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and allow any insoluble matter to settle. Transfer 3.0 mL to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter having a 0.5-µm or finer porosity. Use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; packing L1
[NOTE—A guard column containing packing L1 may be added.]
Column temperature: 50°
Flow rate: 1 mL/min
Injection size: 20–50 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are 0.75 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*
Column efficiency: NLT 750 theoretical plates from the clarithromycin peak, *Standard solution*
Tailing factor: 0.9–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of label claim of $C_{38}H_{69}NO_{13}$ in the portion of Tablets taken:

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

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Buffer solution: ~~Transfer 13.61 g of sodium acetate trihydrate to a 1-L volumetric flask, add water to dissolve, dilute with water to volume. Adjust with 0.1 M acetic acid to a pH of 5.0.~~ ▲Combine portions of a solution containing 13.61 mg/mL of sodium acetate trihydrate in water with portions of a solution containing 5.7 mL of glacial acetic acid/L in water to obtain a pH of 5.0. ~~(the proportion is almost 1:1)~~■ ■2S *(USP33)*▲*USP33*

Medium: *Buffer solution*, 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: Proceed as directed in the *Assay*.
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Mobile phase* to yield a nominal concentration of 125 µg/mL clarithromycin.

Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of $C_{38}H_{69}NO_{13}$ dissolved in the *Medium*, as directed in the *Assay*.
Calculate the percentage of clarithromycin dissolved:

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

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{38}H_{69}NO_{13}$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩**:** Dry a portion of powdered Tablets in vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h: it loses NMT 6.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clarithromycin RS
  USP Clarithromycin Related Compound A RS

In-Process Revision

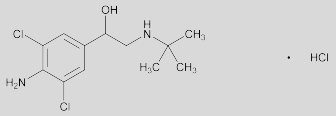
BRIEFING

Clenbuterol Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed based on the Clenbuterol Hydrochloride monograph in the *European Pharmacopoeia's 6.5 Edition* and comments provided to USP regarding its suitability for the article in distribution in the United States. The test for *Organic Impurities* is based on the analyses performed with an Agilent Lichrospher RP 18 brand of L1 column. The typical retention time for clenbuterol peak is about 29 min.

(VET: E. Gonikberg.) RTS—C58037

Add the following:

■Clenbuterol Hydrochloride



$C_{12}H_{18}Cl_2N_2O \cdot HCl$ 313.65

Ethanol, 1-(4-amino-3,5-dichlorophenyl)-2-(*tert*-butylamino), hydrochloride;
4-Amino-α-[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol, hydrochloride [21898-19-1].

DEFINITION

Clenbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{12}H_{18}Cl_2N_2O \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
  [NOTE—Alternatively, *Infrared Absorption* ⟨197A⟩ may be used.]
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* **⟨191⟩:** Meets the requirements

ASSAY

- **PROCEDURE**
  Sample solution: Dissolve 0.25 g in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.
  Analysis: Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Read the volume added between the two points of inflection. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 31.37 mg of $C_{12}H_{18}Cl_2N_2O \cdot HCl$.
  Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%, from 1 g
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

Organic Impurities

- **PROCEDURE**
  Buffer: Dissolve 3.0 g of sodium 1-decanesulfonate and 5.0 g of monobasic potassium phosphate in 900 mL of water, adjust with dilute phosphoric acid (1 in 10) to a pH of 3.0, and dilute with water to 1000 mL.
  Mobile phase: Acetonitrile, methanol, and *Buffer* (2:2:6)
  System suitability solution: 0.2 mg/mL each of USP Clenbuterol Related Compound B RS and Clenbuterol Hydrochloride in *Mobile phase*
  Sample solution 1: 2.0 mg/mL Clenbuterol Hydrochloride in *Mobile phase*
  Sample solution 2: 2.0 µg/mL Clenbuterol Hydrochloride in *Mobile phase*, from *Sample solution 1*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.0-mm × 12.5-cm; 5-µm packing L1
  Temperature: 40°
  Flow rate: 0.5 mL/min
  Injection size: 5 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 4.0 between clenbuterol related compound B and clenbuterol
  Relative standard deviation: NMT 2.0% for the clenbuterol peak
  Analysis
  Samples: *Sample solution 1* and *Sample solution 2*
  Calculate the percentage of impurities in the portion of $C_{12}H_{18}Cl_2N_2O \cdot HCl$ taken:

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

  r_U = peak response for each impurity from *Sample solution 1*
  r_S = peak response for clenbuterol from *Sample solution 2*
  C_S = concentration of Clenbuterol Hydrochloride in *Sample solution 1* (mg/mL)
  C_U = concentration of Clenbuterol Hydrochloride in *Sample solution 2* (mg/mL)
  Acceptance criteria
  Individual impurities: 0.1%
  Total impurities: NMT 0.2%
  [NOTE—The reporting level for impurities is 0.05%.]

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩**
  Sample: 30 mg/mL in water, filter as necessary
  Acceptance criteria: –10° to +10° at 20°
- **pH ⟨791⟩:** 5.0–7.0
  Sample: 50 mg/mL in carbon dioxide-free water
- **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 1.0%

ADDITIONAL REQUIREMENTS

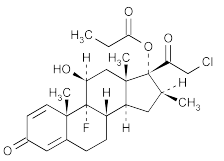
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Clenbuterol Hydrochloride RS
  USP Clenbuterol Related Compound B RS■2S (*USP33*)

BRIEFING

Clobetasol Propionate. *USP 32* page 1976. Because Clobetasol Propionate is practically insoluble in water, it is proposed to specify that *Method II* be used for the *Heavy Metals* test. Additionally, it is proposed to specify that a platinum crucible be used for the *Residue on Ignition* test because porcelain, quartz, or silica crucibles may be eroded by hydrofluoric acid formed when clobetasol propionate is degraded, thereby resulting in aberrant results.

(MD-PS: D. Vicchio.) RTS—C39404

Clobetasol Propionate



$C_{25}H_{32}ClFO_5$ 466.97

Pregna-1,4-diene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (11β,16β)-;
21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [25122-46-7; 25122-41-2].

DEFINITION

Clobetasol Propionate contains NLT 97.0% and NMT 102.0% of $C_{25}H_{32}ClFO_5$, calculated on the dried basis.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨197M⟩

ASSAY

- **PROCEDURE**

Solution A: 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile, methanol, and *Solution A* (19:4:17)

Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol

Standard solution: Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate.

System suitability solution: 0.001 mg of USP Clobetasol Propionate Related Compound A RS and 0.1 mg of USP Clobetasol Propionate RS/mL of *Mobile phase*

Sample solution: Transfer 4 mg of Clobetasol Propionate to a 100-mL volumetric flask, add 40.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

Column efficiency: NLT 5000 theoretical plates for the clobetasol peak

Tailing factor: NMT 2.0 for the clobetasol peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of $C_{25}H_{32}ClFO_5$ in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

Change to read:

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%, ■using a platinum crucible■2S *(USP33)*
- **HEAVY METALS,** ■*Method II*■2S *(USP33)* ⟨231⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE**

Solution A, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sample solution: 0.1 mg/mL of Clobetasol Propionate in *Mobile phase*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Clobetasol Proprionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity

r_T = sum of the areas of all of the peaks

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.5%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** Approximately 196°
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** +98° to +104° at 20°

Sample solution: 10 mg/mL in dioxane

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Clobetasol Propionate RS

USP Clobetasol Propionate Related Compound A RS

BRIEFING

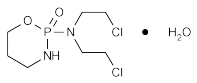
Cyclophosphamide, *USP 32* page 2040. On the basis of comments received, it is proposed to make the following changes:

1. *Identification* test *B* is revised to reflect the change in the *Assay*.
2. The internal standard is removed from the *Assay* and relevant changes are proposed.
3. New *Organic Impurities* section is proposed.
4. New *Limit of Chloride* section is proposed.
5. New *Limit of Phosphate* section is proposed.
6. The sections for *Bacterial Endotoxins Test*, *Sterility Tests*, and *Labeling* are added for sterile Cyclophosphamide.
7. New USP Reference Standards are introduced for the proposed tests.

(MD-OOD: F. Mao. MSA: R. Tirumalai.) RTS—C73270

In-Process Revision

Cyclophosphamide



$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ 279.10
$C_7H_{15}Cl_2N_2O_2P$ 261.09

2H-1,3,2-Oxazaphosphorin-2-amine, *N*,*N*-bis(2-chloroethyl)tetrahydro-, 2-oxide, monohydrate, (±);
(±)-2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate [6055-19-2].
Anhydrous [50-18-0].

DEFINITION

Cyclophosphamide contains NLT 97.0% and NMT 103.0% of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.
[**CAUTION**—Great care should be taken in handling Cyclophosphamide, as it is a potent cytotoxic agent.]

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Change to read:

• **B.** ~~The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.~~■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S (USP33)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Acetonitrile and water (3:7)

~~**Internal standard**~~■**Ethylparaben**■2S (USP33) **solution:** Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.

~~**Standard**~~■**System suitability**■2S (USP33) **solution:** Transfer USP Cyclophosphamide RS, equivalent to 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask. Add 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of ~~Internal standard~~■*Ethylparaben*■2S (USP33) *solution*, and dilute with water to volume. ~~, and mix to obtain a *Standard solution* having a concentration of 0.5 mg/mL of anhydrous cyclophosphamide~~■2S (USP33)

■**Standard solution:** 0.5 mg/mL of USP Cyclophosphamide RS in water■2S (USP33)

Sample solution: ~~Nominally equivalent to 1 mg/mL of anhydrous cyclophosphamide from Cyclophosphamide. Shake for 5 min. Pipet 25 mL of this resultant solution and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, and dilute with water to volume.~~■0.5 mg/mL of Cyclophosphamide in water■2S (USP33)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL

System suitability
Sample: ~~Standard~~■*System suitability*■2S (USP33) *solution*
[NOTE—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between cyclophosphamide and ethylparaben
Relative standard deviation: NMT 2% from six replicate injections, cyclophosphamide peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_7H_{15}Cl_2N_2O_2P$ in the portion of Cyclophosphamide taken:

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$~~

■

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$■2S (USP33)

~~R_U = peak response ratio of cyclophosphamide to the ethylparaben peak in the *Sample solution*~~
~~R_S = peak response ratio of cyclophosphamide to the ethylparaben peak in the *Standard solution*~~
~~C_S = concentration of the *Standard solution* (mg/mL)~~
~~C_U = concentration of Cyclophosphamide in the *Sample solution* (mg/mL)~~

■
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL) [NOTE—Concentration is calculated on the anhydrous basis.]
C_U = concentration of Cyclophosphamide in the *Sample solution* (mg/mL) [NOTE—Nominal concentration is calculated on the anhydrous basis.]
■2S (USP33)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **HEAVY METALS** ⟨231⟩**:** NMT 20 ppm
Sample solution: 40 mg/mL, and filter if necessary

Add the following:

■**Organic Impurities**

• **PROCEDURE 1: LIMIT OF PROPANOLAMINE**
Diluent: Methylene chloride and dehydrated alcohol (17:3)
Standard solution: 12.5 µg/mL of USP Propanolamine RS in *Diluent*. [NOTE—Propanolamine in the *Standard solution* is 0.025% of Cyclophosphamide in the *Sample solution*.]
Sample solution: 50 mg/mL of Cyclophosphamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.1-mm layer of chromatographic silica gel
Application volume: 2 µL
Developing solvent system A: Toluene, methylene chloride, and methanol (5:5:1). Prepare at time of use.
Developing solvent system B: Methanol and glacial acetic acid (9:1)
Solution A: Hydrochloric acid and water (7:18)
Solution B: 5 g/L of potassium permanganate in water
Reagent A: *Solution A* and *Solution B* (1:1). Prepare at time of use.
Reagent B: 100 mg of tetramethylbenzidine in 2.5 mL of methylene chloride and diluted with cyclohexane to 100 mL
Analysis
Samples: *Standard solution* and *Sample solution*
Develop with *Developing solvent system A* over a path of 7 cm followed by air drying for 15 min. Develop again in *Developing solvent system B* over a path of 2 cm following by air drying for NLT 10 min. [NOTE—Transfer *Developing solvent system B* to the chamber 15 min before development.] Dry the plate at 45° under a vacuum for 50 min. Place the plate in a closed chromatography tank containing *Reagent A* and leave the plate in the tank for 10 min. Remove the plate and place it in a fume hood for 10 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Remove it from *Reagent B* and wait for 15 min, evaluate it with a suitable densitometer, equipped with

a filter having its maximum transmittance at 375 nm, and locate and scan the spot produced by propanolamine from the *Standard solution* and any spot from the *Sample solution* having the same R_F as that produced by propanolamine from the *Standard solution*.

Acceptance criteria

Propanolamine: The spot of propanolamine from the *Sample solution* is not more intense than the spot of propanolamine from the *Standard solution* (0.025%).

• **PROCEDURE 2: LIMIT OF DEGRADATION PRODUCTS**

Diluent: Methanol and water (1:1)

Standard solution A: 12 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*

Standard solution B: 12 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*

Standard solution C: 12 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*

Standard solution D: 15 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*.

[NOTE—Cyclophosphamide Related Compound D is free base (M_r = 260.66) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt (M_r = 333.58).]

Standard solution E: 12 µg/mL of USP Cyclophosphamide RS in *Diluent*

Sample solution: 20 mg/mL of Cyclophosphamide in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL. [NOTE—Prepare at time of use.]

Developing solvent system: Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)

Reagent A: 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1). [NOTE—Prepare at time of use.]

Reagent B: Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol and dilute with cyclohexane to 200 mL.

Analysis

Samples: *Standard solution A* to *Standard solution E* and *Sample solution*

[NOTE—Apply *Standard solution E* after the plate development in the *Developing solvent system*, proceed as directed in the *Analysis* below.]

Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in the fresh portion of the *Developing solvent system* over a path of 10 cm following drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate at 50° under a vacuum for 20 min. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank containing *Reagent A* and leave the plate in the tank for 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Examine the plate by visual evaluation.

Acceptance criteria

The spot of cyclophosphamide related compound A from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A*.

The spot of cyclophosphamide related compound B from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B*.

The spot of cyclophosphamide related compound C from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C*.

The spot of cyclophosphamide related compound D from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D*.

The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E*.

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D[a]	0.15	0.06
Cyclophosphamide related compound C[b]	0.20	0.06
Cyclophosphamide related compound B[c]	0.43	0.06
Cyclophosphamide related compound A[d]	0.90	0.06
Any unspecified impurity	—	0.06

[a] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.
[b] 3-Aminopropyl dihydrogen phosphate.
[c] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
[d] Bis(2-chloroethyl)amine hydrochloride.

■2S (*USP33*)

SPECIFIC TESTS

Add the following:

■• **LIMIT OF CHLORIDE**

Sample solution: Dissolve 2.0 g of Cyclophosphamide in 30 mL of water, and add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.

Analysis: Titrate potentiometrically with 0.01 N silver nitrate VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion.

Calculate the percentage of chloride in the portion of Cyclophosphamide taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/[TN \times W \times (100 - A)/100]$$

V = sample titrant volume (mL)
B = blank titrant volume (mL)
N = titrant normality
F = equivalence factor, 0.355 mg of chloride ion/mL of TN
TN = theoretical normality, 0.01 N
W = sample weight (mg)
A = assay correction for water

Acceptance criteria: NMT 0.033%■2S (*USP33*)

Add the following:

■• **LIMIT OF PHOSPHATE**

Diluent: 0.2 g/mL of hydrochloric acid in water

Solution A: Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Standard stock solution: 0.72 g/L of monobasic potassium phosphate. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Prepare immediately before use.

Standard solution: *Standard stock solution* and water (1:49). Prepare immediately before use. [NOTE—This solution contains 100 µg/L of PO_4.]

In-Process Revision

Sample solution: 1 g/L of Cyclophosphamide in water
Analysis: To the *Sample solution* add 4 mL of sulfomolybdic acid TS. Shake and add 0.1 mL of *Solution A*. Prepare a standard in the same manner using the *Standard solution*. After 10 min, compare the colors using 20 mL of each solution in color comparison tubes in diffused daylight, viewing vertically against a white background.
Acceptance criteria: Any color from the *Sample solution* is not more intense than that from the *Standard solution* (NMT 0.01%).■2S *(USP33)*

Add the following:

■• **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** Where the label states that Cyclophosphamide is sterile, it contains NMT 0.0625 USP Endotoxin Unit/mg of cyclophosphamide.■2S *(USP33)*

Add the following:

■• **STERILITY TESTS ⟨71⟩:** Where the label states that Cyclophosphamide is sterile, it meets the requirements.■2S *(USP33)*
- **pH ⟨791⟩:** 3.9–7.1, in a solution (1 in 100), determined 30 min after its preparation
- **WATER DETERMINATION,** *Method I* **⟨921⟩:** 5.7%–6.8%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.

Add the following:

■• **LABELING:** Where the label states that Cyclophosphamide is sterile, the tests for *Bacterial Endotoxins Test* ⟨85⟩ and *Sterility Tests* ⟨71⟩ should be performed.■2S *(USP33)*

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Cyclophosphamide RS
  ■USP Cyclophosphamide Related Compound A RS
  USP Cyclophosphamide Related Compound B RS
  USP Cyclophosphamide Related Compound C RS
  USP Cyclophosphamide Related Compound D RS
  USP Endotoxin RS
  USP Propanolamine RS■2S *(USP33)*

BRIEFING

Diclofenac Sodium Delayed-Release Tablets, *USP 32* page 2124, page 3921 of the *First Supplement*, and page 271 of *PF* 35(2) [Mar.–Apr. 2009]. In the *Buffer Stage* of the *Dissolution* test, it is proposed to correct the concentration of the *Standard solution*.

(BPC: M. Marques.) RTS—C76056

Diclofenac Sodium Delayed-Release Tablets

DEFINITION
Diclofenac Sodium Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION
- **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* **⟨191⟩:** It meets the requirements of the flame test.

ASSAY
- **PROCEDURE**
  Solution A: Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate component to a pH of 2.5 ± 0.2.
  Mobile phase: Methanol and *Solution A* (7:3)
  [NOTE—Increasing the proportion of buffer increases resolution.]
  Diluent: Methanol and water (7:3)
  System suitability solution: 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*
  Standard solution: 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*
  Sample solution: Transfer 20 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of 0.75 mg/mL of diclofenac sodium is obtained. Add *Diluent* to about 70% of the capacity of the flask, and shake by mechanical means for NLT 30 min to disintegrate the Tablets. Cool to room temperature, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Sample solution*.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L7 (end-capped)
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.2 between diethyl phthalate and diclofenac related compound A; NLT 6.5 between diclofenac related compound A and diclofenac, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{14}H_{10}Cl_2NNaO_2$ in the portion of Tablets taken:

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

  r_U = peak response of diclofenac from the *Sample solution*
  r_S = peak response of diclofenac from the *Standard solution*
  C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION ⟨711⟩:** Proceed as directed for *Procedure*, *Apparatus 1 and Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B* to determine the amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved.

In-Process Revision

Acid stage
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm, paddles constructed of (or coated with) polytef being used
Time: 2 h
Detector: UV maxima at about 276 nm
Standard solution: Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with a mixture of 0.1 N hydrochloric acid and 5 N sodium hydroxide (900:20) to volume, and mix. This *Standard solution* contains 13.6 µg/mL of USP Diclofenac Sodium RS.
Sample solution: At the end of 2 h, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessels, and subject them to the test under *Buffer Stage*. To the 0.1 N hydrochloric acid remaining in each vessel, add 20.0 mL of 5 N sodium hydroxide, and stir for 5 min.
Buffer stage
Medium: *pH 6.8 phosphate buffer*; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV maxima at about 276 nm
Solution A: 76 mg/mL of tribasic sodium phosphate
pH 6.8 phosphate buffer: *Solution A* and 0.1 N hydrochloric acid (1:3), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05, if necessary
Standard solution: ~~Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, and dilute with *Medium* to volume. This *Standard solution* contains about 0.02 mg/mL of USP Diclofenac Sodium.~~
■Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask. Add 10.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Buffer stage Medium* to volume, and mix. The final concentration is ~~0.22~~ ■about 0.0204■2S *(USP33)* mg/mL of diclofenac sodium.■1S *(USP33)*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: NLT 75% (Q) of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES
Organic Impurities
• **PROCEDURE**
Solution A, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.8 mg/mL of USP Diclofenac Related Compound A RS in methanol
Standard solution: 4 µg/mL of USP Diclofenac Related Compound A RS from the *Standard stock solution* in *Diluent*
Analysis: Measure the peak responses over a period of 40 min.
Calculate the percentage of diclofenac related compound A in relation to the quantity of diclofenac sodium in the portion of Tablets taken:

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

r_U = peak response of diclofenac related compound A from the *Sample solution*
r_S = peak response of diclofenac related compound A from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)
Calculate the percentage of each other impurity, other than diethyl phthalate, if present, in relation to the diclofenac sodium in the portion of Tablets taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for each impurity obtained from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)
Acceptance criteria
Individual impurities: NMT 0.5% of diclofenac related compound A; NMT 1.0% of any other individual impurity
Total impurities: NMT 1.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Diclofenac Sodium RS
USP Diclofenac Related Compound A RS

BRIEFING

Diclofenac Sodium Extended-Release Tablets, *USP 32* page 2125. On the basis of comments received, it is proposed to revise the *Assay* procedure and add an *Organic Impurities* test to monitor the impurities present. The liquid chromatographic procedure in the *Assay* and in the test for *Organic Impurities* is based on analyses performed with a Zorbax C-8 brand of L7 column. The typical retention time for diclofenac is about 12.5 min.

(MD-CCA: C. Anthony.) RTS—C41893

Diclofenac Sodium Extended-Release Tablets

DEFINITION
Diclofenac Sodium Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION
• **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
Standard solution: 2.0 mg/mL of USP Diclofenac Sodium RS in methanol. [NOTE—Shake by mechanical means for 10 min before makeup to final volume.]
Sample solution: Equivalent to 2.0 mg/mL of diclofenac sodium from a portion of the powder (NLT 10 Tablets) in methanol. [NOTE—Sonicate for 10 min, and shake by mechanical means for 10 min before make up to final volume. Centrifuge this solution, and use the clear supernatant as the *Sample solution*.]
Developing solvent system: Methanol, toluene, and glacial acetic acid (8:12:0.1)

In-Process Revision

ASSAY

Change to read:

- **PROCEDURE**

[NOTE—Protect the *Standard solution*, *System suitability solution*, and *Sample solution* from light.]

Diluent: ~~Acetonitrile and water (43:57)~~■Methanol and water (7:3)■2S *(USP33)*

~~**Solution A:** Dissolve 6.8 g of monobasic potassium phosphate in 950 mL of water, adjust with dilute phosphoric acid or dilute potassium hydroxide solution to a pH of 4.0 ± 0.05, and dilute with water to 1 L.~~

■**Buffer:** 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. Adjust with appropriate component to a pH of 2.5.■2S *(USP33)*

Mobile phase: ~~Acetonitrile, tetrahydrofuran, and *Solution A* (43:2:57)~~■Methanol and *Buffer* (7:3)■2S *(USP33)*

~~**Diclofenac related compound A solution:** 200 µg/mL of USP Diclofenac Related Compound A RS in *Diluent*~~

■■2S *(USP33)*

Standard solution: ~~200~~■0.5■2S *(USP33)* mg/mL of USP Diclofenac Sodium RS in *Diluent*

~~**System suitability solution:** *Standard solution*, *Diclofenac related compound A solution*, and *Diluent* (2:1:1)~~

■**Resolution solution:** 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*■2S *(USP33)*

Sample solution: Powder NLT 20 Tablets, and transfer a weighed portion of the powder, equivalent to 100 mg of diclofenac sodium, to a ~~100~~■200■2S *(USP33)*-mL volumetric flask~~, add 50~~■and add 150■2S *(USP33)* mL of *Diluent*~~., sonicate for 15 min, then shake by mechanical means for 15 min. Add a few drops of methanol to remove the foam, and dilute with *Diluent* to volume. Transfer 10.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with *Diluent* to volume.~~■Heat on a steam bath for 3–5 min, and sonicate for 20 min. Cool to room temperature, and dilute with *Diluent* to volume. Place the flask in an ice bath for 45 min, shaking occasionally to precipitate out any undissolved waxy material. Pass a portion of the chilled solution through a filter of 0.45-µm or finer porosity. Allow the filtrate to reach room temperature before using.■2S *(USP33)*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × ~~15-cm; 5-µm packing L1~~■25-cm; packing L7■2S *(USP33)*

Flow rate: ~~1.5~~■1■2S *(USP33)* mL/min

Injection size: ~~20~~■10■2S *(USP33)* µL

System suitability

Samples: *Standard solution* and ~~*System suitability*~~■*Resolution*■2S *(USP33)* *solution*

~~[NOTE—Inject 40 µL of *System suitability solution*, and 20 µL of *Standard solution*.]~~■■2S *(USP33)*

[NOTE—The relative retention times for ■diethyl phthalate,■2S *(USP33)* diclofenac related compound A, and diclofenac are ~~0.9~~■0.5, 0.6,■2S *(USP33)* and 1.0, respectively.]

Suitability requirements

Resolution: NLT ~~2.0~~■2.2■2S *(USP33)* between the diethyl phthalate and diclofenac related compound A peaks, ~~*System suitability*~~■and NLT 3.8 between the diclofenac related compound A and the diclofenac peaks, *Resolution*■2S *(USP33)* solution

~~**Tailing factor:** NMT 2.0 for the relative diclofenac peak, *Standard solution*~~

■■2S *(USP33)*

Relative standard deviation: NMT 2.0% for diclofenac, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{14}H_{10}Cl_2NNaO_2$ in the portion of Tablets taken:

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

r_U = peak response of diclofenac from the *Sample solution*

r_S = peak response of diclofenac from the *Standard solution*

C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩

Test 1

Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL

Apparatus 2: 50 rpm; use wire sinkers

Times: 1, 5, 10, 16, and 24 h

Detector: UV 276 nm

Standard solution: USP Diclofenac Sodium RS in *Medium*

Analysis: Pass portions of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Tolerances: The percentages of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	15%–35%
5	45%–65%
10	65%–85%
16	75%–95%
24	NLT 80%

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, and **Analysis:** Proceed as directed in *Dissolution Test 1*.

Times: 1, 2, 4, 6, and 10 h

Tolerances: The percentages of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	NMT 28%
2	20%–40%
4	35%–60%
6	50%–80%
10	NLT 65%

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium and **Analysis:** Proceed as directed in *Dissolution Test 1*.

Apparatus 1: 100 rpm

Times: 2, 4, 8, and 16 h

Tolerances: The percentages of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	22%–42%
4	34%–61%
8	52%–82%
16	NLT 73%

•**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium and **Analysis:** Proceed as directed in *Dissolution Test 1*.
Apparatus 1: 100 rpm
Times: 2, 4, 8, and 16 h
Tolerances: The percentages of the labeled amount of $C_{14}H_{10}Cl_2NNaO_2$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	20%–40%
4	35%–55%
8	60%–85%
16	NLT 75%

• (RB 1-Mar-2009)
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Add the following:

▪**Organic Impurities**
• **PROCEDURE**
Diluent, Buffer, Mobile phase, Resolution solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
Standard stock solution: 0.8 mg/mL of USP Diclofenac Related Compound A RS in *Diluent* in methanol
Standard solution: 4 µg/mL of USP Diclofenac Related Compound A RS by diluting a measured volume of *Standard stock solution* with *Diluent*
System suitability solution: 0.5 mg/mL of USP Diclofenac Sodium RS in *Diluent*
System suitability
Samples: *Resolution solution* and *System suitability solution*
[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks, and NLT 3.8 between the diclofenac related compound A and the diclofenac peaks, *Resolution solution*
Standard deviation: NMT 2.0% for the diclofenac peak, *System suitability solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for any impurity from the *Sample solution*
r_S = peak response for USP Diclofenac Related Compound A RS from the *Standard solution*
C_S = concentration (mg/mL) of USP Diclofenac Related Compound A in the *Standard solution*
C_U = concentration (mg/mL) of diclofenac sodium in the *Sample solution*
Acceptance criteria
Total impurities: NMT 1.5%▪2S *(USP33)*

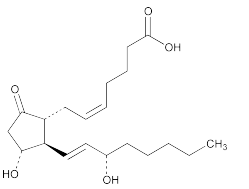
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature, and protect from light.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Diclofenac Related Compound A RS
USP Diclofenac Sodium RS

BRIEFING

Dinoprostone, *USP 32* page 2172. On the basis of comments received that the acceptance criteria for "any other impurity" is overly restrictive, it is proposed to revise *Impurity Table 1* in the test for *Organic Impurities* as follows:
1. Change the term "any other impurity" to "any individual unspecified impurity".
2. Change the acceptance criteria for unspecified impurities from NMT 0.1% total present to NMT 0.10% each.

(MD-PS: D. Vicchio.) RTS—C74667

Dinoprostone



$C_{20}H_{32}O_5$ 352.47
Prosta-5,13-dien-1-oic acid, 11,15-dihydroxy-9-oxo-, (5*Z*,11α,13*E*,15*S*)-;
(*E*,*Z*)-(1*R*,2*R*,3*R*)-7-[3-Hydroxy-2-[(3*S*)-(3-hydroxy-1-octenyl)]-5-oxocyclopentyl]-5-heptenoic acid;
Prostaglandin E_2 [363-24-6].

DEFINITION
Dinoprostone contains NLT 97.0% and NMT 103.0% of $C_{20}H_{32}O_5$.
[NOTE—Prepare all solutions in all tests immediately before use.]

IDENTIFICATION
• **A. INFRARED ABSORPTION ⟨197K⟩**
• **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and 0.2% acetic acid (29:21)
Standard solution: 2.5 mg/mL of USP Dinoprostone RS in *Mobile phase*
Sample solution: 2.5 mg/mL of Dinoprostone in *Mobile phase*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability.*)

In-Process Revision

Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between dinoprostone and any other adjacent peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{32}O_5$ in the portion of Dinoprostone taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dinoprostone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0%

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.5%

Change to read:

Organic Impurities

• **PROCEDURE**

Mobile phase: Proceed as directed in the *Assay*.
Standard stock solution: Prepare as directed for the *Standard solution* in the *Assay*.
Standard solution: Transfer 0.5 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Prepare as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard stock solution* and *Sample solution*
Suitability requirements
Column efficiency: NLT 6000 theoretical plates, *Standard stock solution*
Relative standard deviation: NMT 2.0%, *Standard stock solution*
Resolution: NLT 1.0 between dinoprostone and any other adjacent peak, *Sample solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dinoprostone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for dinoprostone from the *Standard solution*
C_S = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dinoprostone in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1* for values)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
15-Oxo-dinoprostone	0.79	5	—*
15-Epi-dinoprostone	0.85	1.1	—*
8-Isodinoprostone	0.90	1.0	—*
5,6-*trans*-Dinoprostone	1.15	1.0	2.0
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,10,13-triene-1-oic acid	1.80	5	1.0
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,8(12),13-trien-1-oic acid	1.90	1.43	1.0
~~Any other impurity~~■Any individual unspecified impurity■2S *(USP33)*	—	1.0	~~0.1 of total other impurities present~~ ■0.10■2S *(USP33)*

* The sum of these three impurities is NMT 1.0%.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781**⟩**:** –82.0° to –90.0°, at 20°
Sample solution: 5 mg/mL, in alcohol
• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Dinoprostone RS

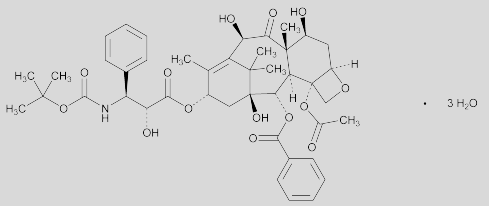
BRIEFING

Docetaxel. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is being proposed. The liquid chromatography procedure for the *Assay* and for *Organic Impurities* is based on analyses performed with a Waters SunFire C18, 3.5-µm brand of L1 column. The typical retention time reported for docetaxel is about 26.5 min.

(MDOOD-05: F. Mao. MSA-05: R. Tirumalai.) RTS—C67219

Add the following:

■Docetaxel



$C_{43}H_{53}NO_{14} \cdot 3H_2O$ 861.93

Benzenepropanoic acid, β-[[(1,1-dimethylethoxy)carbonyl]amino]-α-hydroxy-, 12b-(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl ester trihydrate, [2a*R*-[2aα,4β,4aβ,6β,9α(α*R**,β*S**),11α,12α,12aα,12bα]]-. ;(2*R*,3*S*)-*N*-Carboxy-3-phenylisoserine, *N*-*tert*-butyl ester, 13-ester with 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate [148408-66-6].

DEFINITION

Docetaxel contains NLT 97.5% and NMT 102.0% of $C_{43}H_{53}NO_{14}$, calculated on the anhydrous and solvent-free basis.

[**CAUTION—**Docetaxel is cytotoxic. Great care should be taken to prevent inhaling particles of Docetaxel and exposing the skin to it.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197S**⟩
  Sample solution: 60 mg/mL in methylene chloride
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
  Solution A: Water
  Solution B: Acetonitrile
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	72	28
9.0	72	28
39.0	28	72
39.1	72	28
50	72	28

Diluent: Acetonitrile, water, and acetic acid (100:100:0.1)
Standard solution: 1.0 mg/mL made by transferring a quantity of USP Docetaxel RS to a suitable volumetric flask, dissolving in alcohol, equivalent to about 5% of the final volume, and diluting with *Diluent* to volume
System suitability solution: 1 mg/mL of USP Docetaxel Identification RS in *Diluent*. [NOTE—USP Docetaxel Identification RS contains docetaxel and a small amount of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. See *Impurity Table 1*.]
Sample solution: 1.0 mg/mL made by transferring a quantity of Docetaxel to a suitable volumetric flask, dissolving in alcohol, equivalent to about 5% of the final volume, and diluting with *Diluent* to volume
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Refrigerated autosampler temperature: 10°
Column: 4.6-mm × 15-cm column; 3.5-µm packing L1
Column temperature: 45°
Flow rate: 1.2 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 4 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{43}H_{53}NO_{14}$ in the portion of Docetaxel taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of docetaxel in the *Standard solution* (mg/mL)
C_U = nominal concentration of Docetaxel in the *Sample solution* (mg/mL)
Acceptance criteria: 97.5%–102.0%, calculated on the anhydrous and solvent-free basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS** *Method I* ⟨**231**⟩
  Sample solution: Dissolve 1 g in 20 mL of a mixture of dimethylformamide and water (17:3). To 12 mL of this solution, add 2 mL of *pH 3.5 Acetate Buffer* and mix. Add 1.2 mL of thioacetamide–glycerin base TS and mix.
  Acceptance criteria: NMT 20 ppm

Organic Impurities

- **PROCEDURE**
  Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Sensitivity solution: 0.5 µg/mL of USP Docetaxel RS in *Diluent*, from *Standard solution*
  System suitability
  Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
  Suitability requirements:
  Resolution: NLT 4 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
  Signal-to-noise: NLT 10 for docetaxel peak, *Sensitivity solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Sample: *Sample solution*
  Calculate the percentage of each impurity in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*
r_T = sum of the responses of all peaks from the *Sample solution*
F = relative response factor for each individual impurity, see *Impurity Table 1.*
Acceptance criteria
Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

In-Process Revision

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Limit (%)
2-Debenzoxyl 2-pentenoyl docetaxel[a]	0.97	0.63	0.5
Docetaxel	1.00	—	—
6-Oxodocetaxel [b]	1.08	1.0	0.3
4-Epidocetaxel [c]	1.13	1.0	0.3
4-Epi-6-oxodocetaxel [d]	1.18	1.0	0.2
Any Unspecified Impurity	—	—	0.10

[a] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-[(*E*)-2-methylbut-2-enoate], 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
[b] (2a*R*,4*S*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
[c] (2a*R*,4*R*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
[d] (2a*R*,4*R*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS ⟨61⟩:** The total aerobic microbial limit does not exceed 100 cfu/g. The total yeast and mold count does not exceed 10 cfu/g.
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains not more than 0.3 USP Endotoxin Units/mg.
- **WATER DETERMINATION,** *Method Ic* **⟨921⟩:** 5.0%–7.0%
- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩:** –39° to –41° (t=20°), calculated on the anhydrous and solvent-free basis.
  Sample solution: 10 mg/mL in methanol

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well closed, light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Docetaxel RS
  USP Docetaxel Identification RS
  USP Endotoxin RS■2S *(USP33)*

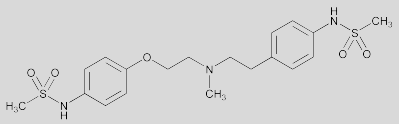
BRIEFING

Dofetilide. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods, is being proposed. The liquid chromatographic procedures in the test for *Organic impurities* are based on analyses performed with a Symmetry brand of 5-µm L1 column. The typical retention time for dofetilide is about 11 min. The liquid chromatographic procedures in the *Assay* are based on analyses performed with a Novapak brand of 4-µm L7 column. The typical retention time for dofetilide is about 17 min.

(MDCV: S. Ramakrishna.) RTS—C62478

Add the following:

■Dofetilide



$C_{19}H_{27}N_3O_5S_2$ 441.56

Methanesulfonamide, *N*-[4-[2-[methyl[2-[4-[(methylsulfonyl)amino]phenoxy]ethyl]amino]ethyl]phenyl]-; *β*-[(*p*-Methanesulfonamidophenethyl)methylamino]methanesulfono-*p*-phenetidide [115256-11-6].

DEFINITION

Dofetilide contains NLT 97.0% and NMT 103.0% of $C_{19}H_{27}N_3O_5S_2$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION, ⟨197K⟩**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Potassium hydroxide solution: 0.56 g/mL of potassium hydroxide
Buffer: Prepare a solution of 1.36 g of monobasic potassium phosphate and 5 mg of ascorbic acid in 1 L of water. Adjust with *Potassium hydroxide solution* to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (1:3)
System suitability solution: 25 µg/mL of USP Dofetilide RS and 0.5 µg/mL of USP Dofetilide Related Compound A RS in *Mobile phase*
Standard solution: 25 µg/mL of USP Dofetilide RS in *Mobile phase*
Sample solution: 25 µg/mL of Dofetilide in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 15-cm; 4-µm packing L7
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements:
Resolution: NLT 8.0 between dofetilide and dofetilide related compound A, *System suitability solution*
Relative standard deviation NMT 2.0%, *Standard solution*
Analysis:
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{27}N_3O_5S_2$ in the portion of Dofetilide taken:

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

r_U = peak area of dofetilide from the *Sample solution*
r_S = peak area of dofetilide from the *Standard solution*
C_S = concentration of dofetilide in the *Standard solution* (µg/mL)
C_U = concentration of Dofetilide in the *Sample solution* (µg/mL)
Acceptance criteria: 97.0%–103.0% on the anhydrous and solvent-free basis

In-Process Revision

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS ⟨231⟩,** *Method II*: NMT 20 ppm

Organic Impurities

- **PROCEDURE**

Buffer: 0.78 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1.
Diluent: Acetonitrile and *Buffer* (22:3)
Solution A: *Buffer*
Solution B: Methanol
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	88	12
5	88	12
25	70	30
30	70	30

System suitability solution: 1.25 µg/mL each of USP Dofetilide RS and USP Dofetilide Related Compound A RS in *Diluent*
Standard solution: 1.25 µg/mL of USP Dofetilide RS in *Diluent*
Diluted standard solution: 0.125 µg/mL of USP Dofetilide RS in *Diluent* from the *Standard solution*
Sample solution: 0.25 mg/mL of Dofetilide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
System suitability requirements:
Samples: *System suitability solution* and *Diluted standard solution*
Resolution: NLT 5.0 between dofetilide and dofetilide related compound A, *System suitability solution*
Column efficiency: NLT 35,000 theoretical plates for the dofetilide peak, *System suitability solution*
Tailing factor: NMT 1.5 for the dofetilide peak, *System suitability solution*
Relative standard deviation: NMT 10.0% for the dofetilide peak, *Diluted standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dofetilide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of dofetilide from the *Standard solution*
C_S = concentration of USP Dofetilide RS in the *Standard solution* (mg/mL)
C_U = concentration of Dofetilide in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dofetilide related compound A [a]	0.9	1.04	0.5
Dofetilide	1.0	—	—
Any other individual unspecified impurity	—	1.00[b]	0.1
Total impurities	—	—	0.5

[a] *N*-[4-(2-(2-[4-(Methanesulfonamido)phenoxy]ethylamino)ethyl)phenyl]-methanesulfonamide.
[b] Unless otherwise determined.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Dofetilide RS
USP Dofetilide Related Compound A RS■2S *(USP33)*

BRIEFING

Estradiol Transdermal System, *USP 32* page 2303, and page 225 of *PF* 33(2) [Mar.–Apr. 2007]. On the basis of comments received, the following revisions are being proposed in the test for *Drug Release*.

1. *Test 1*: The formulas used to calculate the percentage of estradiol dissolved are being revised to take into account that the amount of *Medium* withdrawn at each sampling is not being replaced. The *Tolerances* are being revised to express the results in percentage of the transdermal system label claim.
2. *Test 2*: The formulas used to calculate the percentage of estradiol dissolved and the *Tolerances* are being revised to express the results in percentage of the transdermal system label claim.
3. *Test 3* is being added. The chromatographic procedure in this test was validated with a Symmetry C18 brand of column containing packing L1 for 9 cm² transdermal systems, with estradiol having a retention time of about 9 min; and with a PartiSphere C18 brand of column containing packing L1 for 18, 27, or 36 cm² transdermal systems, with estradiol having a retention time of about 7 min.

(BPC: M. Marques.) RTS—C67685

Estradiol Transdermal System

DEFINITION

Estradiol Transdermal System contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (1:1)

Mobile phase: Acetonitrile and water (11:9)

Standard solution: 0.1 mg/mL of USP Estradiol RS in *Diluent*

Sample solution: Cut 10 Transdermal Systems into pieces, keeping the pieces from each system separate. Remove the protective liners, if any, from the strips, and discard. Transfer the pieces of each system into separate stoppered flasks of suitable size, and add a measured volume of *Diluent* to each flask to obtain solutions having a concentration of 0.1 mg/mL of estradiol. Shake by mechanical means for about 3 h, and sonicate for 15 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; packing L1

Temperature: 35°

Flow rate: 1 mL/min

Injection size: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.9–1.6

Relative standard deviation: NMT 2.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{18}H_{24}O_2$ in each Transdermal System taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Estradiol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Calculate the average quantity of estradiol (mg) in each Transdermal System. Use the individual assays to determine the uniformity of dosage units.

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ALCOHOL** (if present)

Diluent: Acetonitrile and water (1:1)

Internal standard solution: Pipet 4.0 mL of dehydrated methanol into a 100-mL volumetric flask. Dilute with water to volume.

Standard solution: Weigh, by difference, 1.6 mL of dehydrated alcohol into a tared 50-mL volumetric flask containing 15 mL of water. Dilute with *Diluent* to volume, and mix. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pipet 25.0 mL of this solution into a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with water to volume.

Sample solutions: Prepare as directed for the *Sample solutions*, with the following changes. Pipet 25.0 mL of each solution into individual 50-mL volumetric flasks. Add 5.0 mL of *Internal standard solution*, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 2-cm glass column; support S2

Temperature

Column: 100°

Injector and **detector:** 200°

Carrier gas: Helium

Flow rate: 30 mL/min

Injection size: 2 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard and alcohol are 0.4 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 1.5% from the ratios of alcohol peak areas to those of the internal standard

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of alcohol in each Transdermal System taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alcohol to internal standard from the *Sample solution*

R_S = peak response ratio of alcohol to internal standard from the *Standard solution*

C_S = concentration of dehydrated alcohol in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Calculate the average quantity of alcohol in the *Sample solution* taken.

Acceptance criteria: 80%–120% of the labeled amount of C_2H_5OH is found.

PERFORMANCE TESTS

Change to read:

▲• **DRUG RELEASE** ⟨724⟩

Test 1: For products labeled for dosing every 84 h

Medium: Water; 900 mL, deaerated

Apparatus 5: 50 rpm

Times: 24, 48, and 96 h

Mobile phase: Water and acetonitrile (3:2)

Standard solution: 9 µg/mL of USP Estradiol RS in dehydrated alcohol. Dilute this solution with *Medium* to obtain solutions having concentrations of about 0.9, 0.45, and 0.045 µg/mL.

Sample solution: At each sampling time interval, withdraw a 10-mL aliquot of the solution under test.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Fluorimetric, with excitation at 220 nm and emission at 270 nm

Column: 4.6-mm × 3-cm, packing L1

Temperature: 40°

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Tailing factor: 0.9–2.5

Relative standard deviation: NMT 3.0%, using 0.45 µg/mL of the *Standard solution*

Analysis: Plot the peak responses of the *Standard solutions* versus concentration, in µg/mL, of estradiol. From the graph determine the amount, in µg/mL, of estradiol released. ~~using the following formulas~~■Calculate the cumulative release rate as percentage of the labeled amount of estradiol:■2S *(USP33)*

~~µg of estradiol released in the interval 0 to~~■$At_{■2S\ (USP33)}$ ~~24 h:~~

~~$[900(A_1 - b)]/m$~~

■

$$\text{Result} = \{[900(A_1 - b)]/(1000 \times m \times L)\} \times 100_{■2S\ (USP33)}$$

~~µg of estradiol released in the interval 24 to~~■$At_{■2S\ (USP33)}$ ~~48 h:~~

~~$[900(A_1 - b)] - [890(A_{n-1} - b)]/480m$~~

■

$$\text{Result} = \{[890(A_2 - b) + 10(A_1 - b)]/(1000 \times m \times L)\} \times 100_{■2S\ (USP33)}$$

In-Process Revision

~~Cumulative µg of estradiol released:~~

$$\frac{900(A_n - b)}{20m} + \frac{10}{20}\left[\sum_{x=1}^{n=1}\frac{(A_x - b)}{m}\right]$$

■ At 96 h:

$$\text{Result} = \{[880(A_3 - b) + 10(A_2 - b) + 10(A_1 - b)]/(1000 \times m \times L)\} \times 100$$

■2S (*USP33*)

A_1 = peak area of estradiol in the *Sample solution* at the first time interval
A_n = peak area of estradiol in the *Sample solution* at the release interval *n*
m = slope of the calibration curve
b = y-intercept of the calibration curve■
L = Transdermal System label claim (mg)■2S (*USP33*)

Tolerances: ~~The amounts of estradiol released, as percentages of the labeled amount of the dose released to the skin~~ ■The percentage of the labeled amount of estradiol released■2S (*USP33*) at the times specified, conform to ~~*Acceptance Table 4*~~ *Acceptance Table 1*.

Time (h)	Amount Dissolved (Release Rate)
24	~~2.4% – 26.4%~~■1.2%–6.0%■2S (*USP33*)
48	~~4.8% –52.0%~~■3.0%–11.4%■2S (*USP33*)
96	~~10.0% –85.0%~~■5.0%–16.3%■2S (*USP33*)

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.
Medium: 0.005 M phosphate buffer, pH 5.5, containing 0.3% sodium lauryl sulfate; 500 mL
Apparatus 5: 100 rpm. Use a 76-mm stainless steel disk assembly. Adhere the patch to the disk assembly using ~~double-faced adhesive~~ transfer tape. [NOTE—A suitable tape is available as 3 M adhesive transfer tape 927, www.mmm.com.]
Times: 1, 4, 8, and 24 h
Mobile phase: Acetonitrile and water (1:1)
Standard stock solution: 800 µg/mL of USP Estradiol RS in acetone
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution having a known concentration close to that expected in the solution under test, assuming 100% drug release.
Sample solution: At each sampling time interval, withdraw a known volume aliquot of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: ~~4.0 mm~~ 3.9-mm × 30-cm, packing ~~4 µm~~ L1
Flow rate: 1.0 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Tailing factor: NMT 2.0
Relative standard deviation: NMT ~~2.0%~~ 3.0%
Analysis: Calculate the amount of estradiol released at each sampling time:

$$M_i = r_U/r_S \times C_S \times V_i$$

$$m_1 = M_1$$

$$m_2 = M_2 + M_1(V_a/V_1)$$

$$m_3 = M_3 + M_2(V_a/V_2) + M_1(V_a/V_1)$$

$$m_4 = M_4 + M_3(V_a/V_3) + M_2(V_a/V_2) + M_1(V_a/V_1)$$

■ Calculate the percentage of the labeled amount of estradiol released at each sampling time:

$$\text{Result} = (m_i/L) \times 100$$

M_i = amount of estradiol released into the *Medium* at a given sampling time (mg)
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V_i = corrected volume of the *Medium* at a given sampling time (mL)
m_1, m_2, m_3, m_4 = total amounts of estradiol released from the patch at given sampling times (mg)
M_1, M_2, M_3, M_4 = amounts of estradiol released into the *Medium* at given sampling times (mg)
V_a = volume of the aliquot taken from the dissolution vessel at each sampling time (mL)
V_1, V_2, V_3 = volumes of *Medium* at given sampling times (mL)
L = Transdermal System label claim (mg)
■2S (*USP33*)

Tolerances: ~~The amounts of estradiol released, as percentages of the labeled amount of the dose released to the skin,~~ ■The percentage of the labeled amount of estradiol released■2S (*USP33*) at the times specified conform to *Acceptance Table 1*.

Time (h)	Amount Dissolved (Release Rate)
1	15%–40%
4	45%–70%
8	70%–90%
24	NLT 80%

■**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.
Medium: 1% Polysorbate 40 in water; 900 mL
Apparatus 5: 50 rpm
Times: 4, 8, and 24 h
Standard stock solution: Known concentration (mg/mL) of USP Estradiol RS in methanol
Standard solution: Make dilutions of the *Standard stock solution* in *Medium* to obtain at least 5 different concentrations within the range of the expected released amounts of estradiol.

In-Process Revision

Sample solution: At each sampling time interval, withdraw a known volume aliquot of the solution under test.
Mobile phase: Acetonitrile and water (2:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm, 5-µm packing L1 for 9-cm² systems. 4.6-mm × 12.5-cm, 5-µm packing L1 for 18-, 27-, or 36-cm² systems. In any case, a guard column containing packing L1 is used.
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Relative standard deviation: NMT 2.0%
Analysis: Calculate the cumulative release rate as percentage of the labeled amount of estradiol by the formulas used in *Test 1*.
Tolerances: The percentage of the labeled amount of estradiol released at the times specified conform to the acceptance tables shown below.

Time (h)	Amount Dissolved (Release Rate)
4	40%–71%
8	58%–94%
24	NLT 75%

L1 (6 units)

Time (h)	Amount Dissolved (individual values)
4	40%–71%
8	58%–94%
24	NLT 75%

L2 (12 units)

Time (h)	Amount Dissolved (average of 12)	Amount Dissolved (individual values)
4	40%–71%	34%–77%
8	58%–94%	50%–102%
24	NLT 75%	NLT 68%

L3 (24 units)

Time (h)	Amount Dissolved (average of 24)	Amount Dissolved (individual for 22 units of 24)	Amount Dissolved (individual for 24)
4	40%–71%	34%–77%	29%–82%
8	58%–94%	50%–102%	43%–109%
24	NLT 75%	NLT 68%	NLT 60%

■2S (*USP33*)
▲*USP33*

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetic, light-resistant, unit-dose pouches.
- **LABELING:** The label states the total amount of estradiol in the Transdermal System and the release rate, in mg/day, for the duration of application of one system. When more than one *Drug Release Test* is given, the labeling states the *Drug Release Test* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Estradiol RS

BRIEFING

Estradiol and Norethindrone Acetate Tablets, *USP 32* page 2305 and page 4052 of the *First Supplement*. It is proposed to make some revisions to the *Dissolution* test to be in accordance with the conditions approved by FDA.

(BPC: M. Marques.) RTS—C56383

Estradiol and Norethindrone Acetate Tablets

DEFINITION

Estradiol and Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  Standard solution: 0.5 mg/mL of USP Estradiol RS and 0.25 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol
  Sample solution: Place 2 Tablets into a 10-mL vial, and add 0.2 mL of water. When the Tablets are partially disintegrated, add a few glass beads, and shake vigorously to disintegrate. Add 4.0 mL of dehydrated alcohol, and shake. [NOTE—Centrifuge until the supernatant is clear before application to the plate.]
  Adsorbent: 0.25-mm chromatographic silica gel plate
  Application volume: 2 µL
  Developing solvent system: Chloroform and acetone (9:1)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Proceed as directed in the chapter, using the *Developing solvent system*. Apply the samples and develop the plate. After removal of the plate, mark the solvent front, and allow the solvent to evaporate. Place the plate on a heating plate at 100° for 15 min. Allow the plate to cool, and then immerse it in a mixture of dehydrated alcohol and concentrated sulfuric acid (95:5). Place the plate on a piece of thick horizontal paper until it is almost dry. Heat the plate at 100° until it has fully developed. Examine under UV light at 365 nm.
  Acceptance criteria: The color and R_F value of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.
- **B.** The retention time and UV spectrum of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Mobile phase: Acetonitrile and water (11:9)
  Diluent: Dehydrated alcohol and water (1:1)
  Estrone standard stock solution: 0.12 mg/mL of USP Estrone RS in dehydrated alcohol
  Estradiol standard stock solution: 0.25 mg/mL of USP Estradiol RS in dehydrated alcohol
  Norethindrone acetate standard stock solution: 0.15 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol
  Standard solution: 20 µg/mL of USP Estradiol RS from *Estradiol standard stock solution* and 10 µg/mL of USP Norethindrone Acetate RS from *Norethindrone acetate standard stock solution* in *Diluent*

System suitability solution: Combine 800 µL of *Estradiol standard stock solution*, 600 µL of *Norethindrone acetate standard stock solution*, 200 µL of *Estrone standard stock solution*, and 10.0 mL of *Diluent*.
Sample solution: Add 12 Tablets into a measured amount of *Diluent* to obtain a solution having an estradiol concentration of 20 µg/mL and a norethindrone acetate concentration of 10 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: •UV Dual wavelength (254 nm/280 nm) or equivalent•$_6$
[NOTE—Thus, the absorption of estradiol at 280 nm and norethindrone acetate at 254 nm can be included in a single run by altering the wavelength.]
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
[NOTE—Perform an investigational run to determine the retention times for estradiol and norethindrone acetate.]
Injection size: 50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.8 between estradiol and estrone acetate, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Measure the areas for the estradiol and norethindrone acetate peaks.]
Calculate the quantity, as a percentage, of $C_{18}H_{24}O_2$ in each of the Tablets taken:

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

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (µg/mL)
Calculate the quantity, as a percentage, of $C_{22}H_{28}O_3$ in each of the Tablets taken:

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

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of norethindrone acetate in the *Sample solution* (µg/mL)
Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{18}H_{24}O_2$ and 90.0%–110.0% of the labeled amount of $C_{22}H_{28}O_3$

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩
Medium: 0.3% sodium lauryl sulfate; 500 mL
Apparatus 2: 50 rpm
Time: 30 min ■for Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate, and 50 min for Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate.■2S *(USP33)*
Mobile phase: Acetonitrile and water (11:9)
Standard stock solution A: 20 µg/mL of USP Estradiol RS in alcohol ■or in a mixture of alcohol and water■2S *(USP33)*
Standard stock solution B: 10 µg/mL of USP Norethindrone Acetate RS in alcohol ■or in a mixture of alcohol and water■2S *(USP33)*
Standard solution: Dilute suitable quantities of *Standard stock solution A* and *Standard stock solution B* in *Medium* ■or a mixture of alcohol and water■2S *(USP33)* to obtain a final concentration of both analytes similar to the expected concentration of the *Sample solution*.
Sample solution: Portions of the solution under test passed through a 0.45-µm filter
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 241 nm for norethindrone acetate and 280 nm for estradiol
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 150 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT ~~2.0~~■1.5■2S *(USP33)*
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{24}O_2$ and of $C_{22}H_{28}O_3$ ~~in the portion of Tablets taken~~■dissolved: ■2S *(USP33)*

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

r_U = peak response of estradiol or norethindrone acetate from the *Sample solution*
r_S = peak response of estradiol or norethindrone acetate from the *Standard solution*
C_S = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estradiol or norethindrone acetate in the *Sample solution* (mg/mL) (based on the label claim)
Tolerances: ■For Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate:■2S *(USP33)* NLT 75% (Q) of the labeled amounts of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ is dissolved ■in 30 min. For Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate: NLT 75% (Q) of the labeled amounts of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ is dissolved in 50 min.■2S *(USP33)*.

Add the following:

•• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements•$_6$

IMPURITIES

Change to read:

Organic Impurities
• **PROCEDURE**
Solution A: Tetrahydrofuran and water (1:200)
Solution B: Acetonitrile, tetrahydrofuran, and water (160:1:40)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	65	35
35	20	80
49	20	80
50	80	20
60	80	20

In-Process Revision

Diluent: Dehydrated alcohol and water (1:1)
System suitability solution: 240 µg/mL of USP Estradiol RS, 60 µg/mL of USP Norethindrone Acetate RS, and 1 µg/mL of USP Estrone RS in *Diluent*
Estradiol standard stock solution: 250 µg/mL of estradiol from USP Estradiol RS in alcohol
Norethindrone acetate standard stock solution: 150 µg/mL of norethindrone acetate from USP Norethindrone Acetate RS in alcohol
Standard solution: Combine 250 µL of *Estradiol standard stock solution* and 100 µL of *Norethindrone acetate standard stock solution*, and dilute with 50.0 mL of *Diluent*.
Sample solution: A quantity equivalent to 240 µg/mL of estradiol and 120 µg/mL of norethindrone acetate from NLT 20 Tablets in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 and 254 nm
Column: 3.9-mm × 30-cm; 4-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 100 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for estradiol, estrone, and norethindrone acetate are about 1.0, 1.4, and 3.0, respectively.]
Suitability requirements
Resolution: NLT 1.3 between estrone and estradiol, measured at 254 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any estradiol impurity in the portion of Tablets taken:

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

r_U = peak response area at 235 nm for each impurity from the *Sample solution*
r_S = peak response area at 235 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1* or *Impurity Table 2*)

••$_6$ Calculate the percentage of any norethindrone acetate related impurities in the portion of Tablets taken:

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

r_U = peak response area at 254 nm for each impurity from the *Sample solution*
r_S = peak response area at 254 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1* or *Impurity Table 2*)

Acceptance criteria: •The Tablets meet the requirements given in either *Impurity Table 1* or *Impurity Table 2*.

Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6α-Hydroxyestradiol	0.47	1.0	1.0
6β-Hydroxyestradiol	0.51	1.0	1.0
6-Ketoestradiol	0.62	1.0	1.0
6-Dehydroestradiol	0.95	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	0.5
Total estradiol related impurities	—	—	2.0
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate	0.58	1.0	1.0
Norethindrone	0.66	1.0	1.0
6-Ketonorethindrone acetate	0.79	1.8	1.0
6-Dehydronorethindrone acetate	0.97	2.2	1.0
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	0.5
Total norethindrone acetate related impurities	—	—	2.0

Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other single estradiol related impurity	—	1.0	0.5
Total estradiol related impurities	—	—	2.0
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate	0.58	1.0	1.0
Norethindrone	0.66	1.0	1.0
6-Ketonorethindrone acetate	0.79	1.8	1.0
6-Dehydronorethindrone acetate	0.97	2.2	1.0
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	0.5
Total norethindrone acetate related impurities	—	—	2.0

Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6β-Hydroxyestradiol	0.51	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	1.0
Total estradiol related impurities	—	—	2.5
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate	0.58	1.0	1.5
Norethindrone	0.66	1.0	1.0
6-Ketonorethindrone acetate	0.79	1.8	2.5
6-Dehydronorethindrone acetate	0.97	2.2	1.0
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	1.0
Total norethindrone acetate related impurities	—	—	4.0

•$_6$

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. The Tablets meet the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

In-Process Revision

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Estradiol RS
  USP Estrone RS
  USP Norethindrone Acetate RS

BRIEFING

Conjugated Estrogens. *USP 32* page 2309. On the basis of comments received, it is proposed to change the GC column temperature in the *Assay* from 220° to 208° to prevent an unknown component from co-eluting with the internal standard.

(MD-PS: D. Vicchio.) RTS—C66810

Conjugated Estrogens

DEFINITION

Conjugated Estrogens is a mixture of sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from Estrone and Equilin. It contains other conjugated estrogenic substances of the type excreted by pregnant mares. It is a dispersion of the estrogenic substances on a suitable powdered diluent.

Conjugated Estrogens contains NLT 52.5% and NMT 61.5% of sodium estrone sulfate and NLT 22.5% and NMT 30.5% of sodium equilin sulfate, and the total of sodium estrone sulfate and sodium equilin sulfate is NLT 79.5% and NMT 88.0% of the labeled content of Conjugated Estrogens. Conjugated Estrogens contains as concomitant components as sodium sulfate conjugates NLT 13.5% and NMT 19.5% of 17α-dihydroequilin, NLT 2.5% and NMT 9.5% of 17α-estradiol, and NLT 0.5% and NMT 4.0% of 17β-dihydroequilin, of the labeled content of Conjugated Estrogens.

IDENTIFICATION

- **A.** The relative retention times of the 17α-dihydroequilin, estrone, and equilin peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*
- **B.** The chromatogram of Conjugated Estrogens from the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17α-estradiol and 17β-dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-*O*-methylestrone.

ASSAY

Change to read:

- **PROCEDURE**

Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol

Stock solution: 160 µg/mL, 70 µg/mL, and 50 µg/mL each of USP Estrone RS, USP Equilin RS, and USP 17α-Dihydroequilin RS, respectively, in alcohol

Solution A: Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1, if necessary.

Standard solution: Pipet 1.0 mL of the *Stock solution* and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue, add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.

System suitability solution: Pipet 1.0 mL of a 2.0 µg/mL solution of USP Estradiol RS (17β-estradiol) in alcohol, 1.0 mL of *Stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture…".

Sample solution: Transfer a quantity of Conjugated Estrogens, equivalent to 2 mg of total conjugated estrogens, to a 50-mL centrifuge tube, fitted with a polytef-lined screw cap, containing 15 mL of *Solution A* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Place in a sonic bath for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by rapidly passing through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture…".

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 15-m fused silica capillary column; with a 0.25-µm layer of phase G19, and a split injection system

Temperature

Column: ~~220°~~■208°■2S *(USP33)*

Detector: 260°

Injector port: 260°

Carrier gas: Hydrogen

Flow rate: 2 mL/min

Split flow rate: 40–60 mL/min

Injection size: 1 µL

System suitability

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

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak at between 17 and 25 min.]

[NOTE—The relative retention times for 17β-estradiol, 17α-dihydroequilin, estrone, equilin, and 3-*O*-methylestrone are 0.29, 0.30, 0.80, 0.87, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.2 between estrone and equilin

Tailing factor: NMT 1.3 for the estrone peak

Relative standard deviation: NMT 2.0% for the estrone peak ratios, for NLT four injections of the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sodium estrone sulfate and sodium equilin sulfate in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*

R_S = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Standard solution*

C_S = concentration of USP Estrone RS or USP Equilin RS in the *Stock solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

In-Process Revision

Acceptance criteria: 52.5%–61.5% of sodium estrone sulfate and 22.5%–30.5% of sodium equilin sulfate

OTHER COMPONENTS

- **CONTENT OF 17α-DIHYDROEQUILIN, 17β-DIHYDROEQUILIN, AND 17α-ESTRADIOL** (concomitant components)

Internal standard solution, Stock solution, Solution A, System suitability solution, Standard Solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

[NOTE—The relative retention times for 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin are about 0.82, 1.00, and 1.11, respectively.]

Analysis:

Samples: *Standard solution* and *Sample solution*

Identify the peaks due to 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin in the chromatogram of the *Sample solution.* Calculate the quantities, in percentage, of 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
- R_S = ratio of the peak response of 17α-dihydroequilin to that of the internal standard from the *Standard solution*
- C_S = concentration of USP 17α-Dihydroequilin RS in the *Stock solution* (µg/mL)
- C_U = concentration of the *Sample solution* (µg/mL)
- F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

IMPURITIES

Organic Impurities

- **PROCEDURE 1: LIMITS OF 17α-DIHYDROEQUILENIN, 17β-DIHYDROEQUILENIN, AND EQUILENIN** (signal impurities)

Internal standard solution, Stock solution, Solution A, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

[NOTE—The relative retention times for dihydroequilenin, 17β-dihydroequilenin, 3-*O*-methylestrone, and equilenin are 0.56, 0.64, 1.0, and 1.3, respectively.]

Analysis:

Samples: *Standard solution* and *Sample solution*

Identify any peaks due to dihydroequilenin, 17β-dihydroequilenin, 3-*O*-methylestrone, and equilenin in the chromatogram of the *Sample solution.* Calculate the quantities, in percentage, of 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
- R_S = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*
- C_S = concentration of USP Estrone RS in the *Stock solution* (µg/mL)
- C_U = concentration of the *Sample solution* (µg/mL)
- F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: NMT 3.25%, NMT 2.75%, and NMT 5.5% of the labeled content of Conjugated Estrogens for 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin, respectively, as their sodium sulfate salts.

- **PROCEDURE 2: LIMITS OF 17β-ESTRADIOL AND $\Delta^{8,9}$-DEHYDROESTRONE**

Internal standard solution, Stock solution, Solution A, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

[NOTE—The relative retention times of 17β-estradiol, 3-*O*-methylestrone, and $\Delta^{8,9}$-dehydroestrone are about 0.29, 1.0, and 0.9, respectively.]

Analysis:

Samples: *Standard solution* and *Sample solution*

Identify any peaks due to 17β-estradiol, 3-*O*-methylestrone, and $\Delta^{8,9}$-dehydroestrone in the chromatogram of the *Sample solution.* Calculate the quantities, in percentage, of 17β-estradiol and $\Delta^{8,9}$-dehydroestrone as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
- R_S = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*
- C_S = concentration of USP Estrone RS in the *Stock solution* (µg/mL)
- C_U = concentration of the *Sample solution* (µg/mL)
- F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: NMT 2.25% and NMT 6.25% of the labeled content of Conjugated Estrogens for 17β-estradiol and $\Delta^{8,9}$-dehydroestrone, respectively, as their sodium sulfate salts.

- **PROCEDURE 3: LIMIT OF ESTRONE, EQUILIN, AND 17α-DIHYDROEQUILIN** (free steroids)

Internal standard solution, Solution A, Stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Free steroids standard solution: Dilute the *Stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture…".

Sample solution: Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the solution instead of 3.0 mL to the centrifuge tube. Prepare a reagent blank in the same manner.

System suitability: Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, on the basis of NLT two replicate injections.

Analysis:

Samples: *Free steroids standard solution* and *Sample solution*

Calculate the ratio, R_U, of the combined peak areas of estrone, equilin, and 17α-dihydroequilin relative to the area of the internal standard in the *Sample solution*, correcting for any reagent blank peaks.

Acceptance criteria: NMT 0.65 (1.3% of free steroids), for the ratio R_U/R_S, where R_S is the peak response ratio of estrone to that of the internal standard of the *Free steroids standard solution*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Label it to state the content of Conjugated Estrogens on a weight-to-weight basis.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP 17α-Dihydroequilin RS
USP Equilin RS
USP Estradiol RS
USP Estrone RS

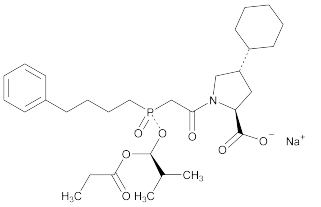
In-Process Revision

BRIEFING

Fosinopril Sodium, *USP 32* page 2449 and page 4059 of the *First Supplement*. On the basis of comments and supporting validation data received, in *Procedure 1* of the test for *Organic Impurities* it is proposed to add a new impurity, Impurity 3, that is well resolved from fosinopril and all other known impurities described in *Impurity Table 1*.

(MD-CV: S. Ramakrishna.) RTS—C73793

Fosinopril Sodium



$C_{30}H_{45}NNaO_7P$ 585.64

L-Proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]acetyl]-, sodium salt, [1[*S**(*R**)],2α,4*β*]-; (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt [88889-14-9].

DEFINITION

Fosinopril Sodium contains NLT 97.5% and NMT 102.0% of $C_{30}H_{45}NNaO_7P$, calculated on the anhydrous basis.

IDENTIFICATION

• **INFRARED ABSORPTION** ⟨**197**⟩

ASSAY

Procedure

Mobile phase: Acetonitrile, phosphoric acid, and water (2000:1:10)

System suitability solution: 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL of USP Fosinopril Related Compound B RS in *Mobile phase*

Standard solution: 0.10 mg/mL of USP Fosinopril Sodium RS in *Mobile phase*

Sample solution: 0.10 mg/mL of Fosinopril Sodium in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.9-mm × 15-cm; packing L3

Temperature: 33°

Flow rate: 1.2 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between fosinopril related compound B and fosinopril sodium

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of $C_{30}H_{45}NNaO_7P$ in the portion of Fosinopril Sodium taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fosinopril Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Fosinopril Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 20 ppm

Change to read:

Organic Impurities

Procedure 1

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL each of USP Fosinopril Related Compound A RS and USP Fosinopril Related Compound B RS in *Mobile phase*

Sample solution: Use the *Sample solution* as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Proceed as directed in the *Assay*, and measure the areas for each component in the chromatogram obtained, carrying out the chromatography to four times the retention time of the fosinopril sodium peak.

Calculate the percentage of each individual related compound:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = individual peak response, other than the fosinopril sodium peak

r_T = sum of all the peak responses

[NOTE—If present, two more diastereomers may not be resolved from fosinopril related compound B by this method. These peaks, appearing at a relative retention time of 0.7, should be integrated together to determine conformance with the limit in *Impurity Table 1*.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Procedure	Acceptance Criteria, NMT (%)
Fosinopril related compound A[a]	2.0	1	0.75
Fosinopril related compound B[b]	0.7	1	1.0
Fosinopril related compound C[c]	1.2	2	0.3

[a] (4*S*)-4-Cyclohexyl-1-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

[b] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester).

[c] Mixture of (4*S*)-4-cyclohexyl-1-[(*S*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt and (4*S*)-4-cyclohexyl-1-[[(*R*)-[(*R*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[d] (4*R*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[e] (4*S*)-4-Phenyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[f] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-propoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester),sodium salt.

[g] (2*S*,4*S*)-4-Cyclohexyl-1-pivaloylpyrrolidine-2-carboxylic acid.

[h] 2-((*RS*)-((*SR*)-2-Methyl-1-(propionyloxy)propoxy)(4-phenylbutyl)phosphinyl)acetic acid.

■[i] (*S*)-4-cyclohexyl-1-(3-oxopentanoyl)-L-proline■ *(USP33)*

In-Process Revision

Impurity Table 1 *(Continued)*

Name	Relative Retention Time	Procedure	Acceptance Criteria, NMT (%)
Fosinopril related compound D[d]	1.3	2	0.3
Fosinopril related compound E[e]	0.8	3	0.3
Fosinopril related compound F[f]	0.9	3	0.3
Impurity 1[g]	0.53	1	0.3
Impurity 2[h]	0.67	1	0.2
■Impurity 3 (if present)[i]	0.37	1	0.15■2S *(USP33)*

[a] (4*S*)-4-Cyclohexyl-1-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.
[b] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester).
[c] Mixture of (4*S*)-4-cyclohexyl-1-[(*S*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt and (4*S*)-4-cyclohexyl-1-[[(*R*)-[(*R*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
[d] (4*R*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
[e] (4*S*)-4-Phenyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
[f] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-propoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester),sodium salt.
[g] (2*S*,4*S*)-4-Cyclohexyl-1-pivaloylpyrrolidine-2-carboxylic acid.
[h] 2-((*RS*)-((*SR*)-2-Methyl-1-(propionyloxy)propoxy)(4-phenylbutyl)phosphinyl)acetic acid.
■[i] (*S*)-4-cyclohexyl-1-(3-oxopentanoyl)-L-proline■2S *(USP33)*

Procedure 2
Mobile phase: Acetonitrile, phosphoric acid, and water (4000:2:15)
Standard solution: Use the *Standard solution* in the *Assay*.
System suitability solution: 0.01 mg/mL each of USP Fosinopril Sodium RS, USP Fosinopril Related Compound C RS, and USP Fosinopril Related Compound D RS in the *Standard solution*
Sample solution: Use the *Sample solution* in the *Assay*.
Chromatographic system
(See *Chromatography,* ⟨621⟩ *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L12
Temperature: 45°
Flow rate: 0.9 mL/min
Injection size: 20 µL
Run time: 2 times the retention time of fosinopril sodium
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between fosinopril sodium and fosinopril related compound C
Analysis
Sample: *Sample solution*
Calculate the percentages of fosinopril related compound C and fosinopril related compound D only:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of fosinopril related compound C or fosinopril related compound D
r_T = sum of all the peak areas

Procedure 3
Solution A: 1-in-500 solution of phosphoric acid
Mobile phase: Acetonitrile and *Solution A* (14:11)
System suitability solution: 0.01 mg/mL each of USP Fosinopril Sodium RS, USP Fosinopril Related Compound E RS, and USP Fosinopril Related Compound F RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Fosinopril Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography,* ⟨621⟩ *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; packing L11
Temperature: 45°
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 4 times the retention time of fosinopril sodium
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between fosinopril related compound F and fosinopril sodium; NLT 1.5 between fosinopril related compound E and fosinopril related compound F
Analysis
Sample: *Sample solution*
Calculate the percentages of fosinopril related compound E and fosinopril related compound F only:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of fosinopril related compound E or fosinopril related compound F
r_T = sum of all the peak areas

Acceptance criteria
Individual impurities: See *Impurity Table 1*. In addition to not exceeding the limits for impurities in *Impurity Table 1*, NMT 0.1% of any other individual impurity is found (calculated as directed in *Procedure 1*).
Total impurities: NMT 1.5% for the total of *Procedure 1, Procedure 2,* and *Procedure 3*

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Fosinopril Sodium RS
  USP Fosinopril Related Compound A RS
  USP Fosinopril Related Compound B RS
  USP Fosinopril Related Compound C RS
  USP Fosinopril Related Compound D RS
  USP Fosinopril Related Compound E RS
  USP Fosinopril Related Compound F RS

BRIEFING

Galantamine Tablets, *USP 32* page 2478, *Interim Revision Announcement* on page 253 of *PF* 35(2) [Mar.–Apr. 2009], and page 1452 of *PF* 34(6) [Nov.–Dec. 2008]. On the basis of comments received, it is proposed to revise the relative response factor value for *N*-Desmethylgalantamine and 6α-Hexahydrogalantamine from 1.0 to 1.1. It is also proposed to delete the specified impurity *O*-Desmethylgalantamine from *Impurity Table 1* as this is a process impurity in the drug substance.

(MD-PP: R. Ravichandran.) RTS—C73352

Galantamine Tablets

DEFINITION
Galantamine Tablets contain an amount of Galantamine Hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of galantamine ($C_{17}H_{21}NO_3$).

In-Process Revision

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩:** The spectrum of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the test for *Uniformity of Dosage Units.*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer solution: 5.34 g/L of dibasic sodium phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 6.5.
Solution A: Methanol and *Buffer solution* (1:19)
Solution B: Acetonitrile and methanol (19:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40.0	75	25
45.0	60	40
46.0	40	60
55.0	40	60
56.0	100	0
61.0	100	0

Diluent: Dissolve 35.4 g of edetate disodium in 950 mL of water, and add 50 mL of methanol. [NOTE—First dissolve in water, then add methanol.]
Standard solution: 0.62 mg/mL of USP Galantamine Hydrobromide RS in *Diluent*
Sample solution: 0.48 mg/mL of galantamine from powdered Tablets (NLT 10) in *Diluent*. Pass through a PTFE filter having a porosity of 0.45 µm or finer.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 3-µm L1 packing
Temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, as a percentage of the label claim, of $C_{17}H_{21}NO_3$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 20 min
Sample solution: Pass portions of the solution under test through a suitable 0.2-µm filter.
Standard solution: Dissolve USP Galantamine Hydrobromide RS in *Medium*, and dilute quantitatively, and stepwise if necessary, to obtain concentrations as follows: 8 µg/mL of galantamine for Tablets labeled to contain 4 mg; 16 µg/mL of galantamine for Tablets labeled to contain 8 mg; and 24 µg/mL of galantamine for Tablets labeled to contain 12 mg.
Spectrometric conditions
Mode: UV
Analytical wavelength: 288 nm
Cell: 5-cm cell for 4-mg and 8-mg Tablets; 1-cm cell for 12-mg Tablets
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{21}NO_3$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Tolerances: NLT 80% (Q) of the labeled amount of galantamine is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements for coated Tablets

Procedure for content uniformity
Standard solution: 0.05 mg/mL of USP Galantamine Hydrobromide RS in 0.1 N hydrochloric acid
Sample solution: Add 1 Tablet to each appropriately sized volumetric flask to obtain a final galantamine concentration of 0.04 mg/mL, add an appropriate amount of 0.1 N hydrochloric acid, equivalent to 75% of the total volume of the volumetric flask, and mechanically shake for 45 min. Dilute with 0.1 N hydrochloric acid to volume. Pass a portion of this solution through a filter having a 0.2-µm or finer porosity, and use the filtrate.
Spectrometric conditions
Mode: UV
Analytical wavelength: Absorption maximum at about 289 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of $C_{17}H_{21}NO_3$ dissolved in filtered portions of the *Sample solution* in comparison with the *Standard solution.*
Calculate the quantity, as a percentage of the label claim, of $C_{17}H_{21}NO_3$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

IMPURITIES

Change to read:

Organic Impurities

- **PROCEDURE**

Buffer solution, Solution A, Solution B, Mobile phase, Diluent, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.

In-Process Revision

System suitability solution: 0.6 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between 6*β*-hexahydrogalantamine and 6*β*-octahydrogalantamine, *System suitability solution*
Relative standard deviation: NMT 2.0% for galantamine, *Standard solution*
[NOTE—Identify the impurities using the approximate relative retention times given in *Impurity Table 1*.]

Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Ignore the peak due to bromide near the void volume.]
Calculate the percentage of each of the ~~galantamine related compounds~~■impurities including the unspecified degradation impurities■2S (USP33) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (100/F)$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of galantamine from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27
F = relative response factor for each of the impurities relative to galantamine (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.5%

●**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethylgalantamine[a]	0.41	~~1.0~~ ■1.1■2S (USP33)	0.5
~~O-Desmethylgalantamine[b]~~	~~0.56~~	~~1.0~~	~~0.5~~■■2S (USP33)
6*β*-Hexahydrogalantamine (also known as galantamine *N*-oxide)[b]	0.73	1.1	0.75
6*β*-Octahydrogalantamine (also known as lycoramine)[c]*	0.86	—	—
Galantamine hydrobromide	1.00	1.0	—
6*α*-Hexahydrogalantamine (also known as epigalantamine)[d]	1.15	~~1.0~~ ■1.1■2S (USP33)	0.5
Tetrahydrogalantamine[e]*	2.09	—	—
Individual, unspecified degradation impurity	—	1.0	0.2

* Impurities are not quantified and are intended for system suitability evaluation only.
[a] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
~~[b] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-3,6-diol.~~
■[b] [4a*S*-(4α,6*β*,8a*R**)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.■2S (USP33)
[c] [4a*S*-(4aα,6*β*,8a*R**)]-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[d] [4a*S*-(4aα,6α,8a*R**)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[e] [4a*S*-(4a*R**,8a*R**)]-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-4a*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.

●2

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Galantamine Hydrobromide RS
  USP Galantamine Hydrobromide Related Compounds Mixture RS

In-Process Revision

BRIEFING

Glucagon, *USP 32* page 2504. The following revisions are proposed.

1. The Glucagon source (see the chemical name) has been changed.
2. The *Definition* has been revised to define Glucagon of a recombinant DNA origin, and potency is to be determined by a validated bioassay approved by a competent authority.
3. *Identification* test *A* has been revised to use the *Assay* retention times. *Identification* test *B*, which uses peptide mapping, has been added.
4. The animal-based *Assay* has been replaced with an HPLC *Assay*. The LC method has been validated using a 3-mm × 15-cm column containing 3-µm packing L1.
5. In *Other Components,* the test for *Nitrogen Determination* has been deleted.
6. The *Inorganic Impurities* section has been deleted (includes *Residue on Ignition* and *Zinc Determination)*.
7. The *Organic Impurities* test has been replaced with a test based on the chromatographic procedure used in the *Assay*.
8. In *Specific Tests,* the *Water Determination* test has been revised, and a *Bacterial Endotoxins* test has been added.
9. The current USP Reference Standards have been deleted, and USP rGlucagon RS and USP Endotoxin RS have been added for use in the revised text. As noted in item 2 above, USP intends to release a USP rGlucagon RS of recombinant origin.

(BB PP: T. Sigambris.) RTS—C54723

Glucagon

Change to read:

HSQGTFTSDY SKYLDSRRAQ DFVQWLMNT

$C_{153}H_{225}N_{43}O_{49}S$ ~~3482.82~~■3482.75■2S *(USP33)*

~~Glucagon (pig);~~■■2S *(USP33)*

~~Glucagon~~■Glucagon (human)■2S *(USP33)* [16941-32-5].

DEFINITION

Change to read:

~~Glucagon is a polypeptide hormone that has the property of increasing the concentration of glucose in the blood. It is obtained from porcine and bovine pancreas glands.~~

■Glucagon is a polypeptide hormone that has the property of increasing the concentration of glucose in the blood. It has the same structure (29 amino acids) as the hormone produced by the α-cells of the human pancreas. Glucagon is produced by microbial processes using recombinant DNA (rDNA) technology. The host cell–derived protein content and/or the host cell–derived or vector-derived DNA content are determined by validated methods. During the course of product development, it must be demonstrated that the manufacturing process produces Glucagon having a biological activity of NLT 1 USP unit/mg, using a validated bioassay approved by a competent authority. It contains NLT 90% and NMT 105% of $C_{153}H_{225}N_{43}O_{49}S$, calculated on the anhydrous basis.■2S *(USP33)*

IDENTIFICATION

Delete the following:

■~~• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Procedure* for *Inorganic Impurities.*~~■2S *(USP33)*

Add the following:

■**A:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S *(USP33)*

Add the following:

■**B:** Determine the peptide fragments, using the following peptide mapping procedure.

Ammonium bicarbonate buffer: Prepare a 1 M solution of ammonium bicarbonate, and adjust the pH with ammonia to 10.3. Prepare a mixture of 1 M ammonium bicarbonate and water (1:9).

Enzyme solution: 2 mg/mL α-chymotrypsin (peptide mapping grade) in *Ammonium bicarbonate buffer*.

Solution A: Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid and 1000 mL of water.

Solution B: Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid, 600 mL of ethanol, and 400 mL of water.

Mobile phase: See *Gradient Table 1* below.

Gradient Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	53	47
45	0	100
46	100	0
75	100	0

Standard digest solution: Prepare a 5 mg/mL solution of USP rGlucagon RS in 0.01 M hydrochloric acid. Mix 200 µL of this solution with 800 µL of *Ammonium bicarbonate buffer*. To this solution add 25 µL of *Enzyme solution*, and place in a closed vial at about 37° for 2 h. Remove the vial, and stop the reaction immediately by the addition of 120 µL of glacial acetic acid.

Sample digest solution: Prepare a 5 mg/mL solution of Glucagon in 0.01 M hydrochloric acid. Proceed as directed for *Standard digest solution*, beginning with "Mix 200 µL of this solution".

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 5-cm; 5-µm or less packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard digest solution*
Suitability requirement: The chromatogram of the *Standard digest solution* corresponds to the chromatogram provided with USP rGlucagon RS.

Analysis
Samples: *Standard digest solution* and *Sample digest solution*
Acceptance criteria: The chromatographic profile of the *Sample digest solution* corresponds to that of the *Standard digest solution*.■2S *(USP33)*

In-Process Revision

ASSAY

Change to read:

- **PROCEDURE**

[NOTE—All buffers have a final pH of 7.4, unless otherwise indicated. The concentration range of the *Standard solutions* and the *Sample solutions* may be modified to fall within the linear range of the *Assay*. The calculations should be adjusted accordingly. Alternatively, full curve analysis using validated nonlinear statistical methods can be used, provided that similarity is demonstrated when comparing the responses of the *Standard solutions* and the *Sample solutions*.]

Hepatocyte Preparation

Calcium-free perfusion buffer with dextrose: Prepare a solution containing, in each L, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 1.80 g of dextrose, 0.19 g of edetic acid, and 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. Oxygenate prior to circulation.

Collagenase buffer: Prepare a solution containing, in each L, 3.62 g of sodium chloride, 23.83 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.35 g of potassium chloride, 0.52 g of calcium chloride, and 1.8 g of dextrose. Adjust to a pH of 7.6, and oxygenate. Immediately before perfusion, dissolve a quantity of collagenase in this solution to obtain a concentration of 0.02% to 0.05%.

Wash buffer: Prepare a solution containing, in each L, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 0.19 g of edetic acid, 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.22 g of calcium chloride, and 0.12 g of magnesium sulfate.

Incubation buffer: Prepare a solution containing, in each L, 6.19 g of sodium chloride, 0.35 g of potassium chloride, 0.22 g of calcium chloride, 0.12 g of magnesium sulfate, 0.16 g of monobasic potassium phosphate, 11.915 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, and 1% bovine serum albumin (BSA). Adjust to a pH of 7.5.

Test animals: Male Sprague-Dawley rats are maintained on a standard rat chow diet and freely given water. On the morning of the test, select a healthy rat weighing approximately 300 g, and administer 100 Units of Heparin Sodium subcutaneously.

Method

[NOTE—Conduct this *Method* in the morning to ensure that the rat has optimal glycogen in its liver.]

Anesthetize the rat with an appropriate anesthetic. Open the abdominal cavity, and isolate the portal vein. Insert an angiocatheter connected to a perfusion pump, and tie into the portal vein at the general location of the lienal branch. Start the perfusion (25 mL/min) in situ with *Calcium-free perfusion buffer with dextrose,* equilibrated with oxygen, at a temperature of 37°. As the liver enlarges, cut the inferior vena cava to allow pressure equilibrium.

[NOTE—300 mL of the perfusate is needed to clear the liver of red blood cells at a flow rate of 30–60 mL/min.]

Then circulate *Collagenase buffer* at a flow rate of 30–60 mL/min for 10 min. The exact concentration of collagenase (within the range of 0.02%–0.05%) is determined empirically for each lot of enzyme. The concentration of collagenase is that necessary to consistently cause a breakdown of the liver 10 min after initial entry of the *Collagenase buffer* into the liver. When the liver significantly increases in size, changes color and consistency, and starts to leak perfusate out of the lobes, change the system to the oxygenated prewarmed *Wash buffer.* 100 mL of *Wash buffer* is needed to wash the liver of collagenase at a flow rate of 25 mL/min. Surgically remove the liver from the animal, and place it in a prewarmed tray containing oxygenated *Wash buffer* (37°). Gently comb the liver with a stainless steel, fine-toothed comb to free the hepatocytes. Wash the hepatocytes with *Wash buffer,* and filter through cheesecloth (or a 150-µm mesh polyethylene net) into a plastic beaker. Centrifuge the cell suspension for 2 min at 25 × g to form a loosely packed pellet. Discard the supernatant, and resuspend the pellet in *Wash buffer.* Repeat the washing procedure twice for a total of three washes. Resuspend the final pellet in 100–200 mL of *Incubation buffer,* depending on cell yield.

[NOTE—If the *Analysis* is interrupted, cool the cells by collecting them in a beaker placed in ice. The cells are washed with ice-cold *Wash buffer,* and stored on ice until ready for use. At that point the cells are pelleted once more, and resuspended in ice-cold *Incubation buffer.*]

Suitability: The concentrations of cells may vary due to the collagenase activity and the viability of the hepatocytes. To check cell viability and to determine viable cell concentration, dilute duplicate 100-µL aliquots of cell suspension with 400 µL of *Wash buffer* and 500 µL of isotonic 0.4% trypan blue. The aliquots are counted in a hemocytometer. The cells are suspended in *Incubation buffer* to obtain a viable cell concentration of NLT 3×10^6/mL. Count several distinct fields.

[NOTE—Viable cells are those cells that exclude the trypan blue.]

Negative control solution: Prepare a solution containing 0.5% BSA in sterile water.

Incubation flasks: Use 25-mL conical flasks, the bottoms of which have been heated and pushed inward to form a conically raised center.

Standard solutions: In duplicate, dissolve a suitable quantity of USP Glucagon RS in 0.01 N hydrochloric acid or other suitable diluent to obtain a solution containing 1.0 USP Glucagon Unit/mL. All dilutions thereafter are made using 0.5% BSA (w/v) in water. Accurately dilute measured volumes of each solution with *Negative control solution* to obtain five concentrations (200, 100, 50, 25, and 12.5 micro-Units/mL) of each solution (*Standard solutions*). Pipet 0.2 mL of each *Standard solution* into separate *Incubation flasks.* Pipet 0.2 mL of *Negative control solution* into each of two flasks (*Negative control solutions 1* and *2*). Then add the hepatocytes into each of the 12 flasks.

Sample solutions: Using quantities of Glucagon, proceed as directed for *Standard solutions.*

D-Glucose Determination

Standard stock solution: Transfer 2.0 g of USP Dextrose RS to a 200-mL volumetric flask, and dissolve in and dilute with saturated benzoic acid solution to volume.

Standard solutions: Transfer suitable quantities of *Standard stock solution* to three flasks, and dilute with saturated benzoic acid solution to obtain solutions having known concentrations of 0.5, 1.0, and 1.5 times the typical sample glucose concentration.

Potassium ferrocyanide solution: Dissolve 1.25 g of trihydrate potassium ferrocyanide in 125 mL of *Sterile Water for Injection* (see *Water for Pharmaceutical Purposes* ⟨1231⟩).

System suitability: Analyze the *Potassium ferrocyanide solution,* the *Standard solutions,* and five replicates of the middle *Standard solution.* Prepare a standard curve using the *Standard solutions* as directed for *Analysis*: the relative standard deviation of the standard curve is NMT 2.0%; the response of the *Potassium ferrocyanide solution* is NMT 30 mg/L; and the relative standard deviation is NMT 2.0% for the replicate analyses of the middle *Standard solution.*

Analysis: Dispense 5 mL of *Hepatocyte Preparation* into the special incubation flasks in sequence from high glucagon concentration to low glucagon concentration, alternating the *Standard solutions* with the *Sample solutions.* The flasks are swirled in an orbiting water bath at 125 rpm at 30° for approximately 30–60 min.

[NOTE—The exact incubation time must be determined to optimize the signal-to-noise ratio.]

Following incubation, place 0.5- to 1.0-mL aliquots, in duplicate, from each incubation flask into labeled tubes, and centrifuge at 12,500 × g. Determine the percentage of glucose concentration in each flask's supernatant.

To conform to the linear range of the instrument being used, it may be necessary to adjust by dilution each of the preparations. Use a glucose analyzer that has demonstrated appropriate specificity, accuracy, precision, and linear response over the range of concentrations being determined.

In-Process Revision

[NOTE—A suitable analyzer may use an immobilized, oxidase-enzyme membrane or jacket-generating hydrogen peroxide, which is then detected at the electrode.]

Perform the glucose analysis in the following sequence: *Negative control solution 1, Standard solutions, Sample solutions,* and *Negative control solution 2*.

Determine the percentage of glucose against the *Negative control solution* for each preparation.

Linearity test: Use an analysis of variance (ANOVA) with one sample assayed against a standard, and using two replicates each, construct a table (see *Table 1*). Compare the value of the ratio $MSNL/MSRES_T$ to a critical value obtained from a table for an F distribution with $m-2$ and $3m-3$ degrees of freedom, where m is the number of dose levels for each preparation. If the ratio $MSNL/MSRES_T$ does not indicate the presence of significant nonlinearity (ratio value is lower than the critical value), then proceed to the test for parallelism. If the ratio exceeds the critical value (significance level of 0.025), the nonlinearity is statistically significant and the test is repeated, discarding the results from either the highest or lowest dose of both the *Standard solutions* and the *Sample solutions* (four dose levels). If the ratio $MSNL/MSRES_T$ does not indicate the presence of significant nonlinearity, then proceed to the test for parallelism.

Parallelism test: Compare the ratio $MSNP/MSRES_2$ to a critical value obtained from an F distribution having 1 and $4m-5$ degrees of freedom. If the ratio $MSNP/MSRES_2$ does not indicate the presence of significant nonparallelism, then the assay is considered valid. Use the appropriate dose levels for the estimation of the relative potency.

Relative potency: Calculate the relative potency, R, of the *Sample solutions* as compared with the *Standard solutions* as follows:

(1) X_j is defined as the $\log_{10}$ of the j^{th} dose of the *Standard solutions* or the *Sample solutions*. The glucagon dose varies from 12.5 to 200×10^{-6} USP Glucagon Units/mL. For ease in the subsequent calculations, these doses are respectively represented by 1 through 5, as shown in the table below:

j	1	2	3	4	5
Dose	12.5	25	50	100	200
X_j	1.10	1.40	1.70	2.00	2.30

(2) To differentiate between the *Standard solutions* and the *Sample solutions* in the calculations, the subscript i will be used, with $i = 1$ to designate the *Standard solutions* and $i = 2$ to designate the *Sample solutions*. Y_{ijk} will denote the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the i^{th} preparation. For example, Y_{1jk} is the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the appropriate *Standard preparation,* Y_{11k} is the glucose concentration associated with the k^{th} replicate of dose 1 of the *Standard preparation,* and Y_{21k} is the glucose concentration associated with the k^{th} replicate of dose 1 of the *Sample solution.* Dose 1 represents a glucose dose of 12.5×10^{-6} USP Glucagon Units/mL. Finally, Y_{132} represents the glucose concentration associated with the 2nd replicate of dose 3 for the *Standard solution.*

(3) Y_S and Y_t denote the average glucose concentrations for the *Standard solutions* and the *Sample solutions,* respectively.

(4) Calculate the least-squares slope estimate, b, for a linear regression relating the Y_{ijk}'s to the X_j's as follows: $b = S_{xy} / S_{xx}$, with S_{xy} and S_{xx} calculated using the equations in *Table 2*.

(5) The log potency, M, is calculated using $M = -1[(Y_S - Y_t)/b]$.

(6) R = antilog (M).

(7) Calculate the confidence limits (upper and lower) for the relative potency, R, using the value $s^2 = MSRES_3$ (see *Table 1* and *Table 2*) as follows. Obtain t from a table for a t distribution having $4m - 4$ degrees of freedom. For the 95% limits, the t values can be obtained from *Design and Analysis of Biological Assays* ⟨111⟩, *Table 9*.

[NOTE—For confidence limits having other probability levels (i.e., 100(1 − a)%), the right tail t critical value having a/2 area to its right is used.]

Calculate $g = t^2 S^2 / b^2 S_{xx}$

and $F = (ts/b)\sqrt{(1/m)(1-g) + (M^2/S_{xx})}$

and calculate

$$M_L = (M - F)/(1 - g)$$

and

$$M_U = (M + F)/(1 - g)$$

where M is the log potency and M_L and M_U are the log potency lower and upper confidence limits. The lower and upper confidence limits for the relative potency, R, are given by:

RL = antilog (M_L)

RU = antilog (M_U)

It meets the requirements if the potency is 0.8–1.25 USP Glucagon Units/mg, and the confidence interval width at P = 0.95 does not exceed 45% of the computed potency. Repeat the assay if the confidence interval width exceeds 45% of the computed potency.

Table 1. ANOVA for the Rat Hepatocyte Assay for Glucagon

Source	Degrees of Freedom	SS (Sum of Squares)	MS (Mean Square)
Preparations	1	SSPREP	MSPREP
Replicates	1	SSREP	MSREP
Linear Slope	1	SSLIN	MSLIN
$Residual_3$	$4m-4$	$SSRES_3$	$MSRES_3$
Nonparallelism	1	SSNP	MSNP
$Residual_2$	$4m-5$	$SSRES_2$	$MSRES_2$
Nonlinearity	$m-2$	SSNL	MSNL
$Residual_T$	$3m-3$	$SSRES_T$	$MSRES_T$
TOTAL	$4m-1$	SST	

[NOTE—This analysis pertains to one sample assayed against a standard, using two replicates each.]

The number of dose levels for each preparation is denoted by *m*.

Table 2 gives the equations for calculating the SS terms.

In each row of the ANOVA table, the MS is obtained by dividing the SS term by the degrees of freedom.

In-Process Revision

Table 2. Equations for Calculating the Sums of Squares in the Analysis of Variance*

$$Y_{i..} = \sum_{jk} \sum y_{ijk}$$

$$Y_{.j.} = \sum_{ik} \sum y_{ijk}$$

$$Y_{..k} = \sum_{ij} \sum y_{ijk}$$

$$CF = \frac{\left(\sum_{ijk} \sum \sum y_{ijk}\right)^2}{4m}$$

$$S_{xy}^S = \sum_{jk} \sum x_j y_{1jk} - \frac{\left(\sum_j x_j\right)(Y_{1..})}{m}$$

$$S_{xy}^T = \sum_{jk} \sum x_j y_{2jk} - \frac{\left(\sum_j x_j\right)(Y_{2..})}{m}$$

$$S_{xy} = S_{xy}^S + S_{xy}^T$$

$$S_{xx}^S = 2\sum_j x_j^2 - \frac{2\left(\sum_j x_j\right)^2}{m}$$

$$S_{xx}^T = S_{xx}^S$$

$$S_{xx} = S_{xx}^S + S_{xx}^T$$

$$SSPREP = \frac{\sum_i Y_{i..}^2}{2m} - CF$$

$$SSREP = \frac{\sum_k Y_{..k}^2}{2m} - CF$$

$$SSLIN = \frac{\left(S_{xy}\right)^2}{S_{xx}}$$

$$SST = \sum_{i\,j\,k} \sum \sum y_{ijk}^2 - CF$$

$$SSRES_3 = SST - SSPREP - SSREP - SSLIN$$

$$SSNP = \frac{\left(S_{xy}^S\right)^2}{S_{xx}^S} + \frac{\left(S_{xy}^T\right)^2}{S_{xx}^T} - SSLIN$$

$$SSRES_2 = SSRES_3 - SSNP$$

$$SSNL = \frac{\sum_j Y_{.j.}^2}{4} - SSLIN - CF$$

$$SSRES_1 = SSRES_2 - SSNL$$

*Refer to *D-Glucose Determination* for the section on *Relative Potency* for the definitions of x_j and x_{ijk}.

■**Solution A:** Dissolve 16.3 g of potassium phosphate, monobasic in 750 mL of water, adjust the pH with phosphoric acid to 2.7 (±0.05), add water to 800 mL, add 200 mL of acetonitrile, and degas.

Solution B: Prepare a degassed solution of acetonitrile and water (4:6).

System suitability solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

Standard solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

Sample solution: 0.5 mg/mL of Glucagon in 0.01 N hydrochloric acid

Mobile phase: See *Gradient Table 2* below. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Gradient Table 2

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25[a]	61	39
29	12	88
30	12	88
31	61	39
70	61	39

[a] The end time of the isocratic elution can be adjusted so that the gradient begins after the fourth desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3-mm × 15-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 15 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: *System suitability solution*: four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible. The resolution between the main peak and the first eluting desamido peak is NLT 1.5.
Tailing factor: NMT 1.8 for the glucagon peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{153}H_{225}N_{43}O_{49}S$ in the portion of Glucagon taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 90% and NMT 105% on the anhydrous basis■2S *(USP33)*

In-Process Revision

OTHER COMPONENTS

Delete the following:

~~■• **NITROGEN DETERMINATION,** *Method II* ⟨461⟩: 16.0%–18.5%, calculated on the anhydrous basis■2S (USP33)~~

IMPURITIES

Delete the following:

~~■**Inorganic Impurities**~~
~~• **RESIDUE ON IGNITION** ⟨281⟩: NMT 2.5%~~
~~• **ZINC DETERMINATION** ⟨591⟩: NMT 0.05%■2S (USP33)~~

Change to read:

Organic Impurities

• **PROCEDURE**

~~**Solution A:** 18.9 mg/mL of monobasic sodium phosphate and 0.327 mg/mL of L-cysteine in water. Before diluting to final volume, adjust with phosphoric acid to a pH of 2.6.~~

~~**Sample solvent:** Acetonitrile and 0.01 N hydrochloric acid (20:80)~~

~~**Mobile phase:** Acetonitrile and *Solution A* (270:730)~~

~~**Standard solution:** 0.5 mg/mL of USP Glucagon RS in *Sample solvent*~~

~~**Sample solution:** 0.5 mg/mL of Glucagon in *Sample solvent*~~

~~**System suitability solution:** 0.5 mg/mL of USP Glucagon RS in *Sample solvent*~~

~~[NOTE—Heat the solution at 75° for at least 3 h to allow the formation of related substance GLU (24) Glucagon.]~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨621⟩, *System Suitability*.)~~

~~**Mode:** LC~~

~~**Detector:** UV 214~~

~~**Column:** 4.6 × 25m, 5-µm packing L7~~

~~**Temperature:** 35°~~

~~**Flow rate:** 1 mL/min~~

~~**Injection size:** 50 µL~~

~~**System suitability**~~

~~**Sample:** *System suitability solution*~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 4.0 between the main peak and the large peak with a relative retention time of 1.3~~

~~**Tailing factor:** NMT 1.7 for the glucagon peak~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of each impurity in the portion of Glucagon taken:~~

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

~~r_i = peak response for each individual impurity obtained from the *Sample solution*~~

~~r_S = sum of the responses of all of the peaks~~

~~C_S = concentration of *Standard solution* (mg/mL)~~

~~C_U = nominal concentration of *Sample solution* (mg/mL)~~

~~**Acceptance criteria**~~

~~**Individual impurities:** NMT 2.5%~~

~~**Total impurities:** NMT 10.0%~~

■**Solution A, Solution B, System suitability solution, Standard solution, Sample solution, Mobile phase, Chromatographic system,** and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon taken:

$$(r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses of all peaks

Acceptance criteria: NMT 2.0% total of all four desamido glucagons is found, and NMT 6.0% of total impurities and related compounds is found.■2S (USP33)

SPECIFIC TESTS

Change to read:

• **WATER DETERMINATION,** ~~*Method I*~~■*Method Ic*■2S (USP33) ⟨**921**⟩: ~~NMT 10.0%~~

■NMT 10%, determined on a 20- to 50-mg sample■2S (USP33)

Add the following:

■• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩: NMT 10 USP EU/mg■2S (USP33)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ~~Preserve in tight glass containers, under nitrogen, in a refrigerator.~~

■Preserve in airtight containers, protected from light, and store in a freezer.■2S (USP33)

Change to read:

• **USP REFERENCE STANDARDS** ⟨**11**⟩

~~USP Dextrose RS~~

~~USP Glucagon RS~~

■USP Endotoxin RS

USP rGlucagon RS■2S (USP33)

BRIEFING

Glucagon for Injection, *USP 32* page 2507. It is proposed to replace the animal-based assay with an HPLC assay. The LC method has been validated using a 3-mm × 15-cm column containing 3-µm packing L1. USP intends to release USP rGlucagon RS of a recombinant origin. The following changes are also proposed:

1. The *Definition* range has been revised to NLT 65% and NMT 110% of the labeled amount.
2. *Identification* test *A* has been added to use the *Assay* retention time comparison.
3. The test for *Chromatographic Purity* has been deleted and replaced with an *Organic Impurities* test based on the same chromatographic procedure as that of the *Assay*.
4. A *Labeling* section has been added to state that the material is of recombinant DNA origin.

(BB PP: T. Sigambris.) RTS—C54723

In-Process Revision

Glucagon for Injection

DEFINITION

Change to read:

~~Glucagon for Injection is a mixture of the hydrochloride of Glucagon with one or more suitable dry diluents. It contains NLT 80.0% and NMT 125.0% of the labeled amount of glucagon ($C_{153}H_{225}N_{43}O_{49}S$).~~

■Glucagon for Injection is a sterile lyophilized mixture of the hydrochloride of glucagon with one or more suitable buffering and stabilizing agents. It contains NLT 65% and NMT 110% of the labeled amount of glucagon.■2S *(USP33)*

IDENTIFICATION

Add the following:

■• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.■2S *(USP33)*

ASSAY

Change to read:

• **PROCEDURE**

~~[NOTE—All buffers have a final pH of 7.4, unless otherwise indicated. The concentration range of the *Standard solutions* and the *Sample solutions* may be modified to fall within the linear range of the *Assay*. The calculations should be adjusted accordingly. Alternatively, full curve analysis using validated nonlinear statistical methods can be used, provided that similarity is demonstrated when comparing the responses of the *Standard solutions* and the *Sample solutions*.]~~

~~**Hepatocyte preparation**~~

~~**Calcium-free perfusion buffer with dextrose:** Prepare a solution containing, in each L, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 1.80 g of dextrose, 0.19 g of edetic acid, and 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. Oxygenate prior to circulation.~~

~~**Collagenase buffer:** Prepare a solution containing, in each L, 3.62 g of sodium chloride, 23.83 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.35 g of potassium chloride, 0.52 g of calcium chloride, and 1.8 g of dextrose. Adjust to a pH of 7.6, and oxygenate. Immediately before perfusion, dissolve a quantity of collagenase in this solution to obtain a concentration of 0.02%–0.05%.~~

~~**Wash buffer:** Prepare a solution containing, in each L, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 0.19 g of edetic acid, 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.22 g of calcium chloride, and 0.12 g of magnesium sulfate.~~

~~**Incubation buffer:** Prepare a solution containing, in each L, 6.19 g of sodium chloride, 0.35 g of potassium chloride, 0.22 g of calcium chloride, 0.12 g of magnesium sulfate, 0.16 g of monobasic potassium phosphate, 11.915 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, and 1% bovine serum albumin (BSA). Adjust to a pH of 7.5.~~

~~**Test animals:** Male Sprague-Dawley rats are maintained on a standard rat chow diet and freely given water. On the morning of the test, select a healthy rat weighing approximately 300 g, and administer 100 Units of Heparin Sodium subcutaneously.~~

~~**Analysis**~~

~~[NOTE—Conduct this Analysis in the morning to ensure that the rat has optimal glycogen in its liver.]~~

~~Anesthetize the rat with an appropriate anesthetic. Open the abdominal cavity, and isolate the portal vein. Insert an angiocatheter connected to a perfusion pump, and tie into the portal vein at the general location of the lienal branch. Start the perfusion (25 mL/min) in situ with a *Calcium-free perfusion buffer with dextrose*, equilibrated with oxygen, at a temperature of 37°. As the liver enlarges, cut the inferior vena cava to allow pressure equilibrium.~~

~~[NOTE—300 mL of the perfusate is needed to clear the liver of red blood cells at a flow rate of 30–60 mL/min.]~~

~~Circulate the *Collagenase buffer* at a flow rate of 30–60 mL/min for 10 min. The exact concentration of collagenase (within the range of 0.02%–0.05%) is determined empirically for each lot of enzyme. The concentration of collagenase is that necessary to consistently cause a breakdown of the liver 10 min after initial entry of the *Collagenase buffer* into the liver. When the liver significantly increases in size, changes color and consistency, and starts to leak perfusate out of the lobes, change the system to the oxygenated prewarmed *Wash buffer*. Use 100 mL of *Wash buffer* to wash the liver of collagenase at a flow rate of 25 mL/min. Surgically remove the liver from the animal and place in a prewarmed tray containing oxygenated *Wash buffer* (37°). Gently comb the liver with a stainless steel, fine-toothed comb to free the hepatocytes. Wash the hepatocytes with *Wash buffer*, and filter through cheesecloth (or a 150-µm mesh polyethylene net) into a plastic beaker. Centrifuge the cell suspension for 2 min at 25 × g to form a loosely packed pellet. Discard the supernatant, and resuspend the pellet in *Wash buffer*. Repeat the washing *Analysis* twice for a total of three washes. Resuspend the final pellet in 100–200 mL of *Incubation buffer*, depending on cell yield.~~

~~[NOTE—If the *Assay Analysis* is interrupted, cool the cells by collecting the cells in a beaker placed in ice. The cells are washed with ice-cold *Wash buffer*, and stored on ice until ready for use. At that point the cells are pelleted once more, and resuspended in ice-cold *Incubation buffer*.]~~

~~**Suitability:** The concentrations of cells may vary due to the collagenase activity and the viability of the hepatocytes. To check cell viability and to determine viable cell concentration, dilute duplicate 100-µL aliquots of cell suspension with 400 µL of *Wash buffer* and 500 µL of isotonic 0.4% trypan blue. The aliquots are counted in a hemocytometer. The cells are suspended in *Incubation buffer* to obtain a viable cell concentration of not less than 3×10^6 per mL. Count several distinct fields.~~

~~[NOTE—Viable cells are those cells that exclude the trypan blue.]~~

~~**Negative control solution:** Prepare a solution containing 0.5% BSA in sterile water.~~

~~**Incubation flasks:** Use 25-mL conical flasks, the bottoms of which have been heated and pushed inward to form a conically raised center.~~

~~**Standard solutions:** In duplicate, dissolve a suitable quantity of USP Glucagon RS in 0.01 N hydrochloric acid or other suitable diluent to obtain a solution containing 1.0 USP Glucagon Unit/mL. All dilutions thereafter are made using 0.5% BSA (w/v) in water. Dilute measured volumes of each solution with *Negative control solution* to obtain five concentrations: 200, 100, 50, 25, and 12.5 micro-Units/mL of each solution (*Standard solutions*). Pipet 0.2 mL of each *Standard solution* into separate *Incubation flasks*. Pipet 0.2 mL of *Negative control solution* into each of two flasks (*Negative control solutions* 1 and 2). Then add the hepatocytes into each of the 12 flasks.~~

~~**Sample solutions:** Using weighed quantities of Glucagon, proceed as directed for *Standard solutions*.~~

~~**D-Glucose determination**~~

~~**Standard stock solution:** Transfer 2.0 g of USP Dextrose RS to a 200-mL volumetric flask, and dissolve in and dilute with saturated benzoic acid solution to volume.~~

~~**Standard solutions:** Transfer suitable quantities of *Standard stock solution* to three flasks, and dilute with saturated benzoic acid solution to obtain solutions having known concentrations of 0.5, 1.0, and 1.5 times the typical sample glucose concentration.~~

In-Process Revision

Potassium ferrocyanide solution: Dissolve 1.25 g of trihydrate potassium ferrocyanide in 125 mL of Sterile Water for Injection.

System suitability: Analyze the *Potassium ferrocyanide solution*, the *Standard solutions*, and five replicates of the middle *Standard solution*. Prepare a standard curve using the *Standard solutions* as directed for the *Analysis*.

Suitability requirements: The relative standard deviation of the standard curve is NMT 2.0%; the response of the *Potassium ferrocyanide solution* is NMT 30 mg/L; and the relative standard deviation is NMT 2.0% for the replicate analyses of the middle *Standard solution*.

Analysis: Dispense 5 mL of *Hepatocyte preparation* into the special *Incubation flasks* in sequence from high glucagon concentration to low glucagon concentration, alternating the *Standard solutions* with the *Sample solutions*. The flasks are swirled in an orbiting water bath at 125 rpm at 30° for approximately 30–60 min.

[NOTE—The exact incubation time must be determined to optimize the signal-to-noise ratio.]

Following incubation, place 0.5- to 1.0-mL aliquots, in duplicate, from each *Incubation flask* into labeled tubes, and centrifuge at 12,500 × g. Determine the percentage of glucose concentration in each flask's supernatant.

To conform to the linear range of the instrument being used, it may be necessary to adjust by dilution each of the preparations. Use a glucose analyzer that has demonstrated appropriate specificity, accuracy, precision, and linear response over the range of concentrations being determined.

[NOTE—A suitable analyzer may use an immobilized, oxidase-enzyme membrane or jacket-generating hydrogen peroxide, which is then detected at the electrode.]

Perform the glucose analysis in the following sequence: *Negative control solution 1*, *Standard solutions*, *Sample solutions*, and *Negative control solution 2*.

Determine the percentage of glucose against the *Negative control solution* for each preparation.

Calculations

Linearity test: Use an analysis of variance (ANOVA) with one sample assayed against a standard, and using two replicates each, construct a table (see *Table 1*). Compare the value of the ratio $MSNL/MSRES_T$ to a critical value obtained from a table for an F distribution with $m - 2$ and $3m - 3$ degrees of freedom, where m is the number of dose levels for each preparation. If the ratio $MSNL/MSRES_T$ does not indicate the presence of significant nonlinearity (ratio value is lower than the critical value), then proceed to the *Parallelism test*. If the ratio exceeds the critical value (significance level of 0.025), the nonlinearity is statistically significant and the test is repeated, discarding the results from either the highest or lowest dose of both the *Standard solutions* and the *Sample solutions* (four dose levels). If the ratio $MSNL/MSRES_T$ does not indicate the presence of significant nonlinearity, then proceed to the *Parallelism test*.

Parallelism test: Compare the ratio $MSNP/MSRES_2$ to a critical value from an F distribution having 1 and $4m - 5$ degrees of freedom. If the ratio $MSNP/MSRES_2$ does not indicate the presence of significant nonparallelism, then the assay is considered valid. Use the appropriate dose levels for the estimation of the *Relative potency*.

Relative potency: Calculate the *Relative potency*, R, of the *Sample solutions* as compared with the *Standard solutions* as follows.

(1) X_j is defined as the $\log_{10}$ of the j^{th} dose of the *Standard solutions* or the *Sample solutions*. The glucagon dose varies from 12.5 to 200 × 10^{-6} USP Glucagon Units/mL. For ease in the subsequent calculations, these doses are represented by 1 through 5, as shown in the table below.

j	1	2	3	4	5
Dose	12.5	25	50	100	200
X_j	1.10	1.40	1.70	2.00	2.30

(2) To differentiate between the *Standard solutions* and the *Sample solutions* in the calculations, the subscript i will be used, with i = 1 to designate the *Standard solutions* and i = 2 to designate the *Sample solutions*. Y_{ijk} will denote the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the i^{th} preparation. For example, Y_{1jk} is the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the appropriate *Standard solution*; Y_{11K} is the glucose concentration associated with the k^{th} replicate of dose 1 of the *Standard solution*; and Y_{21K} is the glucose concentration associated with the k^{th} replicate of dose 1 of the *Sample solution*. Dose 1 represents a glucose dose of 12.5 × 10^{-6} USP Glucagon Units per mL. Finally, Y_{132} represents the glucose concentration associated with the 2nd replicate of dose 3 for the *Standard solution*.

(3) Y_S and Y_T denote the average glucose concentrations for the *Standard solutions* and the *Sample solutions*, respectively.

(4) Calculate the least-squares slope estimate, b, for a linear regression relating the Y_{ijk}'s to the X_j's as follows: $b = S_{XY}/S_{XX}$, with S_{XY} and S_{XX} calculated using the equations in *Table 2*.

(5) The log potency, M, is calculated using $M = -1[(Y_S - Y_T)/b]$.

(6) R = antilog (M).

(7) Calculate the confidence limits (upper and lower) for the relative potency, R, using the value $s^2 = MSRES_3$ (see *Table 1* and *Table 2*) as follows. Obtain t from a table for a t distribution having 4m −4 degrees of freedom. For the 95% limits, the t values can be obtained from *Table 9* under *Design and Analysis of Biological Assays* ⟨111⟩.

[NOTE—For confidence limits having other probability levels (i.e., 100(1 - a)%), the right tail t critical value having a/2 area to its right is used.]

Calculate $g = t^2 S^2 / b^2 S_{xx}$

and $F = (ts/b)\sqrt{(1/m)(1-g) + (M^2/S_{xx})}$

and calculate

$$M_L = (M - F)/(1 - g)$$

and

$$M_U = (M + F)/(1 - g)$$

M = log potency
M_L = log potency lower confidence limit
M_U = log potency upper confidence limit

The lower and upper confidence limits for the *Relative potency*, R, are as follows:

RL = antilog (M_L)
RU = antilog (M_U)

Acceptance criteria: The potency is NLT 80.0% and NMT 125.0% of the labeled amount of glucagon, and the confidence interval width at *P* equals 0.95 does not exceed 45% of the computed potency.

[NOTE—Repeat the *Assay* if the confidence interval width exceeds 45% of the computed potency, or if the potency is less than 80.0% or more than 125.0% of the labeled amount of glucagon.]

Table 1. ANOVA for the Rat Hepatocyte Assay for Glucagon

Source	Degrees of Freedom	SS (Sum of Squares)	MS (Mean Square)
Preparations	1	SSPREP	MSPREP
Replicates	1	SSREP	MSREP
Linear Slope	1	SSLIN	MSLIN
$Residual_3$	4m −4	$SSRES_3$	$MSRES_3$
Nonparallelism	1	SSNP	MSNP
$Residual_2$	4m −5	$SSRES_2$	$MSRES_2$
Nonlinearity	m −2	SSNL	MSNL
$Residual_T$	3m −3	$SSRES_T$	$MSRES_T$
TOTAL	4m −1	SST	

In-Process Revision

~~[NOTE—This analysis pertains to one sample assayed against a standard, using two replicates each.~~
~~The number of dose levels for each preparation is denoted by *m*.~~
~~*Table 2* gives the equations for calculating the SS terms.~~
~~In each row of the ANOVA table, the MS is obtained by dividing the SS term by the degrees of freedom.~~]

Table 2. Equations for Calculating the Sums of Squares in the Analysis of Variance*

$$Y_{i..} = \sum_{jk} \sum y_{ijk}$$

$$Y_{.j.} = \sum_{ik} \sum y_{ijk}$$

$$Y_{..k} = \sum_{ij} \sum y_{ijk}$$

$$CF = \frac{\left(\sum_{ijk} \sum\sum y_{ijk}\right)^2}{4m}$$

$$S_{xy}^S = \sum_{jk} \sum x_j y_{1jk} - \frac{\left(\sum_j x_j\right)(Y_{1..})}{m}$$

$$S_{xy}^T = \sum_{jk} \sum x_j y_{2jk} - \frac{\left(\sum_j x_j\right)(Y_{2..})}{m}$$

$$S_{xy} = S_{xy}^S + S_{xy}^T$$

$$S_{xx}^S = 2\sum_j x_j^2 - \frac{2\left(\sum_j x_j\right)^2}{m}$$

$$S_{xx}^T = S_{xx}^S$$

$$S_{xx} = S_{xx}^S + S_{xx}^T$$

$$SSPREP = \frac{\sum_i Y_{i..}^2}{2m} - CF$$

$$SSREP = \frac{\sum_k Y_{..k}^2}{2m} - CF$$

$$SSLIN = \frac{\left(S_{xy}\right)^2}{S_{xx}}$$

$$SST = \sum_{i\,j\,k} \sum\sum y_{ijk}^2 - CF$$

$$SSRES_3 = SST - SSPREP - SSREP - SSLIN$$

$$SSNP = \frac{\left(S_{xy}^S\right)^2}{S_{xx}^S} + \frac{\left(S_{xy}^T\right)^2}{S_{xx}^T} - SSLIN$$

$$SSRES_2 = SSRES_3 - SSNP$$

$$SSNL = \frac{\sum_j Y_{.j.}^2}{4} - SSLIN - CF$$

$$SSRES_1 = SSRES_2 - SSNL$$

*Refer to the *Calculations* for section on *Relative Potency* for the definitions of x_j and x_{ijk}.

■**Solution A:** Dissolve 16.3 g of monobasic potassium phosphate in 750 mL of water, adjust with phosphoric acid to a pH of 2.7 (± 0.05), add water to 800 mL, add 200 mL of acetonitrile, and degas.

Solution B: Prepare a degassed solution of acetonitrile and water (4:6).

Standard solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

System suitability solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

Sample solution: Dissolve an adequate amount of Glucagon for Injection in order to obtain a 0.5-mg/mL concentration of glucagon in 0.01 N hydrochloric acid.

Mobile phase: See the gradient table below. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Time (min)	Solution A (%)	Solution B (%)	Elution
0	61	39	isocratic
25[a]	61	39	isocratic
29	12	88	linear gradient
30	12	88	isocratic
31	61	39	linear gradient
70	61	39	re-equilibration

[a] The end time of the isocratic elution can be adjusted so that the gradient begins after the 4th desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3-mm × 15-cm; 3-µm or less packing L1
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 15 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, *Standard solution*
Tailing factor: NMT 1.8 for the glucagon peak, *Standard solution*
Resolution: NLT 1.5 between the main peak and the first eluting desamido peak. Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible, *System suitability solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{153}H_{225}N_{43}O_{49}S$ in the amount of Glucagon for Injection taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 65%–110%■2S (*USP33*)

IMPURITIES

Add the following:

■**Organic Impurities**
• **PROCEDURE**
Solution A, Solution B, Standard solution, System suitability solution, Mobile phase, Chromatographic system, and

In-Process Revision

System suitability requirements: Proceed as directed in the *Assay*.
Sample solution: Dissolve the substance to be examined in water in order to obtain a concentration of 0.5 mg/mL of glucagon.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all peaks
Acceptance criteria: NMT 14% total of all four desamido glucagons is found, and NMT 31% of total impurities and related compounds is found.■2S (USP33)

SPECIFIC TESTS

Add the following:

■• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 4.0%■2S (USP33)

Delete the following:

■• ~~**pH AND CLARITY OF SOLUTION:** Dissolve it in the solvent and in the concentration recommended in the labeling: the pH of the solution is between 1.7 and 3.0, and the solution is clear.~~■2S (USP33)

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** ~~It contains NMT 125.0 USP Endotoxin Units/mg of glucagon.~~■It contains NMT 10 USP Endotoxin Units/mg.■2S (USP33)

Change to read:

• **STERILITY TESTS** ⟨**71**⟩**:** ~~Both Glucagon for Injection and the accompanying solvent meet the requirements.~~■Meets the requirements■2S (USP33)
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* ⟨1⟩, *Constituted Solutions*.

Delete the following:

■• ~~**OTHER REQUIREMENTS:** Meets the requirements under *Injections* ⟨1⟩, *Labeling*~~■2S (USP33)

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Injections* ⟨1⟩, *Containers for Sterile Solids*.

Add the following:

■• **LABELING:** The labeling states that the material is of recombinant DNA origin.■2S (USP33)

Change to read:

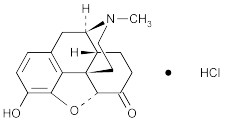
• **USP REFERENCE STANDARDS** ⟨**11**⟩
~~USP Dextrose RS~~
■■2S (USP33)
USP Endotoxin RS
~~USP Glucagon RS~~
■USP rGlucagon RS■2S (USP33)

BRIEFING

Hydromorphone Hydrochloride. *USP 32* page 2588. It is proposed to replace the *Ordinary Impurities* chromatographic test with an *Organic Impurities* procedure which uses a validated HPLC method. The liquid chromatographic procedure is based on analyses performed with a Waters Symmetry brand of L1 column. The typical retention time for hydromorphone hydrochloride is about 21 min. The USP Reference Standards section has also been updated appropriately to reflect the addition of USP Hydromorphone Related Compound A RS.

(MD-CCA: C. Anthony.) RTS—C44976

Hydromorphone Hydrochloride



$C_{17}H_{19}NO_3 \cdot HCl$ 321.80
Morphinan-6-one, 4,5-epoxy-3-hydroxy-17-methyl-, hydrochloride, (5α)-;
4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride [71-68-1].

DEFINITION
Hydromorphone Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 101.0% of $C_{17}H_{19}NO_3 \cdot HCl$.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
• **B. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
Sample solution: 100 µg/mL
Analytical wavelength: 280 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements
Sample solution: 1 in 20

ASSAY
• **PROCEDURE**
Sample: 225 mg, previously dried
Analysis: Transfer the *Sample* to a 250-mL conical flask. Dissolve in 80 mL of glacial acetic acid, warming, if necessary. Cool, and add 5 mL of acetic anhydride and 10 mL of mercuric acetate TS. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 32.18 mg of $C_{17}H_{19}NO_3 \cdot HCl$.
Acceptance criteria: 98.0%–101.0%, dried at 105° for 2 h

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.3%
• **SULFATE**
Sample solution: 100 mg in 5 mL of water
Analysis: To the *Sample solution,* add 0.5 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS.

In-Process Revision

Acceptance criteria: No turbidity is produced.

Change to read:

Organic Impurities

• **~~PROCEDURE: ORDINARY IMPURITIES ⟨466⟩~~**
~~Sample solution:~~ ~~Prepare in water.~~
~~Standard solution:~~ ~~Prepare in water.~~
~~Eluant:~~ ~~Methylene chloride, methanol, and ammonium hydroxide (80:20:1)~~
~~Visualization:~~ ~~3, followed by overspraying with hydrogen peroxide TS and immediate exposure of the plate to iodine vapors for 30 min.~~

■• **PROCEDURE**

Diluent: Phosphoric acid and water (1:1000)

Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:9). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Solution B: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:1). Add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	20
75	94	6
90	94	6

Standard solution: 4 µg/mL USP Hydromorphone Hydrochloride RS in *Diluent*.

System suitability solution: 0.8 mg/mL each of USP Hydromorphone Hydrochloride RS and USP Hydromorphone Related Compound A RS in *Diluent*. [NOTE—The solution should be kept in a cool place protected from light.]

Sample solution: 0.8 mg/mL of Hydromorphone Hydrochloride in *Diluent*.

Chromatographic system:
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Temperature: 45°
Flow rate: 1.0 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.0 between hydromorphone related compound A and hydromorphone peaks, *System suitability solution*
Tailing factor: NMT 1.5 for hydromorphone peak, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Calculate the percentage any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak response for each degradation found, including those in *Impurity Table 1*, from the *Sample solution*
r_S = peak response of hydromorphone from the *Standard solution*
C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding individual specified or unspecified impurity from *Impurity Table 1*.

Acceptance criteria
See *Impurity Table 1*. [NOTE—Disregard peaks corresponding to those obtained from the *Diluent*.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
8-Hydroxyhydromorphone[a]	0.50	1.0	0.15
Dihydromorphine (DHM)[b]	0.61	1.0	0.5
Morphine[c]	0.65	1.8	0.15
Hydromorphone *N*-oxide[d]	0.79	1.0	0.15
Hydromorphone related compound A[e]	0.93	1.4	0.1
Hydromorphone	1.0	—	—
2,2′-Bis hydromorphone dihydrochloride[f]	2.02	1.7	0.15
Individual unspecified impurities	—	1.0	0.1
Total impurities	—	—	1.0

[a] 4,5α-Epoxy-17-methylmorphinan-3,8-diol-6-one.
[b] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.
[c] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.
[d] 4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.
[e] 7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one.
[f] 2,2′-Bihydromorphone.

■2S (*USP33*)

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781**⟩
Sample solution: 50 mg/mL
Acceptance criteria: Between –136° and –139°

• **ACIDITY**
Sample: 300 mg
Analysis: Dissolve the *Sample* in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide VS.
Acceptance criteria: NMT 0.30 mL is required to produce a yellow color.

• **LOSS ON DRYING** ⟨**731**⟩ Dry a sample at 105° for 2 h: it loses NMT 1.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Change to read:

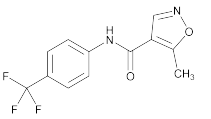
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Hydromorphone Hydrochloride RS
■USP Hydromorphone Related Compound A RS■2S (*USP33*)

BRIEFING

Leflunomide, *USP 32* page 2754. On the basis of comments received, it is proposed to increase the *Loss on Drying* specifications from 0.3% to 0.5% and increase the limit for leflunomide related compound A from 0.01% to 0.02% to be consistent with approved products.

(MD-CCA: C. Anthony.) RTS—C75413; C77281

Leflunomide



$C_{12}H_9F_3N_2O_2$ 270.21

4-Isoxazolecarboxamide, 5-methyl-*N*-[4-(trifluoromethyl)-phenyl]-;

α,α,α-Trifluoro-5-methyl-4-isoxazolecarboxy-*p*-toluidide [75706-12-6].

DEFINITION

Leflunomide contains NLT 98.0% and NMT 102.0% of $C_{12}H_9F_3N_2O_2$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
  Sample: Dry the substance for 10 min at 130°.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.
  Standard solution: 0.5 mg/mL of USP Leflunomide RS in *Mobile phase*. [NOTE—Dissolve USP Leflunomide RS in 5 mL of acetonitrile, and dilute with *Mobile phase*.]
  System suitability solution: 0.5 mg/mL of USP Leflunomide RS, 0.15 mg/mL of USP Leflunomide Related Compound B RS, and 0.05 mg/mL of USP Leflunomide Related Compound C RS in *Mobile phase*. [NOTE—Dissolve the Reference Standards in acetonitrile and dilute with *Mobile phase*.]
  Sample solution: 0.5 mg/mL of Leflunomide in acetonitrile and *Mobile phase* (1:9). [NOTE—First dissolve in acetonitrile. Protect solutions from light.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4-mm × 12.5-cm; packing L1
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for leflunomide related compound B and leflunomide related compound C are 0.2 and 0.9, respectively.]
  Suitability requirements
  Resolution: NLT 1.0 between the leflunomide and leflunomide related compound C peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{12}H_9F_3N_2O_2$ in the portion of Leflunomide taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 20 ppm

Change to read:

Organic Impurities

- **PROCEDURE 1: LIMIT OF LEFLUNOMIDE RELATED COMPOUND A**
  Mobile phase, System suitability solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
  Standard stock solution: 0.125 mg/mL of USP Leflunomide Related Compound A RS, in acetonitrile and *Mobile phase* (1:19)
  Standard solution: 0.25 µg/mL of USP Leflunomide Related Compound A RS, from *Standard stock solution* in *Mobile phase*
  Sample solution: 2.5 mg/mL of Leflunomide, in acetonitrile and *Mobile phase* (1:9)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of leflunomide related compound A in the portion of Leflunomide taken:

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

r_U = peak area of leflunomide related compound A from the *Sample solution*
r_S = peak area of leflunomide related compound A from the *Standard solution*
C_S = concentration of USP Leflunomide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT ~~0.01%~~■0.02 %■2S *(USP33)*

- **PROCEDURE 2**
  Mobile phase, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
  Standard solution: 0.5 µg/mL of USP Leflunomide RS, from the *Standard solution* in *Mobile phase*
  System suitability solution: 0.25 µg/mL of Leflunomide, from the *Standard solution* in *Mobile phase*
  System suitability
  Suitability requirements
  Resolution: NLT 1.0 between leflunomide and leflunomide related compound C
  Signal-to-noise ratio: NLT 10, *System suitability solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  [NOTE—Disregard any peak with an area less than that of the leflunomide peak from the *System suitability solution*. Continue the elution for two times the retention time of the leflunomide peak.]
  Calculate the percentage of each related compound and any unknown impurity (see *Impurity Table 1*) in the portion of Leflunomide taken:

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

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of leflunomide from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

In-Process Revision

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methylisoxazole-carboxylic acid	0.05	1.0	0.1
Leflunomide related compound B	0.22	1.0	0.3
N-(2′-Trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide	0.29	1.0	0.1
2-Cyano-acetic acid-(4′-trifluoromethyl)-anilide	0.36	1.0	0.1
Leflunomide related compound C	0.94	1.0	0.1
Any other individual impurity	—	—	0.1
Total impurities, excluding leflunomide related compound B and leflunomide related compound C	—	—	0.2
Total impurities	—	—	0.4

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE ⟨741⟩:** 164°–168°

Change to read:

• **LOSS ON DRYING ⟨731⟩:** Dry a sample in a vacuum over diphosphorus pentoxide at 60° for 4 h: it loses NMT ~~0.3%~~ ■0.5%■2S *(USP33)* of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in a well-closed container. Store at a temperature not exceeding 30°.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Leflunomide RS
USP Leflunomide Related Compound A RS
USP Leflunomide Related Compound B RS
USP Leflunomide Related Compound C RS

BRIEFING

Leflunomide Tablets, *USP 32* page 2755. On the basis of comments received, it is proposed to delete the test for *Water Determination*. The water content of dosage forms is highly dependent on excipients and may vary widely between formulations. In addition, the excipients used in the formulation are individually limited for water content in their respective *NF* monographs, and this should be sufficient for moisture control.

(MD-CCA: C. Anthony.) RTS—C75414

Leflunomide Tablets

DEFINITION
Leflunomide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
Analytical wavelength: 220–360 nm
Sample solution: 0.01 mg/mL in methanol
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.0.
System suitability solution A: 10 µg/mL of USP Leflunomide Related Compound A RS, 1 mg/mL of USP Leflunomide Related Compound B RS, and 100 µg/mL of USP Leflunomide Related Compound C RS in a minimum amount of acetonitrile, and diluted with *Mobile phase*
System suitability solution B: Transfer 100.0 mg of USP Leflunomide RS to a 100-mL volumetric flask. Dissolve in 2 mL of acetonitrile, add 1 mL of *System suitability solution A* and 80 mL of *Mobile phase*, and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume.
Standard solution: 1 mg/mL of USP Leflunomide RS in a minimum volume of acetonitrile, and diluted in *Mobile phase*
Sample solution: Equivalent to 100 mg of leflunomide from Tablets (finely powder NLT 20 Tablets), to a 100-mL volumetric flask. Add 20 mL of acetonitrile, dilute with *Mobile phase* to volume, and shake by mechanical means for 10 min. Pass through a membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.0-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution B*
[NOTE—The relative retention times for leflunomide related compound A, leflunomide related compound B, leflunomide related compound C, and leflunomide are 0.4, 0.2, 0.9, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between leflunomide related compound C and leflunomide
Tailing factor: NMT 3.0 for leflunomide
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_9F_3N_2O_2$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of leflunomide in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION ⟨711⟩**
Medium:
For Tablets labeled to contain 10 mg or 20 mg: Water, 1000 mL, deaerated
For Tablets labeled to contain 100 mg: Water containing 0.6% of polyoxyethylene lauryl ether; 1000 mL, deaerated
Apparatus 2: 100 rpm
Time: 30 min
Determine the amount of $C_{12}H_9F_3N_2O_2$ dissolved employing one of the following methods:

In-Process Revision

Spectrometric method
Mode: UV
Analytical wavelength: 262 nm
Standard solution: USP Leflunomide RS in *Medium*. [NOTE—A volume of methanol not exceeding 2% of the final volume of the *Standard solution* may be used to dissolve leflunomide.]
Sample solutions: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to *Standard solution*. Pass through a 0.45-µm filter.
Chromatographic method
Mobile phase: Acetonitrile and water (1:1)
Standard solution: Transfer 22 mg of USP Leflunomide RS to a 100-mL volumetric flask. Add 40 mL of acetonitrile, and sonicate until dissolved. Add 40 mL of water, and cool to room temperature. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Use portions of the solution under test passed through a suitable 0.45-µm filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_9F_3N_2O_2$ dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of leflunomide in the *Sample solution* (mg/mL)
Tolerances: NLT 80% (Q) of the labeled amount of $C_{12}H_9F_3N_2O_2$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements
Procedure for content uniformity
Mobile phase, Standard solution, System suitability solutions, and **Chromatographic system:** Prepare as directed in the *Assay*.
Sample solution: Transfer 1 Tablet to a suitable volumetric flask, and prepare a solution having a concentration of 1 mg/mL of leflunomide. Add *Mobile phase* 50% by volume, and shake to disintegrate the Tablet. After the Tablet is completely disintegrated, add acetonitrile 20% by volume, dilute with *Mobile phase* to volume, and shake again. Pass through a membrane filter.
Analysis: Proceed as directed in the *Assay*.

IMPURITIES
Organic Impurities
• **PROCEDURE**
Mobile phase, System suitability solutions, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the related compounds and the leflunomide peak responses from the *Sample solution*

Acceptance criteria
Leflunomide related compound A: NMT 0.1%
Leflunomide related compound B: NMT 3.5%
Leflunomide related compound C: NMT 0.2%
Individual impurities: NMT 0.2%
Total impurities: NMT 4.0%

SPECIFIC TESTS

Delete the following:

■• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** ~~NMT 9.0%.~~■2S *(USP33)*

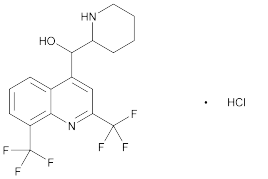
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, and humidity-resistant containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Leflunomide RS
USP Leflunomide Related Compound A RS
USP Leflunomide Related Compound B RS
USP Leflunomide Related Compound C RS

BRIEFING

Mefloquine Hydrochloride, *USP 32* page 2865. On the basis of supporting validation data, it is proposed to revise the nonselective titration method for the *Assay* with a selective HPLC method. The analysis is performed with a Phenomenex Lichrosphere 100 brand of 5-µm, L1 column. The typical retention time for mefloquine is 4.5 min.

(MD-AA: L. Santos, B. Davani.) RTS—C65586

Mefloquine Hydrochloride



$C_{17}H_{16}F_6N_2O \cdot HCl$ 414.77
4-Quinolinemethanol, α-2-piperidinyl-2,8-bis(trifluoromethyl)-, monohydrochloride, (*R**,*S**)- (±)-;
DL-*erythro*-α-2-Piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride [51773-92-3].

DEFINITION
Mefloquine Hydrochloride contains NLT 99.0% and NMT 101.0% of $C_{17}H_{16}F_6N_2O \cdot HCl$, calculated on the anhydrous basis.

In-Process Revision

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨197⟩
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

Change to read:

- **PROCEDURE**

~~**Sample solution:** Dissolve about 0.35 g in 15 mL of anhydrous formic acid, and add 40 mL of acetic anhydride.~~
~~**Analysis:** Place the *Sample solution* in a glass container thermostated at 20°. Titrate with 0.1 N perchloric acid VS. [NOTE—Perform the titration rapidly after the addition of acetic anhydride by predosing with about 60% of the expected titrant, and then slowly titrate.] Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.48 mg of $C_{17}H_{16}F_6N_2O \cdot HCl$.~~
~~**Acceptance criteria:** 99.0%–101.0%~~

■**Solution A:** 1.5 g/L of sodium hydrogen sulfate in water
Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1000-mL mixture of acetonitrile, methanol, and *Solution A* (2:1:2).
Standard solution: 0.2 mg/mL of USP Mefloquine Hydrochloride RS in *Mobile phase*
System suitability solution: 4 µg/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Mefloquine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4-mm × 3-cm; C18
Column: 4.0-mm × 25-cm column; 5-µm packing L1
Column temperature: 25°
Flow rate: 0.8 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between mefloquine related compound A and mefloquine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{16}F_6N_2O \cdot HCl$ in the portion of Mefloquine Hydrochloride taken:

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

r_U = peak response of mefloquine from the *Sample solution*
r_S = peak response of mefloquine from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mefloquine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the anhydrous basis■2S *(USP33)*

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨231⟩**:** NMT 20 ppm

Organic Impurities
- **PROCEDURE**

Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1-L mixture of a 1.5-g/L solution of sodium hydrogen sulfate, acetonitrile, and methanol (2:2:1).
System suitability solution: 4 µg/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase.* [NOTE—Mefloquine related compound A is threo-mefloquine.]
Sample stock solution: 4 mg/mL of Mefloquine Hydrochloride in *Mobile phase*
Sample solution: 4 µg/mL from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4-mm × 2.5-cm; 5-µm packing L1
Column: 4.0-mm × 25-cm column; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 20 µL
[NOTE—Equilibrate the column with *Mobile phase* at a flow rate of 0.8 mL/min for 30 min.]
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between mefloquine related compound A and mefloquine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample stock solution* and *Sample solution*
Record the chromatogram for a time that is 10 times the retention time of the main peak.
Acceptance criteria: The response of the mefloquine related compound A peak in the *Sample stock solution* is NMT twice the area of the main peak of the *Sample solution* (0.2%). The response of any other individual peak, other than the main peak of the *Sample stock solution*, is NMT that of the main peak of the *Sample solution* (0.1%); and the sum of the responses of any such peaks of the *Sample stock solution* is NMT five times the response of the main peak of the *Sample solution* (0.5%). [NOTE—Exclude the main peak and any other peak producing a response of less than 0.2 times (0.02%) the main peak of the *Sample solution*.]

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781⟩**:** –0.2° to +0.2°
  Sample solution: 50 mg/mL, in methanol
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mefloquine Hydrochloride RS
  USP Mefloquine Related Compound A RS

In-Process Revision

BRIEFING

Methylphenidate Hydrochloride Extended-Release Tablets. *USP 32* page 2950. On the basis of comments received, a test for *Organic Impurities* is being added to the monograph. The proposed liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with a Water Symmetry C18, brand of L1 column. The typical retention times are 11.5 min for methylphenidate hydrochloride, 5.4 min for methylphenidate related compound A, and 7.5 min for methylphenidate hydrochloride erythro isomer. *Identification* test *B*, with an HPLC retention time comparison of the major peak in the chromatograms of the *Standard solution* and *Sample solution* in the test for *Organic Impurities* is also proposed.

(MD-PP: H. Ramanathan, R. Ravichandran.) RTS—C42825

Methylphenidate Hydrochloride Extended-Release Tablets

DEFINITION

Methylphenidate Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION

• **INFRARED ABSORPTION**

Sample specimen: Place a portion of powdered Tablets, equivalent to 100 mg of methylphenidate hydrochloride, into a 100-mL beaker. Add 20 mL of chloroform, stir for 5 min, and filter, collecting the filtrate. Evaporate the filtrate to about 5 mL. Add ethyl ether slowly, with stirring, until crystals form. Filter the crystals, wash with ethyl ether, and dry at 80° for 30 min.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the crystals obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methylphenidate Hydrochloride RS.

Add the following:

■• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test under *Organic Impurities*.■2S (*USP33*)

ASSAY

• **PROCEDURE**

Acetate buffer: Dissolve 1.64 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0, and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Acetate buffer* (4:3:3)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: 0.2 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*

Standard solution: Transfer 10.0 mL of *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of *Internal standard solution*, and mix.

Sample stock solution: 0.2 mg/mL of methylphenidate hydrochloride from powdered Tablets (NLT 20 Tablets) in *Mobile phase*. [NOTE—Sonicate for 15 min.] Cool to room temperature. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable membrane filter, discarding the first portion of the filtrate. [NOTE—Avoid the use of glass filters. Polypropylene filters are suitable for use.]

Sample solution: Transfer 10.0 mL of the clear filtrate to a glass-stoppered, 25-mL conical flask, and add 5.0 mL of *Internal standard solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1.5 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 2.0% from the peak response ratios of the analyte to the internal standard

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{19}NO_2 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the analyte to the internal standard from the *Sample solution*
R_S = peak response ratio of the analyte to the internal standard from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨**711**⟩

Test 1
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Times: 1, 2, 3.5, 5, and 7 h
Sample solution: Use portions of the solution under test passed through suitable 0.45-µm filter. [NOTE—Do not use glass fiber filters.]
Analysis: Determine the amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved by using the procedure in the *Assay*, and make any necessary volumetric adjustments.
Tolerances: The percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	25%–45%
2	40%–65%
3.5	55%–80%
5	70%–90%
7	NLT 80

Test 2 (for products labeled for dosing every 24 h): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Acidified water, adjust with phosphoric acid to a pH of 3; 50 mL, at 37 ± 0.5°
Apparatus 7: (see *Drug Release* ⟨724⟩)
30 cycles/min; 2–3 cm amplitude
Use *Sample Preparation A* with a metal coil sample holder (*Figure 4d*). Place 1 Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

In-Process Revision

Times: 1 h intervals for a duration of 10 h
Determine the percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved by using the following method.
Dilution medium: Mixture of acetonitrile and *Medium* (1:3)
Standard stock solution: 0.3 mg/mL USP Methylphenidate Hydrochloride RS in *Dilution medium*
Standard solutions: Prepare at least six solutions by making serial dilutions of the *Standard stock solution* in *Dilution medium* to bracket the expected drug concentration range.
Solution A: Dissolve 2.0 g of 1-octanesulfonic acid sodium salt in 700 mL of water, mix well, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Solution A* (3:7)
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.2-mm × 5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Column temperature: 30°
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Capacity factor: NMT 2
Relative standard deviation: NMT 2% from the peak response of the analyte; NMT 2% of retention time of the analyte
Analysis
Samples: *Standard solutions* and the solution under test
Construct a calibration curve by plotting the peak response versus the concentration of the *Standard solutions*. Determine the amount of $C_{14}H_{19}NO_2 \cdot HCl$ in each interval by linear regression analysis of the standard curve.
Tolerances: The percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	12%–32%
4	40%–60%
10	NLT 85%
Average from 3 to 6	9%–15%/h

Calculate the average percentage released from 3 to 6 h:

$$\text{Result} = (Y - X)/3$$

Y = cumulative drug released from 0 to 6 h
X = cumulative drug released from 0 to 3 h

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Add the following:

■Organic Impurities

• **PROCEDURE**

Mobile phase: Dissolve 2 g of 1-octanesulfonic acid in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.
Solution A: Acidified water. Adjust with phosphoric acid to a pH of 3.
Diluent A: Acetonitrile and *Solution A* (1:3)
Diluent B: Acetonitrile and methanol (1:1)
System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS and 2 µg/mL of USP Methylphenidate Related Compound A RS, in *Diluent A*
Standard solution: 0.2 µg/mL of USP Methylphenidate Hydrochloride RS, 0.5 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS and 1.5 µg/mL of USP Methylphenidate Related Compound A RS, in *Diluent A*
Sample stock solution: Dissolve 10 Tablets in 20% of total volume in *Diluent B*. Stir for 4 h. Dilute with *Solution A* to volume.
Sample solution: 0.1 mg/mL of methylphenidate hydrochloride in *Solution A,* from *Sample stock solution.* [NOTE—Centrifuge before chromatographic analysis.]
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
Temperature: 30°
Run time: 2 times the retention time of methylphenidate
System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 2.0 for methylphenidate peak
Resolution: NLT 6.0 between methylphenidate and erythro isomer peaks
Relative standard deviation: NMT 2.0% for methylphenidate peak and NMT 4.0% each for methylphenidate related compound A and erythro isomer peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylphenidate related compound A or erythro isomer in the portion of Tablets taken:

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

r_U = response of methylphenidate related compound A or erythro isomer from the *Sample solution*
r_S = response of methylphenidate related compound A or erythro isomer from the *Standard solution*
C_S = concentration of USP Methylphenidate Related Compound A RS or methylphenidate hydrochloride erythro isomer, in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of USP Methylphenidate Hydrochloride RS from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1.*
Total impurities: NMT 2.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate related compound A[a]	0.47	1.5
Erythroisomer[b]	0.65	0.5

[a] α-Phenyl-2-piperidineacetic acid.
[b] Methyl (*RS,SR*)-2-phenyl-2-(piperidin-2-yl) acetate.

Impurity Table 1 *(Continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate hydrochloride	1.0	—
Any unspecified degradation product	—	0.2

[a] α-Phenyl-2-piperidineacetic acid.
[b] Methyl (*RS,SR*)-2-phenyl-2-(piperidin-2-yl) acetate.

■2S (*USP33*)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The labeling states the *Dissolution Test* with which the product complies if other than *Test 1*.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Methylphenidate Hydrochloride RS
  ■USP Methylphenidate Hydrochloride Erythro Isomer Solution RS
  USP Methylphenidate Related Compound A RS■2S (*USP33*)

BRIEFING

Morphine Sulfate Extended-Release Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph based on validated methods of analysis is proposed. The liquid chromatographic procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with the Keystone ODS/B brand of L1 column. Typical retention times are about 2.8 min for morphine sulfate related compound A, about 3.7 min for morphine sulfate, and about 5.0 min for morphine sulfate related compound B.

(MD-CCA: C. Anthony. BPC: M. Marques.) RTS—C34840

Add the following:

■Morphine Sulfate Extended-Release Tablets

DEFINITION
Morphine Sulfate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨**191**⟩
  Analysis: Triturate a portion of the composite powder from the *Assay* equivalent to about 250 mg of morphine sulfate with 25 mL of alcohol, sonicate for 10 min, and centrifuge the mixture. Decant the supernatant through a glass-fiber filter. Wash the residue with 15 mL of alcohol, centrifuge, and decant the supernatant through the same filter. Evaporate the combined filtrates to dryness, digest the residue in 10 mL of warm water, and pass through another glass-fiber filter.
  Acceptance criteria: The filtrate (1-mL portions) meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 20.8 mg/mL of monobasic sodium phosphate and 0.87 mg/mL of sodium octanesulfonate in water
  Mobile phase: Methanol, triethylamine, and *Buffer* (150:1:350). Adjust with phosphoric acid to a pH of 4.5. Pass through a 0.45-µm polyvinylidene fluoride membrane filter.
  Solvent: Methanol and water (19:1)
  Diluent: Methanol and water (1:1)
  Standard stock solution A: 0.06 mg/mL of USP Morphine Sulfate Related Compound A RS in *Mobile phase*
  Standard stock solution B: Dissolve a quantity of USP Morphine Sulfate Related Compound B RS in a suitable quantity of 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.06 mg/mL.
  Standard solution: Weigh a quantity of USP Morphine Sulfate RS, and measure quantities of USP Morphine Sulfate Related Compound A RS stock solution and USP Morphine Sulfate Related Compound B RS stock solution. Dissolve in *Mobile phase* to obtain a solution having known concentrations of about 0.12 mg/mL of morphine sulfate, and about 2.4 µg/mL each of morphine sulfate related compound A and morphine sulfate related compound B. Mix well, and pass through a 0.45-µm polyvinylidene fluoride membrane filter, discarding the first 3 mL of solution.
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 30 mg of morphine sulfate, to a 100-mL volumetric flask. Add about 20 mL of *Solvent* and gently swirl to disperse. Allow to stand for 5 min. Add, while swirling, 50 mL of *Diluent.* Sonicate for at least 5 min, and shake mechanically for at least 30 min. Dilute with *Diluent* to volume, mix, and then transfer 4.0-mL into a 10.0-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass through a 0.45-µm polyvinylidene fluoride membrane filter, and discard the first 3 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 15-cm column; 5-µm packing L1
  Flow rate: 1.2 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0 for the morphine sulfate peak
  Resolution: NLT 2 between any two major peaks
  Relative standard deviation: NMT 2.0% for morphine sulfate from replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of morphine sulfate, calculated on anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of of morphine sulfate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of morphine sulfate, 758.83
M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

In-Process Revision

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DRUG RELEASE** ⟨**724**⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Times: 2, 4, and 8 h
Standard solution: 30 µg/mL of USP Morphine Sulfate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a 0.8-µm mixed cellulose ester filter, discarding the first few mL. Replace the volume withdrawn with the same volume of warmed *Medium*.
Detector: UV 286 nm
Blank: *Medium*
Percentage of morphine sulfate dissolved at 2 h:

$$(A_{U2}/A_S) \times (C_S \times V) \times 100/L$$

Percentage of morphine sulfate dissolved at 4 h:

$$(A_{U4}/A_S) \times (C_S \times V) \times 100 + A/L$$

Percentage of morphine sulfate dissolved at 8 h:

$$(A_{U8}/A_S) \times (C_S \times V) \times 100 + A + B/L$$

Amount of morphine sulfate (mg) removed at 2 h (A):

$$A = A_{U2}/A_S \times C_S \times V_2$$

Amount of morphine sulfate (mg) removed at 4 h (B):

$$B = A_{U4}/A_S \times C_S \times V_4$$

A_{U2} = absorbance of the *Sample solution* at 2 h
A_{U4} = absorbance of the *Sample solution* at 4 h
A_{U8} = absorbance of the *Sample solution* at 8 h
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of morphine sulfate, 500 mL
L = Tablet label claim (mg)
V_2 = volume of sample withdrawn at 2 h (mL)
V_4 = volume of sample withdrawn at 4 h (mL)
Tolerances: The percentages of the labeled amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	30%–50%
4	50%–70%
8	NLT 75%

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES
Organic Impurities
• **PROCEDURE**
Buffer, Mobile phase, Solvent, Diluent, Standard solution, Sample solution, Standard stock solution A, and **Standard stock solution B:** Proceed as directed in the *Assay*.
Chromatographic system: Proceed as directed in the *Assay*. (See *Chromatography* ⟨621⟩, *System Suitability*.)
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for each major peak
Resolution: NLT 2 between any two major peaks
Relative standard deviation: NMT 2.0% for morphine sulfate from replicate injections; NMT 3.0% for morphine sulfate related compound A and morphine sulfate related compound B from replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for the individual impurity from the *Standard solution*
C_S = concentration of the related compound in the *Standard solution* (mg/mL)
C_U = concentration of morphine sulfate in the *Sample solution* (mg/mL)
Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Morphine sulfate related compound A[a]	0.8	0.5
Morphine sulfate	1.0	—
Morphine sulfate related compound B[b]	1.4	0.5

[a] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol, *N*-oxide
[b] 2,2′-Bimorphine

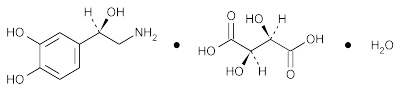
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at or below room temperature.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Morphine Sulfate RS
USP Morphine Sulfate Related Compound A RS
USP Morphine Sulfate Related Compound B RS■2S *(USP33)*

BRIEFING

Norepinephrine Bitartrate, *USP 32* page 3103. On the basis of comments received from FDA, it is proposed to revise the *Residue on Ignition* test under *Inorganic Impurities* by replacing the currently stated limit of "Negligible" with a quantitative limit of NMT 1.0%.

(MD-CV: S. Ramakrishna.) RTS—C68836

Norepinephrine Bitartrate



$C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$ 337.28

$C_8H_{11}NO_3 \cdot C_4H_6O_6$ 319.27

1,2-Benzenediol, 4-(2-amino-1-hydroxyethyl)-, (*R*)-,[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt), monohydrate;
(–)-α-(Aminomethyl)-3,4-dihydroxybenzyl alcohol tartrate (1:1) (salt), monohydrate [69815-49-2].
Anhydrous [51-40-1].

DEFINITION
Norepinephrine Bitartrate contains NLT 97.0% and NMT 102.0% of $C_8H_{11}NO_3 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

In-Process Revision

IDENTIFICATION
- **A. Infrared Absorption** ⟨197K⟩
- **B. Procedure**
  Sample solution: Dissolve 10 mg in 2 mL of water.
  Analysis: Add 1 drop of ferric chloride TS.
  Acceptance criteria: An intensely green color develops.
- **C. Procedure**
  Sample solution: 0.01 µg/mL
  Analysis: Add 1.0 mL of 0.10 N iodine to 10 mL of the *Sample solution*. Allow to stand for 5 min, and add 2.0 mL of 0.10 N sodium thiosulfate.
  Acceptance criteria: The solution is colorless, or has at most a slight pink or slight violet color (epinephrine and isoproterenol at the same pH, about 3.5, give a strong red-brown or violet color).

ASSAY
- **Procedure**
  Sample solution: Dissolve 500 mg of Norepinephrine Bitartrate in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution.
  Analysis: Add 2 drops of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 31.93 mg of $C_8H_{11}NO_3 \cdot C_4H_6O_6$.
  Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

Inorganic Impurities
- **Residue on Ignition** ⟨281⟩: ~~Negligible~~■NMT 0.1%,■2S *(USP33)* from 200 mg

Organic Impurities
- **Procedure: Limit of Arterenone**
  Sample solution: 2 mg/mL in water
  Analysis: Determine the absorptivity of the *Sample solution* (see *Spectrophotometry and Light-Scattering* ⟨851⟩) at 310 nm.
  Acceptance criteria: NMT 0.2

SPECIFIC TESTS
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩: –10° to –12°
  Sample solution: 50 mg/mL
- **Water Determination,** *Method I* ⟨921⟩: 4.5%–5.8%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP Reference Standards** ⟨11⟩
  USP Norepinephrine Bitartrate RS

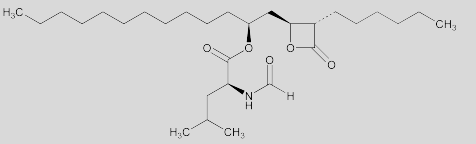
Briefing

Orlistat. Because there is no existing *USP* monograph for this article, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities, Procedure 3* are based on analyses performed with a Nova-Pak brand of L1 column. The typical retention time for orlistat is about 10.5 min. The gas chromatographic procedure in the test for *Limit of Orlistat Related Compound B* is based on analyses performed with a DB-1 brand of column. The typical retention time for orlistat related compound B is about 22 min. The liquid chromatographic procedure in the test for *Limit of Orlistat Related Compound D* is based on analyses performed with a Superspher 60-RP Select B brand of L7 column. The typical retention time for orlistat related compound D is about 35 min. The liquid chromatographic procedure in the test for *Limit of Orlistat Related Compound E* is based on analyses performed with an aminoquant column. The typical retention time for orlistat related compound E is about 15.5 min.

(MD-CCA: C. Anthony.) RTS—C44029

Add the following:

■Orlistat



$C_{29}H_{53}NO_5$ 495.73

L-Leucine, *N*-formyl-, 1-[(3-hexyl-4-oxo-2-oxetanyl)methyl]dodecyl ester, [2*S*-[2α(*R**), 3β]]-;
N-Formyl-L-leucine, ester with (3*S*,4*S*)-3-hexyl-4-[(2*S*)-2-hydroxytridecyl]-2-oxetanone [96829-58-2].

DEFINITION
Orlistat contains NLT 98.0% and NMT 101.5% of $C_{29}H_{53}NO_5$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION
- **A. Infrared Absorption** ⟨197M⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **Procedure**
  [Note—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]
  Mobile phase: Acetonitrile, water, and phosphoric acid (860:140:0.05)
  Standard solution: 0.5 mg/mL of USP Orlistat RS in *Mobile phase*. Inject immediately after preparation or store at 5°.
  Sample solution: 0.5 mg/mL of Orlistat in *Mobile phase*. Inject immediately after preparation or store at 5°.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 195
Column: 3.9-mm × 15-cm column; 4-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{29}H_{53}NO_5$ in the portion of Orlistat taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous, solvent-free basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 20 ppm

Organic Impurities

- **PROCEDURE 1: LIMIT OF ORLISTAT RELATED COMPOUND A**

Standard solution: 0.1 mg/mL of USP Orlistat Related Compound A RS in acetone
Sample solution: 50 mg/mL of Orlistat in acetone
Chromatographic system
(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Toluene and ethyl acetate (4:1)
Detection solution: Transfer 2.5 g of phosphomolybdic acid and 1 g of cerric sulfate into a 100-mL volumetric flask, dissolve in and dilute with methanol to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Remove the plate, and air-dry it thoroughly. Spray the dried plate with *Detection solution*, and place the plate in an oven at 120° for 30 min.
Acceptance criteria: Any secondary spot from the *Sample solution* corresponding to orlistat related compound A is not more intense than the corresponding spot from the *Standard solution* (0.2%).

- **PROCEDURE 2: LIMIT OF ORLISTAT RELATED COMPOUND B**

Standard solution: 0.025 mg/mL of USP Orlistat Related Compound B RS in methylene chloride
Sample solution: 50 mg/mL of Orlistat in methylene chloride
Spiked sample solution: 50 mg/mL of Orlistat in *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm x 30-m fused silica column coated with a 0.25-µm G27 stationary phase
Column temperature: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	4	170	—
170	30	300	30

Injector temperature: 270°
Detector temperature: 280°
Carrier gas: Helium
Flow rate: 30 mL/min
Split ratio: 10:1
Injection size: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Sample solution* and *Spiked sample solution*
Calculate the percentage of orlistat related compound B in the portion of Orlistat taken:

$$\text{Result} = 100(r_U/(r_{SP} - r_U))(C_S/C_T)$$

r_U = peak response of orlistat related compound B from the *Sample solution*
r_{SP} = peak response of orlistat related compound B from the *Spiked sample solution*
C_S = concentration of USP Orlistat Related Compound B RS in the *Standard solution* (mg/mL)
C_T = concentration of Orlistat in the *Spiked sample solution* (mg/mL)

Acceptance criteria: NMT 0.05% of orlistat related compound B is found.

- **PROCEDURE 3**

[NOTE—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 10 µg/mL of USP Orlistat RS, 0.1 µg/mL of USP Orlistat Related Compound C RS, and 0.25 µg/mL of USP Orlistat Related Compound D RS in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Proceed as directed in the *Assay* except to chromatograph the *System suitability solution*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for the orlistat related compound C and orlistat related compound D peaks
Relative standard deviation: NMT 10.0% for the orlistat peak
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Orlistat taken:

$$\text{Result} = 100(r_U/(r_S)(C_S/C_U)(1/F)$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response of USP Orlistat RS from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)
F = relative response factor as given in *Impurity Table 1*

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Formylleucine[a]	0.10	4.0	0.2
Orlistat related compound C	0.13	33	0.05
Orlistat open ring epimer[b]	0.44	1.0	0.2
Orlistat related compound D*	0.90	—	Calculated in *Procedure 4*
Orlistat open ring amide[c*]	0.90	—	Calculated in *Procedure 4*
Orlistat	1.00	—	—
D-Leucine orlistat[d]	1.18	1.0	0.2
Individual unidentified impurity	—	1.0	0.1

*Coelutes in this LC system, determined using *Procedure 4*.
[a]*N*-Formyl-L-leucine.
[b](2*S*,3*R*,5*S*)-5-[(*S*)-2-Formylamino-4-methyl-pentanoyloxy]-2-hexyl-3-hydroxy-hexadecanoic acid.
[c]*N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethyl-carbomoyl]nonyl]-dodecyl ester.
[d]*N*-Formyl-D-leucine (*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester or enantiomer.

- **PROCEDURE 4: LIMIT OF ORLISTAT RELATED COMPOUND D**

Mobile phase: Methanol and water (83:17)
System suitability solution: 4 mg/mL of USP Orlistat RS and 2.4 µg/mL of USP Orlistat Related Compound D RS in acetonitrile, respectively
Standard solution: 5.0 mg/mL of USP Orlistat RS in acetonitrile
Sample solution: 5.0 mg/mL of Orlistat in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 205 nm
Column: 4.0-mm × 25-cm column; 5-µm packing L7
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for the orlistat related compound D peak
Relative standard deviation: NMT 10.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Orlistat taken:

$$\text{Result} = 100(r_U/(r_S)(C_S/C_U)(1/F)$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for USP Orlistat RS from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (µg/mL)
C_U = concentration of Orlistat in the *Sample solution* (µg/mL)
F = relative response factor as obtained in *Impurity Table 2*

Acceptance criteria: See *Impurity Table 2*.

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat related compound D	0.94	1.0	0.2
Orlistat	1.00	—	—
Orlistat open ring amide[e]	1.25	4.3	0.1

[e]*N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethyl-carbomoyl]nonyl]-dodecyl ester.

- **PROCEDURE 5: LIMIT OF ORLISTAT RELATED COMPOUND E**

Solution A: Transfer 4.1 g of sodium acetate trihydrate and 40 mg of ethylenediaminetetraacetic acid (EDTA) into a 1-L volumetric flask, dissolve in 950 mL of water, and adjust the pH to 7.2 with 0.1 N sodium hydroxide. Dilute with water to volume, add 2.5 mL of tetrahydrofuran, and mix. Filter and degas.
Solution B: Transfer 2.7 g of sodium acetate trihydrate and 40 mg of EDTA into a 1-L volumetric flask, dissolve in 200 mL of water, and adjust the pH to 7.2 with 0.1 N sodium hydroxide. Add 800 mL of acetonitrile, filter, and degas.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	96.7	3.3
20	60	40
24	0	100
38	96.7	3.3
45	96.7	3.3

Standard stock solution: Prepare a 2.6 µg/mL solution by dissolving a weighed amount of USP Orlistat Related Compound E RS into a suitable volumetric flask, using a volume of 4 N sodium hydroxide equal to 10% of the volume of the flask. Dilute with water to volume, and mix.
Standard solution: 0.13 µg/mL of USP Orlistat Related Compound E RS in water from *Standard stock solution*
Sample solution: Transfer a weighed quantity of about 50 mg of Orlistat into a 20-mL head-space vial, add 20 mL of 4 N sodium hydroxide, and close the vial. Heat the vial to 100° for 1 h. Allow the solution to cool slowly at room temperature. Transfer 2 mL of the resulting solution into a 50-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence 340 nm (excitation); 450 nm (emission)
Column: 2.1-mm × 20-cm column; packing L1 and 2.1-mm × 2-cm guard column; 50-µm packing L1
Flow rate: 0.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 6.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of this impurity in the portion of Orlistat taken:

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

r_U = peak response for orlistat related compound E in the *Sample solution*
r_S = peak response for USP Orlistat Related Compound E RS in the *Standard solution*

In-Process Revision

C_S = concentration of USP Orlistat Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurity: NMT 0.2% of orlistat related compound E is found.
Total impurities: NMT 1.0% of total impurities is found, the results for *Procedures 1, 2, 3, 4,* and *5* being added.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781**⟩**:** Between –48.0° and –51.0°, at 20°
- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Orlistat RS
  USP Orlistat Related Compound A RS
  USP Orlistat Related Compound B RS
  USP Orlistat Related Compound C RS
  USP Orlistat Related Compound D RS
  USP Orlistat Related Compound E RS■2S (*USP33*)

BRIEFING

Orlistat Capsules. The previous proposal for this *USP* dosage form monograph (see page 1739 of *PF* 32(6) [Nov.–Dec. 2007]) is being canceled, and the monograph is being republished in the new Monograph Redesign format. The liquid chromatographic procedures in the *Assay* and in the tests for *Dissolution* and *Organic Impurities* are based on analyses performed with the Nova-Pak C18 brand of L1 packing. The typical retention time for orlistat is about 10.2 min.

(MD-CCA: C. Anthony, BPC: M. Marques.) RTS—C44029

Add the following:

■Orlistat Capsules

DEFINITION
Orlistat Capsules contains NLT 90.0% and NMT 110.0% of the labeled amount of orlistat ($C_{29}H_{53}NO_5$).

IDENTIFICATION
- The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, water, and phosphoric acid (860:140:0.05)
  Standard solution: 0.6 mg/mL of USP Orlistat RS in *Mobile phase*
  Sample solution: Transfer the contents of not fewer than 10 Capsules into a suitable container, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to 120 mg of orlistat into a 200-mL volumetric flask. Add 140 mL of *Mobile phase*, and sonicate for 1 min. Shake the resulting solution mechanically for 15 min, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, discarding the first few mL of filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)
  Mode: LC
  Detector: 195-nm
  Column: 3.9-mm × 15-cm column; packing L1
  Flow rate: 1.0 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  System suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{29}H_{53}NO_5$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨**711**⟩
  Medium: 3% sodium lauryl sulfate and 0.5% sodium chloride in water. To each 10 L of media, add 1–2 drops of *n*-octanol, and adjust with phosphoric acid to a pH of 6.0; 900 mL.
  Apparatus: 75 rpm, with coil wire sinker
  Time: 45 min
  Mobile phase: Proceed as directed in the *Assay*.
  Standard solution: Transfer about 13 mg of USP Orlistat RS to a 100-mL volumetric flask, dissolve in 2 mL of acetonitrile, and dilute with *Medium* to volume.
  Sample solution: Pass a portion of the solution under test through a suitable 0.2-µm filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)
  Mode, detector, and **column:** Proceed as directed in the *Assay*.
  Flow rate: 2.0 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *Standard solution*
  System suitability requirements
  Relative standard deviation: NMT 2.0%
  Calculate the percentage of $C_{29}H_{53}NO_5$ dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = capsule label claim (mg)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% of the labeled amount of orlistat is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES
Organic Impurities
- **PROCEDURE**
  Mobile phase: Proceed as directed in the *Assay*.
  Standard solution: Proceed as directed for *Standard solution* in the *Assay.*
  Sample solution: Proceed as directed for *Sample solution* in the *Assay.*
  System suitability solution: Transfer 0.025 mg/mL of USP Orlistat Related Compound D RS in *Mobile phase.* Transfer 1 mL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)

In-Process Revision

System suitability
Sample: *System suitability solution*
System suitability requirements
Resolution: NLT 1.4 between USP Orlistat RS and USP Orlistat Related Compound D RS
Relative standard deviation: NMT 2.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/(r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for the individual impurity in the *Sample solution*
r_S = peak response for orlistat in the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of orlistat in the *Sample solution* (mg/mL)
F = the relative response factor (see *Impurity Table 1* for values)

Acceptance criteria: See *Impurity Table 1*.
The limits of impurities are specified in the table below.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat open-ring epimer[a]	0.45	1.0	1.5
Orlistat open ring[b]	0.5	1.0	0.3
Orlistat related compound D	0.9	1.0	1.0
Orlistat	1.0	—	—
Hexyl undecyl pyranone[c]	2.0	0.78	0.2
Henicosenyl leucinate[d]	4.7	0.45	0.3
Any other identified impurity	—	—	0.3
Individual unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

[a](2*S*,3*R*,5*S*)-5-[(*N*-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[b](2*S*,3*S*,5*S*)-5-[(*N*-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[c](*S*)-3-Hexyl-5,6-dihydro-6-undecyl-2*H*-pyran-2-one.
[d](*S*)-[(*S*,*E*)-henicos-7-en-10-yl] *N*-formyl-L-leucinate.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Orlistat RS
  USP Orlistat Related Compound D RS■2S (*USP33*)

BRIEFING

Oxazepam Capsules, *USP 32* page 3154. As part of *USP* monograph modernization efforts, it is proposed to replace the UV-based *Assay* with a stability-indicating HPLC-based method. A test for *Organic Impurities* is also introduced. The proposed HPLC method for the *Assay* and the test for *Organic Impurities* is based on analyses performed with a Zorbax SB-C18 brand of L1 column. The retention time of the oxazepam peak is 6 min.

(MD-PP: H. Ramanathan, R. Ravichandran.) RTS—C53348

Oxazepam Capsules

DEFINITION
Oxazepam Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{15}H_{11}ClN_2O_2$.

IDENTIFICATION
- The solution prepared for measurement of absorbance in the *Assay* exhibits a maximum at 229 ± 2 nm.

ASSAY

Delete the following:

■• ~~**PROCEDURE**~~
~~**Standard solution:** 4 µg/mL USP Oxazepam RS in alcohol~~
~~**Sample solution:** Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh. Transfer a portion of the mixed powder, nominally equivalent to 50 mg of oxazepam, to a medium-porosity, sintered-glass funnel that is fitted into a small suction flask. Add 25 mL of alcohol, mix with the aid of a stirring rod, and after about 5 min apply gentle suction to remove the extract. Repeat the extraction with four additional 25-mL portions of alcohol, transfer the extracts to a 250-mL volumetric flask, and dilute with alcohol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with alcohol to volume.~~
~~**Spectrometric conditions**~~
~~**Detector:** UV 229 nm~~
~~**Cell:** 1 cm~~
~~**Blank:** Alcohol~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Calculate the percentage of $C_{15}H_{11}ClN_2O_2$ in the portion of Capsules taken:~~

~~$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$~~

~~A_U = absorbance of the solution from the Capsules~~
~~A_S = absorbance of the solution from the *Standard solution*~~
~~C_S = concentration of the *Standard solution* (µg/mL)~~
~~C_U = concentration of the *Sample solution* (µg/mL)~~
~~**Acceptance criteria:** 90.0%–110.0%~~■2S (*USP33*)

Add the following:

■• **PROCEDURE**
Diluent: Methanol and water (9:1)
Buffer: 8.5 g/L potassium phosphate, monobasic in water. Adjust with 1 N sodium hydroxide to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (3:2)
Standard solution: 0.1 mg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.1 mg/mL of oxazepam in *Diluent*, from contents of NLT 20 capsules. [NOTE—Sonicate for 15 min and shake for 15 min. Pass through a 0.45–µm filter.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: At least 1.7 times the retention time of oxazepam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{11}ClN_2O_2$ in the portion of Capsules taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxazepam in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0% ■2S (*USP33*)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩
Medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
~~**Standard solution:** USP Oxazapam RS at a known concentration in 0.1 N hydrochloric acid~~
~~[NOTE—A volume of methanol NMT 10% of the final total volume may be used to dissolve the USP Oxazepam RS.]~~
~~**Sample solution:** Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed, and filter.~~
■**Standard solution:** Prepare NMT 30 min before use. Transfer 20 mg of USP Oxazepam RS to a 200-mL volumetric flask, and dilute with methanol to volume. Transfer 10.0 mL to a 100-mL volumetric flask, and dilute with *Medium* to volume. Keep the solution at about 6° for the *Analysis.* This solution is stable for 72 h if kept refrigerated.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Keep it at about 6° for the *Analysis.*■1S (*USP33*)
Mobile phase: Methanol, water, and glacial acetic acid (60:40:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4-mm × 15-cm; packing L7
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the oxazepam peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{11}ClN_2O_2$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Add the following:

■**Organic Impurities**

• **PROCEDURE**
Diluent, Buffer, and **Mobile phase:** Proceed as directed in the *Assay*
Standard solution: 2 µg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.2 mg/mL of oxazepam in *Diluent*, from contents of NLT 20 capsules. [NOTE—Sonicate for 15 min and shake for 15 min. Pass through a 0.45–µm filter.]
Chromatographic system Proceed as directed in the *Assay*
Run time: 3.5 times the retention time of oxazepam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

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

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of oxazepam from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of oxazepam in the *Sample solution* (µg/mL)
Acceptance criteria
Individual impurities: See *Impurity Table 1*. [NOTE—Disregard peaks less than 0.05%.]
Total impurities: NMT 0.5%, not including 2-amino 5-chlorobenzophenone

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxazepam	1.0	—
2-Amino 5-chlorobenzophenone	2.7	0.5
Any individual unspecified degradation product	—	0.1

■2S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Oxazepam RS

BRIEFING

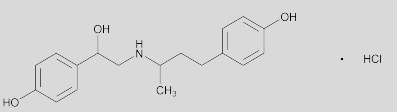
Ractopamine Hydrochloride Suspension. Because there is no *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the *Assay* are based on analyses performed with a Supecosil LC 18-DB brand of L1 column. The typical retention time for ractopamine is about 13 min. The liquid chromatographic procedures in the test for *Organic Impurities* are based on analyses performed with a Supecosil LC 18-DB brand of L1 column. The typical retention time for ractopamine is about 19 min. The liquid chromatographic procedures in the test for *Diastereomer Ratio* are based on analyses performed with a Jones Apex ODS brand of L1 column. Typical retention times for the *RS,SR* diastereoisomer and the *RR,SS* diastereoisomer are about 20 and 21 min, respectively.

(VET: E. Gonikberg.) RTS—C62987

In-Process Revision

Add the following:

▪Ractopamine Hydrochloride Suspension



$C_{18}H_{23}NO_3 \cdot HCl$ 337.84

Benzenemethanol, 4-hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-, hydrochloride;
(±)-all-*rac*-*p*-Hydroxy-α-[[[3-(*p*-hydroxyphenyl)-1-methylpropyl]amino]methyl]benzyl alcohol, hydrochloride [90274-24-1].

DEFINITION

Ractopamine Hydrochloride Suspension contains NLT 10% and NMT 20%, by weight, of ractopamine hydrochloride ($C_{18}H_{23}NO_3 \cdot HCl$) in water. [NOTE—The material partially precipitates out at room temperature to form a slurry, and redissolves to form a clear solution when heated to 50°–60°.]

IDENTIFICATION

• **INFRARED ABSORPTION ⟨197K⟩**

Sample: Dry a portion of Ractopamine Hydrochloride Suspension under vacuum for 3 h at 60°.

ASSAY

• **Procedure**

Solution A: 5.75 mg/mL solution of monobasic ammonium phosphate adjusted to a pH of 4.0 ± 0.1 with 10% phosphoric acid

Solution B: 1.1 mg/mL solution of 1-heptanesulfonic acid sodium salt in *Solution A*

Mobile phase: Stabilizer-free tetrahydrofuran and *Solution B* (3:17)

Diluent: Stabilizer-free tetrahydrofuran and water (3:17).

[NOTE—The *Standard solutions* and *System suitability solution* are stable for up to 72 h at room temperature. The *Sample solution* is stable for up to 90 h at room temperature.]

System suitability solution: 100 µg/mL of USP Ractopamine Hydrochloride RS and 10 µg/mL of USP Raspberry Alcohol RS in *Diluent*

Standard solution A: 0.08 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*

Standard solution B: 0.1mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*

Standard solution C: 0.12 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*

Sample stock solution: Stir Ractopamine Hydrochloride Suspension in a 50°–60° water bath for up to 1 h, to ensure complete dissolution. While hot, transfer 700 mg of the Ractopamine Hydrochloride Suspension dropwise to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Dilute a portion of the *Sample stock solution* with *Diluent* (1:10).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*).

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between raspberry alcohol and ractopamine, *System suitability solution*

Tailing factor: NLT 0.7 and NMT 2.0, for the ractopamine peak, *Standard solution B*

Relative standard deviation: NMT 2.0%, three replicate injections, *Standard solution B*

Analysis

Samples: *Standard solutions* and *Sample solution*

Prepare a calibration curve using the three ractopamine peak responses from *Standard solutions A, B,* and *C* and their corresponding concentrations. From the graph so obtained determine the concentration, C, in mg/mL, of ractopamine hydrochloride in the *Sample solution*.

Calculate the percentage (w/w) of $C_{18}H_{23}NO_3 \cdot HCl$ in the portion of Ractopamine Hydrochloride Suspension taken :

$$\text{Result} = (V/W) \times C \times D \times 100$$

V = volume of the *Sample stock solution*, 100 mL
W = weight of Ractopamine Hydrochloride Suspension taken (mg)
C = as determined above
D = dilution factor to prepare the *Sample solution*, 10

Acceptance criteria: 10%–20% of $C_{18}H_{23}NO_3 \cdot HCl$

IMPURITIES

Organic Impurities

• **PROCEDURE**

Solution A: 5.75 mg/mL of monobasic ammonium phosphate in water; pH NLT 4.4

Solution B: 1.1 mg/mL of 1-heptanesulfonic acid sodium salt in *Solution A*

Solution C: Acetonitrile and *Solution B* (1:9)

Solution D: Acetonitrile and *Solution B* (17:33)

Mobile phase: See the gradient table below.

Time (min)	Solution C (%)	Solution D (%)
0	100	0
22	0	100
32	0	100
37	100	0
55	100	0

Diluent: Acetonitrile and water (1:4)

System suitability solution: 9 µg/mL each of USP Raspberry Ketone RS and USP Ractopamine Hydrochloride RS in *Diluent*

Blank: *Diluent*

Sample solution A: Stir Ractopamine Hydrochloride Suspension in a 50°–60° water bath for up to 1 h, to ensure complete dissolution. While hot, transfer 200 mg of the Ractopamine Hydrochloride Suspension dropwise into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution B: Dilute a portion of *Sample solution A* with *Diluent* (1:100).

[NOTE—The *Sample solutions* are stable for up to 48 h if stored at 5°.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between raspberry ketone and ractopamine

Analysis

Samples: *Blank*, *Sample solution A*, and *Sample solution B*

[NOTE—Disregard any peaks that correspond to those in the *Blank*. Correct the response of the ractopamine peak in *Sample solution B* by subtracting the peak response at the retention time of ractopamine in the *Blank*.]

Calculate the percentage of each individual impurity in the portion of Ractopamine Hydrochloride Suspension taken:

$$\text{Result} = (r_A/r_B) \times 100/D$$

In-Process Revision

r_A = peak response of each individual impurity from *Sample solution A*
r_B = corrected response for the ractopamine peak from *Sample solution B*
D = dilution factor to prepare *Sample solution B,* 100

Acceptance criteria
Individual impurities: See *Impurity Table 1.*
Total impurities: NMT 3.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Octopamine[a]	0.37	0.5
Tyramine[b]	0.55	0.5
N-isopropyloctopamine[c]	0.63	0.5
Piperazinediphenol[d]	0.74	0.5
Aminobutylphenol[e]	0.76	0.5
Raspberry alcohol[f]	0.85	0.5
Raspberry ketone[g]	0.96	1.0
Ractopamine	1.0	—
Deoxyractopamine[h]	1.1	0.5
Ractopamine *O*-methyl[i]	1.2	1.0
Ractopamine *N*-hydroxybenzyl[j]	1.26	1.0
Ractopamine cyclohexyl analog[k]	1.29	0.5
Ractopamine dimer[l]	1.4	1.0
Any individual unspecified impurity	—	0.2

[a] 4-(2-Amino-1-hydroxyethyl)phenol.
[b] 4-(2-Aminoethyl)phenol.
[c] 4-[1-Hydroxy-2-(isopropylamino)ethyl]phenol.
[d] 4,4′-(Piperazine-2, 5-diyl)diphenol.
[e] 4-(3-Aminobutyl)phenol.
[f] 4-(3-Hydroxybutyl)phenol.
[g] 4-(4-Hydroxyphenyl)butan-2-one.
[h] 4-[3-(4-Hydroxyphenethylamino)butyl]phenol.
[i] 4-{3-[2-(4-Hydroxyphenyl)-2-methoxyethylamino]butyl}phenol.
[j] 4-(1-Hydroxy-2-{(4-hydroxybenzyl)[4-(4-hydroxyphenyl)butan-2-yl]amino}ethyl)phenol.
[k] 4-{1-Hydroxy-2-[3-(4-hydoxyphenyl)-5-methylcyclohexylamino]ethyl}phenol).
[l] 4,4′-(1,1′-Oxybis{2-[4-(4-hydroxyphenyl)butan-2-ylamino]ethane-1,1-diyl})diphenol.

SPECIFIC TESTS
- **DIASTEREOMER RATIO**

Solution A: 5.75 mg/mL of monobasic ammonium phosphate in water
Solution B: Add 10 mL of triethylamine to 950 mL of *Solution A,* and adjust with phosphoric acid to a pH of 4.5.
Mobile phase: Acetonitrile and *Solution B* (3:22)
Diluent: Acetonitrile and *Solution A* (1:4)
System suitability solution: 0.4 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*
Sample solution: Stir Ractopamine Hydrochloride Suspension in a 50°–60° water bath for up to 1 h to ensure complete dissolution. While hot, transfer 275 mg of it dropwise into a 100-mL volumetric flask, and dilute with *Diluent* to volume. [NOTE—The *Sample solution* is stable for up to 36 h when stored at ambient conditions.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The elution order is *RS,SR* diastereoisomer followed by *RR,SS* diastereoisomer.]
Suitability requirements
Resolution: NLT 1.25 between the diastereomers
Analysis
Sample: *Sample solution*
Calculate the *RS,SR* diastereomer content, in percentage:

$$\text{Result} = r_A/(r_A + r_B) \times 100$$

r_A = peak area response of the *RS,SR* diastereoisomer from the *Sample solution*
r_B = peak area response of the *RR,SS* diastereoisomer from the *Sample solution*

Acceptance criteria: 45%–49%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at a temperature not exceeding 70°.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ractopamine Hydrochloride RS
USP Raspberry Alcohol RS
USP Raspberry Ketone RS■2S *(USP33)*

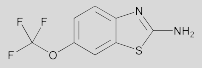
BRIEFING

Riluzole. Because there is no existing *USP* monograph for this drug substance, a new monograph is being proposed. The liquid chromatographic procedures in the *Assay* and the test for *Organic Impurities* were validated with a Waters Symmetry brand of L1 column, in which riluzole elutes at about 3 min.

(MD-PP: D. Vicchio, R. Ravichandran.) RTS—C61270

Add the following:

■Riluzole



$C_8H_5F_3N_2OS$ 234.20
2-Benzothiazolamine, 6-(trifluoromethoxy)-;
2-Amino-6-(trifluoromethoxy)benzothiazole [1744-22-5].

DEFINITION
Riluzole contains NLT 98.0% and NMT 102.0% of the labeled amount of $C_8H_5F_3N_2OS$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (9:11)

Standard solution: 0.05 mg/mL of USP Riluzole RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Riluzole in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 221 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection size: 20 µL. [NOTE—Monitor for 3 times the retention time of riluzole.]

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_8H_5F_3N_2OS$ in the portion of Riluzole taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%, a 1.5-g of sample used

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

• **PROCEDURE**

Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 500 µg/mL of USP Riluzole RS and 0.5 µg/mL USP Riluzole Related Compound A RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Riluzole RS in *Mobile phase*

Sample solution: 500 µg/mL of Riluzole in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Proceed as directed in the *Assay*, and use an injection volume of 100 µL. [NOTE—Monitor for 11 times the retention time of riluzole.]

System suitability

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

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Resolution: NLT 1.5 between riluzole and riluzole related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times shown in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of Riluzole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from *Sample solution*

r_S = peak response of riluzole from the *Standard solution*

C_S = concentration of USP Riluzole RS in the *Standard solution* (µg/mL)

C_U = concentration of Riluzole in the *Sample solution* (µg/mL)

F = relative response factor relative to riluzole

Acceptance criteria

Individual impurities: See *Impurity Table 1*

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Riluzole	1.0	—	—
Riluzole related compound A[a]	1.3	0.16	0.1
Bromoriluzole[b]	1.9	0.54	0.2
Dibromo-trifluoromethoxy-aniline[c]	7.4	0.28	0.1
Any unspecified impurity	—	1.0	0.1

[a] 4-(Trifluoromethoxy)aniline.

[b] 4-Bromo-6-(trifluoromethoxy)benzothiazol-2-amine.

[c] 2,6-Dibromo-4-(trifluoromethoxy)aniline.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Riluzole RS

USP Riluzole Related Compound A RS■2S (*USP33*)

BRIEFING

Riluzole Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph is being proposed. The liquid chromatographic procedures in the tests for *Assay* and *Organic Impurities* were validated with a Waters Symmetry brand of L1 column, in which riluzole elutes at about 6 min.

(MD-PP: D. Vicchio, R. Ravichandran. BPC: M. Marques.)
RTS—C61270

Add the following:

■Riluzole Tablets

DEFINITION

Riluzole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of riluzole ($C_8H_5F_3N_2OS$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩**:** The spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Dissolution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (9:11)

Standard solution: 0.05 mg/mL of USP Riluzole RS in *Mobile phase*

Sample stock solution: Weigh and finely powder not fewer than 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of riluzole, to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, sonicate for about 10 min, and stir for another 10 min. Dilute with *Mobile phase* to volume.

Sample solution: 0.05 mg/mL of riluzole in *Mobile phase*, prepared from *Sample stock solution*. Pass this solution through a PVDF (or equivalent) filter having a 0.45-µm porosity, discard the first 5 mL of filtrate. Use the filtrate for analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 221 nm

Column: 4.6-mm × 15-cm column; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_8H_5F_3N_2OS$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)
C_U = concentration of riluzole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.05 mg/mL of USP Riluzole RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.

Detector: UV 254 nm

Blank: *Medium*

Cell: 0.5 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of riluzole taken:

$$\text{Result} = (A_U/A_S) \times (C_S \times V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of $C_8H_5F_3N_2OS$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Riluzole RS in *Mobile phase*, prepare from *Standard solution* under *Assay*

System suitability solution: 500 µg/mL of USP Riluzole RS and 0.5 µg/mL of USP Riluzole Related Compound A RS in *Mobile phase*

Sample solution: 0.5 mg/mL, *Sample stock solution*

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between riluzole and riluzole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

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

r_U = peak response of the impurity from *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Riluzole RS in the *Standard solution* (µg/mL)
C_U = concentration of riluzole in the *Sample solution* (µg/mL)

Acceptance criteria

Individual impurities: NMT 0.2%

Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Riluzole RS

USP Riluzole Related Compound A RS■2S *(USP33)*

BRIEFING

Sodium Fluoride Gel. Because there is no existing *USP* monograph for this dosage form, a new monograph is being proposed.

(MD-GRE: E. Gonikberg.) RTS—C70458

Add the following:

■Sodium Fluoride Gel

DEFINITION

Sodium Fluoride Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of NaF, in an aqueous medium containing a suitable viscosity-inducing agent. It may contain a suitable buffering agent.

IDENTIFICATION

• **PROCEDURE**

Sample solution: Amount of Gel, equivalent to 500 mg of fluoride ion

Analysis: Place the *Sample solution* in a platinum crucible in a well-ventilated hood, and add 15 mL of sulfuric acid. Cover the crucible with a piece of clear, polished glass, and heat on a steam bath for 1 h. Remove the glass cover, rinse it in water, and dry.

Acceptance criteria: The glass surface exposed to vapors from the crucible is etched.

In-Process Revision

ASSAY

- **PROCEDURE**

[NOTE—Store all solutions, except the *Buffer*, in plastic containers.]

Buffer: Dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo)tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of 5.25 ± 0.25, and dilute with water to 1000 mL.

Standard solution A: 420 µg/mL of USP Sodium Fluoride RS, equivalent to 190 µg/mL of fluoride ion (10^{-2} M)

Standard solution B: 19 µg/mL of fluoride ion (10^{-3} M), from *Standard solution A*

Standard solution C: 1.9 µg/mL of fluoride ion (10^{-4} M), from *Standard solution B*

Sample solution: Equivalent to 20 µg/mL of fluoride from the Gel in water

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Pipet 20 mL of each *Standard solution* and the *Sample solution* into separate plastic beakers each containing a plastic-coated stirring bar. Pipet 20 mL of *Buffer* into each beaker. Concomitantly measure the potentials (see *pH* ⟨791⟩), in mV, of the solutions from the *Standard solutions* and of the solution from the *Sample solution*, with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific, ion-indicating electrode and a suitable reference electrode.

[NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]

Plot the logarithms of the fluoride-ion concentrations, in µg/mL, of the diluted *Standard solutions* versus potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, C, in µg/mL, of fluoride ion in the *Sample solution.*

Calculate the quantity, as a percentage of label claim, of fluoride ion in the portion of Gel taken:

$$\text{Result} = (C/C_U) \times 100$$

C = defined above

C_U = nominal concentration of fluoride ion in the *Sample solution* (µg/mL)

Calculate the quantity, as a percentage of label claim, of sodium fluoride in the portion of Gel taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = defined above

C_U = nominal concentration of sodium fluoride (NaF) in the *Sample solution* (µg/mL)

M_r = molecular weight of sodium fluoride, 41.99

A_r = atomic weight of fluoride, 19.00

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH ⟨791⟩:** 6.0–8.0

Place about 40 mL in a plastic beaker, and determine the pH using a suitable electrode system.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, plastic containers.
- **LABELING:** Label the Gel in terms of the content of sodium fluoride (NaF) and in terms of the content of fluoride ion.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Sodium Fluoride RS■2S *(USP33)*

BRIEFING

Valsartan and Hydrochlorothiazide Tablets, *USP 32* page 3843. On the basis of comments received, it is proposed to revise the calculations in the test for *Organic Impurities* and to clarify that the other impurities are quantitated based on the valsartan peak as per the approved NDA.

(MD-CV: S. Ramakrishna.) RTS—C70461

Valsartan and Hydrochlorothiazide Tablets

DEFINITION

Valsartan and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Sample solution: To an amount of ground Tablets, equivalent in weight to a single Tablet, add 2.0 mL of acetone, sonicate for 15 min, and centrifuge.

Application volume: 2 µL

Developing solvent system: Ethyl acetate, dehydrated alcohol, and 3.6 M of ammonium hydroxide (8:2:1)

Analysis: Proceed as directed in the chapter, except to develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent system* for 15 min before use. Allow the chromatogram to develop until the solvent front has moved at least 7 cm. After removing the plate and marking the solvent front, dry the plate under a current of warm air. The R_F values of the principal spots obtained from the *Sample solution* correspond to those from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (1:1)

Solution A: Acetonitrile, water, and trifluoroacetic acid (10:90:0.1)

Solution B: Acetonitrile, water, and trifluoroacetic acid (90:10:0.1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
25	10	90
27	90	10
40	90	10

Standard solution: Transfer 12.5 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask, and add 12.5J mg of USP Valsartan RS, J being the ratio of the labeled amount, in mg, of valsartan to the labeled amount, in mg, of hydrochlorothiazide/Tablet. Add 100 mL of *Diluent*, sonicate for 15 min, dilute with *Diluent* to 250 mL, and mix. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Dilute with *Diluent* to obtain a solution having a concentration of 0.2 mg/mL of USP Valsartan RS in *Diluent*.

Sample stock solution: To the equivalent of 62.5 mg of hydrochlorothiazide from a number of Tablets add 5 mL of water, and allow to stand for 5 min. Then add 100 mL of *Diluent*, sonicate for 15 min, and shake for 30 min. Dilute with

In-Process Revision

Diluent to 250 mL, and centrifuge a portion of this solution at 3000 rpm. Dilute 25.0 mL of the clear supernatant with *Diluent* to 200.0 mL.
Sample solution: 0.2 mg/mL of valsartan, from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 3.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ in the portion of Tablets taken:

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

r_U = peak response of valsartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of the appropriate USP Reference Standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨**711**⟩
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Detector: UV
Analytical wavelength: 250 nm for valsartan and 272 nm for hydrochlorothiazide
Sample solutions: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution.*
Analysis: Determine the amounts of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ dissolved by using UV absorption on portions of the *Sample solution* passed through a 1-µm glass fiber filter, diluted with *Medium* if necessary, using a 0.2-cm quartz cell.
Calculate the amount of $C_{24}H_{29}N_5O_3$ dissolved in percentage:

$$\text{Result} = [(A_{UT2} \times \alpha H_{272}) - (A_{UT1} \times \alpha H_{250})/(\alpha V_{250} \times \alpha H_{272}) - (\alpha V_{272} \times \alpha H_{250})] \times 12{,}500$$

Calculate the amount of $C_7H_8ClN_3O_4S_2$ dissolved in percentage:

$$\text{Result} = [(A_{UT1} \times \alpha V_{250}) - (A_{UT2} \times \alpha V_{272})/(\alpha H_{272} \times \alpha V_{250}) - (\alpha H_{250} \times \alpha V_{272})] \times 80{,}000$$

A_{UT1} = absorbance of the *Sample solution* at 272 nm
αV_{250} = absorptivity (1%, 0.2 cm, 250 nm) of valsartan in *Medium*
A_{UT2} = absorbance of the *Sample solution* at 250 nm
αV_{272} = absorptivity (1%, 0.2 cm, 272 nm) of valsartan in *Medium*
αH_{272} = absorptivity (1%, 0.2 cm, 272 nm) of hydrochlorothiazide in *Medium*
αH_{250} = absorptivity (1%, 0.2 cm, 250 nm) of hydrochlorothiazide in *Medium*
Tolerances: NLT 80% (Q) of the labeled amounts of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ is dissolved.

• **Uniformity of Dosage Units** ⟨**905**⟩
Procedure for content uniformity
Diluent, Solution A, Solution B, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sample solution: Transfer 1 Tablet to a 200-mL volumetric flask, add 5 mL of water, and allow to stand for 5 min. Add 100 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to 250 mL, mix, and centrifuge a portion of this solution at 3000 rpm. Dilute a volume of the clear supernatant with *Diluent* to obtain a solution having a concentration of 0.2 mg/mL of valsartan.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)
L = labeled quantity of the relevant analyte (mg/Tablet)
Acceptance criteria: Meets the requirements

IMPURITIES

Change to read:

Organic Impurities

• **Procedure**
Diluent, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: Prepare a solution in *Diluent* containing 0.03 mg/mL of USP Benzothiadiazine Related Compound A RS, 0.06 mg/mL of USP Hydrochlorothiazide RS, 0.08 mg/mL of USP Valsartan RS, and 0.2 mg/mL of USP Valsartan Related Compound B RS.
System suitability solution: Dilute 5.0 mL of *Standard stock solution* with *Diluent* to 100 mL.
Standard solution: Dilute 10.0 mL of *System suitability solution* in 100.0 mL of *Diluent*.
Chromatographic system
Mode: LC
Detector: UV 265 nm
Column: 3.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.4 between valsartan related compound B and valsartan, and NLT 1.4 between benzothiadiazine related compound A and hydrochlorothiazide, *System suitability solution*
Relative standard deviation: NMT 10.0% from the valsartan and hydrochlorothiazide peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Disregard the peak, if any, with a retention time of 22 min.
~~Calculate the percentage of each impurity in the portion of Tablets taken:~~

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

~~r_U = peak response from the *Sample solution*~~
~~r_S = peak response from the *Standard solution*~~
~~C_S = concentration of appropriate Reference Standard in the *Standard solution* (mg/mL)~~
~~C_U = nominal concentration of hydrochlorothiazide or valsartan in the *Sample solution*~~

■Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

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

In-Process Revision

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of benzothiadiazine related compound A in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity in the portion of Tablets taken:

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

r_U = peak response of each other impurity from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of valsartan in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan (for calculating other impurities) in the *Sample solution* (mg/mL)

■2S (*USP33*)

Acceptance criteria: NMT 1.0% of benzothiadiazine related compound A; NMT 0.2% of any other impurity, excluding valsartan related compound A; NMT 1.3% of total impurities, excluding valsartan related compound A. [NOTE—Valsartan related compound A is the enantiomer of valsartan and coelutes with valsartan in this test.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 25°; excursions are permitted between 15° and 30°. Protect from moisture and heat.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Benzothiadiazine Related Compound A RS
  USP Hydrochlorothiazide RS
  USP Valsartan RS
  USP Valsartan Related Compound B RS

BRIEFING

Sterile Water for Inhalation, *USP 32* page 3871 and page 883 of *PF* 35(4) [July–Aug. 2009]. In *Water Conductivity* ⟨645⟩, the name of the section for testing this article has been changed from *Packaged Water* to *Sterile Water*. There is no change to the monograph requirements. For a description of companion changes, see the briefings in the chapters *Water Conductivity* ⟨645⟩ and *Water for Pharmaceutical Purposes* ⟨1231⟩, appearing elsewhere in this issue of *PF*.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Sterile Water for Inhalation

DEFINITION

Change to read:

Sterile Water for Inhalation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no ~~antimicrobial agents, except where used in humidifiers or other similar devices and where liable to contamination over a period of time, or other added substances~~■ added antimicrobial agents.■2S (*USP33*)

[NOTE—Do not use Sterile Water for Inhalation for parenteral administration or for other sterile compendial dosage forms.]

SPECIFIC TESTS

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Inhalation in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear.

Change to read:

- **WATER CONDUCTIVITY,** ~~*Packaged*~~■ *Sterile*■2S (*USP33*) *Water* ⟨**645**⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Less than 0.5 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** Label it to indicate that it is for inhalation therapy only and that it is not for parenteral administration.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS

BRIEFING

Sterile Water for Injection, *USP 32* page 3871, and page 803 of *PF* 31(3) [May–June 2005]. In *Water Conductivity* ⟨645⟩, the name of the section for testing this article has been changed from *Packaged Water* to *Sterile Water*. There is no change to the monograph requirements—See briefings for a description of associated changes under *Water Conductivity* ⟨645⟩ and *Water for Pharmaceutical Purposes* ⟨1231⟩.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Sterile Water for Injection

DEFINITION

Sterile Water for Injection is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Injection in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear.

In-Process Revision

Delete the following:

~~■• **CALCIUM**~~
~~**Sample:** 100 mL~~
~~**Analysis:** Add 2 mL of ammonium oxalate TS.~~
~~**Acceptance criteria:** No turbidity is produced.~~■2S *(USP32)*

Delete the following:

~~■• **CARBON DIOXIDE**~~
~~**Sample:** 25 mL~~
~~**Analysis:** Add 25 mL of calcium hydroxide TS.~~
~~**Acceptance criteria:** The mixture remains clear.~~■2S *(USP32)*

Delete the following:

~~■• **SULFATE**~~
~~**Sample:** 100 mL~~
~~**Analysis:** Add 1 mL of barium chloride TS.~~
~~**Acceptance criteria:** No turbidity is produced.~~■2S *(USP32)*

Delete the following:

~~■• **CHLORIDE**~~
~~**Sample:** 20 mL in a color-comparison tube~~
~~**Control:** 20 mL of a 0.5 mg/L chloride solution in *High-Purity Water* (see *Containers—Glass* ⟨660⟩, *Reagents*.)~~
~~**Analysis:** Add 5 drops of nitric acid and 1 mL of silver nitrate TS.~~
~~**Acceptance criteria:** Any turbidity formed within 10 min is not greater than that produced in a similarly treated control, viewed downward over a dark surface with light entering the tubes from the sides.~~■2S *(USP32)*

Delete the following:

~~■• **AMMONIA**~~
~~**Sample solution:** For containers having a fill volume of less than 50 mL, dilute 50 mL of it with 50 mL of *High-Purity Water* (see *Containers—Glass* ⟨660⟩, *Reagents*), and use this dilution as the *Sample solution*; where the fill volume is 50 mL or more, use 100 mL of it as the *Sample solution*.~~
~~**Control:** 30 µg of added ammonia (furnished by adding 1 mL of the final solution prepared by diluting 3.0 mL of ammonia TS with *High-Purity Water* to 100 mL; 1.0 mL of this solution is further diluted to 100 mL) in 100 mL of *High-Purity Water*.~~
~~**Analysis:** Add 2 mL of alkaline mercuric-potassium iodide TS.~~
~~**Acceptance criteria:** Any yellow color produced immediately is not darker than that of the control. This corresponds to a limit of 0.6 mg/L for containers having a fill volume of less than 50 mL and 0.3 mg/L where the fill volume is 50 mL or more.~~■2S *(USP32)*

Delete the following:

~~■• **pH** ⟨791⟩**:** 5.0–7.0, in a solution containing 0.3 mL of saturated potassium chloride solution/100 mL of test specimen~~■2S *(USP32)*

Change to read:

■• **WATER CONDUCTIVITY,** ~~*Packaged*~~■*Sterile*■2S *(USP33)* *Water* ⟨**645**⟩**:** Meets the requirements■2S *(USP32)*
- **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Less than 0.25 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers of not larger than 1-L size. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** Label it to indicate that no antimicrobial or other substance has been added, and that it is not suitable for intravascular injection without first having been made approximately isotonic by the addition of a suitable solute.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS

BRIEFING

Sterile Water for Irrigation, *USP 32* page 3871. In *Water Conductivity* ⟨645⟩, the name of the section for testing this article has been changed from *Packaged Water* to *Sterile Water*. There is no change to the monograph requirements—See briefings for a description of associated changes under *Water Conductivity* ⟨645⟩ and *Water for Pharmaceutical Purposes* ⟨1231⟩.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Sterile Water for Irrigation

DEFINITION
Sterile Water for Irrigation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS
[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]
- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Irrigation in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear.

Change to read:

• **WATER CONDUCTIVITY,** ~~*Packaged*~~■*Sterile*■2S *(USP33)* *Water* ⟨**645**⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Less than 0.25 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may contain a volume of more than 1 L, and may be designed to empty rapidly.
- **LABELING:** Label it to indicate that no antimicrobial or other substance has been added. The designations "For irrigation only" and "Not for injection" appear prominently on the label.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS

In-Process Revision

BRIEFING

Sterile Purified Water, *USP 32* page 3872. In *Water Conductivity* ⟨645⟩, the name of the section for testing this article has been changed from *Packaged Water* to *Sterile Water*. There is no change to the monograph requirements—See briefings for a description of associated changes under *Water Conductivity* ⟨645⟩ and *Water for Pharmaceutical Purposes* ⟨1231⟩.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Sterile Purified Water

H_2O 18.02

DEFINITION
Sterile Purified Water is Purified Water sterilized and suitably packaged. It contains no antimicrobial agents.
[NOTE—Do not use Sterile Purified Water in preparations intended for parenteral administration. For such purposes use Water for Injection, Bacteriostatic Water for Injection, or Sterile Water for Injection.]

SPECIFIC TESTS
[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]
- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Purified Water in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear.

Change to read:

- **WATER CONDUCTIVITY,** ~~*Packaged*~~ ■ *Sterile* ■2S *(USP33)* *Water* ⟨**645**⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨**71**⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in suitable tight containers.
- **LABELING:** Label it to indicate the method for preparation, and to indicate that it is not for parenteral administration.

BRIEFING

Zidovudine Oral Solution, *USP 32* page 3890. It is proposed to use a flexible monograph approach to indicate that manufacturers may perform either *Organic Impurities, Procedure 1* or *Procedure 2*, based on labeling instructions and the impurity profile. The HPLC procedure in *Organic Impurities, Procedure 2* is based on analysis performed with a Hypersil BDS 3-µm brand of L1 column. Two reference standards are added as required to perform *Organic Impurities, Procedure 2*. The typical retention time for zidovudine is 21.6 min. Additionally, it is proposed to omit *Identification* test *A* from the monograph because the procedure was not orthogonal to *Identification* test *B*. An editorial change is proposed for the remaining *Identification* test to provide clarity.

(MD-AA: L. Santos, B. Davani.) RTS—C45529

Zidovudine Oral Solution

DEFINITION
Zidovudine Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

Delete the following:

■• ~~**A. CHROMATOGRAPHY,** *Thin-Layer Chromatography* ⟨**621**⟩~~
~~**Standard solution:** 5 mg/mL in methanol and water (75:25)~~
~~**Sample solution:** 5 mg/mL in methanol and water (75:25)~~
~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having an optimal intensity at 254 nm~~
~~**Application volume:** 5 µL~~
~~**Developing solvent system:** Butyl alcohol, *n*-heptane, acetone, and ammonium hydroxide (40:30:30:10)~~
~~**Analysis:** When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.~~
~~**Acceptance criteria:** The R_F value of the principal spot from the *Sample solution* corresponds to that of the principal spot from the *Standard solution*.~~ ■2S *(USP33)*

Delete the following:

■• ~~**B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.~~ ■2S *(USP33)*

Add the following:

■• The retention time of the zidovudine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ■2S *(USP33)*

ASSAY
- **PROCEDURE**
  Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.040 M sodium acetate (90:10:2:900)
  Standard stock solution: 1 mg/mL of USP Zidovudine RS in *Mobile phase*
  Zidovudine related compound C stock solution: 0.1 mg/mL of USP Zidovudine Related Compound C RS in *Mobile phase* [NOTE—Sonicate for 10 min before making final volume.]
  Standard solution: Transfer 10.0 mL of *Standard stock solution* and 2.0 mL of *Zidovudine related compound C stock solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.
  Sample solution: Equivalent to 0.1 mg/mL of zidovudine in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.0-mm × 12.5-cm; packing L1
  Flow rate: 1 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for zidovudine related compound C and zidovudine are about 0.12 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 4.0 between zidovudine and zidovudine related compound C

In-Process Revision

Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{10}H_{13}N_5O_4$ in the portion of Oral Solution taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

Organic Impurities
■[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*.]■2S *(USP33)*

• **PROCEDURE** ■1■2S *(USP33)*
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.040 M sodium acetate (90:10:2:900)
Standard stock solution: 1 mg/mL of USP Zidovudine RS in *Mobile phase*
Zidovudine related compound C stock solution: 0.1 mg/mL of USP Zidovudine Related Compound C RS in *Mobile phase*
[NOTE—Sonicate for 10 min before making final volume.]
Standard solution: Transfer 10.0 mL of *Standard stock solution* and 2.0 mL of *Zidovudine related compound C stock solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.
Sample solution: Equivalent to 0.1 mg/mL of zidovudine in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*.
System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of zidovudine related compound C (thymine) in the portion of Oral Solution taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)
Acceptance criteria: NMT 3.0%

■• **PROCEDURE 2**
Buffer: 5.44 mg/mL of sodium acetate trihydrate in water. Pass the solution through a suitable 0.45-µm filter.
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Buffer* (50:10:2:940)
Standard stock solution: 0.25 mg/mL of USP Zidovudine RS in *Mobile phase*. [NOTE—Sonicate to dissolve if necessary.]
Standard solution: 0.002 mg/mL of USP Zidovudine RS in *Mobile phase* obtained from the *Standard stock solution*
Impurity stock solution: 0.625 mg/mL of USP Zidovudine Related Compound C RS, 0.375 mg/mL of thymidine, 0.375 mg/mL of USP Stavudine RS, and 0.25 mg/mL of USP Zidovudine Related Compound B RS dissolved initially at 25% of final volume with methanol. Dilute with *Mobile phase* to final volume. [NOTE—Sonicate with intermittent shaking to dissolve, if necessary, and cool to room temperature before diluting to final volume.]
Impurity solution: 0.05 mg/mL of USP Zidovudine Related Compound C RS, 0.03 mg/mL of thymidine, 0.03 mg/mL of USP Stavudine RS, and 0.02 mg/mL of USP Zidovudine Related Compound B RS in *Mobile phase* obtained from the *Impurity stock solution*
System suitability solution: 1 mg/mL of USP Zidovudine RS, 0.005 mg/mL of USP Zidovudine Related Compound C RS, 0.003 mg/mL of thymidine, 0.003 mg/mL of USP Stavudine RS, 0.002 mg/mL of USP Zidovudine Related Compound B RS in *Mobile phase*. [NOTE—Obtained from USP Zidovudine RS and *Impurity solution*.]
Sample solution: Equivalent to 1 mg/mL of zidovudine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 25°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times are listed in *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 1.4 between zidovudine and zidovudine related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_S = peak response of zidovudine in the *Standard solution*
C_S = concentration of USP Zidovudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)
F = relative response factor for each impurity as listed in *Impurity Table 1*

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Acceptance Criteria, NMT (%)
Zidovudine related compound C[a]	0.08	1.9	3.0
Zidovudine (thymidine)[b]	0.14	1.0	0.30
Stavudine[c]	0.27	1.0	0.30
Zidovudine	1.00	—	—
Zidovudine related compound B[d]	1.22	1.0	0.20
Individual unspecified impurity	—	1.0	0.20

[a] 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
[b] [1-(2-Deoxy-β-D-ribofuranosyl)]thymine.
[c] Thymidine, 2′,3′-didehydro-3′-deoxy-.
[d] 3′-Chloro-3′-deoxythymidine.

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 2.0%. [NOTE—All impurities listed in *Impurity Table 1* excluding zidovudine related compound C]■2S *(USP33)*

In-Process Revision

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*
- **pH** ⟨**791**⟩**:** 3.0–4.0, in a mixture containing a volume of Oral Solution equivalent to 150 mg of zidovudine and 5 mL of 0.12 M potassium chloride (3:1)

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements for Oral Solution packaged in multiple-unit containers
- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meets the requirements for Oral Solution packaged in single-unit containers

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Add the following:

■• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.■2S (*USP33*)

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩

■USP Stavudine RS■2S (*USP33*)
USP Zidovudine RS
■USP Zidovudine Related Compound B RS■2S (*USP33*)
USP Zidovudine Related Compound C RS

In-Process Revision

DIETARY SUPPLEMENTS—MONOGRAPHS

BRIEFING

Andrographis; Powdered Andrographis; Powdered Andrographis Extract. *USP* Dietary Supplement monographs are being proposed. The liquid chromatographic procedure in the test for *Content of Diterpene Lactones* is based on analyses performed with the LiChrosphere or Luna C18(2) brand of L1 column, with 5-µm packing. The typical retention times for andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin are about 14.3, 17.2, 19.5, and 23.1 min, respectively.

(DSB: M. Sharaf.) RTS—C66623

Add the following:

▪Andrographis

DEFINITION

Andrographis consists of the dried stems and leaves of *Andrographis paniculata* Nees. (Fam. Acanthaceae). It contains NLT 1.0% of diterpene lactones, calculated on the dried basis as the sum of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide and andrograpanin.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

Standard solution 1: Use *Standard solution A*, prepared as directed in the test for *Content of Diterpene Lactones*.

Standard solution 2: Sonicate an amount of USP Powdered Andrographis Extract RS, equivalent to about 15 mg of diterpene lactones, for 10–15 min in 25 mL of methanol, centrifuge, and use the supernatant.

Sample solution: Use *Sample stock solution*, prepared as directed in the test for *Content of Diterpene Lactones*.

Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 µm (TLC plates)

Application volume: 10 µL, as 5–10 mm bands

Developing solvent system: Chloroform, acetone, and toluene (2:2:1)

Spray reagent: A mixture of 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1)

Analysis

Samples: *Standard solution 1*, *Standard solution 2* and *Sample solution*

Use a saturated chamber. Develop the chromatograms until the solvent front has moved about 90% of the length of the plate. Remove the plate from the chamber, dry, spray with the *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The *Sample solution* exhibits three main grayish blue zones with R_F values of approximately 0.4, 0.6, and 0.8 that correspond in position and color to zones in *Standard solution 2*. *Standard solution 1* exhibits a grayish blue zone due to andrographolide at an R_F of about 0.4. The *Sample solution* exhibits a zone similar in color and R_F value to that due to andrographolide in *Standard solution 1*.

• **B.** The retention time of the main peak of the *Sample solution* obtained in the test for *Content of Diterpene Lactones* corresponds to that of andrographolide in *Standard solution A*. Identify other diterpene lactone peaks in the *Sample solution* by comparison with *Standard solution B* and the reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS. *Sample solution* shows additional peaks corresponding to neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin.

COMPOSITION

• **CONTENT OF DITERPENE LACTONES**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, mix, filter, and degas.

Solution B: Use filtered and degassed acetonitrile.

Standard solution A: Dissolve a weighed quantity of USP Andrographolide RS in methanol to obtain a solution having a known concentration of about 1.0 mg/mL. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Standard solution B: Transfer an amount of USP Powdered Andrographis Extract RS, equivalent to about 25 mg of diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Sample stock solution: Transfer about 2.0 g of finely powdered Andrographis to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 15 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum, and adjust the volume to 50.0 mL using methanol.

Sample solution: Transfer 25.0 mL of *Sample stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

[NOTE—The chromatogram from *Standard solution B* is similar to the Reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS.]

Column efficiency: NLT 5000 theoretical plates, *Standard solution A*

Tailing factor: NMT 1.5 for the andrographolide peak, *Standard solution A*

Relative standard deviation: NMT 2.0%, determined from the andrographolide peak for replicate injections, *Standard solution A*

In-Process Revision

Resolution: NLT 5 between neoandrographolide and 14-deoxy-11,12-didehydroandrographolide peaks, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatogram of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS, identify the retention times of the peaks corresponding to the different diterpene lactones. The approximate relative retention times of the different diterpene lactones are provided in the following table:

Analyte	Relative Retention Time
Andrographolide	1.00
Neoandrographolide	1.16
14-Deoxy-11,12-didehydroandrographolide	1.31
Andrograpanin	1.50

Separately calculate the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin in the portion of Andrographis taken:

$$\text{Result} = (C_S/W) \times (r_U/r_S) \times 10F$$

C_S = concentration of USP Andrographolide RS in *Standard solution A* (mg/mL)

W = weight of Andrographis taken to prepare the *Sample solution* (g)

r_U = peak response for each diterpene lactone from the *Sample solution*

r_S = peak response for andrographolide from *Standard solution A*

F = conversion factor for each analyte (1.00 for andrographolide, 3.90 for neoandrographolide, 1.45 for 14-deoxy-11,12-didehydroandrographolide, and 2.65 for andrograpanin)

Acceptance criteria: NLT 1.0%, on the dried basis, of the sum of the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin

IMPURITIES

Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩**:** NMT 3.0%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1: ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matter* ⟨**561**⟩**:** NMT 2.0%
- **PROCEDURE 2: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

Macroscopic: Stem is dark green, woody, 2–6 mm in diameter, bearing numerous branches, showing slightly swollen nodes, the upper part is distinctly quadrangular with four bulges in the four corners, and the lower part is somewhat rounded; texture fragile, easily broken; branches quadrangular, often narrowly winged in the upper part. Leaves are simple, opposite, short petiolated or nearly sessile; lamina crumpled and easily broken, when whole, lanceolate or ovate-lanceolate, 2–7 cm long, 1–3 cm wide, with acuminate apex, reticulate venation and cuneate-decurrent base, margin entire or undulate; the upper surface green, the lower surface grayish green; both surfaces are glabrous. Pharmacopeial article consists of dry mixtures of crisp, dark-green broken leaves and quadrangular stems; leaves brittle; stems fracture short, fibrous.

Histology

Transverse section of stems: Epidermal layer showing cells containing round, long-elliptical or clavate calcium carbonate deposits (cystoliths), 1–4 celled nonglandular hairs and multicellular, disk-shaped glandular hairs; collenchyma below the epidermis and in the bulges; endodermis is distinct; vascular bundles surround the parenchyma of the central pith; small acicular crystals of calcium oxalate present in the cortex and pith.

Transverse section of leaves: Subsquare or rectangular upper and lower epidermal cells; lower epidermal cells are relatively smaller; both epidermal layers show cells containing cystoliths, nonglandular hairs and glandular hairs similar to those of the stem; mesophyll composed of 1–2 layers of palisade parenchyma and spongy parenchyma; loosely arranged spongy parenchyma appear across the upper part of the midrib; vascular bundles of midrib are collateral and grooved; cells containing cystoliths appear above the xylem.

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of finely powdered Andrographis at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 15%, determined on 1.0 g of finely powdered Andrographis
- **ARTICLES OF BOTANICAL ORIGIN,** *Alcohol-Soluble Extractives, Method 2* ⟨**561**⟩**:** NLT 8.0%
- **MICROBIAL ENUMERATION TESTS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^5 cfu/g; the total combined molds and yeasts count does not exceed 10^3 cfu/g; and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the parts of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Andrographolide RS

USP Powdered Andrographis Extract RS■2S (*USP33*)

BRIEFING

Powdered Andrographis. A new *USP* Dietary Supplement monograph is proposed—See briefing under *Andrographis* published elsewhere in this issue of *PF*.

(DSB: M. Sharaf.) RTS—C66624

Add the following:

■Powdered Andrographis

DEFINITION

Powdered Andrographis is Andrographis reduced to a fine or very fine powder.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩

Standard solution 1: Use *Standard solution A*, prepared as directed in the test for *Content of Diterpene Lactones*.

Standard solution 2: Sonicate an amount of USP Powdered Andrographis Extract RS, equivalent to about 15 mg of

In-Process Revision

diterpene lactones, for 10–15 min in 25 mL of methanol, centrifuge, and use the supernatant.

Sample solution: Use *Sample stock solution*, prepared as directed in the test for *Content of Diterpene Lactones*.

Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 µm (TLC plates)

Application volume: 10 µL, as 5–10 mm bands

Developing solvent system: Chloroform, acetone, and toluene (2:2:1)

Spray reagent: A mixture of 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1)

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Use a saturated chamber. Develop the chromatograms until the solvent front has moved about 90% of the length of the plate. Remove the plate from the chamber, dry, spray with the *Spray reagent*, heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The *Sample solution* exhibits three main grayish blue zones with R_F values of approximately 0.4, 0.6, and 0.8 that correspond in position and color to the main zones of *Standard solution 2*. *Standard solution 1* exhibits a grayish blue zone due to andrographolide at an R_F of about 0.4. The *Sample solution* exhibits a zone similar in color and R_F value to that due to andrographolide in *Standard solution 1*.

• **B.** The retention time of the main peak of the *Sample solution* obtained in the test for *Content of Diterpene Lactones* corresponds to that of andrographolide in *Standard solution A*. Identify other diterpene lactone peaks in the *Sample solution* by comparison with *Standard solution B* and the reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS. The *Sample solution* shows additional peaks corresponding to neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin.

COMPOSITION

• **CONTENT OF DITERPENE LACTONES**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, mix, filter, and degas.

Solution B: Use filtered and degassed acetonitrile.

Standard solution A: Dissolve a weighed quantity of USP Andrographolide RS in methanol to obtain a solution having a known concentration of about 1.0 mg/mL. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Standard solution B: Transfer an amount of USP Powdered Andrographis Extract RS, equivalent to about 25 mg of diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Sample stock solution: Transfer about 2.0 g of Powdered Andrographis to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 15 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum, and adjust the volume to 50.0 mL using methanol. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Sample solution: Transfer 25.0 mL of *Sample stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

[NOTE—The chromatogram from *Standard solution B* is similar to the Reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS.]

Column efficiency: NLT 5000 theoretical plates, *Standard solution A*

Tailing factor: NMT 1.5 for the andrographolide peak, *Standard solution A*

Relative standard deviation: NMT 2.0%, determined from the andrographolide peak for replicate injections, *Standard solution A*

Resolution: NLT 5 between neoandrographolide and 14-deoxy-11,12-didehydroandrographolide peaks, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatogram of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS, identify the retention times of the peaks corresponding to the different diterpene lactones. The approximate relative retention times of the different diterpene lactones are provided in the following table:

Analyte	Relative Retention Time
Andrographolide	1.00
Neoandrographolide	1.16
14-Deoxy-11,12-didehydroandrographolide	1.31
Andrograpanin	1.50

Separately calculate the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin in the portion of Powdered Andrographis taken:

$$\text{Result} = 10(C_S/W)(r_U/r_S) \times F$$

r_U = peak response for each diterpene lactone from the *Sample solution*

r_S = peak response for andrographolide from *Standard solution A*

C_S = concentration of USP Andrographolide RS in the *Standard solution A* (mg/mL)

W = weight of Powdered Andrographis taken to prepare the *Sample solution* (g)

F = conversion factor for each analyte (1.00 for andrographolide, 3.90 for neoandrographolide, 1.45 for 14-deoxy-11,12-didehydroandrographolide, and 2.65 for andrograpanin)

In-Process Revision

Acceptance criteria: NLT 1.0%, on the dried basis, of the sum of the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin

IMPURITIES

Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩**:** NMT 3.0%
- **HEAVY METALS,** *Method II* ⟨ **231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

  Macroscopic: It is a grayish brown powder.

  Histology

  Microscopic examination: It reveals cells of the upper and lower epidermis of the leaves, some cells containing large cystoliths, up to 36 µm in diameter and 180 µm long, with a hilum-shaped scar in the large end; 1–4 celled nonglandular hairs; disk-shaped glandular hairs, 8-celled head and very short stalk; diacytic stomata mostly on the lower epidermis; stem epidermal cells, some cells containing cystoliths, stomata, nonglandular hairs and glandular hairs similar to those of the leaves; thin-walled parenchyma cells; collenchyma cells; acicular phloem fibers; tracheids; vessels, with spiral and scalariform thickening.
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of finely Powdered Andrographis at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 15%, determined on 1.0 g of finely Powdered Andrographis
- **ARTICLES OF BOTANICAL ORIGIN,** *Alcohol-Soluble Extractives***,** *Method 2* ⟨**561**⟩**:** NLT 8.0%
- **MICROBIAL ENUMERATION TESTS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^5 cfu/g; the total combined molds and yeasts count does not exceed 10^3 cfu/g; and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the parts of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

  USP Andrographolide RS

  USP Powdered Andrographis Extract RS■2S *(USP33)*

BRIEFING

Powdered Andrographis Extract. A new *USP* Dietary Supplement monograph is proposed. See briefing under *Andrographis* published elsewhere in this *PF*.

(DSB: M. Sharaf.) RTS—C66625

Add the following:

■Powdered Andrographis Extract

DEFINITION

Powdered Andrographis Extract is prepared from Andrographis by extraction with methanol or alcohol. The ratio of plant material to extract is between 15:1 and 10:1. It contains NLT 90.0% and NMT 110.0% of the labled amount of diterpene lactones, calculated on the dried basis as the sum of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin. The content of 14-deoxy-11,12-didehydroandrographolide is NMT 15% of the total diterpene lactones. It may contain suitable added substances as carriers.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩

  Standard solution 1: Use *Standard solution A*, prepared as directed in the test for *Content of Diterpene Lactones*.

  Standard solution 2: Sonicate for 10–15 min a quantity of USP Powdered Andrographis Extract RS, equivalent to about 15 mg diterpene lactones, in 25 mL of methanol, centrifuge, and use the supernatant.

  Sample solution: Sonicate for 10–15 min a quantity of Powdered Andrographis Extract, equivalent to about 15 mg diterpene lactones, in 25 mL of methanol. Centrifuge, and use the supernatant.

  Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 µm (TLC plates).

  Application volume: 10 µL, as 5–10 mm bands.

  Developing solvent system: Chlorofom, acetone, and toluene (2:2:1).

  Spray reagent: A mixture containing 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1).

  Analysis

  Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

  Use a saturated chamber. Develop until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber. Dry, and spray with the *Spray reagent*. Heat for 5–10 min at 100°, and examine under visible light.

  Acceptance criteria: The *Sample solution* exhibits three main grayish blue zones with R_F values of approximately 0.4, 0.6, and 0.8 that correspond in position and color to zones in *Standard solution 2*. *Standard solution 1* exhibits a grayish blue zone due to andrographolide at an R_F of about 0.4. The *Sample solution* exhibits a zone similar in color and R_F value to that due to andrographolide in *Standard solution 1*.
- **B.** The *Sample solution* in the test for *Content of Diterpene Lactones* shows a main peak at a retention time corresponding to that of andrographolide in *Standard solution A*. Identify other diterpene lactone peaks in the *Sample solution* by comparison with *Standard solution B* and the Reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS. The *Sample solution* shows additional peaks corresponding to neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin.

COMPOSITION

- **CONTENT OF DITERPENE LACTONES**

  Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, mix, filter, and degas.

  Solution B: Use filtered and degassed acetonitrile.

  Standard solution A: Dissolve a weighed quantity of USP Andrographolide RS in methanol to obtain a solution having a known concentration of about 1.0 mg/mL. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

  Standard solution B: Transfer an amount of USP Powdered Andrographis Extract RS, equivalent to about 25 mg diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume,

In-Process Revision

and mix. Before injection, filter through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Sample solution: Transfer a weighed amount of Powdered Andrographis Extract, equivalent to about 25 mg diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume, and mix. Before injection, filter through a membrane filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV, 223 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution A* and *Standard solution B*
Suitability requirements
[NOTE—The chromatogram from *Standard solution B* is similar to the Reference chromatogram provided with the lot of USP Powdered Andrographis Extract RS.]
Column efficiency: NLT 5000 theoretical plates, *Standard solution A*
Tailing factor: NMT 1.5 for the andrographolide peak, *Standard solution A*
Relative standard deviation: NMT 2.0%, determined from the andrographolide peak for replicate injections, *Standard solution A*
Resolution: NLT 5 between neoandrographolide and 14-deoxy-11,12-didehydroandrographolide peaks, *Standard solution B*

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Using *Standard solution A*, *Standard solution B*, and the Reference provided with the lot of USP Powdered Andrographis Extract RS, identify the retention times of the peaks corresponding to different diterpene lactones. The approximate relative retention times of the different diterpene lactones are provided in the following table:

Analyte	Relative Retention Time
Andrographolide	1.00
Neoandrographolide	1.16
14-Deoxy-11,12-didehydroandrographolide	1.31
Andrograpanin	1.50

Separately calculate the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin in the portion of Powdered Andrographis Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times 5F$$

r_U = peak response for each diterpene lactone from the *Sample solution*
r_S = peak response for andrographolide from *Standard solution A*
C_S = concentration of USP Andrographolide RS in *Standard solution A* (mg/mL)
W = weight of Powdered Andrographis Extract taken to prepare the *Sample solution* (g)
F = conversion factor for each analyte (1.00 for andrographolide, 3.90 for neoandrographolide, 1.45 for 14-deoxy-11,12-didehydroandrographolide, and 2.65 for andrograpanin)

Acceptance criteria: 90.0%–110.0%, on the dried basis, of the labeled amount of diterpene lactones calculated as the sum of the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin

IMPURITIES
Inorganic Impurities
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
Organic Impurities
• **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨**731**⟩**:** Dry 2.0 g at 105° for 3 h: it loses NMT 5.0% of its weight.
• **MICROBIAL ENUMERATION TESTS—NUTRITIONAL AND DIETARY SUPPLEMENTS** ⟨**2021**⟩**:** The total aerobic microbial count does not exceed 10^4 cfu/g. The total combined yeast and mold count does not exceed 10^3 cfu/g.
• **MICROBIOLOGICAL PROCEDURES FOR ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
• **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Andrographolide RS
USP Powdered Andrographis Extract RS■2S *(USP33)*

BRIEFING

***Cryptheconidinium cohnii* Oil Capsules.** Because there is no existing *USP* Dietary Supplement monograph for this article, the following new monograph is being proposed. The *Content of DHA* is conducted using the same chromatographic procedure in the USP general test chapter *Fats and Fixed Oils, Omega-3 Fatty Acids Determination and Profile* ⟨401⟩ that became official in the *First Supplement* to *USP 32*.

(DSN: C. Phinney.) RTS—C55686

Add the following:

■*Cryptheconidinium cohnii* Oil Capsules

DEFINITION
Cryptheconidinium cohnii Oil Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22: 6 n-3).

In-Process Revision

IDENTIFICATION

• **FATTY ACID PROFILE**

Fatty Acids as Methyl Esters	Shorthand Notation	Lower Limit Area, (%)	Upper Limit Area, (%)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	0	1.0
Eicosapentanoic acid	20:5 n-3	0	0.1
Docosapentaenoic acid	22:5 n-6	0	0.1
Docosahexanoic acid	22:6 n-3	39.0	47.0

Acceptance criteria: The oil contained in the Capsules meets the following requirements: The area percent for each methyl ester of the fatty acids of the *Test Solution* as obtained in the test for *Content of EPA and DHA* in *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile* meet the requirements for each fatty acid indicated in the table above; and the methyl esters have a common elution order as described in *Fats and Fixed Oils, Fatty Acid Composition* ⟨401⟩.

OTHER COMPONENTS

• **CONTENT OF *Crypthecodinium cohnni* OIL:** Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade or other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied capsules by washing with several small portions of isooctane. Discard the washings, and allow the empty capsules to dry in a current of dry air until the isooctane is completely evaporated. Weigh the empty capsules in the original tared weighing bottle, and calculate the average net weight of *Crypthecodinium cohnni* oil/Capsule.

• **CONTENT OF DHA**

Analysis: Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.

Acceptance criteria: NLT 40.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

• **LIMIT OF ARSENIC**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: Transfer 1 g of ultrapure palladium metal into a Teflon beaker. Add 20 mL of water and 10 mL of nitric acid, and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate into a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (3:2:5). A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed in the test for *Arsenic* ⟨211⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of arsenic.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of arsenic.

Sample solution: For preparation of the *Sample solution*, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [**CAUTION—**Wear proper eye protection and protective clothing and gloves.] Transfer approximately 500 mg of *Crypthecondinium cohnii* oil from Capsules, weighed to the nearest 0.1 mg, into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [NOTE—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with water to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min. Char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min. Cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min. Atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped. Clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration as obtained above

W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of nitric acid and 40 mL of water to dissolve the phosphate. Dilute with deionized water to 100 mL.

In-Process Revision

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.
Solution C: *Solution A*, *Solution B*, and 2% nitric acid (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.
Blank: Nitric acid and water (1:19)
Standard stock solution: Transfer 10.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in the test for *Heavy Metals* ⟨231⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of lead.
Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of lead.
Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*
Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min. char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min. Cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min. Atomize at 2100°, using a 0-s ramp and a 5-s hold with the argon flow stopped. Clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of the *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for lead. Determine the peak area at the lead emission line at 283.3 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of lead, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of lead in each mL of the *Sample solution* by interpolation from the regression line.
Calculate the content of lead in the portion of Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF CADMIUM**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]
Solution A: 10 g of ultrapure monobasic ammonium phosphate in 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.
Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.
Solution C: *Solution A*, *Solution B*, and 2% nitric acid to volume (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate and 0.01 mg of magnesium nitrate.
Blank: Nitric acid and water (1:19)
Standard stock solution A: 0.1372 mg/mL of cadmium nitrate
Standard stock solution B: *Standard stock solution A*, nitric acid, and water (2:1:97). This solution contains 0.10 µg/mL of cadmium. [NOTE—Before make up to final volume dissolve in a portion of water and nitric acid.]
Standard solutions: Dilute *Standard stock solution B* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of cadmium.
Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*
Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min. Char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min. Cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min. Atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped. Clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of the *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for cadmium. Determine the peak area at the cadmium emission line at 228.8 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of cadmium, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of cadmium in each mL of the *Sample solution* by interpolation from the regression line. Calculate the content of cadmium in the Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF MERCURY:** Proceed as directed for *Mercury* ⟨261⟩, *Method IIa*, except to use a *Standard Mercury Solution* having the equivalent of 0.1 µg/mL of mercury.
Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic* combining the two duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution.*
Acceptance criteria: NMT 0.1 µg/g

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0, determined on the contents of the Capsules
- **FATS AND FIXED OILS,** *Free Fatty Acids* ⟨**401**⟩**:** The free fatty acids in 10 g require for neutralization NMT 1.42 mL of 0.1 N sodium hydroxide.
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0, determined on the contents of the Capsules
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26 (determined on the contents of the Capsules), calculated:

$$\text{Result} = (2 \times PV) + AV$$

PV = the peroxide value
AV = the anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 3.5%, determined on the contents of the Capsules
- **SPECIFIC GRAVITY** ⟨**841**⟩**:** 0.91–0.93, determined on the contents of the Capsules

PERFORMANCE TESTS

- **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS** ⟨**2040**⟩**:** Meet the requirements for *Rupture Test for Soft Gelatin Capsules*
- **WEIGHT VARIATION OF DIETARY SUPPLEMENTS** ⟨**2091**⟩**:** Meet the requirements

In-Process Revision

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **LABELING:** The label states the content of docosahexaenoic acid in mg/g. It also states the name and concentration of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Docosahexaenoic Acid Ethyl Ester RS
  USP Eicosapentaenoic Acid Ethyl Ester RS
  USP Methyl Tricosanoate RS■2S (*USP33*)

BRIEFING

Omega-3 Acid Ethyl Esters. Because there is no existing *USP* monograph for this article, the following new monograph is being proposed. The *Assay* is conducted using the same chromatographic procedure in the USP general test chapter *Fats and Fixed Oils, Omega-3 Fatty Acids Determination and Profile* ⟨401⟩ that became official in the *First Supplement* to *USP 32*. Typical retention times obtained using a Macrogol 20000 brand of G16 phase column for eicosapentaenoic acid ethyl ester and docosohexaenoic acid ethyl ester are 20.5 and 25.7 min, respectively.

(DSN: C. Phinney.) RTS—C52079

Add the following:

■Omega-3 Acid Ethyl Esters

DEFINITION

Omega-3 Acid Ethyl Esters are obtained by transesterification of the body oil of fish of families such as *Engraulidae, Carangidae, Clupeidae, Osmeridae, Salmonidae* and *Scombridae* and subsequent purification processes including urea fractionation followed by molecular distillation. The content of eicosapentaenoic acid ethyl ester (EPAee) plus the content of docosohexaenoic acid ethyl ester (DHAee) is NLT 800 mg/g and NMT 880 mg/g, with NTL 430 mg/g and NMT 495 mg/g of EPAee and NLT 347 mg/g and NMT 403 mg/g of DHAee. It contains NLT 90% of the sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION

- The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in the *Test Solution* correspond to those respective compounds in the *Standard Solution*, as obtained in the *Assay*.

ASSAY

- **CONTENT OF EPAEE, DHAEE, AND TOTAL OMEGA-3 ACIDS ETHYL ESTERS**
  (See *Fats and Fixed Oils, Omega-3 Fatty Acids Determination and Profile* ⟨401⟩.)

Analysis

Samples: *Standard Solution* and *Test Solution*

Calculate the content of EPAee and DHAee in the portion of Omega-3 Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak in the *Test Solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak in the *Standard Solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosapentaenoic Acid Ethyl Ester RS in the *Standard Solution* (mg/mL)

C_U = concentration of Omega-3 Ethyl Esters in the *Test Solution* (g/mL)

Calculate the content of total omega-3 acids ethyl esters in the portion of Omega-3 Acid Ethyl Esters taken:

$$\text{Result} = r_{FAn-3ee}\,[(\text{EPAee} + \text{DHAee})/(r_{EPAee} + r_{DHAee})] + \text{EPAee} + \text{DHAee}$$

$r_{FAn-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in the *Test Solution*

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

r_{EPAee} = peak area of the EPAee in the *Test Solution*

r_{DHAee} = peak area of the DHAee in the *Test Solution*

Acceptance criteria: It conforms to the acceptance criteria in *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NLT (mg/g)	Acceptance Criteria, NMT (mg/g)
C18:3 n-3, EE[a]	0.585	—	—
C18:4 n-3, EE[b]	0.608	—	—
C20:4 n-3, EE[c]	0.777	—	—
C20:5 n-3, EE (EPAee)[d]	0.796	430	495
C21:5 n-3, EE[e]	0.889	—	—
C22:5 n-3, EE[f]	0.977	—	—
C22:6 n-3, EE (DHAee)[g]	1.000	347	403
EPAee + DHAee	—	800	880
Total omega-3 acid ethyl esters	—	900	—

[a] Alpha-linolenic acid ethyl ester.
[b] Moroctic acid ethyl ester.
[c] Eicosatetraenoic acid ethyl ester.
[d] Eicosapentaenoic acid ethyl ester.
[e] Heneicosapentaenoic acid ethyl ester.
[f] Docosapentaenoic acid ethyl ester (clupanodonic acid ethyl ester).
[g] Docosahexaenoic acid ethyl ester.

IMPURITIES

Inorganic Impurities

- **FATS AND FIXED OILS** ⟨401⟩**:** NMT 0.1 ppm each of Pb, Cd, As, and Hg

In-Process Revision

Organic Impurities

- **PROCEDURE 1: CHOLESTEROL**

Internal standard stock solution: 3 mg/mL of 5α-cholestane in *n*-heptane

Internal standard solution: 0.3 mg/mL of 5α-cholestane in *n*-heptane. [NOTE—Prepare fresh prior to use.]

Standard stock solution: 3.0 mg/mL of cholesterol in *n*-heptane. [NOTE—This solution is stable for 6 months stored in a freezer.]

Standard solution: Mix 1.0 mL of *Standard stock solution* and 1.0 mL of *Internal standard stock solution* in a 10.0-mL volumetric flask. Dilute with *n*-heptane to volume. [NOTE—Prepare this solution fresh daily.]

Alpha tocopherol stock solution: 1.5–2.0 mg/mL of USP Alpha Tocopherol RS in *n*-heptane. [NOTE—This solution is stable for 12 months stored in a freezer.]

System suitability solution: Mix 1.0 mL of the *Standard stock solution*, 1.0 mL of the *Internal standard stock solution*, and 2.0 mL of *Alpha tocopherol stock solution* in a 50-mL volumetric flask. Evaporate to dryness with the aid of heat, and dilute with ethyl acetate to volume. Dilute 1.0 mL of this solution with ethyl acetate to 10.0 mL. [NOTE—This solution is stable for 6 months stored in a freezer.]

Response factor solution: Mix 1.0 mg/mL of *Standard solution* and 1.0 mL of *Internal standard solution.* Proceed as directed in the *Sample solution* beginning with "Evaporate to dryness".

Sample solution: Transfer 100 mg of Omega-3 Acid Ethyl Esters to a 15-mL centrifuge tube. Add 1.0 mL of *Internal standard solution*. Evaporate to dryness at about 50° with a gentle stream of nitrogen. Add 0.5 mL of 50% potassium hydroxide and 3 mL of alcohol, fill the tube with nitrogen, and cap. Heat the sample at 100° for 60 min, using a heating block. Cool for about 10 min. Add 6 mL of water to the tube, and shake for 1 min. Extract the solution four times with 2.5-mL portions of ethyl ether, using a vortex mixer or suitable shaker for 1 min for each extraction. Transfer and combine the extracts into a large centrifuge tube, and wash with 5 mL of water, mixing completely with gentle inversion. Remove the water phase, and add 5 mL of 0.5 M potassium hydroxide to the ether phase, mixing carefully to avoid an emulsion. Remove the potassium hydroxide, and add another 5 mL of water mixing carefully. Transfer the ether phase into a small centrifuge tube. [NOTE—If an emulsion has occurred, a small amount of sodium chloride may be added to obtain a separation of the phases.] Evaporate the ether phase to dryness under a stream of nitrogen with careful heating. Dissolve the sample in 600 µL of ethyl acetate, and mix well. Transfer 200 µL of this solution to a sample vial, and dilute with ethyl acetate to about 2 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary column coated with a G27 phase of 0.25-µm thickness

Temperature

Injector: 320°

Detector: 300°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	1
170	4	320	1.5

Carrier gas: Helium

Flow rate: 1.3 mL/min

Injection size: 1 µL

Injection type: Splitless injection system

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.2 between alpha tocopherol and cholesterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of total cholesterol in the portion of Omega-3 Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 1000$$

R_U = ratio of the cholesterol peak to the internal standard from the *Sample solution*

R_S = ratio of the cholesterol peak to the internal standard from the *Standard solution*

C_S = concentration of cholesterol in the *Standard solution* (mg/mL)

C_U = concentration of Omega-3 Acid Ethyl Esters in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0 mg/g

- **PROCEDURE 2: OLIGOMERS**

Mobile phase: Tetrahydrofuran

Sample solution 1: 1.00 mg/mL of Omega-3 Acid Ethyl Esters in tetrahydrofuran

Sample solution 2: [NOTE—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shakle vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thorughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase,* with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [NOTE—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: Differential refractometer

Columns: Three concatenated 7.8-mm × 30-cm columns; 7-µm packing L21. Two columns are 50-nm in pore size and the third is 10-nm, arranged so that the 50-nm pore size columns are closer to the injector.

Flow rate: 0.8 mL/min

Injection size: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin, and NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

In-Process Revision

Analysis

Sample: *Sample solution*

Measure the areas of the major peaks. Calculate the percentage of oligomers in the portion of Omega-3 Acid Ethyl Esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the areas of the peaks with a retention time less than that of the ethyl esters peaks

r_T = sum of areas of all peaks

Calculate the percentage of oligomers in the portion of Omega-3 Acid Ethyl Esters taken to prepare *Sample solution 2*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the areas of all peaks with retention times shorter than that of the methyl esters

r_T = sum of areas of all peaks

Acceptance criteria: NMT 1.0% of oligomers

- **PROCEDURE 3: LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS (PCBS):** Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.
  Acceptance criteria: The sum of PCDDs and PCDFs is NMT 1 pg/g of WHO toxic equivalents. The sum of PCBs (polychlorinated biphenyls, IUPAC congeners PCB-28, PCB-52, PCB-101, and PCB-118, PCB-138, PCB-153, PCB-180) is NMT 0.5 ppm.
- **PROCEDURE 4: LIMIT OF DDT, HEXACHLOROBENZENE (HCB), AND LINDANE:** Use a suitable method. The analytical procedure satisfies the following criteria: the method is not susceptible to interference from the components of Omega-3 Acid Ethyl Esters; the concentration of test and reference solutions and the setting of the apparatus are such that the responses used for quantification of the pesticide residues are within the dynamic range of the detector; test solutions containing pesticide residues at a level outside the dynamic range may be diluted within the calibration range provided that the concentration of the matrix in the solution is adjusted in the case where the calibration solutions must be matrix-matched; recovery for HCB, lindane, and DDT is NLT 70% and NMT 110%; the repeatability of the method RSD is NMT 20%; and the reproducibility of the method RSD is NMT 40%.
  Acceptance criteria: NMT 0.05 ppm of HCB, 0.05 ppm of lindane, and 0.05 ppm of DDT expressed as the sum of *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, and *p,p'*-DDD
- **PROCEDURE 5: LIMIT OF TOTAL UNIDENTIFIED FATTY ACIDS ETHYL ESTERS:** From the chromatogram obtained with *Test Solution 4* in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3 Acids Ethyl Esters*, determine the peak area of the largest single unidentified peak with a relative retention time different from those in the following table.

Identified Ethyl Ester	Relative Retention Time
Phytanic acid	0.416
C16:3 n-4	0.431
C16:4 n-1	0.468
C18:3 n-6	0.557
C18:3 n-4	0.574
C18:3 n-3	0.585
C18:4 n-3	0.608
C18:4 n-1	0.618
Furan acid 5	0.691
C19:5	0.710
C20:3 n-6	0.720
C20:4 n-6	0.736
Furan acid 7	0.744
C20:4 n-3	0.777
Furan acid 8	0.783
EPA	0.796
Furan acid 9	0.867
C21:5 n-3	0.889
C22:4	0.917
Furan acid 10	0.922
C22:5 n-6	0.939
Furan acid 11	0.963
C22:5 n-3	0.977
DHA	1.000

Calculate the content of unidentified fatty acids ethyl esters in area percentage:

$$\text{Result} = 100 - \Sigma \text{ Aiee}$$

Aiee = peak area of each identified ethyl ester in the table above

Acceptance criteria: The area of the largest single unidentified peak is NMT 0.5% of the total area. The total area of unidentified peaks as calculted above is NMT 2%.

SPECIFIC TESTS

- **FATS AND FIXED OILS ⟨401⟩,** *Acid Value*: NMT 2
- **FATS AND FIXED OILS ⟨401⟩,** *Anisidine Value*: NMT 15.0
- **FATS AND FIXED OILS ⟨401⟩,** *Peroxide Value*: NMT 10.0
- **ABSORBANCE**
  Sample solution: 0.24 mg/mL in isooctane
  Acceptance criteria: NMT 0.55, determined at 233 nm, with isooctane being used as the blank

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers under a nitrogen atmosphere. Store at controlled room temperature.
- **LABELING:** The label states the average content of DHA and EPA in mg/g, and the content of the total omega-3 acid ethyl esters. It also states the name of any added antioxidant.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Alpha Tocopherol RS
  USP Docosahexaenoic Acid Ethyl Ester RS
  USP Eicosapentaenoic Acid Ethyl Ester RS
  USP Methyl Tricosanoate RS■2S (*USP33*)

BRIEFING

Schizochytrium Oil Capsules. Because there is no *USP* monograph for this dietary supplement ingredient, a new monograph is being proposed. The test for the *Content of DHA* references the gas chromatographic procedure described in the USP general test chapter *Fats and Fixed Oils, Omega-3 Fatty Acids Determination and Profile* ⟨401⟩ that was published on page 3941 of the *First Supplement* to *USP 32*.

(DSN: C. Phinney.) RTS—C55687

In-Process Revision

Add the following:

▪Schizochytrium Oil Capsules

DEFINITION

Schizochytrium Oil Capsules are prepared from Schizochytrium Oil and contain NLT 95.0% and NMT 105.0% of the labeled amount of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22: 6 n–3).

IDENTIFICATION

• **FATTY ACID PROFILE**

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Dihomo-gamma-linolenic acid	20:3 n–6	1.7	2.8
Arachidonic acid	20:4 n–6	0.6	1.3
Eicosapentaenoic acid (EPA)	20:5 n–3	1.3	3.9
Docosapentaenoic acid (DPA n–6)	22:5 n–6	10.5	16.5
Docosahexanoic acid (DHA)	22:6 n–3	30.0	40.0

Acceptance criteria: The retention time of the peaks of the docosahexaenoic acid methyl ester and the eicosapentanoic acid methyl ester of the *Test Solution* corresponds to that of *Standard Solution 2* as obtained in the test for *Content of EPA and DHA* in *Fats and Fixed Oils, Omega-3 Fatty Acids Determination and Profile* ⟨401⟩. The area percentage for the methyl esters of the fatty acids of the *Test Solution* meets the requirements for each fatty acid indicated in the table above.

OTHER COMPONENTS

• **CONTENT OF SCHIZOCHYTRIUM OIL:** Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade or other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied capsules by washing with several small portions of isooctane. Discard the washings, and allow the empty capsules to dry in a current of dry air until the isooctane is completely evaporated. Weigh the empty capsules in the original tared weighing bottle, and calculate the average net weight of schizochytrium oil/Capsule.

• **CONTENT OF DHA**

Analysis: Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.

Acceptance criteria: NLT 32.0% docosahexaenoic acid (DHA)

PERFORMANCE TESTS

• **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS ⟨2040⟩:** Meet the requirements for *Rupture Test for Soft Gelatin Capsules*

• **WEIGHT VARIATION ⟨2091⟩:** Meet the requirements

IMPURITIES

Inorganic Impurities

• **LIMIT OF ARSENIC**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: Transfer 1 g of ultrapure palladium metal into a Teflon beaker. Add 20 mL of water and 10 mL of nitric acid, and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate into a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (3:2:5). A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed in the test for *Arsenic* ⟨211⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of arsenic.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of arsenic.

Sample solution: For preparation of the *Sample solution*, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [**CAUTION—**Wear proper eye protection and protective clothing and gloves.]

Transfer approximately 500 mg of schizochytrium oil from Capsules, weighed to the nearest 0.1 mg, into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [NOTE—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with water to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min; char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of the *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration as obtained above
W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of nitric acid and 40 mL of water to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: 1 g of ultrapure magnesium nitrate, to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution: Transfer 10.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in the test for *Heavy Metals* ⟨231⟩, to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.10 µg/mL of lead.

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of lead.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2100°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of the *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for lead. Determine the peak area at the lead emission line at 283.3 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of lead, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of lead in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of lead in the portion of Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF CADMIUM**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% nitric acid to volume (2:1:2). A volume of 5 µL provides 0.2 mg of phosphate and 0.01 mg of magnesium nitrate.

Blank: Nitric acid and water (1:19)

Standard stock solution A: 0.1372 mg/mL of cadmium nitrate

Standard stock solution B: *Standard stock solution A*, nitric acid, and water (2:1:97). This solution contains 0.10 µg/mL of cadmium. [NOTE—Before make up to final volume dissolve in a portion of water and nitric acid.]

Standard solutions: Dilute the *Standard stock solution B* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of cadmium.

Sample solution: Prepare as directed for *Sample solution* in the test for *Limit of Arsenic.*

Analysis: Program the graphite furnace as follows. Dry at 120°, using a 1-s ramp, a 55-s hold, and an argon flow of 300 mL/min; char the sample at 850°, using a 1-s ramp, a 30-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of the *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrometer equipped with a hollow-cathode lamp for cadmium. Determine the peak area at the cadmium emission line at 228.8 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of cadmium, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, C, in µg/mL, of cadmium in each mL of the *Sample solution* by interpolation from the regression line. Calculate the content of cadmium in the Capsules taken:

$$\text{Result} = 25 \times (C/W)$$

C = concentration, as obtained above
W = weight of Capsules content taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF MERCURY:** Proceed as directed for *Mercury* ⟨261⟩, *Method IIa*, except to use a *Standard Mercury Solution* having the equivalent of 0.1 µg/mL of mercury.

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic* combining the 2 duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution.*

Acceptance criteria: NMT 0.1 µg/g

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0
- **FATS AND FIXED OILS,** *Free Fatty Acids* ⟨**401**⟩**:** The free fatty acids in 10 g require for neutralization NMT 1.42 mL of 0.1 N sodium hydroxide.
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26, calculated:

In-Process Revision

Result = (2 × PV) + AV

PV = the peroxide value
AV = the anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 4.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **LABELING:** The label states the content of docosahexaenoic acid in mg/g. It also states the name and concentration of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Docosahexaenoic Acid Ethyl Ester RS
  USP Eicosapentaenoic Acid Ethyl Ester RS
  USP Methyl Tricosanoate RS■2S *(USP33)*

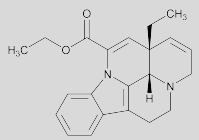
BRIEFING

Vinpocetine. Because there is no existing *USP* monograph for this dietary ingredient, a new monograph is being proposed that is based on the monograph in the *European Pharmacopoeia*. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analysis performed with a Supelco Discovery C18 brand of L1 column. The typical retention time for vinpocetine is about 16 min. Interested parties are encouraged to submit comments.

(DSN: C. Phinney.) RTS—C65492

Add the following:

■Vinpocetine



$C_{22}H_{26}N_2O_2$ 350.45
Eburnamenine-14-carboxylic acid, ethyl ester, (3α,16α)-;
Ethyl apovincamin-22-oate. [42971-09-5].

DEFINITION

Vinpocetine contains NLT 98.5% and NMT 101.5% of $C_{22}H_{26}N_2O_2$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the main peak of the *Sample solution* in the test for *Organic Impurities* corresponds to the retention time of the principal peak for the *Stock standard solution*.

ASSAY

- **PROCEDURE**

Dissolve about 300 mg of Vinpocetine in 50 mL of a mixture of acetic anhydride and acetic acid (1:1), and titrate with 0.1 N perchloric acid VS determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 35.05 mg of $C_{22}H_{26}N_2O_2$.

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 0.001

Organic Impurities

- **PROCEDURE**

Ammonium acetate solution: Transfer 15.4 g of ammonium acetate to a 1-L volumetric flask, dilute with water to volume, and mix.

Mobile phase: Prepare a filtered and degassed mixture of *Ammonium acetate solution* and acetonitrile (45:55). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Sample solution: Transfer about 50.0 mg of Vinpocetine, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Stock standard solution: Prepare a solution of USP Vinpocetine RS accurately weighed in *Mobile phase* having a known concentration of about 0.02 mg per mL.

Standard solution 1: Prepare a solution in *Mobile phase* containing 0.12 mg of USP Vinpocetine Related Compound A RS per mL and 0.10 mg each of USP Vinpocetine Related Compound B RS, USP Vinpocetine Related Compound C RS, and USP Vinpocetine Related Compound D RS per mL.

Standard solution 2: Dilute 1.0 mL of *Stock standard solution* and 1.0 mL of *Standard solution 1* with *Mobile phase* to 20.0 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System suitability.*)
Mode: LC
Detector: 280 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 1.0 mL
Chromatograph the *Stock standard solution* and *Standard solution 2*, and identify the vinpocetine peak and peaks due to the related compounds listed in *Impurity Table 1*. Record the peak responses as directed for *Analysis*: the resolution, *R*, between vinpocetine related compound B and vinpocetine related compound D is NLT 2.0.
Injection size: 15 µL (duplicate equal volumes)

System suitability
Sample: *Standard solution 2*
Suitability requirements
Resolution: NLT 2.0 between vinpocetine related compound B and vinpocetine related compound D

Analysis
Samples: *Sample solution* and *Standard solution 2*
Record the chromatograms for up to a minimum of 3 times the retention time of vinpocetine. Disregard any peak with an area less than 0.5 times the area of the peak due to vinpocetine in *Standard solution 2*. Calculate the percentage of vinpocetine related compounds A, B, C, and D in the portion of Vinpocetine taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 2*
C_S = concentration of each of the known impurities in *Standard solution 2* (mg/mL)
C_U = concentraton of Vinpocetine in *Sample solution* (mg/mL)

Calculate the percentage of any unspecified individual impurity as for vinpocetine by the same formula indicated above, where C_S is the concentration of USP Vinpocetine RS in *Standard solution 2*.

Acceptance criteria: In addition to not exceeding the limits given in *Impurity Table 1*, NMT 1.0% of total impurities is found.

In-Process Revision

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Vinpocetine	1.00	—
Vinpocetine related compound A[1]	0.40	0.6
Vinpocetine related compound B[2]	0.75	0.5
Vinpocetine related compound C[3]	0.83	0.3
Vinpocetine related compound D[4]	0.68	0.5
Unspecified individual impurity	—	0.1

[1]Ethyl (12*RS*,13a*SR*,13b*SR*)-13a-ethyl-12-hydroxy-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (ethyl vincaminate).
[2]Methyl (13a*S*,13b*S*)-13a-ethyl-9-methoxy-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (apovincamine).
[3]Ethyl (13a*S*,13b*S*)-13a-ethyl-9-methoxy-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (methoxyvinpocetine).
[4]Ethyl (12*RS*,13a*RS*,13b*RS*)-13a-ethyl-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (dihydrovinpocetine).

SPECIFIC TESTS

- **Loss on Drying ⟨731⟩:** Dry a sample in a vacuum at 100° for 3 h: it loses NMT 0.5% of its weight.
- **Specific Rotation ⟨781S⟩:** Between +127.0° and +134.0°, determined at 20°.
  Sample solution: 10 mg/mL in dimethylformamide

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP Reference Standards ⟨11⟩**
  USP Vinpocetine RS
  USP Vinpocetine Related Compound A RS
  USP Vinpocetine Related Compound B RS
  USP Vinpocetine Related Compound C RS
  USP Vinpocetine Related Compound D RS■2S (*USP33*)

In-Process Revision

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 27* page 1143, page 4022 of the *First Supplement*, and page 897 of *PF* 35(4) [July–Aug. 2009]. It is proposed to add *Diethyl Sebacate* and *L-Glutamic Acid, Hydrochloride*, to the *Flavors and Perfumes* category*; Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer* to the *Coating Agent and Film-Forming Agent* categories; *Methylpyrrolidone* to the *Solvent* category; and *Hydrogenated Polydextrose* to the *Bulking Agent*, *Coating Agent*, *Humectant*, *Tablet Binder*, and *Suspending and/or Viscosity-Increasing Agent* categories, to complement the proposed new monographs for *Diethyl Sebacate*; *L-Glutamic Acid, Hydrochloride***;** *Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer***;** *Methylpyrrolidone*; and *Hydrogenated Polydextrose*, which appear elsewhere in this issue of *PF.*

(EM1; EM2) RTS—C64233; C65585; C67224; C68840; C69714

Change to read:

Antimicrobial Preservative

Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzethonium Chloride
Benzoic Acid
Benzyl Alcohol
Butylparaben

▲Calcium Propionate▲*NF28*

Cetrimonium Bromide
Cetylpyridinium Chloride
Chlorobutanol
Chlorocresol
Cresol
Dehydroacetic Acid
▲Erythorbic Acid▲*NF27*
Ethylparaben
Methylparaben
Methylparaben Sodium
Phenol
Phenoxyethanol
Phenylethyl Alcohol
Phenylmercuric Acetate
Phenylmercuric Nitrate
Potassium Benzoate
Potassium Sorbate
Propylparaben
Propylparaben Sodium
Sodium Benzoate
Sodium Dehydroacetate
Sodium Propionate
Sorbic Acid
Thimerosal
Thymol

Change to read:

Antioxidant

Ascorbic Acid
Ascorbyl Palmitate
Butylated Hydroxyanisole
Butylated Hydroxytoluene
Stannous Chloride
▲Erythorbic Acid▲*NF27*
Hypophosphorous Acid

■Lactobionic Acid■2S *(NF28)*

Monothioglycerol
Potassium Metabisulfite
Propyl Gallate
Sodium Bisulfite
Sodium Formaldehyde Sulfoxylate
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Sulfur Dioxide
Tocopherol
Tocopherols Excipient

Change to read:

Buffering Agent

Acetic Acid
Adipic Acid
Ammonium Carbonate
Ammonium Phosphate
Boric Acid
Citric Acid, Anhydrous
Citric Acid Monohydrate

■Alpha-Lactalbumin■1S *(NF28)*

Lactic Acid
Phosphoric Acid
Potassium Citrate
Potassium Metaphosphate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Citrate
Sodium Lactate Solution
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Succinic Acid

Change to read:

Bulking Agent for Freeze-Drying

Creatinine

■Alpha-Lactalbumin■1S *(NF28)*

Mannitol
Polydextrose

■Hydrogenated Polydextrose■2S *(NF28)*

Pullulan

■Trehalose■2S *(NF27)*

Change to read:

Coating Agent

Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carboxymethylcellulose, Sodium

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

Cellaburate
Cellacefate (formerly Cellulose Acetate Phthalate)
Cellulose Acetate
Cellulose Acetate Phthalate (see Cellacefate)

■Chitosan■1S *(NF28)*

Coconut Oil
■Hydrogenated Coconut Oil■1S *(NF27)*
Copovidone
Corn Syrup Solids
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Ethylcellulose Aqueous Dispersion

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S *(NF28)*

Gelatin
Glaze, Pharmaceutical
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hydroxypropyl Methylcellulose Phthalate (see Hypromellose Phthalate)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate
Hypromellose Phthalate (formerly Hydroxypropyl Methylcellulose Phthalate)

■Alpha-Lactalbumin■1S *(NF28)*

Maltodextrin

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S *(NF28)*

■Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer■2S *(NF28)*

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S *(NF28)*

Methacrylic Acid Copolymer
Methacrylic Acid Copolymer Dispersion
Methylcellulose
Palm Kernel Oil

■Palm Oil■2S *(NF27)*

■Hydrogenated Palm Oil■1S *(NF27)*

■Hydrogenated Polydextrose■2S *(NF28)*

Polyethylene Glycol

▲Polyvinyl Acetate▲*NF28*

▲Polyvinyl Acetate Dispersion▲*NF28*

Polyvinyl Acetate Phthalate
Pullulan
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Shellac
Starch, Pregelatinized Modified
Sucrose
Titanium Dioxide
Wax, Carnauba
Wax, Microcrystalline
Zein

Change to read:

Complexing Agent

Edetate Calcium Disodium
Edetate Disodium
Edetic Acid

■Alpha-Lactalbumin■1S *(NF28)*

Oxyquinoline Sulfate

Change to read:

Desiccant

Calcium Chloride
Calcium Sulfate

▲Polyvinyl Acetate▲*NF28*

Silicon Dioxide

Change to read:

Emulsifying and/or Solubilizing Agent

Acacia
Carbomer Copolymer
Carbomer Interpolymer
Cholesterol
Stannous Chloride
Coconut Oil

▲Desoxycholic Acid▲*NF28*

Diethanolamine (Adjunct)
Diethylene Glycol Stearates
Ethylene Glycol Stearates
Gamma Cyclodextrin
Glyceryl Distearate
Glyceryl Monolinoleate
Glyceryl Monooleate
Glyceryl Monostearate

■Alpha-Lactalbumin■1S *(NF28)*

Lanolin Alcohols
Lecithin
Mono- and Di-glycerides
Monoethanolamine (Adjunct)
Oleic Acid (Adjunct)
Oleyl Alcohol (Stabilizer)
Oleyl Oleate
Palm Kernel Oil

■Palm Oil■2S *(NF27)*

Poloxamer
Polyoxyethylene 50 Stearate
Polyoxyl 10 Oleyl Ether
Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polyoxyl Lauryl Ether
Polyoxyl Stearyl Ether
Polysorbate 20
Polysorbate 40

In-Process Revision

Polysorbate 60
Polysorbate 80
Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Propylene Glycol Monostearate
Superglycerinated Fully Hydrogenated Rapeseed Oil
Sodium Cetostearyl Sulfate
Sodium Lauryl Sulfate
Sodium Stearate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Stearic Acid

■Sucrose Stearate■1S (*NF28*)

Trolamine
Wax, Emulsifying

Change to read:

Film-Forming Agent

■Chitosan■1S (*NF28*)

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S (*NF28*)

■Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer■2S (*NF28*)

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S (*NF28*)

Change to read:

Flavors and Perfumes

Almond Oil
Anethole
Benzaldehyde

■Diethyl Sebacate■2S (*NF28*)

Ethyl Acetate
Ethyl Vanillin

■L-Glutamic Acid, Hydrochloride■2S (*NF28*)

Lactitol
Maltol
Menthol
Methyl Salicylate
Monosodium Glutamate
Peppermint
Peppermint Oil
Peppermint Spirit
Rose Oil
Rose Water, Stronger
Thymol
Vanillin

Change to read:

Humectant

Corn Syrup Solids
Erythritol
Glycerin
Hexylene Glycol
Inositol
Maltitol
Polydextrose

■Hydrogenated Polydextrose■2S (*NF28*)

Propylene Glycol
Sorbitol
Sorbitol Sorbitan Solution

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

Tagatose

Change to read:

Solvent

Acetone
Alcohol
Alcohol, Diluted
Amylene Hydrate
Benzyl Benzoate
Butyl Alcohol
Canola Oil
Caprylocaproyl Polyoxylglycerides
Corn Oil
Cottonseed Oil
Diethylene Glycol Monoethyl Ether
Ethyl Acetate
Glycerin
Hexylene Glycol
Hydrogenated Polydecene
Isopropyl Alcohol
Lauroyl Polyoxylglycerides
Linoleoyl Polyoxylglycerides
Methyl Alcohol
Methylene Chloride
Methyl Isobutyl Ketone

■Methylpyrrolidone■2S (*NF28*)

Mineral Oil
Oleoyl Polyoxylglycerides
Peanut Oil
Polyethylene Glycol
Polyethylene Glycol Monomethyl Ether
Propylene Glycol
Sesame Oil
Stearoyl Polyoxylglycerides
Water for Injection
Water for Injection, Sterile
Water for Irrigation, Sterile
Water, Purified

Change to read:

Stiffening Agent

Castor Oil, Hydrogenated
Cetostearyl Alcohol
Cetyl Alcohol
Cetyl Esters Wax
Cetyl Palmitate
Hard Fat

■Alpha-Lactalbumin■1S (*NF28*)

Paraffin
Synthetic Paraffin
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Stearyl Alcohol
Wax, Emulsifying
Wax, White
Wax, Yellow

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

▲Carmellose▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

■Chitosan■1S (*NF28*)

▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

■Alpha-Lactalbumin■1S (*NF28*)

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin

■Hydrogenated Polydextrose■2S (*NF28*)

Polyethylene Oxide
Polyvinyl Alcohol
Povidone
Propylene Glycol Alginate
Pullulan
Hydrophobic Colloidal Silica
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca
Starch, Wheat

■Sucrose Palmitate■1S (*NF28*)

Tragacanth
Xanthan Gum

Change to read:

Sweetening Agent

Acesulfame Potassium
Aspartame
Aspartame Acesulfame
▲Corn Syrup▲*NF27*
Corn Syrup Solids
High Fructose Corn Syrup
Dextrates
Dextrose
Dextrose Excipient
Erythritol
Fructose
Galactose
Maltitol
Maltose
Mannitol
Saccharin
Saccharin Calcium
Saccharin Sodium
Sorbitol
Sorbitol Solution

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

Sucralose
Sucrose
Sugar, Compressible
Sugar, Confectioner's
Syrup
Tagatose

■Trehalose■2S (*NF27*)

Change to read:

Tablet Binder

Acacia
Alginic Acid
Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Sodium
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

■Hydrogenated Coconut Oil■1S (*NF27*)
Copovidone
▲Corn Syrup▲*NF27*
Corn Syrup Solids

In-Process Revision

Dextrin
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S *(NF28)*

Gelatin
Glucose, Liquid
Guar Gum
Low-Substituted Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate

■Alpha-Lactalbumin■1S *(NF28)*

Maltodextrin
Maltose
Methylcellulose
■Hydrogenated Palm Oil■1S *(NF27)*

■Hydrogenated Polydextrose■2S *(NF28)*

Polyethylene Oxide

▲Polyvinyl Acetate▲*NF28*

Povidone
Pullulan
Starch, Corn

■Hydrogenated Starch Hydrolysate■1S *(NF28)*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Syrup

■Trehalose■2S *(NF27)*

Change to read:

Tablet and/or Capsule Diluent

Calcium Carbonate
Calcium Phosphate, Dibasic
Calcium Phosphate, Tribasic
Calcium Sulfate
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Cellulose, Powdered
▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrates
Dextrin
Dextrose Excipient
Fructose
Kaolin

■Alpha-Lactalbumin■1S *(NF28)*

Lactitol
Lactose, Anhydrous
Lactose, Monohydrate
Maltitol
Maltodextrin
Maltose
Mannitol
Propylene Glycol Monocaprylate
Pullulan
Sorbitol
Starch
Starch, Corn

■Hydrogenated Starch Hydrolysate■1S *(NF28)*

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Sucrose
Sugar, Compressible
Sugar, Confectioner's

■Trehalose■2S *(NF27)*

Change to read:

Tablet Disintegrant

Alginic Acid
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S *(NF27)*

Croscarmellose Sodium
Crospovidone
Low-Substituted Hydroxypropyl Cellulose
Maltose
Polacrilin Potassium
Pullulan
Sodium Starch Glycolate
Starch
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat

■Trehalose■2S *(NF27)*

Change to read:

Tablet and/or Capsule Lubricant

■Behenoyl Polyoxylglycerides■2S *(NF27)*

Calcium Stearate
■Hydrogenated Coconut Oil■1S *(NF27)*
Glyceryl Behenate
Magnesium Stearate
Mineral Oil, Light
■Hydrogenated Palm Oil■1S *(NF27)*
Polyethylene Glycol
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Sodium Lauryl Sulfate
Sodium Stearyl Fumarate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Starch
Stearic Acid
Stearic Acid, Purified

■Sucrose Stearate■1S *(NF28)*

Talc
Vegetable Oil, Hydrogenated, Type I
Zinc Stearate

Change to read:

Vehicle

FLAVORED AND/OR SWEETENED

Aromatic Elixir
Benzaldehyde Elixir, Compound
Corn Syrup Solids
Dextrose

■Ethyl Maltol■2S *(NF27)*

Peppermint Water
Sorbitol Solution
Syrup

■Trehalose■2S *(NF27)*

OLEAGINOUS

Alkyl (C12-15) Benzoate
Almond Oil
Canola Oil
Corn Oil
Cottonseed Oil
Ethyl Oleate
Hydrogenated Polydecene
Isopropyl Myristate
Isopropyl Palmitate
Mineral Oil
Mineral Oil, Light
Octyldodecanol
Olive Oil
Peanut Oil

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Safflower Oil
Sesame Oil
Soybean Oil
Squalane

SOLID CARRIER

■Chitosan■1S *(NF28)*

Corn Syrup Solids

■Alpha-Lactalbumin■1S *(NF28)*

Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Sugar Spheres

STERILE

▲rAlbumin Human▲*NF27*
Sodium Chloride Injection, Bacteriostatic
Water for Injection, Bacteriostatic

Change to read:

Wetting and/or Solubilizing Agent

Benzalkonium Chloride
Benzethonium Chloride
Cetylpyridinium Chloride
Docusate Sodium
Nonoxynol 9
Octoxynol 9
Poloxamer
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Pullulan
Sodium Lauryl Sulfate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Tyloxapol

NF MONOGRAPHS

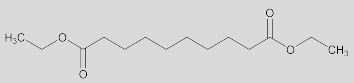
BRIEFING

Diethyl Sebacate. Because there is no existing *NF* monograph for this excipient, a new monograph is proposed based on comments and data received.

(EM2: H. Wang.) RTS—C69714

Add the following:

■Diethyl Sebacate



$CH_3CH_2OOC(CH_2)_8COOCH_2CH_3$

$C_{14}H_{26}O_4$ 258.35

Decanedioic acid, 1,10-diethyl ester;
Diethyl 1,10-decanedioate [110-40-7].

DEFINITION
Diethyl Sebacate consists of the diester of alcohol and sebacic acid. It contains NLT 98.0% and NMT 100.5% of $C_{14}H_{26}O_4$.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197F⟩**
- **B.** It meets the requirements of the test for *Refractive Index*.

ASSAY
- **PROCEDURE**

Analysis: Determine the Ester value, I_E, as directed in *Fats and Fixed Oils, Ester Value* ⟨401⟩.

Calculate the percentage of $C_{14}H_{26}O_4$ in the portion of Diethyl Sebacate taken:

$$\text{Result} = [(I_E \times M_{W1})/(M_{W2} \times N)] \times F \times 100$$

M_{W1} = molecular weight of $C_{14}H_{26}O_4$, 258.35 g/mol
M_{W2} = molecular weight of potassium hydroxide, 56.11 g/mol
N = ester group number per $C_{14}H_{26}O_4$, 2
F = conversion factor, 10^{-3} g/mg

Acceptance criteria: 98.0%–100.5%

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.10%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 20 ppm

SPECIFIC TESTS
- **SPECIFIC GRAVITY ⟨841⟩:** 0.958–0.968 at 20°
- **REFRACTIVE INDEX ⟨831⟩:** 1.435–1.437 at 20°
- **FATS AND FIXED OILS,** *Acid Value* **⟨401⟩:** NMT 0.5
- **FATS AND FIXED OILS,** *Iodine Value* **⟨401⟩:** NMT 0.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool, dry, and well-ventilated place.

- **USP REFERENCE STANDARDS ⟨11⟩**
USP Diethyl Sebacate RS■2S *(NF28)*

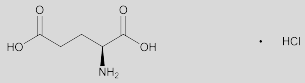
BRIEFING

L-Glutamic Acid, Hydrochloride. Because there is no existing *NF* monograph for this excipient, a new monograph is being proposed, based on the monograph appearing in *Food Chemicals Codex, 6th Edition*. Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver. NOM: A. Wilk.) RTS—C64233

Add the following:

■L-Glutamic Acid, Hydrochloride



$C_5H_9NO_4 \cdot HCl$ 183.59

L-2-Aminoglutaric acid, hydrochloride;
2-Aminopentanedioic acid, hydrochloride [138-15-8].

DEFINITION
L-Glutamic Acid, Hydrochloride, contains NLT 98.5% and NMT 101.5% of $C_5H_9NO_4 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION
- **INFRARED ABSORPTION ⟨197K⟩**

ASSAY
- **PROCEDURE**

Sample: 100 mg of L-Glutamic Acid, Hydrochloride, previously dried

Analysis: Dissolve the *Sample* in 0.5 mL of water, add 15.0 mL of 0.1 N perchloric acid VS, and heat on a water bath for 30 min. After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 N sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 18.36 mg of $C_5H_9NO_4 \cdot HCl$.

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.25%
- **HEAVY METALS,** *Method I* **⟨231⟩:** NMT 5 ppm

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩:** +25.2° to +25.8°, determined at 20°

Sample solution: 100 mg/mL, in 2 N hydrochloric acid
- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 80° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP L-Glutamic Acid, Hydrochloride RS■2S *(NF28)*

In-Process Revision

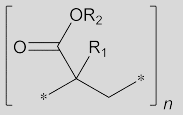
BRIEFING

Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer. Because there is no existing *NF* monograph for this excipient, a new monograph is being proposed, based on validated methods of analysis and to align with the *European Pharmacopoeia* monograph, Methacrylic Acid-Ethyl Acrylate Copolymer (1:1), Type B. The liquid chromatographic procedure in the test for *Limit of Methacrylic Acid and Ethyl Acrylate* employs a Thermo-Hypersil Aquasil C18 or Zorbax SB-AQ RP 18 brand of column that contains 5-µm packing L1. The typical retention times for methacrylic acid and ethyl acrylate are 2.8 min and 6.3 min, respectively.

(EM2: H. Wang.) RTS—C67224

Add the following:

▪Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer



$R_1 = CH_3$; R_2 = H or

$R_1 = CH_3$; R_2 = Na or

R_1 = H; $R_2 = C_2H_5$

Partially-Neutralized Poly(methacrylic acid, ethyl acrylate);
Partially-Neutralized Methacrylic acid-ethyl acrylate copolymer [25212-88-8].

DEFINITION

Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer consists of methacrylic acid and ethyl acrylate monomers arranged in a random distribution, some units of methacrylic acid in the copolymer are neutralized by sodium hydroxide. The non-neutralized methacrylic acid units in the partially-neutralized methacrylic acid and ethyl acrylate copolymer are NLT 43.2% and NMT 47.6%, calculated on the dried basis. It may contain suitable emulsifiers.

IDENTIFICATION

- **A. INFRARED ABSORPTION**

Sample: 100 mg of Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer

Analysis: Dissolve the *Sample* in 1 mL of dehydrated alcohol, and place 2 drops of the solution on a sodium chloride (or potassium bromide) plate. Dry to evaporate the solvent and allow to form a film and cover with another sodium chloride (or potassium bromide) plate.

Acceptance criteria: The IR absorption spectrum of Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer exhibits maxima corresponding to the same wavelengths as that of a similar preparation of USP Partially-Neutralized Methacrylic and Ethyl Acrylate Copolymer (1:1) RS, treated in the same manner.

- **B.** It meets the requirements of the *Assay*.

ASSAY

- **PROCEDURE**

Sample: 1 g, previously dried

Analysis: Dissolve the *Sample* in 40 mL of water and 60 mL of 2-propanol, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 8.609 mg of methacrylic acid ($C_4H_6O_2$) units.

Acceptance criteria: 43.2%–47.6% for Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** 0.5%–3.0%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 20 ppm

Organic Impurities

- **PROCEDURE: LIMIT OF METHACRYLIC ACID AND ETHYL ACRYLATE**

Phosphoric acid solution: 0.1% phosphoric acid prepared from phosphoric acid

Mobile phase: Methanol and *Phosphoric acid solution* (3:7)

Standard solution: 1.0 µg/mL each of methacrylic acid and ethyl acrylate in methanol

Sample solution: Transfer 0.5 g of Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer to a 25-mL volumetric flask, and dissolve in 20 mL of methanol. Add *Phosphoric acid solution* dropwise to precipitate the polymer while continuously stirring until the volume of 25 mL is reached. [NOTE—Stir with a magnetic stirrer for 10 min. Any volume deviation caused by the precipitation is negligible for contents in the ppm range. Use a magnetic stirrer of appropriate size to avoid a large variance from the final volume of the *Sample solution*.] As soon as the solid matter has settled, filter the supernatant through a 0.45-µm filter. [NOTE—Solution that cannot be filtered is centrifuged at NLT 20,000 × *g* for NLT 30 min.] Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.0-mm × 12.5-cm analytical column, 5-µm packing L1 or 4.6-mm × 15.0-cm analytical column, 5-µm packing L1

Flow rate: 1.2 mL/min

Injection size: 20 µL

[NOTE—Where appropriate, the volume must be adapted to the sensitivity of the detector.]

[NOTE—Column switching system may be used for extension of column lifetime.]

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methacrylic acid and ethyl acrylate are 1.0 and 2.2, respectively.]

Suitability requirements

Resolution: NLT 5.0 between methacrylic acid and ethyl acrylate

Relative standard deviation: NMT 5.0%

In-Process Revision

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each monomer (methacrylic acid or ethyl acrylate) in the portion of Methacrylic Acid and Ethyl Acrylate Copolymer taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times V_F \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
W = weight of Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer taken to prepare the *Sample solution* (g)
V_F = final volume of the *Sample solution*, 25 mL
F = conversion factor, 10^{-6} g/µg

Acceptance criteria: NMT 0.01% for the total amount of monomers

SPECIFIC TESTS

- **Loss on Drying ⟨731⟩:** Dry a sample at 110° for 6 h: it loses NMT 5.0% of its weight.
- **Viscosity ⟨911⟩:** Weigh 400 g of water and transfer to a short form, 600-mL beaker (about 80 mm in internal diameter and 120 mm high). After determining the *Loss on Drying*, weigh a quantity of undried Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer, equivalent to 100 g on the dried basis. Transfer the sample to the beaker very slowly under gentle stirring (avoid lumps). Ensure a homogeneous solution by gently stirring at room temperature for 3 h and taking care to avoid mixing in excess air. Afterwards allow the container to stand for 1 h, control the temperature to 23 ± 0.1°, and let the entrapped air dissipate. [NOTE—Ensure that the concentration of this solution is 20% (w/w).] Determine the viscosity of this solution at 23 ± 0.1° using a suitable rotational viscometer with a cylindrical spindle 1.9 cm in diameter and 6.5 cm high, attached to a shaft 0.3 cm in diameter.[1] The spindle rotates at 50 rpm at an immersion depth of 8.1 cm. Follow the instrument manufacturer's directions to measure the apparent viscosity.
  Acceptance criteria: 20–100 mPa · s

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **Labeling:** Label it to indicate the range of non-neutralized methacrylic acid units. The labeling also indicates the name and quantity of any emulsifier if the content is 0.10% or greater.
- **USP Reference Standards ⟨11⟩**
  USP Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer (1:1) RS■2S *(NF28)*

BRIEFING

Methylpyrrolidone. Because there is no existing *NF* monograph for this excipient, a new monograph, based on the monograph appearing in *European Pharmacopoeia, 6.3 Edition*, is being proposed. Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver. NOM: A. Wilk.) RTS—C65585

[1]A suitable spindle is available from Brookfield as an LV1 spindle, or the equivalent.

Add the following:

■Methylpyrrolidone

C_5H_9NO 99.1

1-Methyl-2-pyrrolidinone;
N-Methyl-2-pyrrolidone;
N-Methylpyrrolidone;
1-Methyl-2-Pyrrolidone;
Pyrrolidin, 1-methyl-2-one-;
1-Methylpyrrolidin-2-one;
N-Methyl-γ-butyrolactam;
N-Methyl tetrahydropyrrolone;
1-Methyl-2-oxopyrrolidine;
N-Methyl-1-oxotetramethyleneamine;
2-Methyl-2-azacyclopentanone [872-50-4].

IDENTIFICATION

- **Infrared Absorption ⟨197F⟩**

IMPURITIES

Inorganic Impurities

- **Heavy Metals ⟨231⟩:** NMT 10 ppm

Organic Impurities

- **Procedure**
  Standard solution: To 1 mL of USP Methylpyrrolidone RS, add 1 mL of pyrrolidone, and dilute with methylene chloride to 20 mL.
  Sample solution: Methylpyrrolidone (neat)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 0.32-mm × 30-m fused-silica capillary column, 5-µm of phase G2
  Temperature
  Injector: 280°
  Detector: 280°
  Column: See the temperature program table.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	0
100	3	170	30

  Carrier gas: Nitrogen
  Linear velocity: 20 cm/s
  Split ratio: 100:1
  Injection size: 1 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 2.0 between pyrrolidone and methylpyrrolidone
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each impurity, excluding any solvent peaks and peaks NMT 0.02%, in the portion of Methylpyrrolidone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

  Acceptance criteria: NMT 0.1% of any individual impurity; and NMT 0.3% of total impurities

In-Process Revision

SPECIFIC TESTS

- **ALKALINITY**

Bromothymol blue solution: Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of alcohol, and dilute with water to 100 mL.

Sample: Methylpyrrolidone (neat)

Analysis: Add 0.5 mL of *Bromothymol blue solution* as indicator to 50 mL of water and adjust with 0.02 M potassium hydroxide or 0.02 M hydrochloric acid until a yellow color is obtained. Add 50 mL of *Sample*. Titrate with 0.02 M hydrochloric acid to the initial coloration.

Acceptance criteria: NMT 8.0 mL of 0.02 M hydrochloric acid is required.

- **CLARITY OF SOLUTION**

[NOTE—The *Sample* is to be compared to the *Reference suspension* in diffused daylight 5 min after preparation of the *Reference suspension*.]

Hydrazine solution: 10 mg/mL of hydrazine sulfate. [NOTE—Allow to stand 4–6 h before use.]

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension

[NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask.

[NOTE—Allow to stand for 24 h.]

Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. [NOTE—This suspension should not be used beyond 24 h after preparation.]

Reference suspension: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.

Sample: Methylpyrollidone (neat)

Analysis: Transfer a sufficient portion of the *Sample* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension* and water to separate matching test tubes. Compare the *Sample, Reference suspension*, and water in diffused daylight, viewing vertically against a black background (see *Spectrophotometry and Light-Scattering* ⟨851⟩*, Visual Comparison*.)

[NOTE—The diffusion of light must be such that the *Reference suspension* can readily be distinguished from water.]

Acceptance criteria: The *Sample* shows the same clarity as that of water, or its opalescence is not more pronounced than that of the *Reference suspension.*

- **COLOR OF SOLUTION**

Sample Methylpyrollidone (neat)

Analysis: Transfer a sufficient portion of the *Sample* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer a portion of water to a separate matching test tube. Compare the color of the *Sample* with that of water in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* ⟨851⟩*, Visual Comparison*).

Acceptance criteria: The *Sample* has the color of water.

- **WATER,** *Method Ic* ⟨**921**⟩**:** NMT 0.1%, determined on 1.0 g

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Methylpyrrolidone RS■2S *(NF28)*

BRIEFING

Polydextrose, *NF 27* page 1306. On the basis of the comments and data received, it is proposed to make the following revisions.

1. Use an HPLC method to replace the current procedures for the *Assay*, a nonselective colorimetric assay in sulfuric acid medium for quantitation of polydextrose, and for the *Limit of Monomers*, a silyation in combination with GC determination of residual monomers. The submitted HPLC procedure in the *Assay* and in the test for *Limit of Monomers* is based on analyses performed using the Bio-Rad Aminex Ion Exclusion HPX-87H brand of L17 column; polydextrose elutes at approximately 5.8 min on this system.
2. USP Polydextrose RS is introduced into this monograph.
3. In the test for *Molecular Weight Limit*, the HPLC column designation is changed from L25 to L39. The subsections for *System suitability* and *Analysis* are revised.
4. In the test for *Limit of Lead*, the recovery spec is added in the monograph.

(EM2: H. Wang.) RTS—C72332

Polydextrose

[68424-04-4]

DEFINITION

Polydextrose is a randomly branched polymer prepared by melting and subsequent condensation of the ingredients, which consist of approximately 90 parts dextrose, 10 parts sorbitol, and up to 1 part citric acid or 0.1 part phosphoric acid. The 1,6-glycosidic linkage predominates in the polymer but other linkages are present. It contains NLT 90.0% of dextrose polymer units, calculated on the anhydrous and ash-free basis. It contains small quantities of free dextrose, sorbitol, and 1,6-anhydro-D-glucose (levoglucosan), with traces of citric acid or phosphoric acid.

IDENTIFICATION

- **A.** To 1 drop of a solution (1 in 10) add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS: a deep yellow to orange color is produced.
- **B.** With vigorous swirling, add 1 mL of acetone to 1 mL of a solution (1 in 10): the solution remains clear.
- **C.** With vigorous swirling, add 2 mL of acetone to the solution obtained in *Identification* test *B*: a heavy, milky turbidity develops immediately.
- **D.** To 1 mL of a solution (1 in 50) add 4 mL of alkaline cupric citrate TS. Boil vigorously for 2–4 min. Remove from heat, and allow the precipitate (if any) to settle: the supernatant is blue or blue-green.

ASSAY

Change to read:

- **PROCEDURE**

~~**Phenol solution:** Add 20 mL of water to 80 g of phenol.~~

~~**Dextrose stock standard solution:** 0.2 mg/mL of USP Dextrose RS~~

~~**Standard solution A:** 5 µg/mL of dextrose, from *Dextrose stock standard solution*~~

~~**Standard solution B:** 10 µg/mL of dextrose, from *Dextrose stock standard solution*~~

~~**Standard solution C:** 20 µg/mL of dextrose, from *Dextrose stock standard solution*~~

~~**Standard solution D:** 40 µg/mL of dextrose, from *Dextrose stock standard solution*~~

In-Process Revision

~~**Standard solution E:** 50 µg/mL of dextrose, from *Dextrose stock standard solution*~~
~~**Sample solution:** Transfer 10 mg of Polydextrose, weighed and calculated on the anhydrous and ash-free basis, to a 250-mL volumetric flask, and dilute with water to volume.~~
~~**Spectrometric conditions**~~
~~**Mode:** UV-Vis~~
~~**Analytical wavelength:** 490 nm~~
~~**Cell:** 1 cm~~
~~**Blank:** Prepare as directed under *Analysis* below.~~
~~**Analysis:** *Standard solution A, Standard solution B, Standard solution C, Standard solution D,* and *Standard solution E*~~
~~**Samples:** *Sample solution* and *Blank*~~
~~Freshly pipet 2.0 mL of each of the *Standard solutions* into a separate acetone-free, 15-mL vial equipped with a screw-cap. Transfer 2.0 mL of the *Sample solution* to a separate acetone-free, 15-mL vial equipped with a screw-cap, and transfer 2.0 mL of water to a separate acetone-free, 15-mL vial equipped with a screw-cap to provide the *Blank*. To each vial, add 0.12 mL of the *Phenol solution*, cap the vial, and mix gently. Uncap each vial, and rapidly add 5.0 mL of sulfuric acid. Immediately recap each vial, and shake vigorously.~~
~~[**CAUTION**—Wear rubber gloves and a safety shield while adding sulfuric acid.]~~
~~Allow the vials to stand at room temperature for 45 min. Determine the absorbances of the solutions using the *Spectrometric system*, using the *Blank* to set the instrument. Prepare a standard curve by plotting the absorbances of the solutions from the *Standard solutions* versus their contents of dextrose, in µg/mL. Draw the straight line best fitting the five points, and extrapolate the line until it intercepts the absorbance axis (y axis). From the curve, determine the concentration C_D, in µg/mL, of dextrose in the *Sample solution*.~~
~~Calculate the percentage of dextrose polymer units in the Polydextrose taken:~~

~~$$\text{Result} = F_T[25C_D/W_T - P_G - (F_2 \times P_L)]$$~~

~~F_T = experimentally derived correction factor to account for the polymer (which also contains a small amount of sorbitol) not giving the exact amount of color given by an equivalent amount of glucose monomers, 1.05~~
~~W_T = weight of the Polydextrose taken to prepare the *Sample solution* (mg)~~
~~P_G = percentage of dextrose determined in the test for *Limit of Monomers*~~
~~P_L = percentage of 1,6-anhydrous-D-glucose (levoglucosan) determined in the test for *Limit of Monomers*~~
~~F_2 = conversion factor from 1,6-anhydrous-D-glucose, which gives an equivalent amount of color to an equivalent weight of glucose, 1.11~~

■**Mobile phase:** 0.001 N sulfuric acid. Pass this solution through a filter having a 0.5-µm or finer porosity, and degas.
Standard solution: 4.0 mg/mL of USP Polydextrose RS, calculated on the anhydrous and ash-free basis, in *Mobile phase*
Sample solution: 4.0 mg/mL of Polydextrose, calculated on the anhydrous and ash-free basis, in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Detector temperature: 35 ± 0.1°
Guard column: 4.6-mm × 3.0-cm guard column, packing L17
Analytical column: 7.8-mm × 30-cm analytical column, packing L17
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dextrose polymer units in the portion of Polydextrose taken:

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

r_U = peak response for dextrose polymer units from the *Sample solution*
r_S = peak response for dextrose polymer units from the *Standard solution*
C_S = concentration of USP Polydextrose RS in the *Standard solution* (mg/mL)
C_U = concentration of Polydextrose in the *Sample solution* (mg/mL)

■2S (*NF28*)
Acceptance criteria: NLT 90.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.3%
- **LIMIT OF LEAD**

[NOTE—Use reagent-grade chemicals with as low a lead content as is practicable, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with Purified Water.]
Matrix modifier solution: Prepare a solution in water containing 100.0 mg of dibasic ammonium phosphate per 10 mL of solution.
Lead nitrate stock solution: Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.
Standard lead solution: On the day of use, dilute 10.0 mL of *Lead nitrate stock solution* with water to 100.0 mL. Each mL of *Standard lead solution* contains the equivalent of 10 µg of lead.
Standard solution A: 0.02 µg/mL of lead, from *Standard lead solution* in water
Standard solution B: 0.05 µg/mL of lead, from *Standard lead solution* in water
Standard solution C: 0.1 µg/mL of lead, from *Standard lead solution* in water
Standard solution D: 0.2 µg/mL of lead, from *Standard lead solution* in water
Standard solution E: 0.5 µg/mL of lead, from *Standard lead solution* in water
Sample solution: Transfer 1.0 g of Polydextrose, weighed and calculated on the anhydrous and ash-free basis, into a 10-mL volumetric flask, and dissolve in and dilute with water to volume.
Spiked sample solution: Transfer 1.0 g of Polydextrose, weighed and calculated on the anhydrous and ash-free basis, into a 10-mL volumetric flask, and dissolve in water. Add 100 µL of the *Standard lead solution*, and dilute with water to volume. This solution contains 0.1 µg/mL of added lead.
Spectrometric conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: Graphite furnace atomic absorption spectrophotometer, equipped with a pyrolytic tube with a platform and a lead hollow-cathode lamp, using a slit width of 0.7 mm (set low) and a deuterium arc lamp for background correction
Analytical wavelength: Lead emission line of 283.3 nm
Injection size: See *Samples* under *Analysis* below.
Temperature: The drying temperature of the furnace is maintained at 130° for 40 s after a 20-s ramp time using

In-Process Revision

an argon flow rate of 300 mL/min, the ashing temperature is maintained at 800° for 40 s after a 20-s ramp time using an argon flow rate of 300 mL/min, and the atomization temperature is maintained at 2400° for 6 s using an argon flow rate of 50 mL/min. Clean the graphite furnace at 2600° for 5 s after a 1-s ramp time using an argon flow rate of 300 mL/min, and recharge the graphite furnace at 20° for 20 s after a 2-s ramp time using an argon flow rate of 300 mL/min.

Analysis

Samples: 10-µL aliquots of the five *Standard solutions*, a mixture of 10 µL of the *Matrix modifier solution* and 10 µL of the *Sample solution*, and a mixture of 10 µL of the *Matrix modifier solution* and 10 µL of the *Spiked sample solution*.

Concomitantly determine the absorbances of the *Samples* using the *Spectrometric conditions* described above. Plot the absorbance of each *Standard solution*, compensated for background correction, versus its content of lead, in µg/mL, and draw the straight line best fitting the five points. From this plot, determine the concentrations, C_T and C_{ST}, in µg/mL, of lead in the *Sample solution* and the *Spiked sample solution*, respectively.

Calculate the percentage recovery taken:

$$\text{Result} = [(CST - CT)/A] \times 100$$

A = amount of lead added to the *Spiked sample solution*, 0.1 µg/mL

Calculate the content, in µg/g, of lead in the portion of Polydextrose taken:

$$\text{Result} = (CT/W) \times V$$

W = weight of Polydextrose taken to prepare the *Sample solution* (g)

V = volume of the *Sample solution*, 10 mL

Acceptance criteria: NMT 0.5 µg/g on the anhydrous and ash-free basis. The recovery is 80%-120%.

Change to read:

Organic Impurities

- **PROCEDURE 1: LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED COMPOUNDS**

Sample solution: 1.0 g of Polydextrose, weighed and calculated on the anhydrous and ash-free basis, diluted with water to 100 mL

Analysis: Determine the absorbance of the *Sample solution* in a 1-cm quartz cell at 283 nm, with a suitable spectrophotometer, using water as the blank.

Calculate the percentage of 5-hydroxymethylfurfural and related compounds in the portion of Polydextrose taken:

$$\text{Result} = (V \times M_r \times A)/(\varepsilon_{283} \times L \times W) \times 100$$

V = volume of the *Sample solution*, 0.1 L

M_r = molecular weight of 5-hydroxymethylfurfural, 126 g/mol

A = absorbance of the *Sample solution*

ε_{283} = molar extinction coefficient of 5-hydroxymethylfurfural at a wavelength of 283 nm, 16,830 L/mol cm

L = path length of the spectrophotometer cell (cm)

W = weight of Polydextrose taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1%

- **PROCEDURE 2: LIMIT OF MONOMERS**

~~**Internal standard solution:** 0.5 mg/mL of *n*-octadecane in pyridine~~

~~**Standard stock solution:** 0.5 mg/mL of USP Dextrose RS, 0.4 mg/mL of USP Sorbitol RS, and 0.35 mg/mL of 1,6-anhydro-D-glucose RS in pyridine~~

~~**Standard solution:** Transfer 1.0 mL of *Standard stock solution* to a reaction vial equipped with a screw cap. Add 1.0 mL of *Internal standard solution* and 0.5 mL of *N*-(trimethylsilyl) imidazole to the reaction vial, seal the vial with the screw cap, and mix. Place the vial in an ultrasonic bath at 70° for 60 min, remove, and allow the contents to cool.~~

~~**Sample solution:** Transfer 20 mg of Polydextrose, weighed and calculated on the anhydrous and ash-free basis, into a reaction vial equipped with a screw cap. Add 1.0 mL of *Internal standard solution*, 1.0 mL of pyridine, and 0.5 mL of *N*-(trimethylsilyl) imidazole to the reaction vial, seal the vial with the screw cap, and mix. Place the vial in an ultrasonic bath at 70° for 60 min, remove, and allow the contents to cool.~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨621⟩*, System Suitability*.)~~

~~**Mode:** GC~~

~~**Detector:** Flame ionization~~

~~**Column:** 2-mm × 250-cm glass column; packed with 3% phase G2 on support S1AB~~

~~**Temperature**~~

~~**Column:** 175°~~

~~**Detector:** 230°~~

~~**Injection port:** 210°~~

~~**Carrier gas:** Helium~~

~~**Flow rate:** 30 mL/min~~

~~**Injection size:** 1 µL~~

~~**Injection type:** Splitless mode~~

~~**System suitability**~~

~~**Sample:** *Standard solution*~~

~~[NOTE—The relative retention times for the D-anhydroglucose (levoglucosan) pyranose form, D-anhydroglucose furanose form (not present in the standard), *n*-octadecane, alpha-D-glucose, D-sorbitol, and beta-D-glucose are 0.3, 0.4, 0.45, 0.8, 1.0, and 1.2, respectively.]~~

~~**Analysis**~~

~~**Samples:** *Standard solution* and *Sample solution*~~

~~Calculate the percentage of each monomer in Polydextrose taken:~~

~~$$\text{Result} = (R_U/R_S) \times (100C_S/W)$$~~

~~R_U = ratio of the peak areas of respective monomer silyl derivative peak and the internal standard peak of the *Sample solution*~~

~~R_S = ratio of the peak areas of monomer silyl derivative peak and the internal standard peak of the *Standard solution*~~

~~C_S = concentration of the respective monomer in the *Standard stock solution* (mg/mL)~~

~~W = weight of Polydextrose taken to prepare the *Sample solution* (mg)~~

~~**Acceptance criteria:** NMT 4.0% of 1,6-anhydrous-D-glucose, and NMT 6.0% of dextrose and sorbitol~~

~~[NOTE—In the case of glucose, the peak areas for the alpha- and beta-epimers are combined, and in the case of 1,6-anhydro-D-glucose, the peak areas for the pyranose and furanose forms are combined.]~~

■**Mobile phase, Sample solution,** and **Chromatographic system:** Prepare as directed in the *Assay*.

Standard solution: 0.08 mg/mL of each of USP 1,6-Anhydro-D-glucose RS and USP Sorbitol RS, and 0.16 mg/mL of USP Dextrose RS, in *Mobile phase*

System suitability

Sample: *Standard solution*

[NOTE—For relative retention times, see *Table 1* below.]

In-Process Revision

Table 1

Component	Relative Retention Time
Dextrose (glucose)	0.7
Sorbitol	0.8
An isomer of 1,6-anhydro-D-glucose (D-anhydroglucose furanose form)	0.9
1,6-Anhydro-D-glucose (D-anhydroglucose furanose form)	1.0

Suitability requirements
Resolution: NLT 1.0
Relative standard derivation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Use peak response of USP 1,6-Anhydro-D-glucose RS in the *Standard solution* for calculation of percentage of the isomer of 1,6-anhydro-D-glucose in the *Sample solution*. Calculate the percentage of each monomer in the portion of Polydextrose taken:

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

r_U = peak response for the respective monomer from the *Sample solution*
r_S = peak response for the respective monomer from the *Standard solution*
C_S = concentration of the respective standard monomer in the *Standard solution* (mg/mL)
C_U = concentration of Polydextrose in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4.0% of 1,6-anhydro-D-glucose, NMT 4.0% for dextrose, and NMT 2.0% for sorbitol. [NOTE—In the case of 1,6-anhydro-D-glucose, the peak areas for the pyranose and furanose forms are combined.]
■2S (*NF28*)

SPECIFIC TESTS

Change to read:

- **MOLECULAR WEIGHT LIMIT**

Mobile phase: Dissolve 35.0 g of sodium nitrate and 1.0 g of sodium azide in 100 mL of water. Dilute with water to 4 L. Pass through a filter having a 0.45-µm or finer porosity, and degas by applying an aspirator vacuum for 30 min. The resulting *Mobile phase* is 0.1 N sodium nitrate containing 0.025% sodium azide.
Standard solution: Transfer 20 mg each of USP Dextrose RS, stachyose, and 5800-; 23,700-; and 100,000-molecular weight (MW) pullulan standards into a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Pass through a syringe filter having a 0.45-µm or finer porosity into a suitable autosampler vial, and seal.
Sample solution: Transfer 50 mg of Polydextrose into a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Pass through a syringe filter having a 0.45-µm or finer porosity into a suitable autosampler vial, and seal.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index set at a sensitivity of 4×10^{-6} refractive index units full scale and maintained at a temperature of $35 \pm 0.1°$
Column: 7.8-mm × 30-cm; packing ~~L25~~■L39■2S (*NF28*)
Column temperature: 45°
Flow rate: 0.8 mL/min
[NOTE—After installation of a new column, pump *Mobile phase* through the column overnight at a rate of 0.3 mL/min. Before calibration or analysis, increase the flow slowly over a 1-min period to 0.8 mL/min. Continue to pump *Mobile phase* through the column at this flow rate for at least 1 h before the first injection. Check the flow gravimetrically, and adjust it if necessary. Reduce the flow rate to about 0.1 mL/min when the system is not in use.]
Injection size: 50 µL
~~**System suitability:** Chromatograph replicate injections of the *Standard solution*, allowing 15 min between injections, and record the retention times of the components of the *Standard solution*.~~
~~**Suitability requirements:** The retention times for each component determined on replicate injections agree within ±2 s, and dextrose and stachyose are baseline resolved from one another and from the 5800-MW pullulan standard.~~
~~[NOTE—Elevated valleys are usually observed between the peaks for the three pullulan standards.]~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Plot the average retention time, in s, of each component in the *Standard solution* versus its molecular weight, in g/mol. Draw the best cubic line fitting the five points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of NLT 0.99. From the molecular weight distribution graph, generate an MW distribution profile of Polydextrose.~~
■**System suitability**
Sample: *Standard solution*
Chromatograph five replicate injections of the *Standard solution*, allowing 15 min between injections, and record the retention times of the components of the *Standard solution*.
Insert the average retention time along with the molecular weight of each component in the *Standard solution* into the calibration table of the molecular weight distribution software. Check the regression results for a cubic fit of the calibration points, and obtain a correlation coefficient, R, for the line.
Suitability requirements
Retention time: The retention times for each component determined on replicate injections agree within ±2 s.
Resolution: Dextrose and stachyose are baseline resolved from one another and from the 5800-MW pullulan standard.
[NOTE—Prominent negative baseline valleys are usually observed between the peaks for the 5800-; 23,700-; and 100,000-MW pullulan standards.]
Correlation coefficient R: NLT 0.9999
Analysis
Samples: *Standard solution* and *Sample solution*
Use the molecular weight distribution software of the data reduction system to generate a molecular weight distribution plot of Polydextrose.■2S (*NF28*)
Acceptance criteria: No measurable peak above a molecular weight of 22,000 is found.

- **pH** ⟨**791**⟩**:** 2.5–5.0, in a solution (1 in 10)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 4.0%, anhydrous pyridine being used in place of methanol in the titration vessel

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cool, dry place.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP 1,6-Anhydro-D-glucose RS
USP Dextrose RS
■USP Polydextrose RS■2S (*NF28*)
USP Sorbitol RS

In-Process Revision

BRIEFING

Hydrogenated Polydextrose. Because there is no existing *NF* monograph for this excipient, a new monograph is being proposed, based on validated methods of analysis. The HPLC procedure in the *Assay* and *Limit of Monomers* is based on analyses performed using the Bio-Rad Aminex Ion Exclusion HPX-87H brand of L17 column. Hydrogenated Polydextrose elutes at approximately 5.8 min on this system.

(EM2: H. Wang.) RTS—C68840

Add the following:

▪Hydrogenated Polydextrose

DEFINITION

Hydrogenated Polydextrose is obtained by transition metal catalytic hydrogenation of Polydextrose in aqueous solution. It contains NLT 90.0% of dextrose polymer units, calculated on the anhydrous and ash-free basis. The polymer chain end groups are mainly sorbitol-terminated.

IDENTIFICATION

- **A.** To 1 drop of a solution (1 in 10), add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS: a deep yellow to orange color is produced.
- **B.** With vigorous swirling, add 1 mL of acetone to 1 mL of a solution (1 in 10): the solution remains clear.
- **C.** With vigorous swirling, add 2 mL of acetone to the solution obtained in *Identification* test *B*: a heavy, milky turbidity develops immediately.
- **D.** To 1 mL of a solution (1 in 50), add 4 mL of alkaline cupric citrate TS. Boil vigorously for 2–4 min. Remove from heat, and allow the precipitate (if any) to settle: the supernatant is blue or blue-green.
- **E.** Meets the requirements for dextrose under *Limit of Monomers*

ASSAY

- **PROCEDURE**

Mobile phase: 0.001 N sulfuric acid. Pass through a filter having a 0.5-µm or finer porosity, and degas.

Standard solution: 4.0 mg/mL of USP Polydextrose RS, calculated on the anhydrous and ash-free basis, in *Mobile phase*

Sample solution: 4.0 mg/mL of Hydrogenated Polydextrose, calculated on the anhydrous and ash-free basis, in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: Refractive index

Detector temperature: 35 ± 0.1°

Guard column: 4.6-mm × 3.0-cm guard column; packing L17

Analytical column: 7.8-mm × 30-cm analytical column; packing L17

Flow rate: 0.6 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dextrose polymer units in the Hydrogenated Polydextrose taken:

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

r_U = peak response for dextrose polymer units from the *Sample solution*

r_S = peak response for dextrose polymer units from the *Standard solution*

C_S = concentration of USP Polydextrose RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydrogenated Polydextrose in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 90.0% on the anhydrous and ash-free basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.3%
- **LIMIT OF LEAD**

[NOTE—Use reagent-grade chemicals with a lead content of as low as possible, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with Purified Water.]

Matrix modifier solution: 10.0 mg/mL of dibasic ammonium phosphate

Lead nitrate stock solution: Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard lead solution: On the day of use, dilute 10.0 mL of *Lead nitrate stock solution* with water to 100.0 mL. Each mL of *Standard lead solution* contains the equivalent of 10 µg of lead.

Standard solution A: 0.02 µg/mL of lead, from *Standard lead solution* in water

Standard solution B: 0.05 µg/mL of lead, from *Standard lead solution* in water

Standard solution C: 0.1 µg/mL of lead, from *Standard lead solution* in water

Standard solution D: 0.2 µg/mL of lead, from *Standard lead solution* in water

Standard solution E: 0.5 µg/mL of lead, from *Standard lead solution* in water

Sample solution: Transfer 1.0 g of Hydrogenated Polydextrose, weighed and calculated on the anhydrous and ash-free basis, into a 10-mL volumetric flask, dissolve in and dilute with water to volume.

Spiked sample solution: Transfer 1.0 g of Hydrogenated Polydextrose, weighed and calculated on the anhydrous and ash-free basis, into a 10-mL volumetric flask, and dissolve in water. Add 100 µL of the *Standard lead solution*, and dilute with water to volume. This solution contains 0.1 µg/mL of added lead.

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: Graphite furnace atomic absorption spectrophotometer, equipped with a pyrolytic tube with a platform

Lamp: A lead hollow-cathode lamp, using a slit width of 0.7 mm (set low) and a deuterium arc lamp for background correction

Analytical wavelength: Lead emission line of 283.3 nm

Injection size: See *Samples* in the *Analysis* below.

Temperature: The drying temperature of the furnace is maintained at 130° for 40 s after a 20-s ramp time using an argon flow rate of 300 mL/min; the ashing temperature

In-Process Revision

is maintained at 800° for 40 s after a 20-s ramp time using an argon flow rate of 300 mL/min; and the atomization temperature is maintained at 2400° for 6 s using an argon flow rate of 50 mL/min. Clean the graphite furnace at 2600° for 5 s after a 1-s ramp time using an argon flow rate of 300 mL/min, and recharge the graphite furnace at 20° for 20 s after a 2-s ramp time using an argon flow rate of 300 mL/min.

Analysis

Samples: 10-µL aliquots of the five *Standard solutions*; a mixture of 10 µL of the *Matrix modifier solution* and 10 µL of the *Sample solution*; and a mixture of 10 µL of the *Matrix modifier solution* and 10 µL of the *Spiked sample solution*

Concomitantly determine the absorbances of the *Samples* using the *Spectrometric conditions* described above. Plot the absorbance of each *Standard solution*, compensated for background correction, versus its content of lead, in µg/mL, and draw the best straight line fitting the five points. From this plot, determine the concentrations, C_T and C_{ST}, in µg/mL, of lead in the *Sample solution* and the *Spiked sample solution*, respectively.

Calculate the percentage recovery taken:

$$\text{Result} = [(C_{ST} - C_T)/A] \times 100$$

A = quantity of lead added to the *Spiked sample solution*, 0.1 µg/mL

Calculate the content, in µg/g, of lead in Hydrogenated Polydextrose taken:

$$\text{Result} = (C_T/W) \times V$$

W = weight of Hydrogenated Polydextrose taken to prepare the *Sample solution* (g)

V = volume of the *Sample solution*, 10 mL

Acceptance criteria: NMT 0.5 µg/g; recovery is 80%-120%

- **LIMIT OF NICKEL**

[NOTE—All glassware used must be soaked in *1% Nitric acid* for at least 2 h and then rinsed with water.]

1% Nitric acid: Cautiously add 10 mL of nitric acid to a 1000-mL volumetric flask containing about 500 mL of water. Mix, and dilute with water to volume.

Blank solution: Use *1% Nitric acid*.

Nickel stock standard solution: Immediately before use, dilute an appropriate amount of nickel standard* with *1% Nitric acid* to prepare a solution containing the equivalent of 10 µg/mL of nickel.

Standard solutions: Into four identical 100-mL volumetric flasks, introduce respectively 1.0, 2.0, 5.0, and 10.0 mL of *Nickel stock standard solution*. Dilute with *1% Nitric acid* to volume, and mix. These standards contain 0.1, 0.2, 0.5, and 1.0 µg/mL of nickel.

Sample solution: Weigh 5 g of Hydrogenated Polydextrose into a 100-mL volumetric flask. Dissolve in and dilute with *1% Nitric acid* to volume, and mix.

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: Atomic absorption spectrophotometer equipped with an air–acetylene flame

Lamp: Nickel hollow-cathode

Analytical wavelength: 352.0 nm

System suitability

Sample: *Standard solution* of 0.2 µg/mL of nickel

Suitability requirements

Relative standard deviation: NMT 20%

Analysis

Samples: *Standard solutions* and *Sample solution*

Use the *Blank solution* to zero the instrument. Concomitantly determine the absorbances of the *Samples* at least three times each. Record the average of the steady readings for each of the *Samples*. Clear the nebulizer using the *Blank solution*, and aspirate each of the *Samples* in turn. The standard chosen for reslope should be run every four to five samples. If there is a significant change in its response, reslope and repeat the previous samples.

Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of nickel, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of nickel in the *Sample solution*. Calculate the quantity, in µg, of nickel in each g of Hydrogenated Polydextrose taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution*, 100 mL

W = weight of Hydrogenated Polydextrose taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 2 µg/g

Organic Impurities

- **PROCEDURE 1: LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED COMPOUNDS**

Sample solution: 1.0 g of Hydrogenated Polydextrose, weighed and calculated on the anhydrous and ash-free basis, diluted with water to 100 mL

Analysis: Determine the absorbance of the *Sample solution* in a 1-cm quartz cell at 283 nm, with a suitable spectrophotometer, using water as the blank.

Calculate the percentage of 5-hydroxymethylfurfural and related compounds in the Hydrogenated Polydextrose taken:

$$\text{Result} = (V \times M_r \times A)/(\varepsilon_{283} \times L \times W) \times 100$$

V = volume of the *Sample solution*, 0.1 L

M_r = molecular weight of 5-hydroxymethylfurfural, 126 g/mol

A = absorbance of the *Sample solution*

ε_{283} = molar extinction coefficient of 5-hydroxymethylfurfural at a wavelength of 283 nm, 16830 $L \cdot mol^{-1} \cdot cm^{-1}$

L = path length of the spectrophotometer cell (cm)

W = weight of Hydrogenated Polydextrose taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1%

- **PROCEDURE 2: LIMIT OF MONOMERS**

Mobile phase, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.

Standard solution: 0.08 mg/mL of each for USP 1,6-Anhydro-D-glucose RS and USP Sorbitol RS, and 0.04 mg/mL for USP Dextrose RS, in *Mobile phase*

System suitability

Sample: *Standard solution*

[NOTE—See the relative retention times table below.]

Name	Relative Retention Time
Dextrose (glucose)	0.7
Sorbitol	0.8
An isomer of 1,6-anhydro-D-glucose (D-anhydroglucose furanose form)	0.9
1,6-Anhydro-D-glucose (levoglucosan) (D-anhydroglucose pyranose form)	1.0

Suitability requirements

Resolution: NLT 1.0

Relative standard derivation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Use the peak response of USP 1,6-Anhydro-D-glucose RS in the *Standard solution* for calculation of the percentage of the isomer of 1,6-anhydro-D-glucose in the *Sample solution*. Calculate the percentage of each monomer in the portion of Hydrogenated Polydextrose taken:

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

*Suitable nickel standards are available from e.g., Fisher Scientific, Fair Lawn, NJ (nickel, reference standard solution, 1000 ppm ±1%, certified, application for atomic absorption) or RICCA Chemical Company, Arlington, TX (nickel standard, 1000 ppm Ni, for atomic absorption).

r_U = peak response for the respective monomer from the *Sample solution*
r_S = peak response for the respective monomer from the *Standard solution*
C_S = concentration of the respective standard monomer in the *Standard solution* (mg/mL)
C_U = concentration of Hydrogenated Polydextrose in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4.0% of 1,6-anhydro-D-glucose, NMT 5.75% for sorbitol and NMT 0.25% for dextrose

[NOTE—In the case of 1,6-anhydro-D-glucose, the peak areas for the pyranose and furanose forms are combined.]

SPECIFIC TESTS

- **MOLECULAR WEIGHT LIMIT**

Mobile phase: Dissolve 35.0 g of sodium nitrate and 1.0 g of sodium azide in 100 mL of water. Dilute with water to 4 L. Pass through a filter having a 0.45-µm or finer porosity, and degas by applying an aspirator vacuum for 30 min. The resulting *Mobile phase* is 0.1 N sodium nitrate containing 0.025% sodium azide.

Standard solution: Transfer 20 mg each of USP Dextrose RS, stachyose, and 5800-, 23,700-, and 100,000-molecular weight (MW) pullulan standards into a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Pass through a syringe filter having a 0.45-µm or finer porosity into a suitable autosampler vial, and seal.

Sample solution: Transfer 50 mg of Hydrogenated Polydextrose into a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Pass through a syringe filter having a 0.45-µm or finer porosity into a suitable autosampler vial, and seal.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index set at a sensitivity of 4×10^{-6} refractive index units full scale and maintained at a temperature of $35 \pm 0.1°$

Column: 7.8-mm × 30-cm; packing L39

Column temperature: 45°

Flow rate: 0.8 mL/min

[NOTE—After installation of a new column, pump *Mobile phase* through the column overnight at a rate of 0.3 mL/min. Before calibration or analysis, increase the flow slowly over a 1-min period to 0.8 mL/min. Continue to pump *Mobile phase* through the column at this flow rate for at least 1 h before the first injection. Check the flow gravimetrically, and adjust it if necessary. Reduce the flow rate to about 0.1 mL/min when the system is not in use.]

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Chromatograph five replicate injections of the *Standard solution*, allowing 15 min between injections, and record the retention times of the components of the *Standard solution.* Insert the average retention time along with the molecular weight of each component in the *Standard solution* into the calibration table of the molecular weight distribution software. Check the regression results for a cubic fit of the calibration points, and obtain a correlation coefficient, R, for the line.

Suitability requirements

Retention time: The retention times for each component determined on replicate injections agree within ±2 s.

Resolution: Dextrose and stachyose are baseline resolved from one another and from the 5800-MW pullulan standard.

[NOTE—Prominent negative baseline valleys are usually observed between the peaks for the 5800-, 23,700-, and 100,000-MW pullulan standards.]

Correlation coefficient R: NLT 0.9999

Analysis

Samples: *Standard solution* and *Sample solution*

Use the molecular weight distribution software of the data reduction system to generate a molecular weight distribution plot of Hydrogenated Polydextrose.

Acceptance criteria: No measurable peak above a molecular weight of 22,000 is found.

- **pH** ⟨**791**⟩**:** 5.0–7.0, in a solution (1 in 10)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 4.0%. Use anhydrous pyridine in the titration vessel.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cool and dry place.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

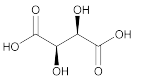
USP 1,6-Anhydro-D-glucose RS
USP Dextrose RS
USP Polydextrose RS
USP Sorbitol RS■2S *(NF28)*

BRIEFING

Tartaric Acid, *NF 27* page 1368. As part of USP modernization efforts, it is proposed to replace the existing nonspecific *Identification* test *B* with a more specific infrared absorption test employing a USP Reference Standard for comparison. Interested parties are encouraged to comment.

(EM1: R. Lafaver.) RTS—C74455

Tartaric Acid



$C_4H_6O_6$ 150.09

Butanedioic acid, 2,3-dihydroxy-;
Butanedioic acid, 2,3-dihydroxy-, [*R*-(*R**,*R**)]-;
Tartaric acid; L-(+)-Tartaric acid [526-83-0]; [87-69-4].

DEFINITION

Tartaric Acid, dried over phosphorus pentoxide for 3 h, contains NLT 99.7% and NMT 100.5% of $C_4H_6O_6$.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨**191**⟩**:** It meets the requirements.

Change to read:

- **B. ■INFRARED ABSORPTION** ⟨**197K**⟩■2S *(NF28)*~~When ignited, it gradually decomposes, emitting an odor resembling that of burning sugar (distinction from citric acid).~~■2S *(NF28)*

ASSAY

- **PROCEDURE**

Sample: 2 g of Tartaric Acid, previously dried, in a conical flask

In-Process Revision

Analysis: Dissolve *Sample* in 40 mL of water, add phenolphthalein TS, and titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 75.04 mg of $C_4H_6O_6$.
Acceptance criteria: 99.7%–100.5%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩
  Sample solution: 10 mL of a 1-in-100 solution of Tartaric Acid
  Analysis: Add to *Sample solution* 3 drops of hydrochloric acid and 1 mL of barium chloride TS
  Acceptance criteria: No turbidity is produced.
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +12.0° to +13.0°
  Sample solution: 200 mg/mL in water
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry over phosphorus pentoxide for 3 h: it loses NMT 0.5% of its weight.
- **LIMIT OF OXALATE**
  Sample solution: 10 mL of a 1-in-10 solution of Tartaric Acid
  Analysis: Nearly neutralize the *Sample solution* with 6 N ammonium hydroxide, and add 10 mL of calcium sulfate TS.
  Acceptance criteria: No turbidity is produced.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Add the following:

■• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Tartaric Acid RS■2S *(NF28)*

In-Process Revision

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

⟨**1**⟩ **Injections,** *USP 32* page 31 and page 601 of *PF* 35(3) [May–June 2009]. As currently written, the *Volume in Container* section addresses the determination of volume in container only for liquid parenteral products. Therefore, the Parenteral Products—Industrial Expert Committee is proposing to add text that provides guidance for determining the volume in container for sterile solid formulations, currently absent from the compendia. In addition, it is proposed to change the section title *Volume in Container* to *Container Content*.

(PPI: D. Hunt) RTS—C74658

Change to read:

INGREDIENTS

Vehicles and Added Substances

Aqueous Vehicles—The vehicles for aqueous Injections meet the requirements of the *Pyrogen Test* ⟨151⟩ or the *Bacterial Endotoxins Test* ⟨85⟩, whichever is specified. *Water for Injection* generally is used as the vehicle, unless otherwise specified in the individual monograph. Sodium chloride may be added in amounts sufficient to render the resulting solution isotonic; and *Sodium Chloride Injection,* or *Ringer's Injection,* may be used in whole or in part instead of *Water for Injection,* unless otherwise specified in the individual monograph. For conditions applying to other adjuvants, see *Added Substances* in this chapter.

Other Vehicles—Fixed oils used as vehicles for nonaqueous Injections are of vegetable origin, are odorless or nearly so, and have no odor suggesting rancidity. They meet the requirements of the test for *Solid paraffin* under *Mineral Oil,* the cooling bath being maintained at 10°, have a *Saponification Value* between 185 and 200 (see *Fats and Fixed Oils* ⟨401⟩), have an *Iodine Value* between 79 and 141 (see *Fats and Fixed Oils* ⟨401⟩), and meet the requirements of the following tests.

~~*Unsaponifiable Matter*—Reflux on a steam bath 10 mL of the oil with 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of alcohol, with occasional shaking until the mixture becomes clear. Transfer the solution to a shallow dish, evaporate the alcohol on a steam bath, and mix the residue with 100 mL of water: a clear solution results.~~

~~*Free Fatty Acids*—The free fatty acids in 10 g of oil require for neutralization not more than 2.0 mL of 0.020 N sodium hydroxide (see *Fats and Fixed Oils* ⟨401⟩).~~

■*Unsaponifiable Matter* (see *Fats and Fixed Oils* ⟨401⟩): not more than 1.5%.

Acid Value (see *Fats and Fixed Oils* ⟨401⟩): not more than 0.2.

Peroxide Value (see *Fats and Fixed Oils* ⟨401⟩): not more than 5.0.

Water, Method I ⟨921⟩: not more than 0.1%, using 50 mL of chloroform instead of 35 to 40 mL of methanol as the solvent.

Limit of Copper, Iron, Lead, and Nickel—Proceed as directed in the section *Trace Metals* under *Fats and Fixed Oils* ⟨401⟩. Not more than 1 ppm of copper is found; not more than 1 ppm of iron is found; not more than 1 ppm of lead is found; and not more than 1 ppm of nickel is found.■2S *(USP32)*

Synthetic mono- or diglycerides of fatty acids may be used as vehicles, provided they are liquid, remain clear when cooled to 10°, and have an *Iodine Value* of not more than 140 (see *Fats and Fixed Oils* ⟨401⟩).

These and other nonaqueous vehicles may be used, provided they are safe in the volume of Injection administered, and also provided they do not interfere with the therapeutic efficacy of the preparation or with its response to prescribed assays and tests.

Added Substances—Suitable substances may be added to preparations intended for injection to increase stability or usefulness, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No coloring agent may be added, solely for the purpose of coloring the finished preparation, to a solution intended for parenteral administration (see also *Added Substances* under *General Notices* and *Antimicrobial Effectiveness Testing* ⟨51⟩).

Observe special care in the choice and use of added substances in preparations for injection that are administered in a volume exceeding 5 mL. The following maximum limits prevail unless otherwise directed: for agents containing mercury and the cationic, surface-active compounds, 0.01%; for chlorobutanol, cresol, phenol, and similar types of substances, 0.5%; and for sulfur dioxide, or an equivalent amount of the sulfite, bisulfite, or metabisulfite of potassium or sodium, 0.2%.

A suitable substance or mixture of substances to prevent the growth of microorganisms must be added to preparations intended for injection that are packaged in multiple-dose containers, regardless of the method of sterilization employed, unless one of the following conditions prevails: (1) there are different directions in the individual monograph; (2) the substance contains a radionuclide with a physical half-life of less than 24 hours; and (3) the active ingredients are themselves antimicrobial. Such substances are used in concentrations that will prevent the growth of or kill micro-organisms in the preparations for injection. Such substances also meet the requirements of *Antimicrobial Effectiveness Testing* ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩. Sterilization processes are employed even though such substances are used (see also *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩). The air

In-Process Revision

in the container may be evacuated or be displaced by a chemically inert gas. Where specified in a monograph, information regarding sensitivity of the article to oxygen is to be provided in the labeling.

Change to read:

PACKAGING

Containers for Injections

Containers, including the closures, for preparations for injections do not interact physically or chemically with the preparations in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use. The container is made of material that permits inspection of the contents. The type of glass preferable for each parenteral preparation is usually stated in the individual monograph. Unless otherwise specified in the individual monograph, plastic containers may be used for packaging injections (see *Containers—Plastics* ⟨661⟩).

For definitions of single-dose and multiple-dose containers, see *Containers* in the *General Notices and Requirements.* Containers meet the requirements under *Containers—Glass* ⟨660⟩ and *Containers—Plastics* ⟨661⟩.

Containers are closed or sealed in such a manner as to prevent contamination or loss of contents. Validation of container integrity must demonstrate no penetration of microbial contamination or chemical or physical impurities. In addition, the solutes and the vehicle must maintain their specified total and relative quantities or concentrations when exposed to anticipated extreme conditions of manufacturing and processing, and storage, shipment, and distribution. Closures for multiple-dose containers permit the withdrawal of the contents without removal or destruction of the closure. The closure permits penetration by a needle and, upon withdrawal of the needle, closes at once, protecting the container against contamination. Validation of the multiple-dose container integrity must include verification that such a package prevents microbial contamination or loss of product contents under anticipated conditions of multiple entry and use.

Piggyback containers are usually intravenous infusion containers used to administer a second infusion through a connector of some type or an injection port on the administration set of the first fluid, thereby avoiding the need for another injection site on the patient's body. Piggyback containers are also known as secondary infusion containers.

Potassium Chloride for Injection Concentrate

The use of a black closure system on a vial (e.g., a black flip-off button and a black ferrule to hold the elastomeric closure) or the use of a black band or series of bands above the constriction on an ampul is prohibited, except for *Potassium Chloride for Injection Concentrate.*

Neuromuscular Blocking and Paralyzing Agents

All injectable preparations of neuromuscular blocking agents and paralyzing agents must be packaged in vials with a cautionary statement printed on the ferrules or cap overseals. Both the container cap ferrule and the cap overseal must bear in black or white print (whichever provides the greatest color contrast with the ferrule or cap color) the words: "Warning: Paralyzing Agent" or "Paralyzing Agent" (depending on the size of the closure system). Alternatively, the overseal may be transparent and without words, allowing for visualization of the warning labeling on the closure ferrule.

Containers for Sterile Solids

Containers, including the closures, for dry solids intended for parenteral use do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use.

A container for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained.

Where the *Assay* in a monograph provides a procedure for the *Assay preparation,* in which the total withdrawable contents are to be withdrawn from a single-dose container with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle not less than 2.5 cm (1 inch) in length, with care being taken to expel any air bubbles, and discharged into a container for dilution and assay.

~~Volume in Container~~

▪Container Content▪2S (*USP33*)

~~Each container of an injection is filled with sufficient excess of the labeled "size" or that volume which is to be withdrawn. See *Injections* under *Pharmaceutical Dosage Forms* ⟨1151⟩.~~

▪Each container of an injection contains sufficient excess to allow withdrawal of the labeled quantity of drug. Such withdrawal shall be performed according to labeled directions, if provided.▪2S (*USP33*)

DETERMINATION OF VOLUME OF INJECTION IN CONTAINERS

▪This section is harmonized with the corresponding texts of the European Pharmacopoeia and/or the Japanese Pharmacopoeia. These pharmacopeias have undertaken not to make any unilateral change to this harmonized section. A portion of the present text (see below) is national USP text, and therefore not part of the harmonized text; it is marked with symbols (◆ ◆) to specify this fact.▪2S (*USP33*)

Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to 20°–25°C before measuring the volume.

■◆Sterile solid formulations must be constituted according to labeled directions before removing the contents. Contents are then to be measured following the procedures for suspensions, emulsions, or solutions, as appropriate.◆■2S (*USP33*)

Single-Dose Containers—Select 1 container if the volume of the container is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured and fitted with a 21-gauge needle not less than 2.5 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively, the volume of the contents in mL may be calculated as the mass, in g, divided by the density. For containers with a nominal volume of 2 mL or less, the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement, provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by means of opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in the case of containers examined individually or, in the case of containers with a nominal volume of 2 mL or less, is not less than the sum of the nominal volumes of the containers taken collectively.

Multi-Dose Containers—For Injections in multiple-dose containers labeled to yield a specific number of doses of a stated volume, select 1 container, and proceed as directed for single-dose containers, using the same number of separate syringe assemblies as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

Injections in Cartridges or Prefilled Syringes—Select 1 container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in mL, calculated as the mass, in g, divided by the density.

The volume measured for each of the containers is not less than the nominal volume.

Large-Volume Intravenous Solutions—For intravenous solutions, select 1 container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

Labeling on Ferrules and Cap Overseals

Only cautionary statements are to appear on the top (circle) surface of the ferrule or cap overseal of a vial containing an injectable product. A cautionary statement is one intended to prevent an imminent life-threatening situation if the injectable drug is used inappropriately. Examples of such statements include but are not limited to the following: ''Warning'', ''Dilute Before Using'', ''Paralyzing Agent'', ''I.M. Use Only'', and ''Chemotherapy''.

The text must be in contrasting color and conspicuous under ordinary conditions of use. The cautionary statement may appear solely on the ferrule, provided the cap overseal is constructed so as to allow the cautionary statement beneath the cap to be readily legible.

Identifying numbers or letters, such as code numbers, lot numbers, etc., may appear on the side (skirt) surface of the ferrule on vials containing injectable products. The appearance of such identifying data on the skirt surface of the ferrule, placed where it does not detract from, or interfere with, the cautionary statement on the top surface, should be considered to be a beneficial attribute of the in-process quality control of a product throughout the manufacturing process. Any anticounterfeiting scheme must not detract from or interfere with the cautionary statements.

Under no circumstances would advertising such as company names, logos, or product names be permitted to appear on the top (circle) surface of any ferrule or cap overseal.

(Official February 1, 2009)

Packaging and Storage

The volume of injection in single-dose containers provides the amount specified for parenteral administration at one time and in no case is more than sufficient to permit the withdrawal and administration of 1 L.

Preparations intended for intraspinal, intracisternal, or peridural administration are packaged only in single-dose containers.

Unless otherwise specified in the individual monograph, a multiple-dose container contains a volume of Injection sufficient to permit the withdrawal of not more than 30 mL.

The following injections are exempt from the 1-L restriction of the foregoing requirements relating to packaging:

1. Injections packaged for extravascular use as irrigation solutions or peritoneal dialysis solutions
2. Injections packaged for intravascular use as parenteral nutrition or as replacement or substitution fluid to be administered continuously during hemofiltration

Injections packaged for intravascular use that may be used for intermittent, continuous, or bolus replacement fluid administration during hemodialysis or other procedures, unless excepted above, must conform to the 1-L restriction.

Injections labeled for veterinary use are exempt from packaging and storage requirements concerning the limitation to single-dose containers and the limitation on the volume of multiple-dose containers.

Change to read:

FOREIGN AND PARTICULATE MATTER

All articles intended for parenteral administration shall be prepared in a manner designed to exclude particulate matter as defined in *Particulate Matter in Injections* ⟨788⟩ and other foreign matter. Each final container of all parenteral preparations shall be inspected to the extent possible for the presence of observable foreign and particulate matter (hereafter termed ''visible particulates'') in its contents. The inspection process shall be designed and qualified to ensure that every lot of all parenteral preparations is essentially free from visible particulates. Qualification of the inspection process shall be performed with reference to particulates in the visible range of a type that might emanate from the manufacturing or filling process. Every container whose contents shows evidence of visible particulates shall be rejected. The inspection for visible particulates may take place when inspecting for other critical defects, such as cracked or defective containers or seals, or when characterizing the appearance of a lyophilized product.

Where the nature of the contents or the container-closure system permits only limited capability for the inspection of the total contents, the 100% inspection of a lot shall be supple-

mented with the inspection of constituted (e.g., dried) or withdrawn (e.g., dark amber container) contents of a sample of containers from the lot.

All large-volume Injections for single-dose infusion and small-volume Injections are subject to the light obscuration or microscopic procedures and limits for subvisible particulate matter set forth in *Particulate Matter in Injections* ⟨788⟩, unless otherwise specified in the individual monograph. An article packaged as both a large-volume and a small-volume Injection meets the requirements set forth for small-volume Injections where the container is labeled as containing 100 mL or less, if the individual monograph states a test for *Particulate Matter in Injections* ⟨788⟩; it meets the requirements set forth for large-volume Injections for single-dose infusion where the container is labeled as containing more than 100 mL. ~~Injections administered exclusively by the intramuscular or subcutaneous route or packaged and labeled for use as irrigating solutions are exempt from requirements for *Particulate Matter in Injections* ⟨788⟩.~~

■Solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenteral products for which the labeling specifies the use of a final filter prior to administration are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.■1S (*USP33*)

BRIEFING

⟨**11**⟩ **USP Reference Standards,** *USP 32* page 35, page 3924 of the *First Supplement,* and page 507 of *PF* 31(2) [Mar.–Apr. 2005], page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 1161 of *PF* 32(4) [July–Aug. 2006], page 95 of *PF* 33(1) [Jan.–Feb. 2007], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 332 of *PF* 34(2) [Mar.–Apr. 2008], page 680 of *PF* 34(3) [May–June 2008], page 1021 of *PF* 34(4) [July–Aug. 2008], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], page 144 of *PF* 35(1) [Jan.–Feb. 2009], page 330 of *PF* 35(2) [Mar–Apr. 2009], page 612 of *PF* 35(3) [May–June 2009], and page 913 of *PF* 35(4) [July–Aug. 2009].

(HDQ) RTS—C34840; C40936; C42827; C43135; C44029; C52657; C54723; C58037; C61270; C62478; C62987; C63092; C64233; C65585; C66623; C67219; C67224; C69714; C72116; C72332; C73270; C74455

Add the following:

■**USP Andrographolide RS.**■2S (*USP33*)

Add the following:

■**USP Powdered Andrographis Extract RS.**■2S (*USP33*)

Add the following:

■**USP 3-Acetyl-11-keto-β-Boswellic Acid RS.**■2S (*USP33*)

Add the following:

■**USP Boswellia Serrata Extract RS.**■2S (*USP33*)

Add the following:

■**USP Fetal Bovine Serum RS.**■2S (*USP33*)

Add the following:

■**USP Clenbuterol Hydrochloride RS.**■2S (*USP33*)

Add the following:

■**USP Clenbuterol Related Compound B RS [**1-(4-amino-3,5-dichlorophenyl)-2-*tert*-butyl-aminoethanone hydrochloride**]**($C_{12}H_{16}Cl_2N_2O \cdot HCl$ ✧ 311.64).■2S (*USP33*)

Add the following:

■**USP Cyclophosphamide Related Compound A RS [**bis(2-chloroethyl)amine hydrochloride**]** ($C_4H_9Cl_2N \cdot HCl$ ✧ 178.49).■2S (*USP33*)

Add the following:

■**USP Cyclophosphamide Related Compound B RS [**3-(2-chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane**]** ($C_7H_{16}ClN_2O_3P$ ✧ 242.64).■2S (*USP33*)

Add the following:

■**USP Cyclophosphamide Related Compound C RS [**3-aminopropyl dihydrogen phosphate**]** ($C_3H_{10}NO_4P$ ✧ 155.09).■2S (*USP33*)

Add the following:

■**USP Cyclophosphamide Related Compound D RS [**3-[2-(2-chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride**]** ($C_7H_{18}ClN_2O_4P \cdot 2HCl$ ✧ 333.58).■2S (*USP33*)

Add the following:

▪USP Diethyl Sebacate RS.▪2S (*USP33*)

Add the following:

▪USP Docetaxel RS.▪2S (*USP33*)

Add the following:

▪USP Docetaxel Identification RS—Contains docetaxel and small amount of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel.▪2S (*USP33*)

Add the following:

▪USP Dofetilide RS.▪2S (*USP33*)

Add the following:

▪USP Dofetilide Related Compound A RS [*N*-[4-(2-{2-[4-(methanesulfonamido)phenoxy]ethylamino}ethyl) phenyl]methanesulfonamide**]** (427.54).▪2S (*USP33*)

Add the following:

▪USP rGlucagon RS ($C_{153}H_{225}N_{43}O_{49}S$).▪2S (*USP33*)

Add the following:

▪USP L-Glutamic Acid, Hydrochloride RS.▪2S (*USP33*)

Add the following:

▪USP rHuman Interleukin 4 RS.▪2S (*USP33*)

Add the following:

▪USP Levetiracteam RS.▪2S (*USP33*)

Add the following:

▪USP Levetiracetam Related Compound A RS [(*S*)-*N*-(1-amino-1-oxobutan-2-yl)-4-chlorobutanamide**]** ($C_8H_{14}ClNO_3$ ✧ 207.65).▪2S (*USP33*)

Add the following:

▪USP Levetiracteam Related Compound B RS [(*S*)-2-aminobutanamide**]** ($C_4H_{10}N_2O$ ✧ 102.13).▪2S (*USP33*)

Add the following:

▪USP Levetiracetam Racemic Mixture RS—A 1 : 1 mixture of levetiracetam *S*-enatiomer-(2*S*)-2-(2-oxopyrrolidin-1-yl)butanamide and levetiracetam *R*-enantiomer (2*R*)-2-(2-oxopyrrolidin-1-yl)butanamide.▪2S (*USP33*)

Add the following:

▪USP Human Acellular Dermal Matrix Reference Photomicrographs.▪2S (*USP33*)

Add the following:

▪USP Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer (1 : 1) RS.▪2S (*USP33*)

Add the following:

▪USP Methylpyrrolidone RS.▪2S (*USP33*)

Add the following:

▪USP Morphine Related Compound B RS [2,2′-bimorphine**]** ($C_{34}H_{36}N_2O_6$ ✧ 568.66).▪2S (*USP33*)

Add the following:

▪USP Orlistat RS.▪2S (*USP33*)

Add the following:

▪USP Orlistat Related Compound A RS [(3*S*, 4*S*)-3-hexyl-4-[(2*R*)-2-hydroxytridecyl]-2-oxetanone**]** ($C_{22}H_{42}O_3$✧ 354.57).▪2S (*USP33*)

Add the following:

▪USP Orlistat Related Compound B RS [diisopropyl hydrazine-1,2 dicarboxylate**]** ($C_8H_{16}N_2O_4$ ✧ 204.22).▪2S (*USP33*)

Add the following:

▪USP Orlistat Related Compound C RS [triphenylphosphine oxide**]** ($C_{18}H_{15}OP$ ✧ 278.28).▪2S (*USP33*)

Add the following:

▪USP Orlistat Related Compound D RS [*N*-formyl-L-leucine (3*S*,4*R*,6*S*)-3-hexyl-2-oxo-6-undecyltetrahydro-2*H*-pyran-4-yl ester**]** ($C_{29}H_{53}NO_5$) ✧495.73).▪2S (*USP33*)

In-Process Revision

Add the following:

▪USP Orlistat Related Compound E RS [*N*-formyl-*S*-isoleucin (*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]-methyl]dodecyl ester**]** ($C_{29}H_{53}NO_5$ ✧ 495.73).▪2S (*USP33*)

Add the following:

▪USP Polydextrose RS.▪2S (*USP33*)

Add the following:

▪USP Propanolamine RS.▪2S (*USP33*)

Add the following:

▪USP Ractopamine Hydrochloride RS.▪2S (*USP33*)

Add the following:

▪USP Raspberry Ketone RS [4-(4-hydroxyphenyl) butan-2-one**]** ($C_{10}H_{12}O_2$ ✧ 164.20).▪2S (*USP33*)

Add the following:

▪USP Raspberry Alcohol RS [4-(3-hydroxybutyl) phenol**]** ($C_{10}H_{14}O_2$ ✧ 166.22).▪2S (*USP33*)

Add the following:

▪USP Riluzole RS.▪2S (*USP33*)

Add the following:

▪USP Riluzole Related Compound A RS [4-trifluoromethoxyphenylamine; 4-trifluoromethoxyanaline**]** ($C_7H_6F_3NO$ ✧ 177.12).▪2S (*USP33*)

Add the following:

▪USP Tartaric Acid RS.▪2S (*USP33*)

Add the following:

▪USP Tramadol Related Compound B RS[2-(dimethylaminomethyl)-1-cyclohexanone hydrochloride**].**▪2S (*USP33*)

Biological Tests and Assays

BRIEFING

⟨90⟩ Fetal Bovine Serum—Quality Attributes and Functionality Tests. Fetal Bovine Serum (FBS) and other types of bovine sera described in general information chapter *Bovine Serum* ⟨1024⟩ provide important guidelines for the biotechnology industry. This general information chapter provides analysts with (a) procedures to measure the FBS quality attributes and (b) functionality assays to assess performance of different types and lots of FBS.

(BB CGT: F. Atouf) RTS—C63092

Add the following:

▪⟨90⟩ FETAL BOVINE SERUM—QUALITY ATTRIBUTES AND FUNCTIONALITY TESTS

PROCESSING

Fetal bovine serum (FBS) is the light-brown liquid fraction of clotted fetal bovine blood. It is depleted of cells, fibrin, and clotting factors. Although the complete composition of FBS is undefined, FBS contains high levels of growth factors and low levels of immunoglobulins. In addition, it contains other key ingredients that are essential in supporting proliferation of cells in culture. This product is used both in life science basic research and industrial manufacturing. FBS is a by-product of the meat industry and is collected from bovine fetuses removed from cattle found to be pregnant at slaughter. FBS is harvested from abattoirs that are inspected by the competent authority in the country of origin. Dedicated trained personnel following written and approved procedures should perform collection and processing. The processing is performed aseptically to minimize risk of acquiring contaminating agents. The raw serum should be processed quickly to prevent hemolysis. The serum usual-

ly is allowed to clot and then is centrifuged in a refrigerated centrifuge; centrifugation should preferably be done at 4°. Serum typically is then removed from the clot, transferred to labeled containers, and frozen. All manufacturers employ sterile filtration before final packaging. Additionally, gamma irradiation provides the highest assurance of the absence of viral activity. Gamma irradiation doses of 25–40 kGy provide significant log reduction of viral and other adventitious agents while preserving cellular growth performance.

The screening of FBS for viral contamination is accomplished by using all applicable testing described in the Code of Federal Regulations 9 CFR 113.53 (known as full 9 CFR testing). Mycoplasma assays are performed by direct cultivation methods that employ aerobic and anaerobic incubations of solid medium in plates and semisolid broth in tubes. In addition, the absence of detectable mycoplasma is determined microscopically using the indirect method of bisbenzimide DNA staining. Large-volume sampling is important to detect low levels of mycoplasma.

FETAL BOVINE SERUM QUALITY ATTRIBUTES

Packaging and Storage—Store in sealed containers at a temperature of –10° or below.

Labeling—Label it to indicate that contents are Fetal Bovine Serum, and indicate lot number, expiration date, and storage conditions.

USP Reference Standards ⟨11⟩—*USP Endotoxin RS*. *USP Fetal Bovine Serum RS*.

pH ⟨791⟩—Between 7.00 and 8.00 in undiluted serum samples.

Osmolality ⟨758⟩—Between 280 and 360 mOsmol/kg.

Bacterial Endotoxins ⟨85⟩—It contains not more than 10 USP Endotoxin Units/mL of serum.

Total Protein Content ⟨1057⟩—Between 30 and 45 mg/mL.

Sterility ⟨71⟩—Meets requirements.

Identification—Radial Immunodiffusion

Reagents—

- FBS test samples
- Horse serum, negative control samples
- Bovine IgG calibrator (500 mg/L)
- Sheep Albumin Diluent (1% Sheep Albumin, 0.18% EDTA, 1.75% NaCl, and 1.21% Tris/HCl pH 7.4).

Materials/Apparatus—Ring measuring device is calibrated in 0.1-mm increments. Radial immunodiffusion (RID) plates are commercially available and contain anti-bovine IgG antiserum in a 1.5% agarose gel, 0.1 M phosphate buffer, pH 7.0, 0.1% sodium azide as bacteriostatic agent, and 1 μg/mL amphotericin B as an antifungal agent. Store at 2°–8°. Use RID plates that can measure bovine IgG in the range of 50–500 mg/L.

Standard Curve—Use the bovine IgG calibrators for system suitability and for generation of a calibration curve. Prepare two dilutions from a 500 mg/L bovine IgG stock solution. Dilute 120 μL of the 500 mg/L stock with 80 μL of diluent (medium dilution) and 25 μL of the 500 mg/L stock with 225 μL diluent (low dilution). Label each dilution respectively as 300 mg/L and 50 mg/L calibrators. Use the 500 mg/L, 300 mg/L, and 50 mg/L solutions to generate the standard curve, and load 5 μL of each sample into the 2.5-mm wells of the plate. At 72 h of incubation, measure ring diameters to the nearest 0.1 mm using the ring measuring device. Record the results and proceed to the generation of a standard curve.

The ring diameter should develop to completion at room temperature for 72 h. Using the result from each data point of the standard curve, generate a single linearity plot where y is the squared diameter (mm^2) of precipitin ring around the well and x is the Bovine IgG

concentration (mg/L). Calculate the linear least-squares-fit regression line of the form $y = m(x) + b$ with the help of suitable software and determine the values for slope (m), y-intercept (b), and coefficient of determination (R^2). The standard curve for the method is linear if R^2 is $\geq$0.98.

Procedure—Frozen undiluted samples of FBS are thawed and tested within 24 h if stored at 4°. Testing of FBS test and USP Fetal Bovine Serum RS samples is performed in triplicate. Prepare RID plates containing anti-bovine IgG to be tested for the various types of sera. Allow plates and reagents to equilibrate to room temperature before use by leaving the plates open for 10–15 min at room temperature to allow any condensation in the wells or on the gel surface to evaporate. Samples should not be applied to wells where moisture is visible. Prepare serial dilutions, if necessary, of FBS test and USP Fetal Bovine Serum RS samples in diluent. Dilute the negative control horse serum in diluent. Load 5 µL of each sample into the 2.5-mm wells of the plate, and incubate at room temperature for 72 h. [NOTE—The test samples and the negative control are loaded on the same plate.]

Calculation—After 72 h, measure the diameters of the rings using the ring measuring device, and record the results. Using the regression equation developed under standard curve deviation, calculate the concentration of bovine IgG in FBS samples. Concentration is expressed as mg/L.

Acceptance Criteria—Horse serum is negative (should not give a precipitation ring). FBS test and USP Fetal Bovine Serum RS samples are positive and should contain not more than 500 mg/L of IgG.

Hemoglobin Content—See *Spectrophotometry and Light Scattering* ⟨851⟩.

Sample Preparation—FBS samples are thawed, are stored at 4°, and are tested within the same day.

Procedure—Determine the absorbance of the serum sample using a spectrophotometric cell of 1-cm path length at the wavelengths of absorbance at 576, 623, and 700 nm and using water as a blank. Calculate the concentration of hemoglobin in mg/dL by the formula:

$$(Abs_{576} \times 115) - (Abs_{623} \times 102) - (Abs_{700} \times 39.1)$$

Acceptance Criteria—Contains not more than 30 mg/dL.

FBS FUNCTIONALITY TESTS

In the absence of a user-defined functionality assay, the following tests are suitable to determine the functionality of specific lots of FBS and to aid in the optimization of the growth conditions of mammalian cell cultures in the presence of FBS. For valid functionality confirmation independent of user-specific applications, tests are performed on the specified cell lines. For in-house validation of specialized cell culture applications, cell line(s) specific to those applications should be used and characterized. Use appropriate tissue culture vessels. Two tests described in this chapter are the *Growth-Promotion Curve* and the *Clonal Assay*. The decision about which type of test or the number of tests to be performed to assess suitability of a specific lot of FBS depends on the type of cell line used. For adherent cell lines, the number of colonies at the end of the culturing period represents a good assessment of the capacity of these cells, at low concentration, to grow in the presence of a specific lot of FBS. For cell lines growing in suspension cultures, the optimum growth kinetics is measured by counting viable cells after 7 days of culture.

Cell Lines—Five cell lines are recommended for use: (1) HFL1 (ATCC CCL-153) normal lung, fibroblast; (2) Mv1 Lu (ATCC CCL-64) mink lung, epithelial; (3) HL-60 (ATCC CCL-240) peripheral blood promyeloblast, suspension; (4) VERO (ATCC CCL-81) Monkey Kidney Fibroblast;

and (5) CHO (CCL-61) Chinese Hamster Ovary. The functionality tests described are to be performed on three cell lines, two of which are drawn from the five recommended cell lines and the third of which is the cell line relevant to the user's application. Cell lines are cultured with specific media as recommended by ATCC.

Materials—

- Suitable growth vessel/container
- Biological Safety Cabinet Class II, Type A
- Cell counter/hemocytometer
- Inverted microscope with digital camera accessory.
- Tissue culture vessels: T25 cm^2.

Preparation of Cells for Assays—Quick-thaw a vial in a 37° water bath, and determine cell count and viability. Prepare multiple cultures from each cell line in serum-supplemented growth medium. Incubate the cultures at 37° following instructions provided by ATCC for each of the cell lines used for the test. Examine the prepared cultures under a microscope to ensure uniform, near-confluent monolayers or suspensions. Expand cells until there are enough for assay (about 1×10^7 total cells; $>$90% viability).

Harvesting of Cultures—

1. Remove and discard the growth medium, and then rinse each culture with media lacking FBS.
2. For adherent cells, add 1 mL of Trypsin/EDTA for a few minutes for cells to disperse. Incubate at 37°, if necessary. Neutralize with 1 mL culture medium containing at least 10% FBS.
3. Spin down the cells in a centifuge. Aspirate off wash media, and resuspend cells in an appropriate volume for seeding.

Seeding of Cells—

1. On day 0: For the three cell lines to be tested prepare multiple cultures using seeding densities that ranges between 2×10^3 and 2×10^4 viable cells/mL. (Different inocula are chosen initially to determine optimum growth conditions. Once the appropriate inoculum is chosen, that condition is used to propagate the cells.) Following are the recommended seeding densities:

   Low seeding density: 2×10^3 viable cells/mL

   Mid seeding density: 6×10^3 viable cells/mL

   High seeding density: 2×10^4 viable cells/mL
2. Prepare cultures in triplicate for at least five time points (in days or hours according to the cell line), to determine the seeding density that will yield the optimal growth conditions for each cell line used.
3. Incubate the cultures at 37° in a humidified incubator saturated with 5% CO_2.
4. For each time point of measurement (days 0, 1, 2, 3, 4, and 7), take a photograph of each culture, in triplicate, for both the FBS test material and the USP Fetal Bovine Serum RS at each of the three concentrations for each cell line, and record the percentage of confluency for each of the conditions. [NOTE—Perform this step before trypsinization and cell counting.]
5. Harvest the cells from the three different seeding density cultures for each specific time point. For adherent cultures, harvest cells as described above.
6. Perform and record total cell count and viability for each of the nine cultures for the FBS test and the USP Fetal Bovine Serum RS for each cell line using an appropriate cell counter or hemacytometer. [NOTES—The schedule for counting may have to be changed for fast-growing cell lines or large cells that would become confluent before day 7 and/or for slow-growing lines that need to be in culture 8–10 days before reaching a plateau. Some adherent cell lines will never reach confluency.]

Growth-Promotion Curve

Measurements of cell proliferation rates often are used to determine the response of cells to exogenous stimuli. Quantitative assessment of cell growth conditions is an important factor in monitoring consistency of culture conditions. The optimal cell concentration range for subculturing, optimum inoculum, and doubling time are parameters that can be quantified and trended. Information about the growth kinetics of a culture is critical in the design of cell-based experiments. Cultures vary significantly in their growth properties from lag phase, log phase, and stationary phase. Document the growth characteristics of the culture during the three growth stages to determine population doubling time and cell cycle time. Cells that have entered the stationary phase may demonstrate reduced growth potential and change in morphology. Cells may become polarized and may secrete more extracellular matrix, making them difficult to remove from the substratum. Cells at the end of the log phase give the highest yield and greatest reproducibility.

Reagents—

- Growth media without FBS
- FBS test samples
- Growth medium + 10% FBS
- Trypsin/EDTA solution (0.25%/0.53 mM) in Hank's Balanced Salt Solution (HBSS)

Procedure—Once the cells have reached the end of the log phase, subculture the cells for the test. Follow the procedure described under *Seeding Cells* and prepare multiple cultures for the USP Fetal Bovine Serum RS, and test FBS for different cell lines at three seeding densities for which at least one growth curve displays a lag phase, log phase, and stationary phase and for which the log phase is linear at three or more time points.

Viable cell counts are determined on days 0, 1, 2, 3, 4, and 7.

Calculation and *Data Analysis*—Calculate the mean viable count [cells/cm^2 (adherent) or cells/mL (suspension)] and the mean percent viability for each data point. Plot the data on a semi-log scale graph with the viable count on the log scale on the *y*-axis and days (or hours) in culture on an arithmetic scale on the *x*-axis. Estimate the doubling time using a growth curve that is linear over three or more time points.

Acceptance Criteria—The R^2 value of the line should be equal to or greater than 0.98 in order to support calculation of a valid doubling time. The doubling time of the test sample should be no less than 90% of the doubling time of USP Fetal Bovine Serum RS.

Clonal Assay

This assay is designed to assess the optimal growth for adherent cell lines. Plating efficiency or colony formation at low cell density is a preferred method for analyzing the proliferative capacity and survival of single cells under optimal growth conditions. This is a very sensitive test and is often used for assessing the quality of serum lots. This technique reveals differences in the growth rate within the cell population and is capable of distinguishing between changes in growth rate (colony size) and cell survival (colony number). Because of the heterogeneous cell population of some cell cultures, remember that cells grow differently as isolated colonies at low densities. Consequently, few cells survive even under ideal conditions because all cell interaction is lost. Cloning is a survival assay that is also used for optimizing growth conditions (selection of medium and serum). If it can be confirmed that a single colony arose from a single cell, then cloning efficiency can be determined.

Reagents—

- Growth medium + 10% FBS (test serum)—Eagle minimum essential medium (EMEM) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium carbonate, 0.1 mM nonessential amino acids, and sodium pyruvate containing 100 U/mL penicillin and 100 g/mL streptomycin plus 10% FBS.
- Trypsin/EDTA solution (0.25%/0.53 mM) in HBSS.
- Dulbecco's Phosphate Buffer Saline without calcium or magnesium.
- Carbol Fuchsin–Methylene Blue Solution—Mix 20 g carbol fuchsin stock in 2 L methanol and stir for 10 min (1% carbol fuchsin). Mix 50 g methylene blue in 5 L methanol, and stir for 10 min (1% methylene blue). Prepare Carbol Fuschin–Methylene Blue working solution by mixing methylene blue, methanol, and carbol fuchsin in a ratio of 3 : 2 : 1. Mix for 20 min and filter through four folds of cheesecloth in a funnel. Aliquot and store in brown glass bottles at 15° to 25°.

Preparation of Test Sample—Multiple lots of FBS are used for this assay. For each lot of serum to be tested, add 20 mL of FBS to 180 mL of EMEM, and use the same test sample for the entire test. Sterilize using 0.22-m low protein binding filter units. Store growth medium at 4° until ready to use.

Preparation of Cells for Assay—This test is only for adherent cultures and is performed with the adherent cell lines described under *Cell Lines* (HFL1 and Mv 1 Lu). One week before testing serum, expand the cell lines as described under *Seeding of Cells*, change the medium every 2–3 days, and subculture the cells when they are about 90% confluent. Determine the cell count and viability (viability should be >90%) before performing the assay. Harvest cells as described under *Harvesting of Cultures*, wash twice, and resuspend cells in basal EMEM.

Procedure—The procedure involves plating single-cell suspension at low densities (2–50 cells/cm^2) from which discrete colonies will form. To count the colonies at the end of the assay, fix, stain, and count the number of colonies.

1. For each cell line label ten 60-mm × 15-mm tissue culture dishes for each serum lot that will be tested. Label the side of the lower half of each dish, including controls.
2. Transfer 5 mL of medium containing 10% of the appropriate test serum (10 replicates). Add 400 cells per culture dish (aim for a cell concentration of about 800 cells/mL).
3. Incubate for 10 to 14 days at 37° in a humidified incubator saturated with 5% CO^2.
4. Remove the supernatant and add enough Carbol Fuchsin–Methylene Blue Solution to cover each of the culture dishes for 10 min.
5. Remove the stain; rinse the culture dishes with several changes of distilled water; invert the dishes on paper towels; and allow to dry.
6. Count and record (1) the number of colonies and (2) the total surface of stained colonies (mm^2). Calculate means and standard deviations.

Acceptance Criteria—Percent plating efficiency is expressed by counting the number of colonies in a defined area divided by the number of cells seeded multiplied by 100. Compare results between lots of FBS, and select a serum lot that is good for various types of cells and optimal for a specific cell culture application.■2S (*USP33*)

In-Process Revision

OTHER TESTS AND ASSAYS

BRIEFING

〈**381**〉 **Elastomeric Closures for Injection,** *USP 32* page 145, the *Revision Bulletin* official May 1, 2009, and page 614 of *PF* 35(3) [May–June 2009]. On the basis of comments received and discussion by the Parenteral Products—Industrial Expert Committee, editorial changes to the *Introduction* are being proposed.

(PPI: D. Hunt.) RTS—C74657

Change to read:

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in the general test chapter *Injections* 〈1〉 are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

•This chapter applies to closures used for long-term storage of preparations defined in the general test chapter *Injections* 〈1〉. Such closures are typically used as part of a vial, bottle, or pre-fill syringe package system.• (RB 1-May-2009)

This chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer; however, it does apply to closures treated with silicone (e.g., Dimethicone, *NF*). When performing the tests in this chapter, it is not required that closures be treated with silicone, although there is no restriction prohibiting the use of siliconized closures.

•This chapter also applies to closures coated with other lubricious materials (e.g., materials chemically or mechanically bonded to the closure) that are not intended to, and in fact do not provide, a barrier to the base elastomer. When performing the tests, closures with lubricious nonbarrier coatings are to be tested in their coated state.

The following comments relate solely to closures laminated or coated with materials intended to provide, or in fact function as, a barrier to the base elastomer (e.g., PTFE or lacquer coatings). It is not permissible to use a barrier material in an attempt to change a closure that does not meet compendial requirements to one that does conform. Therefore, all *Physicochemical Tests* apply to the base formula of such closures, as well as to the coated or laminated closure. To obtain *Physicochemical Tests* results, the tests are to be performed on uncoated or nonlaminated closures of the same elastomeric compound, as well as to the laminated or coated closure. The *Functionality Tests* apply to and are to be performed using the laminated or coated elastomeric closure. *Biological Tests* apply to the lamination or coating material, as well as to the base formula. *Biological Tests* may be performed on the laminated or coated closure, or they may be performed on the laminate/coating material and the uncoated or nonlaminated closures of the same elastomeric compound. In the latter case, the results are to be reported separately. The base formula used for physicochemical or biological tests intended to support the compendial compliance of a barrier-coated closure should be similar to the corresponding coated closure in configuration and size.

For all *Elastomeric Closures for Injection* 〈381〉 tests performed on any closure type, it is important to document the closure being tested, including a full description of the elastomer, and any lubrication, coating, laminations, or treatments applied.• (RB 1-May-2009)

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are those used for aqueous preparations. Type II closures are typically intended for nonaqueous preparations and are those which, having properties optimized for special uses, may not meet all requirements listed for Type I closures because of physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements, but still meets the Type II requirements for the test(s), the closure is assigned a final classification of Type II.

■All elastomeric closures suitable for use with injectable preparations must comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criterion for the selection of such closures.■1S *(USP33)*

~~This chapter is intended as an initial screen to identify elastomeric closures that might be appropriate for use with injectable preparations on the basis of their biological compatibility,~~

■It is appropriate to use this chapter when identifying elastomeric closures that might be acceptable for use with injectable preparations on the basis of their biological reactivity,■1S *(USP33)* their aqueous extract physicochemical properties, and their functionality. ~~All elastomeric closures suitable for use with injectable preparations comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.~~

■■1S *(USP33)*
The following closure evaluation requirements are beyond the scope of this chapter:

— The establishment of closure identification tests and specifications
— The verification of closure–product physicochemical compatibility
— The identification and safety determination of closure leachables found in the packaged product
— The verification of packaged product closure functionality under actual storage and use conditions

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, compatibility testing must be repeated, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and,

■for aseptic processes,■2S *(USP33)* sterilized prior to use in packaging injectable products.

In-Process Revision

Physical Tests and Determinations

BRIEFING

⟨**645**⟩ **Water Conductivity,** *USP 32* page 240. While the majority of water used for production is *Purified Water* and *Water for Injection* that is produced on site (referred to as ''bulk water''), the Pharmaceutical Water Expert Committee recognizes the need for and the use of commercially-available ''packaged'' *Purified Water* and *Water for Injection* in some production environments. Also, there are sterile waters such as *Sterile Purified Water* and *Sterile Water for Injection* (and *Inhalation* and *Irrigation*). As the tests/limits for these various types of waters have been updated in recent years, some of the terminology regarding ''bulk'', ''sterile'', and ''packaged'' needs to be updated and/or clarified. The conductivity test method and limits for *Purified Water* and *Water for Injection*, whether in a packaged or bulk form, are directed to the ''*Bulk Water*'' section of this general test chapter. This does not represent a change because these monographs direct the analyst to this section already. The section ''*Packaged Water*'' is renamed ''*Sterile Water*,'' because the tests in the section are intended for *Sterile Purified Water*, *Sterile Water for Injection*, etc., and not the packaged form of the bulk article. There is no change to the test limits or methods. This is a change to the names of sections and to be consistent with monographs and related general chapters. There are associated changes in *Water for Pharmaceutical Purposes* ⟨1231⟩ and the monographs for *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation***,** and *Sterile Water for Irrigation***.** All changes align the use of these terms.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Change to read:

Electrical conductivity in water is a measure of the ion-facilitated electron flow through it. Water molecules dissociate into ions as a function of pH and temperature and result in a very predictable conductivity. Some gases, most notably carbon dioxide, readily dissolve in water and interact to form ions, which predictably affect conductivity as well as pH. For the purpose of this discussion, these ions and their resulting conductivity can be considered intrinsic to the water.

Water conductivity is also affected by the presence of extraneous ions. The extraneous ions used in modeling the conductivity specifications described below are the chloride and sodium ions. The conductivity of the ubiquitous chloride ion (at the theoretical endpoint concentration of 0.47 ppm when it was a required attribute test in *USP XXII* and earlier revisions) and the ammonium ion (at the limit of 0.3 ppm) represent a major portion of the allowed water impurity level. A balancing quantity of cations, such as sodium ions, is included in this allowed impurity level to maintain electroneutrality. Extraneous ions such as these may have significant impact on the water's chemical purity and suitability for use in pharmaceutical applications. The procedure ~~described~~

■■2S (*USP33*)
in the section *Bulk Water* is ~~designed~~

■specified■2S (*USP33*)

for measuring the conductivity of waters such as Purified Water, Water for Injection, Water for Hemodialysis, and the condensate of Pure Steam. ~~produced in bulk. For water packaged in bulk but manufactured elsewhere or for~~

■The procedure in the section Sterile Water is specified

for measuring the conductivity of waters such as■2S (*USP33*) Sterile Purified Water, Sterile Water for Injection, Sterile Water for Inhalation, and Sterile Water for Irrigation. ~~some additional conductivity tests may be required. Such tests are described in the section *Packaged Water.*~~

■■2S (*USP33*)
Online conductivity testing provides real-time measurements and opportunities for real-time process control, decision, and intervention. Precaution should be taken while collecting water samples for off-line conductivity measurements. The sample may be affected by the sampling method, the sampling container, and environmental factors such as ambient carbon dioxide concentration and organic vapors.

Change to read:

INSTRUMENT SPECIFICATIONS AND OPERATING PARAMETERS

Water conductivity must be measured accurately using calibrated instrumentation. The conductivity cell constant, a factor that represents the geometrical properties of the conductivity sensor, must be known within ±2%. The cell constant can be verified directly by using a solution of known or traceable conductivity, or indirectly by comparing the instrument reading taken with the conductivity sensor in question to readings from a conductivity sensor of known or traceable cell constant.

Meter calibration is accomplished by replacing the conductivity sensor with NIST (or equivalent local national authority) -traceable precision resistors (accurate to ±0.1% of the stated value) or an equivalently accurate adjustable resistance device, such as a Wheatstone Bridge, to give a predicted instrument response. Each scale on the meter may require separate calibration prior to use. The frequency of recalibration is a function of instrument design, degree of use, etc. However, because some multiple-scale instruments have a single calibration adjustment, recalibration may be required between each use of a different scale. Excluding the conductivity sensor cell constant accuracy, the instrument accuracy must be ±0.1 μS/cm.

In order to increase the measurement accuracy on the conductivity ranges used, which can be large, and to ensure a complete equipment calibration, it is suggested that periodic verification of the entire equipment be performed. This could be done by comparing the conductivity/resistivity values displayed by the measuring equipment with those of an external calibrated conductivity-measuring device. The two nontemperature-compensated conductivity or resistivity values must be equivalent to within ±20% of each other, or at a difference that is acceptable on the basis of product water criticality and/or the water conductivity ranges in which the measurements are taken. The two conductivity sensors should be positioned close enough together to measure the same water sample in the same environmental conditions.

In addition to the verification method performed in non-temperature-compensated mode, a similar verification performed in temperature-compensated mode could be performed to ensure an appropriate accuracy of the equipment when such a mode is used for trending or other purposes.

Because temperature has a substantial impact on conductivity readings of specimens at high and low temperatures, many instruments automatically correct the actual reading to display the value that theoretically would be observed at the nominal temperature of 25°. This is typically done using a temperature sensor embedded in the conductivity sensor and an algorithm in the instrument's circuitry. This temperature compensation al-

In-Process Revision

gorithm may not be accurate. Conductivity values used in this method are nontemperature-compensated measurements. Temperature measurement is required for the performance of the *Stage 1* test. It may be made using the temperature sensor embedded in the conductivity cell sensor. An external temperature sensor positioned near the conductivity sensor is also acceptable. Accuracy of the temperature measurement must be $\pm 2^{\circ}$.

■The procedures below shall be performed using instrumentation that has been calibrated, has conductivity sensor cell constants that have been accurately determined, and has temperature compensation function that has been disabled. For both online and offline measurements, the suitability of instrumentation for quality control testing is also dependent on the sampling location(s) in the water system. The selected sampling instrument location(s) must reflect the quality of the water used.■2S (*USP33*)

Change to read:

BULK WATER

~~The procedure below shall be performed using instrumentation that has been calibrated, has conductivity sensor cell constants that have been accurately determined, and has temperature compensation function that has been disabled. For both online and offline measurements, the suitability of instrumentation for quality control testing is also dependent on the sampling location(s) in the water system. The selected sampling instrument location(s) must reflect the quality of the water used.~~

■The procedure and test limits in this section are intended for Purified Water, Water for Injection, Water for Hemodialysis, the condensate of Pure Steam, and any other monographs which specify this section.■2S (*USP33*)

The combined conductivities of the intrinsic and extraneous ions vary as a function of pH and are the basis for the conductivity specifications described in the *Stage 3—pH and Conductivity Requirements* table and used when performing *Stage 3* of the test method. Two preliminary stages are included in the test method. If the test conditions and conductivity limits are met at either of these preliminary stages, the water meets the requirements of this test. Proceeding to the third stage of the test in these circumstances is unnecessary. Only in the event of failure at the final test stage is the sample judged noncompliant with the requirements of the test.

Procedure

STAGE 1

Stage 1 is intended for online measurement or may be performed offline in a suitable container.

1. Determine the temperature of the water and the conductivity of the water using a nontemperature-compensated conductivity reading.

2. Using the *Stage 1—Temperature and Conductivity Requirements* table, find the temperature value that is not greater than the measured temperature, i.e., the next lower temperature. The corresponding conductivity value on this table is the limit. [NOTE—Do not interpolate.]

3. If the measured conductivity is not greater than the table value, the water meets the requirements of the test for conductivity. If the conductivity is higher than the table value, proceed with *Stage 2.*

Stage 1—Temperature and Conductivity Requirements
(for nontemperature-compensated conductivity measurements only)

Temperature	Conductivity Requirement (μS/cm)
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

STAGE 2

4. Transfer a sufficient amount of water (100 mL or more) to a suitable container, and stir the test specimen. Adjust the temperature, if necessary, and, while maintaining it at $25 \pm 1^{\circ}$, begin vigorously agitating the test specimen while periodically observing the conductivity. When the change in conductivity (due to uptake of atmospheric carbon dioxide) is less than a net of 0.1 μS/cm per 5 minutes, note the conductivity.

5. If the conductivity is not greater than 2.1 μS/cm, the water meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 μS/cm, proceed with *Stage 3.*

STAGE 3

6. Perform this test within approximately 5 minutes of the conductivity determination in Step 5, while maintaining the sample temperature at $25 \pm 1^{\circ}$. Add a saturated potassium chloride solution to the same water sample (0.3 mL per 100 mL of the test specimen), and determine the pH to the nearest 0.1 pH unit, as directed under *pH* ⟨791⟩.

7. Referring to the *Stage 3—pH and Conductivity Requirements* table, determine the conductivity limit at the measured pH value. If the measured conductivity in Step 4 is not greater than the conductivity requirements for the pH determined in Step 6, the water meets the requirements of the test for conductivity. If either the measured conductivity is greater than this value or the pH is outside the range of 5.0 to 7.0, the water does not meet the requirements of the test for conductivity.

Stage 3—pH and Conductivity Requirements
(for atmosphere- and temperature-equilibrated samples only)

pH	Conductivity Requirement (μS/cm)
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

Change to read:

~~**PACKAGED**~~

▪STERILE▪2S (*USP33*)

WATER

The procedure and test limits are intended for ~~water packaged in bulk but manufactured elsewhere or for~~

▪▪2S (*USP33*)
Sterile Purified Water, Sterile Water for Injection, Sterile Water for Inhalation, and Sterile Water for Irrigation,

▪and any other monographs which specify this section.▪2S (*USP33*)
~~All these~~

▪The sterile▪2S (*USP33*)
waters are derived from Purified Water or Water for Injection, and therefore have been determined to be compliant with the *Bulk Water* requirements before being stored in the container. The specification provided represents the maximum allowable conductivity value, taking into consideration the limitation of the measurement method and reasonable container leaching. Such specification and the sampling volume choices should be defined and validated on the basis of the intended purpose of the water.

Procedure

Transfer a sufficient amount of water to a suitable container, and stir the test specimen. Adjust the temperature, if necessary, and, while maintaining it at 25 ± 1°, begin vigorously agitating the test specimen while periodically observing the conductivity. When the change in conductivity (due to uptake of ambient carbon dioxide) is less than a net of 0.1 μS/cm per 5 minutes, note the conductivity.

For containers with a nominal volume of 10 mL or less, if the conductivity is not greater than 25 μS/cm, the water meets the requirements. For containers with a nominal volume greater than 10 mL, if the conductivity is not greater than 5 μS/cm, the water meets the requirements.

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1059**⟩ **Excipient Performance.** The following general information chapter has been prepared by the Excipient Performance Joint Advisory Panel of the Excipient General Chapters, Excipient Monographs 1 and Excipient Monographs 2 Expert Committees. A pharmaceutical dosage form typically consists of both active ingredient(s) and excipients. The latter play a critical role in manufacturing, stability, and performance. The properties of excipients that ensure satisfactory and consistent performance depend on the dosage form, the product, the manufacturing process, and the performance requirements. Excipient monographs in the *National Formulary (NF)* provide methods and specifications that ensure excipient identity, quality, and purity. General tests, procedures, and acceptance criteria provided in the *NF* are used to evaluate material attributes of excipients.

Excipient properties that are important to dosage form performance may not be identified or specified in compendial monographs. The proposed chapter resolves this deficiency by commenting on a number of functional categories identified in the *USP–NF*. This information includes a summary of the functional capabilities of an excipient, as well as information about the physical and chemical properties of excipients that may be useful in ensuring consistent and desirable excipient performance. Additional sections will be added as needed.

The tests, procedures, and acceptance criteria delineated in the proposed chapter will not be compendial requirements, but may provide useful information to support private agreements between buyers and suppliers and also, if needed, regulatory requirements for specified excipients and drug products. The expert committee seeks input from readers of *Pharmacopeial Forum* and *USP–NF* regarding the format and content of the chapter.

(EGC: K. Moore) RTS—C76303

Add the following:

▪⟨1059⟩ EXCIPIENT PERFORMANCE

INTRODUCTION

Excipients are used in virtually all drug products and are essential to product performance. Thus, the successful manufacture of a robust product requires the use of well-defined excipients and processes that together yield

In-Process Revision

a consistent product. Typically, excipients are manufactured and supplied to comply with compendial standards. The development, manufacture, and performance of pharmaceutical dosage forms often depend upon the physical and chemical properties of excipients that may not be provided in *National Formulary (NF)* monographs. Also, functionality tests are usually not provided in *NF* monographs.

An excipient may have different functional purposes and may possess various required characteristics (e.g., particle size, particle size distribution, or surface area), depending on its use in a formulation or manufacturing process. A listing of excipients grouped by functional category summarizes the most typically identified purpose these excipients serve in drug products. The list of excipients included in each category is not comprehensive and is not intended to limit in any way the choice or use of the excipient. For the complete list, refer to the *USP* and *NF* Excipients, Listed by Category in the *National Formulary*, under *Contents*.

Excipient functionality is a broad, qualitative, and descriptive term for the purpose or role an excipient serves in a formulation. Of greater importance, however, are the quantitative performance requirements (e.g., critical material attributes) of excipients that must be evaluated and controlled to ensure consistent performance throughout the product life cycle. Not all critical material attributes of an excipient may be identified or evaluated by tests, procedures, and acceptance criteria in *NF* monographs. Excipient suppliers and users therefore at times wish to identify and control critical excipient attributes that go beyond monograph specifications. This requires a thorough understanding of the formulation, the manufacturing processes, and the physical and chemical properties of each ingredient. Manufacturers should anticipate lot-to-lot and supplier-to-supplier variability in excipient properties and should have in place appropriate controls if needed to ensure consistent excipient performance.

This general chapter provides an overview of the key functional categories of excipients, tests that may assess excipient functionality, and test procedures that may not be presented in compendial monographs. The functional categories have been organized by their most typical use in common pharmaceutical dosage forms (Tablets and Capsules; Oral Liquids; Semisolids, Topicals and Suppositories; Parenterals; and Aerosols) to provide a greater level of specificity for each functional category. Several functional categories (e.g., antioxidant) can apply to multiple dosage form types. The association of a functional category with a particular dosage form in this chapter is not absolute and does not limit use of an excipient to a single type of dosage form. Because of the complex nature and interplay of formulation ingredients, processing, and dosage form performance requirements, the information provided in this chapter should not be viewed as either restrictive or completely comprehensive. Each functional category includes a general description; the mechanisms by which the excipients achieve their activity; physical properties common to these excipients; chemical properties; and a list of pharmacopeial general chapters that may be useful in the development of specific tests, procedures, and acceptance criteria that help ensure that the critical material attributes are adequately monitored and controlled.

TABLETS AND CAPSULES

Functional Category: Diluent

Description: Diluents are components that are incorporated into tablet or capsule dosage forms to increase dosage form volume or weight. Sometimes referred to as fillers, diluents often comprise a significant proportion

of the dosage form, and the quantity and type of diluent selected often depend on its physical and chemical properties. Because the diluent may comprise a large portion of the dosage form, successful and robust manufacturing and dosage form performance depend on the measurement and control of the critical material attributes.

Functional Mechanism: Among the most important functional roles diluents play is to impart desirable manufacturing properties (e.g., powder flow, tablet compaction strength, wet or dry granule formation, homogeneity) and performance (e.g., content uniformity, disintegration, dissolution, tablet integrity, friability, physical and chemical stability). Some diluents (e.g., microcrystalline cellulose) are occasionally referred to as dry binders because of the high degree of tablet strength they impart to the final compressed tablet.

Physical Properties: The primary physical properties relevant to tablet/capsule diluents are those that can have a direct effect on diluent and formulation performance. These include: (1) particle size and size distribution, (2) particle shape, (3) bulk/tapped/true density, (4) specific surface area, (5) crystallinity, (6) moisture content, (7) powder flow, (8) solubility, and (9) compaction properties for tablet dosage forms.

Chemical Properties: Tablet diluents comprise a large and diverse group of materials that include inorganics (e.g., dibasic calcium phosphate, calcium carbonate), single-component organic materials (e.g., lactose monohydrate, mannitol), and multicomponent or complex organics (e.g., microcrystalline cellulose, starch). They may be soluble or insoluble in water, and they may be neutral, acidic, or alkaline in nature. These chemical properties of diluents may affect drug substance physical or chemical stability and performance positively or negatively. Appropriate selection of excipients with desirable physical and chemical properties can enhance the physical and chemical stability as well as the performance of the drug substance and dosage form. The detailed composition of an excipient may be important because excipient function could be influenced by the presence of minor concomitant components that are essential for proper performance. Pharmaceutical scientists may need to control the presence of undesirable components (e.g., heavy metals or peroxides) to ensure adequate dosage form stability and performance.

General Chapters: The following general chapters may be useful to ensure consistency in diluent functions: *Bulk Density and Tapped Density* ⟨616⟩, *Density of Solids* ⟨699⟩, *Crystallinity* ⟨695⟩, *Crystallinity Determination by Solution Calorimetry* ⟨696⟩, *Loss on Drying* ⟨731⟩, *Water Determination* ⟨921⟩, *Optical Microscopy* ⟨776⟩, *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩, *Light Diffraction Measurement of Particle Size* ⟨429⟩, *Powder Fineness* ⟨811⟩, *Specific Surface Area* ⟨846⟩, and *Powder Flow* ⟨1174⟩.

Functional Category: Binder

Description: Tablet/capsule binders are incorporated into formulations to facilitate the agglomeration of powder into granules during mixing with a granulating fluid such as water, hydroalcoholic mixtures, or other solvents. The binder may be either dissolved or dispersed in the granulation liquid or blended in a dry state; other components and the granulation liquid may be added separately during agitation. Following evaporation of the granulation liquid, binders typically produce dry granules that achieve the desired properties such as granule size, size distribution, shape, content, mass, and active content. Wet granulation facilitates the further processing of the granules by improving one or more

granule properties such as: flow, handling, strength, resistance to segregation, dustiness, appearance, solubility, compaction, or drug release.

Functional Mechanism: Binders are soluble or partially soluble in the granulating solvent or, as in the case of native starches, can be made soluble. Concentrated binder solutions also have adhesive properties. Upon addition of liquid, binders typically facilitate the production of moist granules (agglomerates) by altering interparticle adhesion. They may also modify interfacial properties, viscosity, and/or other properties. During drying they may produce solid bridges that yield significant residual dry granule strength.

Physical Properties: Dispersion or dissolution of binder in the granulation liquid depends on its physical properties: surface tension, particle size, size distribution, solubility, and viscosity are among the important properties depending on the application. Homogeneous incorporation of binder into a dry blend also depends on its physical properties such as particle size, shape, and size distribution. Viscosity often is an important property to consider for binders and, for polymers, is influenced by the nature of the polymer structure, molecular weight, and molecular weight distribution. Polymeric binders may form gels.

Chemical Properties: Tablet/capsule binders may be categorized as: (1) natural polymers, (2) synthetic polymers, or (3) sugars. The chemical nature of polymers, including polymeric structure, monomer properties and sequence, functional groups, degree of substitution, and cross-linking influence the complex interactions that can occur during granulation. Natural polymers in particular may exhibit greater variation in their properties because of variations in their sources and therefore composition.

General Chapters: The following general chapters may be useful to ensure consistency in binder functions: *Bulk Density and Tapped Density* ⟨616⟩, *Crystallinity* ⟨695⟩, *Density of Solids* ⟨699⟩, *Loss on Drying* ⟨731⟩, *Particle Size Distribution Estimation by Analytical Sieving* ⟨786 ⟩, *Specific Surface Area* ⟨846⟩, *Viscosity* ⟨911⟩, *Powder Flow* ⟨1174⟩, and *Chromatography* ⟨621⟩.

Functional Category: Disintegrant

Description: Disintegrants are functional components that are added to formulations to promote rapid disintegration into smaller units and to allow a drug substance to dissolve more rapidly. Disintegrants are natural, synthetic, or chemically modified natural polymeric substances. When disintegrants come in contact with water or stomach or intestinal fluid they function by absorbing liquid and start to swell, dissolve, or form gels. This causes the tablet structure to rupture and disintegrate, producing increased surfaces for enhanced dissolution of the drug substance.

Functional Mechanism(s): The ability to interact strongly with water is essential to disintegrant function. Four major mechanisms describe the function of the various disintegrants: volume increase by swelling, deformation, capillary action (wicking), and repulsion. In tablet formulations, the function of disintegrants is best described as a combination of two or more of these effects. The onset and degree of the locally achieved actions depend on various parameters of a disintegrant, such as its chemical nature and its particle size distribution and particle shape, as well as some important tablet parameters such as hardness and porosity.

Physical Properties: The primary physical properties relevant to a disintegrant are those that describe the product's particle structure as a dry powder or its structure

when in contact with water. These properties include (1) particle size distribution, (2) water absorption rate, (3) swelling ratio or swelling index, and (4) the characterization of the resulting product whether it is still particulate or a gel is formed.

Chemical Properties: Polymers used as disintegrants are either non-ionic or anionic with counterions such as sodium, calcium, or potassium. Non-ionic polymers are natural or physically modified polysaccharides such as starches, celluloses, pullulan, or cross-linked polyvinylpyrrolidone. The anionic polymers mainly are chemically modified cellulose products or low-crosslinked polyacrylates. These chemical properties should be considered in the case of ionic polymers. Disintegration performance will be affected by pH changes in the gastrointestinal tract or by complex formation with ionic active pharmaceutical ingredients (APIs).

General Chapters: The following general chapters may be useful to ensure consistency in disintegrant functions: *Light Diffraction Measurement of Particle Size* ⟨429⟩, *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩, *Optical Microscopy* ⟨776⟩, and *Powder Flow* ⟨1174⟩.

Functional Category: Lubricant

Description: Lubricants typically are used to reduce the frictional forces between particles, and between particles and metal contact surfaces of manufacturing equipment such as tablet punches and dies used in the manufacture of solid dosage forms. Liquid lubricants may be absorbed into the granule matrix before compaction. Liquid lubricants also may be used to reduce metal–metal friction on manufacturing equipment.

Functional Mechanism: Boundary lubricants function by adhering to solid surfaces (granules and machine parts) and reducing the particle–particle friction or the particle–metal friction. The orientation of the adherent lubricant particles is influenced by the properties of the substrate surface. For optimal performance, the boundary lubricant particles should be composed of small, plate-like crystals or stacks of plate-like crystals. Fluid-film lubricants melt under pressure and thereby create a thin fluid film around particles and on the surface of punches and dies in tablet presses, which helps to reduce friction. Fluid-film lubricants re-solidify after the pressure is removed. Liquid lubricants are released from the granules under pressure and create a fluid film. They do not re-solidify when the pressure is removed but are reabsorbed or redistributed through the tablet matrix over the course of time.

Physical Properties: The primary physical properties that may be important for the function of boundary lubricants include particle size, surface area, hydration state, and polymorphic form. Purity (e.g., stearate:palmitate ratio) and moisture content also may be important. The primary physical properties of possible importance for fluid-film lubricants are particle size and solid state/thermal behavior. Purity may also be important.

Chemical Properties: Lubricants can be classified as boundary lubricants, fluid-film lubricants, or liquid lubricants. Boundary lubricants are salts of long-chain fatty acids (e.g., magnesium stearate) or fatty acid esters (e.g., sodium stearyl fumarate) with a polar head and fatty acid tail. Fluid-film lubricants are solid fats (e.g., hydrogenated vegetable oil, type 1), glycerides (glyceryl behenate and distearate), or fatty acids (e.g., stearic acid) that melt when subjected to pressure. Liquid lubricants are liquid materials that are released from granules under pressure.

General Chapters: The following general chapters may be useful to ensure consistency in lubricant functions: *Light Diffraction Measurement of Particle Size* ⟨429⟩, *Parti-*

cle Size Distribution Estimation by Analytical Sieving ⟨786⟩, *Specific Surface Area* ⟨846⟩, *X-ray Diffraction* ⟨941⟩, *Loss on Drying* ⟨731⟩, *Water Determination* ⟨921⟩, *Crystallinity* ⟨695⟩, *Crystallinity Determination by Solution Calorimetry* ⟨696⟩, *Optical Microscopy* ⟨776⟩, and *Thermal Analysis* ⟨891⟩.

Other Information: Certain lubricants, particularly those used in effervescent dosage forms, do not fall into the chemical categories defined above. These materials are used in special situations, and they are not suitable for universal application. Talc is an inorganic material that may have some lubricant properties. It is generally used in combination with fluid-film lubricants to reduce sticking to punches and dies.

Functional Category: Glidant and/or Anticaking Agent

Description: Glidants and anticaking agents are used to promote powder flow and to reduce the caking or clumping that can occur when powders are stored in bulk. In addition, glidants and anticaking agents reduce the incidence of bridging during the emptying of powder hoppers and during powder processing.

Functional Mechanism: Glidants are thought to work by a combination of adsorption onto the surface of larger particles and reduction of particle–particle adhesive and cohesive forces, thus allowing particles to move more easily relative to one another. In addition, glidants may be dispersed between larger particles and thus may reduce friction between larger particles. Anticaking agents may absorb free moisture that otherwise would allow the development of particle–particle bridges that are implicated in caking phenomena.

Physical Properties: Primary physical properties of potential importance for glidants and anticaking agents are particle size, particle size distribution, and surface area. They may be slightly hygroscopic.

Chemical Properties: Glidants and anticaking agents typically are finely divided inorganic materials. They are insoluble in water but are not hydrophobic. Some of these materials are complex hydrates.

General Chapters: The following general chapters may be useful to ensure consistency in glidant or anticaking agent functions: *Light Diffraction Measurement of Particle Size* ⟨429⟩, *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩, *Specific Surface Area* ⟨846⟩, *Loss on Drying* ⟨731⟩, and *Water Determination* ⟨921⟩.

Functional Category: Coloring Agent

Description: Coloring agents are incorporated into dosage forms in order to produce a distinctive appearance that may serve to differentiate a particular formulation from others that have a similar physical appearance. These substances are subdivided into dyes (water-soluble substances), lakes (insoluble forms of a dye that result from its irreversible adsorption onto a hydrous metal oxide), inorganic pigments (substances such as titanium dioxide or iron oxides), and natural colorants (colored compounds not considered dyes per se, such as riboflavin). Coloring agents are subject to federal regulations, and consequently the current regulatory status of a given substance must be determined before its use.

The Federal Food, Drug, and Cosmetic Act defines three categories of coloring agents:

- FD&C colors: those certifiable for use in coloring foods, drugs, and cosmetics

- D&C colors: dyes and pigments considered safe in drugs and cosmetics when in contact with mucous membranes or when ingested
- Ext. D&C colors: colorants that, because of their oral toxicity, are not certifiable for use in ingestible products but are considered safe for use in externally applied products.

Functional Mechanism: Water-soluble dyes usually are dissolved in a granulating fluid for use, although they may also be adsorbed onto carriers such as starch, lactose, or sugar from aqueous or alcoholic solutions. These latter products are often dried and used as formulation ingredients. Because of their insoluble character, lakes are almost always blended with other dry excipients during formulation. For this reason, direct-compression tablets are often colored with lakes.

Physical Properties: Particle size and size distribution of dyes and lakes can influence product processing times (blending and dissolution), color intensity, and uniformity of appearance.

Chemical Properties: The most important properties of a coloring agent are its depth of color and resistance to fading over time. Substances can be graded on their efficiency in reflecting desired colors of visible light, as well as on their molar absorptivities at characteristic wavelengths. A coloring agent should be physically and chemically nonreactive with other excipients and the drug substances. The quality of a coloring agent ordinarily is measured by a determination of its strength, performance, or assay. The impurity profile is established by measurements of insoluble matter, inorganic salt content, metal content, and organic impurities.

General Chapters: Two general chapters are useful to ensure consistency in selected coloring agent functions: *Color—Instrumental Measurement* ⟨1061⟩ and *Light Diffraction Measurement of Particle Size* ⟨429⟩. Instrumental methods should be used to determine the absolute color of a coloring agent.

Other Information: Coloring agents are subject to federal regulations, and consequently the current regulatory status of a given substance must be determined before it is used. Following is a list of coloring agents and currently applicable sections of the Code of Federal Regulations (CFR).

Color	CFR
Ferric Oxides	21 CFR 73.1200
Titanium Dioxide	21 CFR 73.575 & 21 CFR 73.1575
FD&C Blue #1/Brilliant Blue FCF Aluminum Lake	21 CFR 82.51 & 21 CFR 82.101
FD&C Blue #2/Indigo Carmine Aluminum Lake	21 CFR 82.51 & 21 CFR 82.102
FD&C Red #40/Allura Red AC Aluminum Lake	21 CFR 74.340 & 21 CFR 74.1340
FD&C Yellow #5/Tartrazine Aluminum Lake	21 CFR 82.51 & 21 CFR 82.705
FD&C Yellow #6/Sunset Yellow FCF Aluminum Lake	21 CFR 82.51 & 21 CFR 82.706
D&C Yellow #10 Aluminum Lake	21 CFR 82.1051 & 21 CFR 82.1710
D&C Red #30/Helendon Pink Aluminum Lake	21 CFR 82.1051 & 21 CFR 82.1330
D&C Red #7/Lithol Rubin B Calcium Lake	21 CFR 82.1051 & 21 CFR 82.1307
D&C Red #27/Phloxine Aluminum Lake	21 CFR 82.1051 & 21 CFR 82.1327

Functional Category: Capsule Shell

Description: The word capsule is derived from the Latin capsula, which means a small container. Among other benefits, capsules enable pharmaceutical powders and liquids to be formulated for dosing accuracy as well as ease of transportation. The capsule material should be compatible with all other ingredients in the drug product. Hard capsules typically consist of two parts: both are cylindrical, and one part is slightly longer than other and is called the body. The cap fits closely on the body to enclose the capsule. In contrast, the soft capsule is a one-piece unit that may be seamed along an axis or may be seamless. The capsule material may be derived from hydrolysis of collagen that originates from porcine, bovine, or fish sources, or it can be of non-animal origin, e.g., cellulosic or polysaccharide chemical entities. The capsule shell also contains other additives such as plasticizers, colorants, and preservatives. In some cases, capsule shells are sterilized to prevent microbial growth. The capsule shell is an integral part of the formulation, and therefore robust manufacturing and formulation performance depends on the measurement and control of critical attributes.

Functional Mechanism: Capsules can enclose solid as well as semisolid and liquid formulations. Capsules have a variety of benefits including: masking unpleasant taste, facilitating blinding in clinical studies, promoting ease of swallowing, and presenting a unique appearance. Conventional capsule shells should dissolve rapidly at 37° in biological fluids such as gastric and intestinal media. However, the solubility properties of the shell can be modified, e.g., with enteric and controlled-release polymers, to control the release of capsule contents.

Physical Properties: The primary physical properties relevant to the capsule shell are those that can have a direct effect on product performance: 1) moisture content, 2) gas permeability, 3) stability on storage, 4) disintegration, 5) compactness, and 6) brittleness. The moisture content varies with the type of capsule. Hard gelatin capsules typically contain 13%–16% water compared to hypromellose (hydroxypropyl methylcellulose/HPMC) capsules that typically contain 4%—7% water content. Soft gelatin capsules contain 6%–8% water. Moisture content has a significant impact on capsule brittleness. Equilibrium water content also may be crucial to dosage form stability because water migration will take place between the shell and capsule contents. Gas permeability may be important and generally is greater for HPMC capsules than gelatin capsules because of the presence of open structures. Gelatin capsules may undergo cross-linking upon storage at elevated temperature and humidity (e.g. 40°/75% RH), but under these conditions HPMC capsules do not cross-link. The aldehyde content in the powder fill should be considered because it can promote cross-linking of gelatin shell material. Gelatin capsules should disintegrate within 15 min when exposed to 0.5% HCl at 36°–38° but not below 30°. HPMC capsules also can disintegrate below 30°.

Chemical Properties: Gelatin is a commercial protein derived from native protein collagen. The product is obtained by partial hydrolysis of collagen derived from skin, white connective tissue, and bones of animals. Type A gelatin is derived by acid treatment, and Type B gelatin is derived from base treatment. The common sources of commercial gelatin are pigskin, cattle hide, cattle bone, cod skin, and tilapia skin. The gelatin capsule shell also typically contains coloring agents, plasticizers such as polyhydric alcohols, natural gums and sugars, and preservatives such as sodium metabisulfite and esters of para-hydroxy benzoic acid. The more commonly used nongelatin capsules today are made from HPMC. Different capsule types contain different moisture levels and may thus influence drug product stability. The detailed composition of an excipient may be important because

the shell function can be influenced by small amounts of impurities in the excipients (e.g., peroxides in oils or aldehydes in lactose and starches) that can cause capsule cross-linking. The presence in capsule shells of undesirable materials such as metals, odorants, water-insoluble substances, and sulfur dioxide should be evaluated to ensure stability and performance.

General Chapters: The following general chapters may be useful to ensure consistency in selected capsule shell functions: *Microbial Enumeration Tests* ⟨61⟩, *Tests for Specified Organisms* ⟨62⟩, *Residue on Ignition* ⟨281⟩, *Arsenic* ⟨211⟩, *Heavy Metals* ⟨231⟩, *Water Determination* ⟨921⟩, *Color Instrumental Method* ⟨1061⟩, *Disintegration* ⟨701⟩, *Dissolution* ⟨711⟩, and *Gel Strength of Gelatin* ⟨1081⟩.

Functional Category: Coating Agent

Description: Reasons for coating pharmaceutical dosage forms include masking unpleasant tastes or odors, improving ingestion and appearance, protecting active ingredients from the environment, and modifying the release of the active ingredient (e.g., controlled-release rates or gastrointestinal targeting). The materials used as coating agents include natural, semisynthetic, and synthetic materials. These may be powders or colloidal dispersions (latexes or pseudolatexes) that usually are applied as solutions or dispersions in aqueous or nonaqueous systems. Waxes and lipids may be applied as coatings in the molten state without the use of solvents.

Functional Mechanism: Coating agents are composed of film-forming materials that impart desirable pharmaceutical properties such as appearance, patient acceptance, and ease of swallowing. Coating agents also may serve other functional purposes such as providing a barrier against undesirable chemical reactions or untimely release of a drug from its components. After intake, the coating may dissolve by processes such as hydration, solubilization, or disintegration, depending on the nature of the material used. Enteric coatings are insoluble in acidic (low pH) media but dissolve readily in neutral pH conditions. However, most common coatings do not have pH-specific solubility. The coating thickness may vary by application and the nature of the coating agents. In the coating process, the polymer chains spread out on the core surface and coalesce into a continuous film as the solvent evaporates. Plastic polymers, waxes, and lipid-based coatings may be applied without solvents by melting and atomization. Molten fluid droplets, upon impact on the surface of the fluidized drug particles, spread and re-solidify to form film layers. Therefore, coating materials generally have the ability to form a complete and stable film around the substrate. The coating preparation typically is applied uniformly and is carefully dried to ensure that a consistent product is produced. Suitable plasticizers may be required to lower the minimum film-forming temperature of the polymer, and their potential effect on drug release should be considered.

Physical Properties: Film coating is a complex process, and the characteristics of a film-forming polymer play an important role: the particle size of colloidal dispersions varies with their origin (latex, pseudolatex, or re-dispersed powder) and may have an effect on the film-forming mechanism. Polymer solutions or dispersions with a low viscosity and high pigment-binding capacity reduce the coating time and facilitate relatively simple and cost-effective manufacturing. The concentration–viscosity relationship for the film-forming agent should be evaluated for process optimization. The surface tension of coating preparations can influence the spray pattern in the manufacturing process. The applied coating must withstand mechanical stress during coating or packaging operations. Therefore, the film should possess high elasticity and sufficient mechanical strength. It can be useful to analyze tensile properties of isolated films. For coatings

that are applied in a molten state without solvents (plastic polymers, waxes, and lipid-based coatings), melting range and melt viscosity are the properties of prime consideration.

Chemical Properties: Film-forming agents are of natural, semisynthetic, or synthetic origin and are available in different chemical grades. *NF* monographs often describe classes of polymeric materials that allow a considerable range of composition, structure, or molecular weight. These factors should be considered when pharmaceutical scientists identify and quantitate critical material attributes to ensure consistent performance.

General Chapters: The following general chapters may be useful in ensuring consistency in selected excipient functions: *Viscosity* ⟨911⟩, *Tensile Strength* ⟨881⟩, *Light Diffraction Measurement of Particle Size* ⟨429⟩, *Fats and Fixed Oils* ⟨401⟩, *Thermal Analysis* ⟨891⟩, and *Dissolution* ⟨711⟩.

Additional Information: Additives often are included in a coating formulation. Fillers (e.g., sugar alcohols, microcrystalline cellulose) may be added to increase the solids content of the coating agent without increasing viscosity. Stearic acid can be used to improve the protective function/moisture barrier of a coating. Water-soluble or -insoluble ingredients may be added to create pores in the film to adjust the release pattern of sustained-release formulations. Coloring agents (e.g., titanium dioxide, kaolin) may be added to modify appearance.

Functional Category: Plasticizer

Description: A plasticizer is a low molecular weight substance that, when added to another material—usually a polymer—makes the latter flexible, resilient, and easier to handle. Modern plasticizers are synthetic organic chemicals, the majority of which are esters such as citrates and phthalates. They are key components that determine the physical properties of polymeric pharmaceutical systems such as tablet film coatings and capsule shells.

Functional Mechanism: Plasticizers function by increasing the intermolecular and intramolecular mobility of the macromolecules that comprise polymeric materials. They achieve this by interfering with the normal intermolecular and intramolecular bonding mechanisms in such systems. The most effective plasticizers exert their effect at low concentrations, typically less than 5% w/w. Plasticizers commonly are added to film coatings (aqueous and nonaqueous systems) and capsule shells (hard and soft varieties) to improve their workability and mechanical ruggedness. Without the addition of plasticizers, such materials can split or fracture prematurely. Plasticizers also are added to semisolid pharmaceutical preparations such as creams and ointments to enhance their rheological properties.

Physical Properties: The most common plasticizers are low molecular weight (< 500 Da) solids or liquids. They typically have low melting points ($< 100^\circ$) and can be volatile (i.e., exert an appreciable vapor pressure) at ambient temperature. Plasticizers can significantly reduce the glass transition temperature of the system to which they are added.

Chemical Properties: As noted, many modern plasticizers are synthetic esters such as citrates and phthalates. Traditional pharmaceutical plasticizers include oils, sugars, and their derivatives.

General Chapters: The following general chapters may be useful in ensuring consistency in selected excipient functions: *Melting Range or Temperature* ⟨741⟩, *Water Determination* ⟨921⟩, *Organic Volatile Impurities* ⟨467⟩, *Specific Gravity* ⟨841⟩, *Refractive Index* ⟨831⟩, and *Thermal Analysis* ⟨891⟩.

Other Information: The choice of an appropriate plasticizer often is guided by reference to its "solubility parameter," which is related to its cohesive energy density. Solubility parameter values for many common materials are tabulated in standard reference texts. To ensure maximum effectiveness, the solubility parameter of the plasticizer and the polymeric system being plasticized should be matched as closely as possible.

ORAL LIQUIDS

Functional Category: pH Modifier (Acidifying/Alkalizing/Buffering Agent)

Description: The hydrogen ion concentration, $[H^+]$, in an aqueous solution is expressed as $pH = -\log(H^+)$. The pH of pure water is 7 at 25°. An aqueous solution is acidic at $pH < 7$ and alkaline at $pH > 7$. An acid may be added to acidify a solution. Similarly, a base may be used to alkalize a solution. A buffer is a weak acid (or base) and its salt. When a buffer is present in a solution, the addition of small quantities of strong acid or base leads to only a small change in solution pH. Buffer capacity is influenced by salt/acid (or base/salt) ratio and total concentration of acid (or base) and salt. The pH of pharmaceutical solutions typically is controlled using acidifying/alkalizing and buffering agents to: 1) maintain a pH close to that of relevant body fluid to avoid irritation; 2) improve drug stability that is pH dependent; 3) control equilibrium solubility of weak acids or bases; and 4) maintain consistent ionization state of molecules during chemical analysis, e.g., high-performance liquid chromatography (HPLC).

Functional Mechanism: The ionization equilibria of weak bases, weak acids, and water are the key to the functions of acidifying, alkalizing, and buffering agents. The autoprotolytic reaction of water can be expressed as

$$H_2O + H_2O \leftrightarrow H_3O^+ + OH^-$$

The autoprotolysis constant (or ion product) of water is $K_w = 1 \times 10^{-14}$ at 25° and varies significantly with temperature. Because the concentrations of hydrogen and hydroxyl ions in pure water are equal, each has the value of approximately 1×10^{-7} mole/L, leading to the neutral pH of 7 at 25°. When an acid, base, or salt of weak acid (or base) is added, the ionization equilibrium of water is shifted so that $[H^+][OH^-]$ remains constant, thus resulting a solution pH that is different from 7.

Physical Properties: The ionization equilibrium of a weak acid, HA, can be written as

$$HA + H_2O \leftrightarrow H_3O^+ + A^-$$

The ionization constant of a weak acid (or conjugate acid of a base) is commonly expressed as $pK_a = -\log(K_a)$, where $K_a = [H_3O^+][A^-]/[HA]$. A lower pK_a corresponds to a stronger acid. Similarly, the ionization constant of a weak base (or conjugate base of an acid) is expressed as $pK_b = -\log(K_b)$. The ionization equilibrium of water ($pK_a + pK_b = pK_w$) equals 14 at 25°. Buffers and pH modifiers influence solution osmolality, osmolarity, and water conductivity.

Chemical Properties: When used in chemical analysis, buffers must be chemically compatible with the reagents and test substance. Buffers, when used in physiological systems, should not interfere with pharmacological activity of the medicament or normal function of the organism.

In-Process Revision

General Chapters: The following general chapters may be useful to ensure consistency in selected pH modifier or buffering agent functions: *Water Conductivity* ⟨645⟩, *pH* ⟨791⟩, and *Osmolality and Osmolarity* ⟨785⟩.

Functional Category: Wetting and/or Solubilizing Agent

Description: Solubilizers can be used to dissolve insoluble molecules. They function by facilitating spontaneous phase transfer to yield a thermodynamically stable solution. A number of solubilizers are available commercially. Acceptable solubilizers for pharmaceutical applications have been fully evaluated in animals for safety and toxicology.

Functional Mechanism: Solubilizers comprise a variety of different chemical structures/classes. Some solubilizers may have unique chemical structures. For example, a hydrophilic moiety may be tethered with a hydrophobic moiety to yield distinct micelle shapes and morphologies in water, thus facilitating solubilization. The mechanism of solubilization often is associated with a favorable interaction of the insoluble agent and the interior core of the solubilizer assembly (e.g. micelles). In other cases, unique hydrophobic sites that are capable of forming inclusion complexes are present. Other types of solubilizers utilize a range of polymeric chains that interact with hydrophobic molecules to increase solubility by dissolving the insoluble agent into the polymeric chains.

Physical Properties: Solubilizers are solid, liquid, or waxy materials. Their physical properties depend on their chemical structures. The physical properties and performance of the solubilizers, however, depend on the surface-active properties of the solubilizers and on the hydrophilic–lipophilic balance (HLB). Solubilizers with lower HLB values behave as emulsifiers, and those with higher HLB values behave as solubilizers. For example, sodium lauryl sulfate (HLB 40) is hydrophilic and highly water soluble and, upon dispersion in water, spontaneously forms micelles.

The unique hydrophilicity and hydrophobicity properties of solubilizers are characterized by their aggregate numbers or critical micelle concentrations (CMC). The CMC value is unique to an individual solubilizer bearing hydrophilic, lipophilic, and/or hydrophobic chains. CMC is a measure of the concentration at which the surface-active molecule aggregates and solubilizes the solute by incorporating part into the hydrophobic interior and accommodating the rest in the hydrophilic exterior aqueous layer. Such interactions with the insoluble molecule further stabilize the molecules in the entire assemblies without precipitation to yield a continuous solution.

Chemical Properties: The chemical and surface-active properties depend on the structures of the solubilizers. Because of the complex nature of solute–solvent–solubilizer interactions, pharmaceutical scientists must carefully consider, identify, and control the critical material attributes of solubilizers.

General Chapters: The following general chapters may be useful to ensure consistency in selected solubilizing agent function: *Fats and Fixed Oils* ⟨401⟩, *Specific Gravity* ⟨841⟩, *pH* ⟨791⟩, *Specific Surface Area* ⟨846⟩, *Thermal Analysis* ⟨891⟩, *Spectrophotometry and Light-Scattering* ⟨851⟩, *Scanning Electron Microscopy* ⟨1181⟩, *Viscosity* ⟨911⟩, and *Light Diffraction Measurement of Particle Size* ⟨429⟩.

Functional Category: Antimicrobial Preservative

Description: Antimicrobial preservatives are used to kill or prevent growth of bacteria, yeast, and mold in the dosage form.

Functional Mechanism: Preservatives work by a variety of mechanisms to control microbes. Most of them work at the cell membrane, causing membrane damage and cell leakage. Other modes of action include transport inhibition, protein precipitation, and proton-conducting uncoupling. Some preservatives are -cidal (kill bacteria or yeast and mold); some are -static (inhibit growth of microorganisms); and others are sporicidal (kill spores). Several of the preservatives can act synergistically (e.g., combinations of parabens).

Physical Properties: Antimicrobials generally are soluble in water at concentration ranges at which they are effective. The vapor pressure of these agents is important, especially if the dosage form is intended to be lyophilized or spray dried. Several of these agents are flammable. Understanding of an excipient's partition coefficient is important because partitioning of a preservative into an oil phase will diminish the preservative's concentration in the aqueous phase, which in turn can reduce its value as a preservative.

Chemical Properties: Phenolic preservatives can undergo oxidation and color formation. Incompatibilites of preservatives (cationic and anionic mixtures, adsorption to tubes or filters, binding to surfactants and proteins) should be taken into account during product development.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Injections* ⟨1⟩, *Antimicrobial Effectiveness Testing* ⟨51⟩, *Microbial Limit Tests* ⟨61⟩, and *Antimicrobial Agents—Content* ⟨341⟩.

Other Information: Be aware of safety and labeling requirements, specifically for benzalkonium chloride (eye and skin irritation), benzoic acid and benzoate salts (risk of jaundice in newborn babies), benzyl alcohol (should not given to premature babies or neonates and may cause allergic reactions in children aged 3 years or less), bronopol (may cause skin reaction), chlorocresol (allergic reactions), organic mercury compounds (allergic reaction), parabens (allergic reactions), and sorbic acid and salts (skin reactions). Because of the risk of organic mercury toxicity, thimerosal should not be used. Use of preservative is contraindicated in parenteral products in which the fill volume is greater than 30 mL or comes in contact with cerebrospinal fluid. Antioxidants and chelating agents tend to potentiate antimicrobial efficacy.

Functional Category: Chelating and/or Complexing Agents

Description: Chelating/complexing agents form soluble complex molecules with certain metal ions (e.g., copper, iron, manganese, lead, and calcium) and essentially remove the ions from solution to minimize or eliminate their ability to react with other elements and/or to precipitate. The agents are used in pharmaceuticals (oral, parenteral, and topical formulations), cosmetics, and foods to sequester ions from solution and to form stable complexes. Chelating agents are also referred to as chelants, chelators, or sequestering agents.

Functional Mechanism: Chelating/complexing agents are used to sequester undesirable metal ions from solution. Their chemical structure acts as a ''claw'' to associate with the metal atom by forming a heterocyclic ring structure. Complexing agents function similarly but mechanistically do not (by definition) require a two-point claw structure because they can associate via one or more binding sites. All chelating agents are complexing agents, but not all complexing agents are chelating agents. As excipients, chelating agents are used as antioxidant synergists, antimicrobial synergists, and water softeners. By ''removing '' metal ions from solution, chelating agents reduce the propensity for oxidative reactions. Chelating agents also have the ability to enhance antimicrobial effectiveness by forming a metal ion deficient environment that otherwise could feed microbial growth.

Physical Properties: Chelating and complexing agents are freely soluble in water. Various salt (disodium and calcium disodium) and hydrated forms (anhydrous, dihydrate, and trihydrate) of edetic acid exist. Edetic acid and its derivatives appear as white to off-white crystalline solids. Oxyquinoline sulfate appears as a pale yellow crystalline powder. *USP–NF* recognizes that chelating/complexing agents are stable below 100°, but dehydration and/or decomposition can occur at higher temperatures. Chelating agents exhibit different degrees of hygroscopicity. Because low proportions of chelating agents are used in formulations (typically not more than 0.2%), they are not expected to significantly affect the bulk solid mechanical and flow properties of solid formulations. Because these agents are used in very low levels, their particle size distribution is important to enable acceptable dosage form content uniformity.

Chemical Properties: Chelating/complexing agents complex with metal ions via any combination of ionic and covalent bonds. Dilute aqueous solutions may be neutral, acidic, or alkaline. Edetic acid and its salts are incompatible with strong oxidizers, strong bases, and polyvalent metal ions (e.g., copper and nickel). Specific agents are selected for a formulation based on their solubility, affinity for the target metal ion, and stability. Edetate salts are more soluble than the free acid. Unlike other edetate salts and the free acid, edetate calcium disodium does not sequester calcium and therefore is preferred to prevent hypocalcemia. It is also preferred to chelate heavy metals with the release of calcium ions. Alternatively, disodium edetate can be used to treat hypercalcemia. Edetic acid will decarboxylate if heated above 150°.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Antimicrobial Effectiveness Testing* ⟨51⟩; *Microbial Enumeration Tests* ⟨61⟩; *Heavy Metals* ⟨231⟩; *Iron* ⟨241⟩; *Lead* ⟨251⟩; *Antimicrobial Agents—Content* ⟨341⟩; *Light Diffraction Measurement of Particle Size* ⟨429⟩; *Loss on Drying* ⟨731⟩; *pH* ⟨791⟩; *Water Determination* ⟨921⟩; *Biotechnology-derived Articles* ⟨1045⟩; and *Cell and Gene Therapy Products* ⟨1046⟩, *Manufacturing of Cell Therapy Products*.

Functional Category: Antioxidant

Description: This category applies to antioxidants used as in vitro stabilizers of pharmaceutical preparations to mitigate oxidative processes. Antioxidants used for their biological activity in vivo may be regarded as active ingredients with therapeutic effects and are not discussed. Antioxidants delay the onset and/or significantly reduce the rate of complex oxidative reactions that could otherwise have a detrimental impact on the drug substance. Antioxidants also can be considered for protecting nonactive components like unsaturated oils, pegylated lipids, flavors, and essential oils. Thus antioxidants preserve the overall integrity of the dosage form

against oxidative stress. Antioxidants are most effective when incorporated in the formula to prevent or delay the onset of chain reactions and to inhibit free radicals and hydroperoxides from engaging in the cascading processes described above. Effective application of antioxidants and evaluation of their efficacy necessitate an understanding of oxidative mechanisms and the nature of the by-products they generate. Autoxidation is initiated when oxygen reacts with a substrate to form highly reactive species known as free radicals ($RH \rightarrow R \cdot$). After ''initiation'' the free radicals in the presence of oxygen can trigger chain reactions ($R \cdot + O_2 \rightarrow ROO \cdot$ and $ROO \cdot + RH \rightarrow R \cdot + ROOH$) to form peroxy radicals, hydroperoxides, and new alkyl radicals that can initiate and then propagate their own chain reactions. The cascading reactions during the propagation phase can be accelerated by heat, light, and metal catalysts. In the presence of trace amounts of metal catalysts (Cu^{+}, $Cu2^{+}$, $Fe2^{+}$, and $Fe3^{+}$), hydroperoxides (ROOH) readily decompose to $RO \cdot$ and $ROO \cdot$ and can subsequently trigger reactions with the API and/or the excipients (e.g., hydrocarbons) to form hydroxyl acids, keto acids, and aldehydes that can have further undesirable effects. Note that hydroperoxides are not solely the reaction products of oxidative mechanisms within a formulation. Residual amounts of hydroperoxides can also be found in commonly used excipients like polyethylene glycols (PEG), polyvinlypyrrolidone (PVP), and polysorbates. The initiation phase generally is slow and has limited impact on the quality of the finished product. The propagation phase, in contrast, involves rapid, irreversible degradation of chemical species.

Functional Mechanism: Antioxidants can be grouped by their mode of action. Phenolic antioxidants that block free radical chain reactions are also known as true or primary antioxidants. This group consists of monohydroxy or polyhydroxy phenol compounds with ring substitutions. They have very low activation energy to donate hydrogen atom(s) in exchange for the radical electrons that are rapidly delocalized by free radicals. By accepting the radical electrons they stabilize free radicals. The reaction yields antioxidant free radicals that can also react with lipid free radicals to form other stable compounds. Thus they can block oxidative chain reactions both in the initiation and propagation stages. Because of their solubility behavior, phenolic antioxidants are most effective in protecting oils and oil-soluble actives against oxidative stress. Reducing agents generally are water-soluble antioxidants (e.g., L-ascorbic acid) with lower redox potential than the drug or the excipient they are protecting. They delay the onset and the rate of oxidative reactions by sacrificially reacting with oxygen and other reactive species. The oxygen-scavenging potential of the reducing agents may be sensitive to pH and can also be negatively affected in the presence of trace metals. Chelating agents bind with free metals (Cu+, Cu2+, Fe2+, and Fe3+) that may be present in trace amounts in the formulation. The newly formed complex ions are nonreactive. Chelating agents therefore remove the capacity of the metal catalysts to participate in oxidative reactions that occur during the propagation stage.

The utility of antioxidants can be maximized by synergistic use of one or two primary antioxidants along with reducing and chelating agents. The combined effect is often greater than the sum of the individual effects of each antioxidant (synergistic effect).

Physical Properties: Solubility of the antioxidant should be greatest in the formulation phase (oily, aqueous, or emulsion interface) where the drug substance is most soluble. The temperature at which the antioxidant decomposes is critical for autoclaved preparations where loss of antioxidant activity may occur. Stability of the antioxidant also must be considered and may be a function of pH and processing conditions. Metal ions may react with

propyl gallate to form colored complexes. At alkaline pH, certain proteins and sodium salts may bring about discoloration of *tert*-butylhydroquinone (TBHQ).

Chemical Properties: Activation energy, oxidation-reduction potential, stability at different formulation (e.g., pH), and processing (e.g., heat) conditions are important chemical properties. If the dosage form's expected shelf life depends on the antioxidant's function, the concentration must be factored in and periodically assessed to ensure a sufficient amount of antioxidant remains but does not exceed safety limits.

General Chapters: The following general chapters may be useful for assessing selected excipient antioxidant functions: *Specific Surface Area* ⟨846⟩, *Crystallinity* ⟨695⟩, *Chromatography* ⟨621⟩, *Water Determination* ⟨921⟩, *Melting Range or Temperature* ⟨741⟩, and *Iron* ⟨241⟩.

Functional Category: Sweetening Agent

Description: Sweetening agents are used to sweeten oral dosage forms and to mask unpleasant flavors.

Functional Mechanism: Sweetening agents bind to receptors on the tongue that are responsible for the sensation of sweetness. The longer the sweetener molecule remains attached to the receptor, the sweeter the substance is perceived to be. The standard for sweetness is sucrose.

Physical Properties: The primary physical properties relevant to sweeteners relate to their compatibility with the other ingredients in the formulation (e.g., acidic ingredients), processing conditions (e.g., heating), particle size and distribution, moisture content, isomerism, sweetness, and taste-masking capability. These properties may be formulation dependent.

Chemical Properties: Sweeteners can be divided into three main groups: sugars (which have a ring structure), sugar alcohols (sugars that do not have a ring structure), and artificial sweeteners. All sweeteners are water soluble. The stability of many sweeteners is affected by pH and other ingredients in the formulation. Some sweeteners may catalyze the degradation of some active ingredients, especially in liquids and in cases when the manufacturing processes involve heating.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Optical Rotation* ⟨781⟩, *Specific Rotation*; *Water Determination* ⟨921⟩; *Loss on Drying* ⟨731⟩; and *Melting Range or Temperature* ⟨741⟩.

Other Information: Products that contain aspartame must include a warning on the label stating that the product contains phenylalanine. Sugar alcohols have a glycemic index well below that of glucose. However, sorbitol is slowly metabolized to fructose and glucose, which raises blood sugar levels. Sugar alcohols in quantities generally greater than 20 g/day act as an osmotic laxative, especially when they are contained in a liquid formulation. Preservative systems should be carefully chosen to avoid incompatibility with the sweetener; some sweeteners are incompatible with certain preservatives.

SEMISOLIDS, TOPICALS, AND SUPPOSITORIES

Functional Category: Suppository Base

Description: Suppository bases are used in the manufacture of suppositories (for rectal administration) and pessaries (for vaginal administration). They can be hydrophobic or hydrophilic.

Functional Mechanism: Suppositories should melt at just below body temperature (37°), thereby allowing the drug to be released either by erosion and partition if the drug is dissolved in the base or by erosion and dissolution if the drug is suspended in the base. Hard fat suppository bases melt at approximately body temperature. Hydrophilic suppository bases also melt at body temperature and typically also dissolve or disperse in aqueous media. Thus release takes place via a combination of erosion and dissolution.

Physical Properties: The important physical characteristic of suppository bases is melting range. In general suppository bases melt between 27° and 45°. However, individual bases usually have a much narrower melting range within these temperature boundaries, typically 2°–3°. The choice of a particular melting range is dictated by the influence of the other formulation components on the melting range of the final product.

Chemical Properties: Hard fat suppository bases are mixtures of semisynthetic triglyceride esters of longer-chain fatty acids. They may contain varying proportions of mono- and di-glycerides and may also contain ethoxylated fatty acids. They are available in many different grades that are differentiated by melting range, hydroxyl number, acid value, iodine value, solidification range, and saponification number.

Hydrophilic suppository bases are mixtures of hydrophilic semisolid materials that in combination are solid at room temperature and yet release the drug by melting, erosion, and dissolution when administered to the patient. Hydrophilic suppository bases have much higher levels of hydroxyl groups or other hydrophilic groups than do hard fat suppository bases. Polyethylene glycols that show appropriate melting behavior are examples of hydrophilic suppository bases.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Fats and Fixed Oils* ⟨401⟩, *Congealing Temperature* ⟨651⟩, *Melting Range or Temperature* ⟨741⟩, and *Pharmaceutical Dosage Forms* ⟨1151⟩.

Other Information: Some materials included in suppositories based on hard fats have much higher melting ranges. These materials typically are microcrystalline waxes that help stabilize molten suspension formulations. Suppositories may also be manufactured from glycerinated gelatin.

Functional Category: Suspending and/or Viscosity-increasing Agent

Description: Suspending and/or viscosity-increasing agents are used in pharmaceutical formulations to stabilize disperse systems (e.g., suspensions or emulsions), to reduce the rate of solute or particulate transport, or to decrease the fluidity of liquid formulations.

Functional Mechanism(s): A number of mechanisms contribute to the dispersion stabilization or viscosity-increasing effect of these agents. The most common is the increase in viscosity–due to the entrapment of solvent by macromolecular chains or clay platelets—and the disruption of laminar flow. Other mechanisms include gel formation via a three-dimensional network of excipient molecules or particles throughout the solvent continuum and steric stabilization wherein the macromolecular or mineral component in the dispersion medium adsorbs to the surfaces of particles or droplets of the dispersed phase. The latter two mechanisms increase formulation stability by immobilizing the dispersed phase.

Physical Properties: Each of the mechanisms—increased viscosity, gel formation, or steric stabilization—is a manifestation of the rheological character of the excipient.

Because of the molecular weights and sizes of these excipients, the rheological profiles of their dispersions are non-Newtonian. Dispersions of these excipients display viscoelastic properties. The molecular weight distribution and polydispersity of the macromolecular excipients in this category are important criteria for their characterization.

Chemical Properties: The majority of the suspending and/or viscosity-increasing agents are (a) hydrophilic carbohydrate macromolecules (acacia, agar, alginic acid, carboxymethylcellulose, carrageenans, dextrin, gellan gum, guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hypromellose, maltodextrin, methylcellulose, pectin, propylene glycol alginate, sodium alginate, starch, tragacanth, and xanthan gum) and (b) noncarbohydrate hydrophilic macromolecules, including gelatin, povidone carbomers, polyethylene oxide, and polyvinyl alcohol. Minerals (e.g., attapulgite, bentonite, magnesium aluminum silicate, and silicon dioxide) comprise the second-largest group of suspending and/or viscosity-increasing agents. Aluminum monostearate is the one nonmacromolecular, non-mineral excipient in this functional category. It consists chiefly of variable proportions of aluminum monostearate and aluminum monopalmitate.

General Chapter: The following general chapter may be useful to ensure consistency in selected excipient functions: *Viscosity* ⟨911⟩.

Functional Category: Ointment Base

Description: An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. An ointment base is the major component of an ointment and controls its physical properties.

Functional Mechanism: Ointment bases serve as vehicles for topical application of medicinal substances and also as emollients and protective agents for skin.

Physical Properties: Ointment bases are liquids with a relatively high viscosity so that solids can be suspended as a stable mixture.

Ointment bases are classified as: (a) oleaginous ointment bases that are anhydrous, do not absorb water readily, are insoluble in water, and are not removable by water (e.g., petrolatum); (b) absorption ointment bases that are anhydrous and absorb some water but are insoluble in water and are not water removable (e.g., lanolin); (c) emulsion ointment bases that are water-in-oil or oil-in-water emulsions and are hydrous, absorb water, and are insoluble in water (e.g., creams of water, oils, waxes, and/or paraffins); and (d) water-soluble ointment bases that are anhydrous and absorb water and are soluble in water and are water removable (e.g., polyethylene glycol).

Chemical Properties: Ointment bases are selected to be inert and chemically stable.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Viscosity* ⟨911⟩ and *Congealing Temperature* ⟨651⟩.

Functional Category: Stiffening Agent

Description: A stiffening agent is an agent or a mixture of agents that increases the viscosity or hardness of a preparation, especially in ointments and creams.

Functional Mechanism: In general, stiffening agents are high melting point solids that increase the melting point of ointments or increase the consistency or body of

creams. Stiffening agents can be either hydrophobic (e.g., hard fat or paraffin) or hydrophilic (e.g., polyethylene glycol, high molecular weight).

Physical Properties: The primary physical property relevant to stiffening agents is their high melting point or melting range. Typical melting ranges for stiffening agents range from 43° to 47° (cetyl esters wax), 53° to 57° (glyceryl distearate), 69° to 74° (glyceryl behenate), and 85° to 88° (castor oil, hydrogenated).

Chemical Properties: Stiffening agents comprise a diverse group of materials that include glycerides of saturated fatty acids, solid aliphatic alcohols, esters of saturated fatty alcohols and saturated fatty acids, saturated hydrocarbons, blends of fatty alcohols and a polyoxyethylene derivative of a fatty acid ester of sorbitan, and higher ethylene glycol polymers.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Melting Range or Temperature* ⟨741⟩, *Congealing Temperature* ⟨651⟩, and *Viscosity* ⟨911⟩.

Other Information: Some of the materials included as stiffening agents increase the water-holding capacity of ointments (e.g., petrolatum) or function as co-emulsifiers in creams. Examples include stearyl alcohol and cetyl alcohol.

Functional Category: Emollient

Description: Emollients are excipients used in topical preparations to impart lubrication, spreading ease, texture, and softening of the skin and to counter the potentially drying/irritating impact of surfactants on the skin.

Functional Mechanism: Emollients help form a protective film and maintain the barrier function of the epidermis. Their efficacy may be described by three mechanisms of action: protection against the delipidizing and drying effects of surfactants, humectancy due to occlusion (by providing a layer of oil on the surface of the skin, emollients slow water loss and thus increase the moisture-retention capacity of the stratum corneum), and lubricity, adding slip or glide to the preparation.

Physical Properties: Emollients impart one or more of the following attributes to a pharmaceutical preparation: spreading capacity, pleasant feel to the touch, softness of the skin, and indirect moisturization of the skin by preventing trans-epidermal water loss.

Chemical Properties: Emollients are either oils or are derived from components of oils as esters of fatty acids. Depending on the nature of its fatty acid ester, an emollient may be liquid, semisolid, or solid at room temperature. Generally, the higher the molecular weight of the fatty acid moiety (carbon chain length) the richer the feel and softness of the touch. Fluidity generally is imparted by shorter chain length and higher degree of unsaturation in the fatty acid moiety. The degree of branching of ester bonds also influences the emollient properties.

General Chapter: The following general chapter may be useful to ensure consistency in selected excipient functions: *Fats and Fixed Oils* ⟨401⟩.

PARENTERALS

Functional Category: Pharmaceutical Water

Description: Water is used as a solvent, vehicle, diluent, or filler for many drug products, especially those supplied in liquid form. These can include injectible drugs, ophthalmic drugs, oral solutions, inhalation solutions, and others. Water is also a vehicle for buffers and antimicrobial agents and is a volume expander for infusion solu-

In-Process Revision

tions. Its use in dosage form preparation also can include granulation preparation for solid oral dosage forms and applications in the preparation of ointments and gels.

USP includes monographs for eight grades of pharmaceutical waters. One of these types of USP water is always the water of choice when pharmaceutical scientists prepare a pharmaceutical dosage form for human or animal use. However, USP also contains references to other types of water, such as distilled water, deionized water, and others according to specific use as summarized in general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.

Functional Mechanism: A solvent is able to dissolve materials because it is able to disrupt the intermolecular attractive forces and to allow the individual molecules to become dispersed throughout the bulk solvent. Water is a favored solvent and vehicle in the majority of applications because it is easy to handle, safe, and inexpensive.

Physical Properties: Water is liquid at normal temperature and pressure. It forms ice at the freezing temperatures of 0°; or lower, and it vaporizes at a normal boiling temperature of 100°, depending upon atmospheric pressure. Vaporized water in the form of steam is used for sterilization purposes because the latent heat of steam is significantly higher than that of boiling water.

Chemical Properties: Water in its pure form is neutral in pH and has very low conductivity and total organic carbon (TOC). However, pH, conductivity, and TOC are affected by storage conditions and exposure of water to gases in the air. Exposure of water to atmospheric CO_2 lowers the pH of water. Storage of water in plastic containers may increase the TOC content of water over time. Water stored in glass containers may result in an increase in pH and conductivity of water over time.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Injections* ⟨1⟩, *Water for Pharmaceutical Purposes* ⟨1231⟩, *Water for Health Applications* ⟨1230⟩, *Bacterial Endotoxins Test* ⟨85⟩, *Total Organic Carbon* ⟨643⟩, and *Water Conductivity* ⟨645⟩.

Functional Category: Diluent

Description: Diluents or bulking agents used in lyophilized pharmaceuticals include various saccharides, sugar alcohols, amino acids, and polymers. The primary functions of bulking agents are to provide a pharmaceutically elegant lyophized cake with non-collapsed structural integrity and to prevent drug loss due to blow-out. In addition, bulking agents are selected to facilitate efficient drying and to provide a physically and chemically stable formulation matrix. Frequently, complementary combinations of bulking agents are used to improve functionality.

So-called ''good cake forming'' excipients, such as mannitol, are frequently used because they tend to crystallize during freezing, thereby allowing efficient drying and the formation of a structurally robust cake. For some active ingredients, crystallization during lyophilization helps improve stability. Therefore the use of bulking agents that promote crystallization during lyophilization is important. Amino acids and cosolvents have been used to achieve this effect. Most biopolymer active ingredients remain amorphous upon freeze-drying, and bulking agents such as disaccharides may function as lyoprotectants by helping to maintain a stable amorphous phase during freezing and drying to prevent denaturation. Solubility enhancement of an insoluble crystalline active ingredient is sometimes achieved with the use of a biopolymer that enhances solubility or prevents crystallization of the active ingredient during lyophilization or

subsequent reconstitution. Bulking agents are also selected on the bases of biocompatiability, buffering capability, and tonicity-modifying properties.

Functional Mechanisms: A bulking agent that readily crystallizes during lyophilization helps maintain the structural integrity of the cake formed during primary drying, thereby preventing macroscopic collapse and pharmaceutical inelegance. Microscopic collapse of amorphous components in the formulation may still occur (with some potentially undesirable results) but will not result in macroscopic collapse if the bulking agent properties and concentration are adequate. The bulking agent also should possess a high eutectic melting temperature with ice to permit relatively high primary drying temperatures with commensurate rapid and efficient drying.

Lyoprotectant properties of lyophilization diluents (i.e., those that protect the drug substance during lyophilization) typically are achieved by the formation of a highly viscous glassy phase that includes the biopolymer drug substance in combination with low molecular weight amorphous saccharides such as sucrose, trehalose, or certain amino acids. A typical approach for protein pharmaceutical formulation is to combine a sugar alcohol that readily crystallizes and an amorphous diluent; this mixture acts as a lyoprotectant.

Physical Properties: Bulk agents are dissolved in aqueous solution before lyophilization. Therefore chemical purity and the absence of bioburden and pyrogenic materials are essential properties of the bulk excipient. However, the physical form and particle properties of the bulk excipient are generally not relevant to the final properties of the lyophilized formulation.

The physical properties that are essential to product performance during and after lyophilization include the glass transition temperature of the amorphous frozen concentrate before drying, the glass transition temperature of the final dried formulation cake, and the eutectic melting temperature of the crystalline bulking agent with ice. The glass transition temperature of the formulation depends on the glass transition temperatures of the individual components, concentrations, and interactions. Although approximations can be made based on reported transition temperatures for individual components, current practice includes the measurement of formulation glass transition temperatures by thermal analysis or freeze-drying microscopy.

The physical states of the bulking agent during and after lyophilization are important physical properties. Both formulation composition and processing parameters play roles in determining whether the bulking agent is amorphous or takes a specific crystalline form. For example, although mannitol is easily crystallized during lyophilization, it can also be amorphous based on formulation composition or can crystallize as a hydrate or metastable polymorph. Rate of freezing, drying temperatures, and annealing are among the important process parameters used to control the physical state of the formulation and its components. Moisture retention and adsorption after lyophilization also may contribute to formulation stability and performance.

Chemical Properties: Reactivity of the bulking agent with respect to other formulation components, especially the active ingredient, may be critical. Reducing sugars are well known to react with aromatic and aliphatic amines. Glycols may contain trace peroxide levels that can initiate oxidative degradation. The ability of saccharides and polyhydric alcohols to form hydrogen bonds to biopolymers may play a role in their lyoprotection effects.

General Chapters: The following general chapters may be useful to ensure consistency in selecting bulking agent functions: *Injections* ⟨1⟩; *Biotechnology-Derived Articles* ⟨1045⟩, *Product Formulation*; *Crystallinity* ⟨695⟩; *Crystallin-*

ity Determination by Solution Calorimetry ⟨696⟩; *Pharmaceutical Dosage Forms* ⟨1151⟩; and *Water—Solid Interactions in Pharmaceutical Systems* ⟨1241⟩.

Functional Category: Tonicity Agent

Description: To avoid crenation or hemolysis of red blood cells and to mitigate pain and discomfort if solutions are injected or introduced into the eyes and nose, solutions should be made isotonic. This requires that the effective osmotic pressure of solutions for injection is approximately the same as that in the blood. When drug products are prepared for administration to membranes such as eyes or nasal or vaginal tissues, solutions should be made isotonic with respect to these tissues.

Functional Mechanism: Tonicity is equal to the sum of the concentrations of the solutes that have the capacity to exert an osmotic force across a membrane and thus reflects overall osmolality. Tonicity applies to the impermeant solutes within a solvent–in contrast to osmolarity, which takes into account both permeant and impermeant solutes. For example, urea is a permeant solute, meaning that it can pass through the cell membrane freely and is not factored when determining the tonicity of a solution. In contrast, NaCl is impermeant and cannot pass through a membrane without the help of a concentration gradient and will therefore contribute to a solution's tonicity.

Physical Properties: Solutions of sodium chloride, dextrose, and Lactated Ringer's are common examples of pharmaceutical preparations that contain tonicity agents. Not all solutes contribute to the tonicity, which in general depends only on the number of solute particles present in a solution, not the kinds of solute particles. For example, mole for mole, sodium chloride solutions display a higher osmotic pressure than do glucose solutions of the same molar concentration. This is because when glucose dissolves it remains one particle, but when NaCl dissolves, it becomes two particles: Na^+ and Cl^- .

Chemical Properties: Tonicity agents may be present as ionic and/or nonionic types. Examples of ionic tonicity agents are alkali metal or earth metal halides such as $CaCl_2$, KBr, KCl, LiCl, NaI, NaBr or NaCl, Na_2SO_4, or boric acid. Non-ionic tonicity agents include glycerol, sorbitol, mannitol, propylene glycol, or dextrose.

General Chapters: The following general chapters may be useful to ensure ensure consistency in selected excipient functions: *Injections* ⟨1⟩; *Biotechnology-Derived Articles* ⟨1045⟩, *Product Formulation*; *Pharmaceutical Dosage Forms* ⟨1151⟩, *Ophthalmic Preparations*; and *Pharmaceutical Calculations in Prescription Compounding* ⟨1160⟩.

AEROSOLS

Functional Category: Propellant

Description: Propellants are compounds that are gaseous under ambient conditions. They are used in pharmaceuticals (nasal sprays and respiratory and topical formulations), cosmetics, and foods to provide force to expel contents from a container.

Functional Mechanism: Propellant substances are low boiling point liquids that are relatively inert toward active ingredients and excipients. They can be characterized by three properties: whether they form a liquid phase at ambient temperatures and useful pressures, their solubility and/or miscibility in the rest of the formulation, and their flammability. Their performance is judged by their ability to provide adequate and predictable pressure throughout the usage life of the product.

Propellants that have both a liquid and gas phase in the product provide consistent pressures as long as there is liquid phase present—the pressure in the headspace is maintained by the equilibrium between the two phases. In contrast, the pressure provided by propellants that have no liquid phase may change relatively rapidly as the contents of the container are expelled. As the headspace becomes larger, the pressure within the container falls proportionately. Propellants that have no liquid phase but have significant pressure-dependent solubility in the rest of the formulation have performance characteristics between the other two systems. In such cases, as the headspace increases, the propellant comes out of solution to help to maintain the pressure of the system.

In metered-dose inhalers the propellant has a liquid phase that is an integral part of the dispensed pharmaceutical product. Actuating the metering valve dispenses a defined volume of the liquid contents. The propellant spontaneously boils and provides atomizing and propulsive force. A predictable change in active concentration occurs from the beginning to the end of the container life cycle as the liquid phase of the propellant vaporizes to reestablish the equilibrium pressure of the system as the headspace increases.

Physical Properties: Propellants have boiling points well below ambient temperatures. Density and solubility properties are significant considerations when one selects a propellant. Apaflurane and norflurane have liquid-phase densities that are greater than that of water. Hydrocarbon propellants (butane, isobutene, and propane) and dimethyl ether have liquid-phase densities that are less than that of water.

Chemical Properties: Propellants typically are stable materials that contribute to long shelf lives of formulations. However, the hydrocarbon propellants (butane, isobutene, and propane) and dimethyl ether are all flammable materials. Apaflurane, carbon dioxide, nitrogen, and norflurane are nonflammable. Nitrous oxide is not flammable but supports combustion. Chlorofluorocarbon propellants are considered to be ozone-depleting substances. Their use in foods, drugs, devices, or cosmetics is regulated by 21 CFR 2.125. Albuterol metered-dose inhalers formulated with chlorofluorocarbon propellants have not been available in the United States since January 1, 2009.

General Chapters: The following general chapters may be useful to ensure consistency in selected excipient functions: *Aerosols, Nasal Sprays, Metered-dose Inhalers, and Dry Powder Inhalers* ⟨601⟩, *Chromatography* ⟨621⟩, and *Water Determination* ⟨921⟩.■2S (*USP33*)

BRIEFING

⟨**1072**⟩ **Disinfectants and Antiseptics,** *USP 32* page 517. On the basis of internal deliberations of the Expert Committee, changes are proposed to enhance clarity.

(MSA: R. Tirumalai.) RTS—C77452

Change to read:

CLASSIFICATION OF DISINFECTANTS

Chemical disinfectants are classified by their chemical type. This includes aldehydes, alcohols, halogens, peroxides, quaternary ammonium compounds, and phenolic compounds (see *Table 2*).

Table 2. General Classification of Antiseptics, Disinfectants, and Sporicidal Agents

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% Isopropyl alcohol, 70% alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite
Phenolics	General purpose disinfectant	500 μg per g Chlorocresol, 500 μg per g chloroxylenol
Ozone	Sporicidal agent	8% Gas by weight

In-Process Revision

Table 2. General Classification of Antiseptics, Disinfectants, and Sporicidal Agents *(Continued)*

Chemical Entity	Classification	Example
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 μg per g H_2O_2 vapor, 10%–25% solution, 3% solution
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 μg per g peracetic acid
Ethylene oxide	Vapor-phase sterilant	600 μg per g Ethylene oxide
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	~~200 μg per g~~ ■Concentration dependent on application, ■2S *(USP33)* Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 μg per g β-Propiolactone

The effectiveness of a disinfectant depends on its intrinsic biocidal activity, the concentration of the disinfectant, the contact time, the nature of the surface disinfected, the hardness of water used to dilute the disinfectant, the amount of organic materials present on the surface, and the type and the number of microorganisms present. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Environmental Protection Agency (EPA) registers chemical disinfectants marketed in the United States and requires manufacturers to supply product information on the use dilution, type of microorganisms killed, and the necessary contact time. Certain liquid chemical sterilizers intended for use on critical or semicritical medical devices are defined and regulated by the U.S. Food and Drug Administration (FDA).

Change to read:

SELECTION OF A DISINFECTANT FOR USE IN A PHARMACEUTICAL MANUFACTURING ENVIRONMENT

When selecting a disinfectant for use in a pharmaceutical manufacturing area, the following points should be considered: the number and types of microorganisms to be controlled; the spectrum of activity of commercially available disinfectants; ~~the reputation of the disinfectant supplier;~~

■■2S *(USP33)*
the claims as a sterilant; the disinfectant or sanitizer supported by the EPA registrations; the concentration, application method, and contact time of the disinfectant; the nature of the surface material being disinfected and its compatibility with the disinfectant; the amount of organic compounds on the surface that may inactivate a disinfectant; the possible need to maintain a residual bactericidal activity of the disinfectant on the surface; the corrosiveness of the disinfectant to equipment with repeated application; the safety considerations for operators applying the disinfectant; the compatibility of the disinfectant with cleaning agents and other disinfectants; the planned disinfectant rotation; and the steps that need to be taken to avoid the contamination of pharmaceutical products by a disinfectant.[2]

Change to read:

THEORETICAL DISCUSSION OF DISINFECTANT ACTIVITY

Plots of the log of the number of microorganisms per mL surviving in a disinfectant solution indicate that first-order kinetics can be applied as a gross approximation to the reduction in microbial count with respect to time. In practice, the plots show a more sigmoid curve with a slower initial reduction in numbers followed by an increasing rate with respect to time.

The rate constant, *K,* for the disinfection process can be calculated by the formula:

$$(1 / t)(\log N_O / N)$$

in which t is the time, in minutes, for the microbial count to be reduced from N_O to N; N_O is the initial number of organisms, in cfu per mL; and N is the final number, in cfu per mL, of organisms.

As with a first-order chemical reaction, the same concentration of disinfectant reduces the number of organisms more rapidly at elevated temperatures. This can be expressed as a temperature, *T,* coefficient per 10° rise in temperature, Q_{10}, calculated by the formula:

$$\text{Time to decontamination at } T^\circ / \text{Time to decontamination at } T$$

in which T is $T^\circ - 10$.

Further evidence that a first-order reaction is an inadequate description of disinfection is that the Q_{10} values for chemical and enzyme reactions are 2 to 3, while the common disinfectants phenol and alcohol have a Q_{10} of 4 and 45, respectively.

Critical to the successful employment of disinfectants is an understanding of the effect of disinfectant concentration on microbial reduction. A plot of the log of the time to reduce the microbial population in a standard inoculum to zero against the log of the disinfectant concentration is a straight line with the slope of the line termed the concentration exponent, *n*. The relationship can be expressed as follows:

$$n = (\text{log of the kill time at concentration } C_2) - (\text{log of the kill time at concentration } C_1) / (\log C_1 - \log C_2)$$

in which C_1 and C_2 are the higher and lower disinfectant concentrations, respectively.

The wide differences in concentration exponents, *n,* have practical consequences in picking the use dilution of different disinfectants and in using dilution to neutralize a disinfectant in disinfectant-effectiveness testing and routine microbial monitoring of the manufacturing environment. For example, mercuric chloride has a concentration exponent of 1, so a 3-fold dilution will reduce the disinfectant activity by 3^1 (or by one-third), while phenol with a concentration exponent of 6 will have a 3^6 (or a 729-fold) reduction in disinfectant activity. Disinfectants with a larger concentration exponent or dilution coefficient rapidly lose activity when diluted. The concentration exponents for some disinfectants are listed in *Table 3*.

[2] Denny, V.F.; Marsik, F.J. Current Practices in the Use of Disinfectants within the Pharmaceutical Industry. PDA J. of Pharmaceutical Sci. and Tech., 1997, 51, (6), 227–228.

Table 3. Concentration Exponents of Common Antiseptics, Disinfectants, and Sterilants

Disinfectant	Concentration Exponents
Hydrogen peroxide	0.5
Sodium hypochlorite	0.5
Mercuric chloride	1
Chlorhexidine	2
Formaldehyde	1
Alcohol	9
Phenol	6
Quaternary ammonium compounds	0.8 to 2.5
Aliphatic alcohols	6.0 to 12.7
Phenolic compounds	4 to 9.9

Another important consideration may be the pH of the disinfectant. Many disinfectants are more active in the ionized form, while others are more active in the nonionized form. The degree of ionization will depend on the pK_a of the agent and the pH of the disinfection environment. For example, phenol, with a pK_a of 10, will be more effective at a pH below 7 where it is nonionized.~~, while acetic acid will be more effective at a pH below 4 where it is ionized~~

■■2S (*USP33*)

Change to read:

MECHANISM OF DISINFECTANT ACTIVITY

Table 4 lists the sites and modes of action of some representative disinfectants.

Table 4. Mechanism of Disinfectant Activity Against Microbial Cells

Target	Disinfectant
Cell wall	Formaldehyde, hypochlorite, and ~~mercurials~~ ■glutaraldehyde■2S (*USP33*)
Cytoplasmic membrane, action on membrane potential	Anilides and hexachlorophene
Membrane enzymes, action on electron-transport chain	Hexachlorophene
Action on ATP	Chlorhexidine and ethylene oxide
Action on enzymes with –SH groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, hypochlorite, ~~iodine, and mercurials~~ ■and iodine■2S (*USP33*)
Action on general membrane permeability	Alcohols, chlorhexidine, and quaternary ammonium compounds
Cell contents, general coagulation	Chlorhexidine, aldehydes, ~~hexachlorophene,~~ ■■2S (*USP33*) and quaternary ammonium compounds
Ribosomes	Hydrogen peroxide ~~and mercurials~~ ■■2S (*USP33*)
Nucleic acids	Hypochlorites

Table 4. Mechanism of Disinfectant Activity Against Microbial Cells *(Continued)*

Target	Disinfectant
Thiol groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, and hypochlorite~~, mercurials~~ ■■2S (*USP33*)
Amino groups	Ethylene oxide, glutaraldehyde, and hypochlorite
General oxidation	~~Ethylene oxide, glutaraldehyde, and hypochlorite~~ ■Hypochlorite■2S (*USP33*)

Change to read:

MICROBIAL RESISTANCE TO DISINFECTANTS

The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely

■to occur at significant levels,■2S (*USP33*) as disinfectants are more powerful biocidal agents than antibiotics. ~~and are~~

■In addition, they are normally■2S (*USP33*) applied in high concentrations against low populations of microorganisms usually not growing actively, so the selective pressure for the development of resistance is less profound. However, the most frequently isolated microorganisms from an environmental monitoring program may be periodically subjected to use-dilution testing with the agents used in the disinfection program to confirm their susceptibility,

■as there are real differences among different species in resistance to the lethal effects of different sanitizers.■2S (*USP33*)

Change to read:

DISINFECTANT CHALLENGE TESTING

Under FIFRA, the EPA requires companies that register public health antimicrobial pesticide products including disinfectants, sanitization agents, sporicidal agents, and sterilants to ensure the safety and effectiveness of their products before they are sold or distributed. Companies registering these products must address the chemical composition of their product, include toxicology data to document that their product is safe if used as directed on the label, include efficacy data to document their claims of effectiveness against specific organisms and to support the directions for use provided in the labeling, and provide labeling that reflects the required elements for safe and effective use. While these directions provide valuable information, they may not be helpful in terms of the products' use as disinfectants in a manufacturing environment.

In the United States, the official disinfectant testing methods are published by AOAC International[3] and include the Phenol-Coefficient Test, Use-Dilution Method Test, Hard Surface Carrier Method, and Sporicidal Carrier Test. A scientific study submit-

[3] AOAC International Official Methods of Analysis, 15th, 16th, and 17th editions. Arlington, VA.

In-Process Revision

ted for EPA review in support of disinfectant registration must be conducted at a laboratory facility that follows the Good Laboratory Practices (GLP) regulations (21 CFR 58). To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests: (1) use-dilution tests (screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates); (2) surface challenge tests (using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to surfaces at the selected use concentration with a specified contact time, and determining the log reduction of the challenge microorganisms); and (3) a statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of a new disinfectant. This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated, and the EPA registration requirements do not address how disinfectants are used in the pharmaceutical, biotechnology, and medical device industries. For the surface challenge tests, the test organisms are enumerated using swabs, surface rinse, or contact plate methods. Neutralizers that inactivate the disinfectants should be included in either the diluent or microbiological media used for microbial enumeration or both. ~~(see *Table 5*). Additional information~~

■Information ■2S *(USP33)*
on disinfectant neutralization may be found in *Validation of Microbial Recovery from Pharmacopeial Articles* ⟨1227⟩.

~~Table 5. Neutralizing Agents for Common Disinfectants~~

~~Disinfectant~~	~~Neutralizing Agent~~
~~Alcohols~~	~~Dilution or polysorbate 80~~
~~Glutaraldehyde~~	~~Glycine and sodium bisulfite~~
~~Sodium hypochlorite~~	~~Sodium thiosulfate~~
~~Chlorhexidine~~	~~Polysorbate 80 and lecithin~~
~~Mercuric chloride and other mercurials~~	~~Thioglycolic acid~~
~~Quaternary ammonium compounds~~	~~Polysorbate 80 and lecithin~~
~~Phenolic compounds~~	~~Dilution or polysorbate 80 and lecithin~~

~~Universal neutralizer broths may be formulated to contain a range of neutralizing agents. For example, Dey/Engley (D/E) broth contains 0.5% polysorbate 80, 0.7% lecithin, 0.1% sodium thioglycolate, 0.6% sodium thiosulfate, 0.25% sodium bisulfite, 0.5% tryptone, 0.25% yeast extract, and 1.0% dextrose; letheen broth contains 0.5% polysorbate 80, 0.07% lecithin, 1.0% peptamin, 0.5% beef extract, and 0.5% sodium chloride; and Tryptone–Azolectin–Tween (TAT) broth-base + tween 20 contains 4.0% (v/v) polysorbate 20, 0.5% lecithin, and 2.0% tryptone.~~

■The disinfectant efficacy test must have realistic acceptance criteria. ■2S *(USP33)*
In practice, sufficient organisms need to be inoculated on a 2-inch × 2-inch square of the surface being decontaminated, i.e., a coupon, to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a predetermined contact time (i.e., 10 minutes over and above the recovery observed with a control disinfectant application). The efficacy of the neutralizers and their ability to recover inoculated microorganisms from the material should be demonstrated during the use-dilution or surface-challenge studies. Points to remember are that disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers that are found in clean rooms (see *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* ⟨1116⟩); that inocula from the log growth phase that are typically employed in laboratory tests are more resistant, with the exception of spores formed during the static phase, than those from a static or dying culture or stressed organisms in the environment; and that microorganisms may be physically removed during actual disinfectant application in the manufacturing area.

Although not all inclusive, typical challenge organisms that may be employed are listed in ~~*Table 6*~~ ■*Table 5*. ■2S *(USP33)*

~~Table 6~~

■Table 5. ■2S *(USP33)*
Typical Challenge Organisms

AOAC Challenge Organisms	Typical Environmental Isolates
Bactericide: *E.coli,* ATCC 11229; *S. aureus,* ATCC 6538; *P. aeruginosa,* ATCC 15442	Bactericide: *M.luteus*, *S.epidermidis*, *Coynebacterium jeikeium*, *P.vesicularis*
Fungicide: *C. albicans,* ATCC 10231or 2091*Penicillium chrysogenum*, ATCC 11709;*A.brasiliensis*, ATCC 16404	Fungicide: *P. chrysogenum, A. brasiliensis*
Sporicide: *B. subtilis,* ATCC 19659	Sporicide: *B. sphaericus*, *B. thuringiensis*

Because a wide range of different materials of construction are used in clean rooms and other controlled areas, each material needs to be evaluated separately to validate the efficacy of a given disinfectant. ~~*Table 7*~~

■*Table 6* ■2S *(USP33)*
contains a list of common materials used in clean room construction.

~~Table 7~~

■Table 6. ■2S *(USP33)*
Typical Surfaces to be Decontaminated by Disinfectants in a Pharmaceutical Manufacturing Area

Material	Application
Stainless steel 304L and 316L grades	Work surfaces, filling equipment, and tanks
Glass	Windows and vessels
Plastic, vinyl	Curtains
Plastic, polycarbonate	Insulation coating
Lexan® (plexiglass)	Shields
Epoxyl-coated gypsum	Walls and ceilings
Fiberglass-reinforced plastic	Wall paneling
Tyvek®	Equipment wraps
Terrazzo tiles	Floors

Change to read:

DISINFECTANTS IN A CLEANING AND SANITIZATION PROGRAM

The selection of suitable disinfectants and the verification of their effectiveness in surface challenge testing is critical in the development of a cleaning and sanitization program.

Issues associated with the successful implementation of such a program are the development of written procedures, staff training, decisions on disinfectant rotation, institution of application methods and contact times, environmental monitoring to demonstrate efficacy, and personnel safety.

The cGMP 21 CFR 211.67, *Equipment Cleaning and Maintenance,* details the requirements for written procedures for cleaning, maintenance, and sanitization of pharmaceutical manufacturing equipment. These procedures should address the assignment of responsibility, establishment of schedules, details of cleaning operations, protection of clean equipment prior to use, inspection for cleanliness immediately prior to use, and maintenance of cleaning and sanitization records.

Staff involved in disinfection require training in microbiology, industry practices for cleaning and sanitization, safe handling of concentrated disinfectants, the preparation and disposal of disinfectants, and appropriate application methods. It should be emphasized that the preparation of the correct dilutions is critical because many disinfectant failures can be attributed to use of disinfectant solutions that are too dilute. Typically disinfectants used in aseptic processing and filling areas are diluted with Sterile Purified Water, and are prepared aseptically. Alternately, the disinfectant may be diluted with Purified Water, and then sterile filtered to eliminate microorganisms that may potentially persist in a disinfectant. Diluted disinfectants must have an assigned expiration dating justified by effectiveness studies.

~~Because it is theoretically possible that the selective pressure of the continuous use of a single disinfectant could result in the presence of disinfectant-resistant microorganisms in a manufacturing area, in some quarters the rotation of disinfectants has been advocated. However, the literature supports the belief that the exposure of low numbers of microorganisms on facility and equipment surfaces within a clean room where they are not actively proliferating will not result in the selective pressure that may be seen with the antibiotics.~~

■The rotation of an effective disinfectant with a sporicide is encouraged.■2S (*USP33*)
It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent. The daily application of sporicidal agents is not generally favored because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data. Disinfectants applied on potential product contact surfaces are typically removed with 70% alcohol wipes. The removal of residual disinfectants should be monitored for effectiveness as a precaution against the possibility of product contamination.

The greatest safety concerns are in the handling of concentrated disinfectants and the mixing of incompatible disinfectants. For example, concentrated sodium hypochlorite solutions (at a concentration of more than 5%) are strong oxidants and will decompose on heating, on contact with acids, and under the influence of light, producing toxic and corrosive gases including chlorine. In contrast, dilute solutions (at a concentration of less than 0.5%) are not considered as hazardous. Under no circumstances should disinfectants of different concentrations be mixed. Material Safety Data Sheets for all the disinfectants used in a manufacturing area should be available to personnel handling these agents. Appropriate safety equipment such as face shields, safety glasses, gloves, and uniforms must be issued to personnel handling the disinfectant preparation, and personnel must be trained in the proper use of this equipment. Safety showers and eye wash stations must be situated in the work area where disinfectant solutions are prepared.

BRIEFING

⟨**1086**⟩ **Impurities in Official Articles,** *USP 32* page 546. As a result of comments received and discussions by the Monograph Redesign Committee and the General Chapters Expert Committee, it is proposed to incorporate terminology changes to align with the monograph redesign process. Discussions from the international harmonization process have been considered. Other minor changes are also introduced.

(GC: A. Hernandez-Cardoso) RTS—C77480

Change to read:

⟨1086⟩ IMPURITIES IN ~~OFFICIAL ARTICLES~~

■DRUG SUBSTANCES AND DRUG PRODUCTS■2S (*USP33*)

Change to read:

INTRODUCTION

This general information chapter ~~covers impurities or degradation products in drug substances and degradation products in drug products~~

■is intended to provide common terminology for impurities and degradation products that may be present in compendial drug substances and drug products.■2S (*USP33*)
Impurities or degradation products in drug substances can arise during the manufacturing process or during storage of the drug substance. The degradation products in drug products can arise from drug substances or reaction products of the drug substance with

■the environment, with■2S (*USP33*)
an excipient or ~~an immediate container–closure system~~

■as a result of the interaction with the container–closure system materials that are in intimate contact with the product.■2S (*USP33*)
Biological and biotechnological

■products, fermentation products and semisynthetic products derived therefrom,■2S (*USP33*)
and radiopharmaceutical products are not covered in this chapter.

~~Concepts about purity change with time and are inseparable from developments in analytical chemistry. If a material previously considered to be pure can be resolved into more than one component, that material can be redefined into new terms of purity and impurity. Inorganic, organic, biochemical, isomeric,~~

In-Process Revision

~~or polymeric components can all be considered impurities. Microbiological species or strains are sometimes described in similar terms of resolving into more than one component.~~

■■2S (*USP33*)

Communications about impurities ~~or~~

■and■2S (*USP33*)
degradation products in compendial articles may be improved by including in this Pharmacopeia the definitions of terms and the contexts in which these terms are used. (See *Definitions* below.) There has been much activity and discussion in recent years about the definition of terms. Certain industry-wide concerns about terminology and context deserve widespread publication and ready retrievability and are included here. See ~~Foreign Substances and Impurities~~

■section *5.60, Impurities and Foreign Substances*■2S (*USP33*) in section ~~*Tests and Assays*~~

■*5, Monograph Components*■2S (*USP33*)
under *General Notices and Requirements,* as well as the general chapter *Ordinary Impurities* ⟨466⟩. Some other general chapters added over the years have also addressed topics of purity or impurity as these have come into focus or as analytical methodology has become available. Analytical aspects are enlarged upon in the chapter *Validation of Compendial Procedures* ⟨1225⟩.

~~Monographs for drug substances usually cite one of three types of purity tests: (1) a chromatographic purity test coupled with a nonspecific assay; (2) a chromatographic purity indicating method that serves as the assay; or (3) a specific test and limit for a known impurity, an approach that usually requires a Reference Standard for that impurity. Modern separation methods clearly play a dominant role in scientific research today because these methods simultaneously separate and measure components and fulfill the analytical ideal of making measurements only on purified specimens. Nevertheless, the more classical methods based on titrimetry, colorimetry, spectrophotometry, single or multiple partitions, or changes in physical constants (or any other tests or assays) lose none of their previous validities. The *purity profile* of a specimen that is constructed from the results of experiments using a number of analytical methods is the ultimate goal.~~

■■2S (*USP33*)

Purity or impurity measurements for drug products present a challenge to Pharmacopeial standards-setting. Where degradation of a drug product over time is at issue, the same analytical methods that are stability-indicating are also purity-indicating. Resolution of the active ingredient(s) from the excipients necessary to the preparation presents the same qualitative problem. Thus, many monographs for Pharmacopeial preparations feature chromatographic assays. Where more significant impurities are known, some monographs set forth specific limit tests. In general, however, this Pharmacopeia does not repeat impurity tests in subsequent preparations where these appear in the monographs of drug substances and where these impurities are not expected to increase. It is presumed that adequate retention specimens are in storage for the exact batch of drug substances used in any specific lot of a drug product. Whenever analysis of an official article raises a question of the official attributes of any of the drug substances used, subsequent analysis of retention specimens is in order.

~~Pharmaceutical manufacturers interact with regulatory agencies in developing new drug substances and new drug products, and cooperate with the compendia in writing official monographs for the compendial articles the manufacturers produce. Establishment of impurity limits in drug substances and drug products should proceed on a rational basis so that everyone involved in the development and approval phases can carry on their work in a predictable manner. Although drug development in the United States is the primary focus of this section of the chapter, the subject also has broad applicability across national boundaries.~~

~~Manufacturers share with regulatory agencies and with the compendia the goal of making available to the public high-quality products that are both safe and efficacious. This goal continues to be achieved through rational approaches to the complex process of drug development. Tests used at all stages of drug development and marketing should not be interpreted individually but as a whole. Controls on raw materials and on manufacturing as well as those on drug substances, along with toxicological and clinical studies performed, ensure the safety and efficacy of drug products. It is more rational to identify impurities or degradation products and to set limits based on the factors detailed here, relying on the scientific judgments of manufacturers, the compendia, and regulators to arrive at sets of acceptable limits for identified and unidentified impurities or degradation products.~~

~~Limits are set for impurity levels or degradation products as one of the steps in ensuring the identity, strength, quality, and chemical purity of drug substances or drug products. The ultimate goal is to produce a final drug product of high quality that is safe and efficacious and remains so throughout its shelf life. The setting of limits for impurities or degradation products in drug substances is a complex process that considers a number of factors:~~

~~(1) the toxicology of a drug substance containing typical levels of impurities and/or the toxicology of impurities relative to a drug substance;~~

~~(2) the route of administration, e.g., oral, topical, parenteral, or intrathecal;~~

~~(3) the daily dose, i.e., frequency and amount (micrograms or grams) administered of a drug substance;~~

~~(4) the target population (age and disease state), e.g., neonates, children, or senior citizens;~~

~~(5) the pharmacology of an impurity, when appropriate;~~

~~(6) the source of a drug substance, e.g., synthetic, natural product, or biotechnology;~~

~~(7) the duration of therapy, i.e., administration over a long period (treatment of chronic conditions) versus administration intended for a short duration (treatment of acute conditions); and~~

~~(8) the capability of a manufacturer to produce consistently high-quality material.~~

~~Concepts for setting impurity or degradation product limits in drug substances are based on chemistry and safety concerns. As such, limits for organic and inorganic impurities and residual solvents should be established for drug substances. The basic tenet for setting limits is that levels of impurities or degradation products in a drug substance must be controlled to ensure its safety and quality throughout its development to use in a drug product.~~

~~For drug products the concept for setting degradation product limits is based on sound scientific judgment as applied to available data on the safety and stability of the drug product, which may include the degradation pathways of the drug substance, the manufacturing process, known excipient interactions, any safety assessment studies, stability studies conducted under the recommended storage conditions, and ancillary studies that may provide additional information on the stability profile of the drug product. A rationale should be provided for exclusion of those impurities that are not degradation products (e.g., process impurities from drug substance and impurities arising from excipients).~~

~~For drug substances and drug products, limits are appropriately set no higher than the level that can be justified (e.g., safety data, literature references, etc.) and no lower than the level achievable by the manufacturing process and analytical capability. Where there is no safety concern, limits should be based on (a) data generated on actual batches of the drug substance or drug product, allowing sufficient latitude to deal with normal process and analytical variation, and (b) stability characteristics.~~

~~Documented evidence that the analytical procedure used to evaluate impurities or degradation products is validated and suitable for the detection and quantification of impurities or degradation products should be established.~~

~~The setting of limits on impurities or degradation products is an evolutionary process, beginning in the United States before an investigational new drug (IND) is filed and continuing until well after the approval of a new drug application (NDA). Therefore, it is appropriate to address different stages in drug development as separate issues. There are three points in the drug development process where the setting of limits may be significantly different: (1) at the initial IND application, (2) at the filing of the NDA, and (3) after NDA approval. The filing of an abbreviated new drug application (ANDA) is another activity in which limits are set on impurities or degradation products. Since the approach may vary from that of filing an NDA, it is addressed as a separate issue. The underlying assumption is that the analytical methods used to evaluate impurities or degradation products are suitable for their intended purpose at each stage in the development.~~

▪▪2S (*USP33*)

Delete the following:

~~▪INITIAL IND FILING~~

~~**Drug Substances**—At the initial IND filing, the chemical nature of a drug substance has generally been defined. The manufacturing process normally is in an early stage of development, and materials may be produced on a laboratory scale. Usually few batches have been made and, therefore, little historical data is available. The reference materials of a drug substance may be relatively impure. Limits for the purity of a drug substance are set to indicate drug quality. The setting of limits on related substances and process contaminants can be characterized as follows.~~

~~(1) Limits are set on total impurities, and an upper limit may be set on any single impurity. The limit for total impurities should maintain, if possible, a nominal composition material balance.~~

~~(2) Impurity profiles are documented. These are profiles of the lots of drug substances used in clinical studies and in toxicological studies that establish the safety of drug substances. The lots used in these studies should be typical products of the manufacturing process in use at that time.~~

~~(3) Limits for residual solvents are based on the known toxicology of the solvents and on the manufacturing capabilities and dosing regimens.~~

~~(4) General inorganic impurities are monitored by appropriate tests such as a heavy metals limit test and/or a test for residue on ignition. Traditional compendial limits are applied unless otherwise indicated. Specific residual metals that appear during manufacturing should be monitored by appropriate analytical techniques, and limits should be set based on the toxicological properties of these metals.~~

~~(5) Appropriate limits are set for impurities known to be toxic.~~

~~(6) If appropriate, enantiomeric purity is controlled.~~

~~Although water is not classified as an impurity, limits for water content may be needed to ensure the stability or ease of processing a drug substance.~~

~~**Drug Product**—At the initial IND filing, the dosage form of a drug product has been identified, which is appropriate for early clinical studies (and may or may not be representative of the drug product that will eventually be marketed). Usually, few batches have been made and, therefore, little historical data is available.~~

~~(1) Qualitative and quantitative limits on degradation products may not be established at this point due to the limited data available. Typically, degradation products will be monitored as part of the stability evaluation of the drug product.~~

~~(2) Dating for use of a drug product in clinical studies will be based on data from ongoing stability studies. If the data indicate the presence of degradation products, dating and storage conditions are controlled to ensure any degradation products are controlled within industry accepted limits or within limits established through safety assessment studies.~~

~~(3) Limits for residual solvents, if appropriate, are based on the known toxicology of the solvents and on the manufacturing capabilities and dosing regimens. When amendments to the IND are filed, limits for impurities in drug substance and degradation products in drug substance or drug product may be updated based on additional data as they become available.~~ ▪▪2S (*USP33*)

Add the following:

▪DRUG SUBSTANCE

Classification of Impurities—Impurities can be classified into the following categories:

1. Organic impurities (process- and drug-related)
2. Inorganic impurities
3. Residual solvents

Organic impurities can arise during the manufacturing process and/or storage of the drug substance. They can be identified or unidentified, volatile or nonvolatile, and include the following:

1. Starting materials
2. Byproducts
3. Intermediates
4. Degradation products
5. Reagents, ligands, and catalysts
6. Geometric and stereoisomers

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include the following:

1. Reagents, ligands, and catalysts
2. Heavy metals or other residual metals
3. Inorganic salts
4. Other materials (e.g. filter aids, charcoal)

Residual solvents are organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a drug substance. Because these are generally of known toxicity, the selection of appropriate controls is easily accomplished (see *Residual Solvents* ⟨467⟩).

Concepts for setting impurity or degradation product limits in drug substances are based on chemistry and safety concerns. As such, limits for organic and inorganic impurities and residual solvents should be established for drug substances. The basic tenet for setting limits is that levels of impurities or degradation products in a drug substance must be controlled throughout its development to ensure its safety and quality for use in a drug product.

Documented evidence that the analytical procedure used to evaluate impurities or degradation products is validated and suitable for the detection and quantification of impurities or degradation products should be established.■2S (*USP33*)

Delete the following:

~~▪NDA FILING~~

~~**Drug Substances**—During the IND phases of drug development, the manufacturing process for a drug substance may undergo a number of revisions. Generally, the scale will have changed from laboratory size and will approach or reach full production batch size. A number of batches will normally have been produced, and a historical data base of the results of testing for impurities will exist. When significant changes in a manufacturing process are made, the impurity profile should be reviewed to determine if the toxicological studies are still supportive.~~

~~At the NDA stage a reference standard of defined purity is available, analytical methods have been validated, impurity and degradation profiles are known, and enantiomeric purity has been evaluated. The setting of limits on related substances and process contaminants can be characterized as follows:~~

~~(1) Consistency of the impurity profile of a drug substance has been established.~~
~~(2) IND limits for total and individual impurities (identified and unidentified) are reviewed and adjusted based on manufacturing experience and toxicological data.~~
~~(3) Impurities present in significant amounts are identified and individual limits are set. However, it is not always possible to identify or prepare authentic substances for impurities. The labile nature of some impurities precludes this possibility. Limits may be set on these substances based on comparison of lots produced and used in toxicological and clinical studies.~~
~~(4) The impurity profiles of the lots designated for marketing should not be significantly higher than those of the lot(s) used for toxicological and clinical studies.~~
~~(5) The composition material balance should be used, if possible, to evaluate the adequacy of the controls.~~
~~(6) Limits for residual solvents are based on the known toxicology of the solvents and on the manufacturing capabilities and dosing regimens.~~
~~(7) Limits are set for inorganic impurities by appropriate tests such as a heavy metals limit test and/or by a test for residue on ignition. Traditional compendial limits are applied unless otherwise indicated. Based on toxicological properties, limits may be set for specific residual metals that appear during manufacturing.~~
~~(8) Additional guidance for setting limits can be found in various ICH and FDA guidance documents.~~

~~**Drug Product**—Similarly, for the drug product, the dosage form may change, the number or scale of batches may increase, and more stability data will have become available. Methods will have been validated. The setting of limits can be characterized as follows:~~

~~(1) IND limits for total and individual degradation products (identified and unidentified) are reviewed and adjusted based on manufacturing experience, stability data, and toxicological data.~~
~~(2) Degradation products present in significant amounts are identified and individual limits are set. However, it is not always possible to identify or prepare authentic substances for degradation products.~~
~~(3) The degradation product profiles of the lots designated for marketing should not be significantly higher than those of the lot(s) used for toxicological and clinical studies.~~
~~(4) The mass balance should be used, if possible, to evaluate the adequacy of the controls.~~
~~(5) Limits for residual solvents, if appropriate, are based on the known toxicology of the solvents and on the manufacturing capabilities and dosing regimens.~~
~~(6) Additional guidance for setting limits can be found in various ICH and FDA guidance documents.~~■2S (*USP33*)

Add the following:

▪DRUG PRODUCT

The specification for a drug product should include a list of degradation products expected to occur during manufacture of the commercial product and under recommended storage conditions. Stability studies, knowledge of degradation pathways, product development studies, and laboratory studies should be used to characterize the degradation profile. The selection of degradation products in the drug product specification should be based on the degradation products found in batches manufactured by the proposed commercial process.

This rationale should include a discussion of the degradation profiles observed in the safety and clinical development batches and in stability studies, together with a consideration of the degradation profile of batches manufactured by the proposed commercial process. For de-

gradation products known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the level at which the degradation products should be controlled.

For drug products the concept for setting degradation product limits is based on sound scientific judgment as applied to available data on the safety and stability of the drug product, data that may include the degradation pathways of the drug substance, the manufacturing process, known excipient interactions, any safety assessment studies, stability studies conducted under the recommended storage conditions, and ancillary studies that may provide additional information on the stability profile of the drug product. Impurities that are not degradation products (e.g. process impurities from the drug substance) are often not controlled in the drug product, as they are typically controlled in the drug substance and these impurities are not expected to increase over time. Additional guidance for setting limits can be found in various ICH and FDA guidance documents, as well as in the USP monograph submission guidelines.

Documented evidence that the analytical procedure used to evaluate impurities or degradation products is validated and suitable for the detection and quantification of impurities or degradation products should be established.

Drug products should contain levels of residual solvents no higher than can be supported by safety data (see *Residual Solvents* ⟨467⟩).■2S (*USP33*)

Delete the following:

▪~~POST NDA APPROVAL~~

~~After approval and marketing of a pharmaceutical product, significant changes may be made in manufacturing the drug substance. There may be technological, ecological, economic, or safety reasons for these changes. If they occur, the Pharmacopeial and NDA impurity and degradation product limits and rationale should be reviewed; the limits should be revised when indicated to ensure similar or improved quality of the drug substance or drug product.~~■2S (*USP33*)

Delete the following:

▪~~ANDA FILING~~

~~The drug substance for a pharmaceutical product eligible for ANDA status is frequently an official article and should be well characterized analytically. Drug substances are typically available from multiple sources, and each source may have a different manufacturing process. Therefore, it is essential that the dosage form manufacturer evaluate each supplier's drug substance impurity or degradation profiles. Limits can then be set based on the more detailed concepts described for NDA filing, including review of compendial monographs for appropriateness.~~■2S (*USP33*)

Change to read:

DEFINITIONS

Concomitant Components—Concomitant components are characteristic of many drug substances and are not considered to be impurities in the Pharmacopeial sense. Limits on contents, or specified ranges, or defined mixtures are set forth for concomitant components in this Pharmacopeia. Examples of concomitant components are geometric and optical isomers (or racemates) and antibiotics that are mixtures. Any component that can be considered a toxic impurity because of significant undesirable biological effect is not considered to be a concomitant component.

▪**Degradation Product**—An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container–closure system.■2S (*USP33*)

Foreign Substances (Extraneous Contaminants)~~— Foreign substances (extraneous contaminants), which are introduced by contamination or adulteration, are not consequences of the synthesis or preparation of compendial articles and thus~~

▪An impurity that arises from any source extraneous to the manufacturing process and that is introduced by contamination or adulteration. These impurities■2S (*USP33*) cannot be anticipated when monograph tests and assays are selected. The presence of objectionable foreign substances not revealed by monograph tests and assays constitutes a variance from the official standard. Examples of foreign substances include ephedrine in Ipecac or a pesticide in an oral liquid analgesic. Allowance is made in this Pharmacopeia for the detection of foreign substances by unofficial methods. (See ~~Foreign Substances and Impurities~~

▪section *5.60, Impurities and Foreign Substances*■2S (*USP33*) in section ~~*Tests and Assays*~~

▪*5, Monograph Components*■2S (*USP33*) under *General Notices and Requirements*.)

In-Process Revision

Identified Impurities and Identified Degradation Products—Impurities or degradation products for which structural characterizations have been achieved.

▪**Impurity—**Any component of a drug substance that is not the chemical entity defined as the drug substance and in addition, for a drug product, any component that is not a formulation ingredient.▪2S *(USP33)*

Inorganic Impurities—Inorganic impurities can result from the manufacturing process (e.g., residual metals, inorganic salts, filter aids, etc.). Inorganic impurities are typically controlled by tests such as *Heavy Metals* ⟨231⟩ and *Residue on Ignition* ⟨281⟩. Information found in *Plasma Spectrochemistry* ⟨730⟩ and *Ion Chromatography* ⟨1065⟩ may also be of value.

▪**Intermediate—**A material that is produced during steps of the synthesis of a drug substance and that undergoes further chemical transformation before it becomes a drug substance. The intermediate is often isolated during the process.▪2S *(USP33)*

Ordinary Impurities—Some monographs make reference to ordinary impurities. For more details see *Ordinary Impurities* ⟨466⟩.

▪**Other impurities—**See section *5. Monograph Components* under *General Notices and Requirements.*

Polymorphs—Different crystalline forms of the same drug substance. These can include solvation or hydration products (also knows as pseudopolymorphs) and amorphous forms. Although polymorphs are not impurities by definition, an understanding of the crystalline forms, hydration or solvation states, or amorphous nature is critical to the overall characterization of the drug substance.▪2S *(USP33)*

Process Contaminants—Process contaminants are identified or unidentified substances (excluding related substances and water), including reagents, catalysts, other inorganic impurities (e.g., heavy metals, chloride, or sulfate); and may also include foreign substances (extraneous contaminants). These contaminants may be introduced during manufacturing or handling procedures.

▪**Reagent—**A substance other than a starting material, intermediate, or solvent that is used in the manufacture of a drug substance.▪2S *(USP33)*

Related Substances—Related substances are structurally related to a drug substance. These substances may be (a) identified or unidentified impurities arising from synthesis manufacturing process such as

▪starting materials,▪2S *(USP33)*
intermediates or by-products and do not increase on storage or (b) identified or unidentified degradation products that result from drug substance or drug product manufacturing processes or arise during storage of a material.

Residual Solvents—

▪An organic liquid used as a vehicle for the preparation of solutions or suspensions in the synthesis of a drug substance▪2S *(USP33)*
(see *Residual Solvents* ⟨467⟩).

Specified Impurities and Specified Degradation Products—Previously referred to as Signal Impurities, specified impurities or specified degradation products are impurities or degradation products that are individually listed and limited with specific acceptance criteria in individual monographs as applicable. Specified impurities or specified degradation products can be identified or unidentified.

▪**Starting Material—**A material that is used in the synthesis of a drug substance and is incorporated as an element into the structure of an intermediate and/or of the drug substance. Starting materials are often commercially available and have well-defined chemical and physical properties and structure.

Stereomeric Impurity—A compound with the same 2–dimensional chemical structure as the drug substance but differs in the 3–dimensional orientation of substituents at chiral centers within that structure. In those cases where all chiral centers are in the opposite orientation, the impurity is an enantiomer (enantiomeric impurity). Determinations of impurities in this category often require special chiral chromatographic approaches. Diastereomeric or epimeric impurities occur when only some of the chiral centers are present in the opposite orientation. ▪2S *(USP33)*

Toxic Impurities—Toxic impurities have significant undesirable biological activity, even as minor components, and require individual identification and quantification by specific tests. These impurities may arise out of the synthesis, preparation, or degradation of compendial articles. Based on validation data, individualized tests and specifications are selected. These feature comparison to a Reference Standard of the impurity, if available. It is incumbent on the manufacturer to provide data that would support the classification of such impurities as toxic impurities.

Unidentified Impurities and Unidentified Degradation Products—Impurities or degradation products for which structural characterizations have not been achieved and that are identified solely by qualitative analytical properties (e.g., chromatographic retention times).

Unspecified Impurities and Unspecified Degradation Products—Impurities or degradation products that are limited by general acceptance criteria but not individually listed with their own specific acceptance criteria in individual monographs.

BRIEFING

⟨**1151**⟩ **Pharmaceutical Dosage Forms,** *USP 32* page 663. This general information chapter is being revised in its entirety to represent current compendial thinking with respect to official preparations. The proposed revision incorporates concepts outlined in a Stimuli to the Revision Process article, *Development of a Compendial Taxonomy and Glossary for Pharmaceutical Dosage Forms*, authored by an Ad Hoc Committee composed of the chairs of the Pharmaceutical Dosage Forms Expert Committee, Biopharmaceutics Expert Committee, Nomenclature and Labeling Expert Committee, and the Council of Experts for the revision cycle, 2000—2005, and published in *PF* 29(5). The Stimuli article proposed a tiered categorization for pharmaceutical dosage forms proceeding from route of administration to physical form and ultimately release pattern. This proposed general information chapter emphasizes the second tier of the compendial taxonomy, the physical dosage form, rather than the route of administration, with the intention of avoiding redundancy for dosage forms given by multiple routes.

The proposed revision is organized into four sections providing discussion of general considerations, product quality tests, dosage form monographs, and a glossary. General considerations include dose uniformity, stability, bioavailability, manufacture, and route of administration. The discussion of product quality tests reflects the universally applied as well as dosage form specific testing that help assure safety and efficacy from manufacture through shelf life. The dosage form monographs provide general descriptions, discussion of general principles of their manufacturing or compounding, and recommendations for proper use and storage. The glossary is intended to provide guidance in selection of official names for official articles but also as a resource to provide definitions beyond those used in official names for dosage forms. The glossary clearly distinguishes preferred from not preferred terminology.

The revised general information chapter presents current concepts relating to the naming of dosage forms. Outdated forms such as elixirs, spirits, tinctures, and syrups are herein recognized as solutions. Lotions are defined as emulsions typically for topical use. While inserts are defined as solid dosage forms for placement within body cavities, suppositories are differentiated as only for placement within the rectum.

This general information chapter is intended to be supplemented by more detailed discussion of characteristics, quality tests, and other considerations based on route of administration. Early drafts of such concepts relating to topical and transdermal dosage forms are presented in *PF* 35(3) [May–June 2009]. Proposed general test chapter ⟨3⟩ *Topical and Transdermal Drug Products—Product Quality Tests*, providing quality testing procedures, complements the performance testing proposed in ⟨725⟩ *Topical and Transdermal Drug Products—Product Performance Tests*. Additional revision proposals of similar standards for the oral (gastro-intestinal), mucosal, by inhalation, and by injection routes are planned.

(BPC: W. Brown.) RTS—C69686

Change to read:

⟨1151⟩ PHARMACEUTICAL DOSAGE FORMS

~~Dosage forms are provided for most of the Pharmacopeial drug substances, but the processes for the preparation of many of them are, in general, beyond the scope of the Pharmacopeia. In addition to defining the dosage forms, this section presents the general principles involved in the manufacture of some of them, particularly on a small scale. Other information that is given bears on the use of the Pharmacopeial substances in extemporaneous compounding of dosage forms.~~

~~BIOAVAILABILITY~~

~~Bioavailability, or the extent to which the therapeutic constituent of a pharmaceutical dosage form intended for oral or topical use is available for absorption, is influenced by a variety of factors. Among the inherent factors known to affect absorption are the method of manufacture or method of compounding; the particle size and crystal form or polymorph of the drug substance; and the diluents and excipients used in formulating the dosage form, including fillers, binders, disintegrating agents, lubricants, coatings, solvents, suspending agents, and dyes. Lubricants and coatings are foremost among these. The maintenance of a demonstrably high degree of bioavailability requires particular attention to all aspects of production and quality control that may affect the nature of the finished dosage form.~~

~~TERMINOLOGY~~

~~Occasionally it is necessary to add solvent to the contents of a container just prior to use, usually because of instability of some drugs in the diluted form. Thus, a solid diluted to yield a suspension is called [DRUG] *for Suspension;* a solid dissolved and diluted to yield a solution is called [DRUG] *for Solution;* and a solution or suspension diluted to yield a more dilute form of the drug is called [DRUG] *Oral Concentrate*. After dilution, it is important that the drug be homogeneously dispersed before administration.~~

~~AEROSOLS~~

~~Pharmaceutical aerosols are products that are packaged under pressure and contain therapeutically active ingredients that are released upon activation of an appropriate valve system. They are intended for topical application to the skin as well as local application into the nose (nasal aerosols), mouth (lingual aerosols), or lungs (inhalation aerosols). These products may be fitted with valves enabling either continuous or metered dose delivery; hence, the terms "[DRUG] Metered Topical Aerosols," "[DRUG] Metered Nasal Aerosols," etc.~~

~~The term "aerosol" refers to the fine mist of spray that results from most pressurized systems. However, the term has been broadly misapplied to all self-contained pressurized products, some of which deliver foams or semisolid fluids. In the case of *Inhalation Aerosols*, the particle size of the delivered medication must be carefully controlled, and the average size of the particles should be under 5 µm. These products are also known as metered-dose inhalers (MDIs). Other aerosol sprays may contain particles up to several hundred micrometers in diameter.~~

~~The basic components of an aerosol system are the container, the propellant, the concentrate containing the active ingredient(s), the valve, and the actuator. The nature of these components determines such characteristics as particle size distribution, uniformity of dose for metered valves, delivery rate, wetness and temperature of the spray, spray pattern and velocity or plume geometry, foam density, and fluid viscosity.~~

~~Types of Aerosols~~

~~Aerosols consist of two-phase (gas and liquid) or three-phase (gas, liquid, and solid or liquid) systems. The two-phase aerosol consists of a solution of active ingredients in liquefied propellant and the vaporized propellant. The solvent is composed of the propellant or a mixture of the propellant and cosolvents such as alcohol, propylene glycol, and polyethylene glycols, which are often used to enhance the solubility of the active ingredients.~~

In-Process Revision

Three-phase systems consist of a suspension or emulsion of the active ingredient(s) in addition to the vaporized propellants. A suspension consists of the active ingredient(s) that may be dispersed in the propellant system with the aid of suitable excipients such as wetting agents and/or solid carriers such as talc or colloidal silicas.

A foam aerosol is an emulsion containing one or more active ingredients, surfactants, aqueous or nonaqueous liquids, and the propellants. If the propellant is in the internal (discontinuous) phase (i.e., of the oil in water type), a stable foam is discharged; and if the propellant is in the external (continuous) phase (i.e., of the water in oil type), a spray or a quick breaking foam is discharged.

Propellants

The propellant supplies the necessary pressure within an aerosol system to expel material from the container and, in combination with other components, to convert the material into the desired physical form. Propellants may be broadly classified as liquefied or compressed gases having vapor pressures generally exceeding atmospheric pressure. Propellants within this definition include various hydrocarbons, especially halogenated derivatives of methane, ethane, and propane, low molecular weight hydrocarbons such as the butanes and pentanes, and compressed gases such as carbon dioxide, nitrogen, and nitrous oxide. Mixtures of propellants are frequently used to obtain desirable pressure, delivery, and spray characteristics. A good propellant system should have the proper vapor pressure characteristics consistent with the other aerosol components.

Valves

The primary function of the valve is to regulate the flow of the therapeutic agent and propellant from the container. The spray characteristics of the aerosol are influenced by orifice dimension, number, and location. Most aerosol valves provide for continuous spray operation and are used on most topical products. However, pharmaceutical products for oral or nasal inhalation often utilize metered dose valves that must deliver a uniform quantity of spray upon each valve activation. The accuracy and reproducibility of the doses delivered from metering valves are generally good, comparing favorably to the uniformity of solid dosage forms such as tablets and capsules. However, when aerosol packages are stored improperly, or when they have not been used for long periods of time, valves must be primed before use. Materials used for the manufacture of valves should be inert to the formulations used. Plastic, rubber, aluminum, and stainless steel valve components are commonly used. Metered dose valves must deliver an accurate dose within specified tolerances.

Actuators

An actuator is the fitting attached to an aerosol valve stem, which when depressed or moved, opens the valve, and directs the spray containing the drug preparation to the desired area. The actuator usually indicates the direction in which the preparation is dispensed and protects the hand or finger from the refrigerant effects of the propellant. Actuators incorporate an orifice that may vary widely in size and shape. The size of this orifice, the expansion chamber design, and the nature of the propellant and formulation influence the delivered dose as well as the physical characteristics of the spray, foam, or stream of solid particles dispensed. For inhalation aerosols, an actuator capable of delivering the medication in the proper particle size range and with the appropriate spray pattern and plume geometry is utilized.

Containers

Aerosol containers usually are made of glass, plastic, or metal, or a combination of these materials. Glass containers must be precisely engineered to provide the maximum in pressure safety and impact resistance. Plastics may be employed to coat glass containers for improved safety characteristics, or to coat metal containers to improve corrosion resistance and enhance stability of the formulation. Suitable metals include stainless steel, aluminum, and tin plated steel. Extractables or leachables (e.g., drawing oils, cleaning agents, etc.) and particulates on the internal surfaces of containers should be controlled.

Manufacture

Aerosols are usually prepared by one of two general processes. In the "cold fill" process, the concentrate (generally cooled to a temperature below 0°) and the refrigerated propellant are measured into open containers (usually chilled). The valve actuator assembly is then crimped onto the container to form a pressure tight seal. During the interval between propellant addition and crimping, sufficient volatilization of propellant occurs to displace air from the container. In the "pressure fill" method, the concentrate is placed in the container, and either the propellant is forced under pressure through the valve orifice after the valve is sealed, or the propellant is allowed to flow under the valve cap and then the valve assembly is sealed ("under the cap" filling). In both cases of the "pressure fill" method, provision must be made for evacuation of air by means of vacuum or displacement with a small amount of propellant vapor. Manufacturing process controls usually include monitoring of proper formulation and propellant fill weight and pressure testing, leak testing, and valve function testing of the finished aerosol. Microbiological attributes should also be controlled.

Extractable Substances

Since pressurized inhalers and aerosols are normally formulated with organic solvents as the propellant or the vehicle, leaching of extractables from the elastomeric and plastic components into the formulation is a potentially serious problem. Thus, the composition and the quality of materials used in the manufacture of the valve components (e.g., stem, gaskets, housing, etc.) must be carefully selected and controlled. Their compatibility with formulation components should be well established so as to prevent distortion of the valve components and to minimize changes in the medication delivery, leak rate, and impurity profile of the drug product over time. The extractable profiles of a representative sample of each of the elastomeric and plastic components of the valve should be established under specified conditions and should be correlated to the extractable profile of the aged drug product or placebo, to ensure reproducible quality and purity of the drug product. Extractables, which may include polynuclear aromatics, nitrosamines, vulcanization accelerators, antioxidants, plasticizers, monomers, etc., should be identified and minimized wherever possible.

Specifications and limits for individual and total extractables from different valve components may require the use of different analytical methods. In addition, the standard USP biological testing (see the general test chapters *Biological Reactivity Tests, In Vitro* ⟨87⟩ and *Biological Reactivity Tests, In Vivo* ⟨88⟩) as well as other safety data may be needed.

Labeling

Medicinal aerosols should contain at least the following warning information on the label as in accordance with appropriate regulations.

Warning—Avoid inhaling. Avoid spraying into eyes or onto other mucous membranes.

In-Process Revision

NOTE—The statement "Avoid inhaling" is not necessary for preparations specifically designed for use by inhalation. The phrase "or other mucous membranes" is not necessary for preparations specifically designed for use on mucous membranes.

Warning—Contents under pressure. Do not puncture or incinerate container. Do not expose to heat or store at temperatures above 120° F (49° C). Keep out of reach of children.

In addition to the aforementioned warnings, the label of a drug packaged in an aerosol container in which the propellant consists in whole or in part of a halocarbon or hydrocarbon shall, where required under regulations of the FDA, bear either of the following warnings:

Warning—Do not inhale directly; deliberate inhalation of contents can cause death.

Warning—Use only as directed; intentional misuse by deliberately concentrating and inhaling the contents can be harmful or fatal.

BOLUSES

Boluses are large elongated tablets intended for administration to animals (see *Tablets*).

CAPSULES

Capsules are solid dosage forms in which the drug is enclosed within either a hard or soft soluble container or "shell." The shells are usually formed from gelatin; however, they also may be made from starch or other suitable substances. Hard shell capsule sizes range from No. 5, the smallest, to No. 000, which is the largest, except for veterinary sizes. However, size No. 00 generally is the largest size acceptable to patients. Size 0 hard gelatin capsules having an elongated body (known as size OE) also are available, which provide greater fill capacity without an increase in diameter. Hard gelatin capsules consist of two, telescoping cap and body pieces. Generally, there are unique grooves or indentations molded into the cap and body portions to provide a positive closure when fully engaged, which helps prevent the accidental separation of the filled capsules during shipping and handling. Positive closure also may be affected by spot fusion ("welding") of the cap and body pieces together through direct thermal means or by application of ultrasonic energy. Factory filled hard gelatin capsules may be completely sealed by banding, a process in which one or more layers of gelatin are applied over the seam of the cap and body, or by a liquid fusion process wherein the filled capsules are wetted with a hydroalcoholic solution that penetrates into the space where the cap overlaps the body, and then dried. Hard shell capsules made from starch consist of two, fitted cap and body pieces. Since the two pieces do not telescope or interlock positively, they are sealed together at the time of filling to prevent their separation. Starch capsules are sealed by the application of a hydroalcoholic solution to the recessed section of the cap immediately prior to its being placed onto the body.

The banding of hard shell gelatin capsules or the liquid sealing of hard shell starch capsules enhances consumer safety by making the capsules difficult to open without causing visible, obvious damage, and may improve the stability of contents by limiting O_2 penetration. Industrially filled hard shell capsules also are often of distinctive color and shape or are otherwise marked to identify them with the manufacturer. Additionally, such capsules may be printed axially or radially with strengths, product codes, etc. Pharmaceutical grade printing inks are usually based on shellac and employ FDA approved pigments and lake dyes.

In extemporaneous prescription practice, hard shell capsules may be hand filled; this permits the prescriber a latitude of choice in selecting either a single drug or a combination of drugs at the exact dosage level considered best for the individual patient. This flexibility gives hard shell capsules an advantage over compressed tablets and soft shell capsules as a dosage form. Hard shell capsules are usually formed from gelatins having relatively high gel strength. Either type may be used, but blends of pork skin and bone gelatin are often used to optimize shell clarity and toughness. Hard shell capsules also may be formed from starch or other suitable substances. Hard shell capsules may also contain colorants, such as D&C and FD&C dyes or the various iron oxides, opaquing agents such as titanium dioxide, dispersing agents, hardening agents such as sucrose, and preservatives. They normally contain between 10% and 15% water.

Hard gelatin capsules are made by a process that involves dipping shaped pins into gelatin solutions, after which the gelatin films are dried, trimmed, and removed from the pins, and the body and cap pieces are joined. Starch capsules are made by injection molding a mixture of starch and water, after which the capsules are dried. A separate mold is used for caps and bodies, and the two parts are supplied separately. The empty capsules should be stored in tight containers until they are filled. Since gelatin is of animal origin and starch is of vegetable origin, capsules made with these materials should be protected from potential sources of microbial contamination.

Hard shell capsules typically are filled with powder, beads, or granules. Inert sugar beads (nonpareils) may be coated with active ingredients and coating compositions that provide extended release profiles or enteric properties. Alternatively, larger-dose active ingredients themselves may be suitably formed into pellets and then coated. Semisolids or liquids also may be filled into hard shell capsules; however, when the latter are encapsulated, one of the sealing techniques must be employed to prevent leakage.

In hard gelatin capsule filling operations, the body and cap of the shell are separated prior to dosing. In hard starch shell filling operations, the bodies and caps are supplied separately and are fed into separate hoppers of the filling machine. Machines employing various dosing principles may be employed to fill powders into hard shell capsules; however, most fully automatic machines form powder plugs by compression and eject them into empty capsule bodies. Accessories to these machines generally are available for the other types of fills. Powder formulations often require adding fillers, lubricants, and glidants to the active ingredients to facilitate encapsulation. The formulation, as well as the method of filling, particularly the degree of compaction, may influence the rate of drug release. The addition of wetting agents to the powder mass is common where the active ingredient is hydrophobic. Disintegrants also may be included in powder formulations to facilitate deaggregation and dispersal of capsule plugs in the gut. Powder formulations often may be produced by dry blending; however, bulky formulations may require densification by roll compaction or other suitable granulation techniques.

Powder mixtures that tend to liquefy may be dispensed in hard shell capsules if an absorbent such as magnesium carbonate, colloidal silicon dioxide, or other suitable substance is used. Potent drugs are often mixed with an inert diluent before being filled into capsules. Where two mutually incompatible drugs are prescribed together, it is sometimes possible to place one in a small capsule and then enclose it with the second drug in a larger capsule. Incompatible drugs also can be separated by placing coated pellets or tablets, or soft shell capsules of one drug into the capsule shell before adding the second drug.

Thixotropic semisolids may be formed by gelling liquid drugs or vehicles with colloidal silicas or powdered high molecular weight polyethylene glycols. Various waxy or fatty compounds may be used to prepare semisolid matrices by fusion.

Soft shell capsules made from gelatin (sometimes called softgels) or other suitable material require large scale production methods. The soft gelatin shell is somewhat thicker than that of hard shell capsules and may be plasticized by the addition of a polyol such as sorbitol or glycerin. The ratio of dry plasticizer to dry gelatin determines the "hardness" of the shell and may be varied to accommodate environmental conditions as well as the nature of the contents. Like hard shells, the shell composition may include approved dyes and pigments, opaquing agents such as titanium dioxide, and preservatives. Flavors may be added and up to 5% sucrose may be included for its sweetness and to produce a chewable shell. Soft gelatin shells normally contain 6% to 13% water. Soft shell capsules

also may be printed with a product code, strength, etc. In most cases, soft-shell capsules are filled with liquid contents. Typically, active ingredients are dissolved or suspended in a liquid vehicle. Classically, an oleaginous vehicle such as a vegetable oil was used; however, nonaqueous, water miscible liquid vehicles such as the lower molecular weight polyethylene glycols are more common today due to fewer bioavailability problems.

Available in a wide variety of sizes and shapes, soft-shell capsules are both formed, filled, and sealed in the same machine; typically, this is a rotary die process, although a plate process or reciprocating die process also may be employed. Soft-shell capsules also may be manufactured in a bubble process that forms seamless spherical capsules. With suitable equipment, powders and other dry solids also may be filled into soft-shell capsules.

Liquid-filled capsules of either type involve similar formulation technology and offer similar advantages and limitations. For instance, both may offer advantages over dry-filled capsules and tablets in content uniformity and drug dissolution. Greater homogeneity is possible in liquid systems, and liquids can be metered more accurately. Drug dissolution may benefit because the drug may already be in solution or at least suspended in a hydrophilic vehicle. However, the contact between the hard- or soft-shell and its liquid content is more intimate than exists with dry-filled capsules, and this may enhance the chances for undesired interactions. The liquid nature of capsule contents presents different technological problems than dry-filled capsules in regard to disintegration and dissolution testing. From formulation, technological, and biopharmaceutical points of view, liquid-filled capsules of either type have more in common than liquid-filled and dry-filled capsules having the same shell composition. Thus, for compendial purposes, standards and methods should be established based on capsule contents rather than on whether the contents are filled into hard- or soft-shell capsules.

DELAYED-RELEASE CAPSULES

Capsules may be coated, or, more commonly, encapsulated granules may be coated to resist releasing the drug in the gastric fluid of the stomach where a delay is important to alleviate potential problems of drug inactivation or gastric mucosal irritation. The term "delayed-release" is used for Pharmacopeial monographs on enteric-coated capsules that are intended to delay the release of medicament until the capsule has passed through the stomach, and the individual monographs include tests and specifications for *Drug release* (see *Drug Release* ⟨724⟩) or *Disintegration* (see *Disintegration* ⟨701⟩).

EXTENDED-RELEASE CAPSULES

Extended-release capsules are formulated in such manner as to make the contained medicament available over an extended period of time following ingestion. Expressions such as "prolonged-action," "repeat-action," and "sustained-release" have also been used to describe such dosage forms. However, the term "extended-release" is used for Pharmacopeial purposes and requirements for *Drug release* (see *Drug Release* ⟨724⟩) typically are specified in the individual monographs.

CONCENTRATE FOR DIP

Concentrate for Dip is a preparation containing one or more active ingredients usually in the form of a paste or solution. It is used to prepare a diluted suspension, emulsion, or solution of the active ingredient(s) for the prevention and treatment of ectoparasitic infestations of animals. The diluted preparation (Dip) is applied by complete immersion of the animal or, where appropriate, by spraying. Concentrate for Dip may contain suitable antimicrobial preservatives.

CREAMS

Creams are semisolid dosage forms containing one or more drug substances dissolved or dispersed in a suitable base. This term has traditionally been applied to semisolids that possess a relatively fluid consistency formulated as either water-in-oil (e.g., *Cold Cream*) or oil-in-water (e.g., *Fluocinolone Acetonide Cream*) emulsions. However, more recently the term has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water-washable and more cosmetically and aesthetically acceptable. Creams can be used for administering drugs via the vaginal route (e.g., *Triple Sulfa Vaginal Cream*).

ELIXIRS

See *Solutions*.

EMULSIONS

Emulsions are two-phase systems in which one liquid is dispersed throughout another liquid in the form of small droplets. Where oil is the dispersed phase and an aqueous solution is the continuous phase, the system is designated as an oil-in-water emulsion. Conversely, where water or an aqueous solution is the dispersed phase and oil or oleaginous material is the continuous phase, the system is designated as a water-in-oil emulsion. Emulsions are stabilized by emulsifying agents that prevent coalescence, the merging of small droplets into larger droplets and, ultimately, into a single separated phase. Emulsifying agents (surfactants) do this by concentrating in the interface between the droplet and external phase and by providing a physical barrier around the particle to coalescence. Surfactants also reduce the interfacial tension between the phases, thus increasing the ease of emulsification upon mixing.

Natural, semisynthetic, and synthetic hydrophilic polymers may be used in conjunction with surfactants in oil-in-water emulsions as they accumulate at interfaces and also increase the viscosity of the aqueous phase, thereby decreasing the rate of formation of aggregates of droplets. Aggregation is generally accompanied by a relatively rapid separation of an emulsion into a droplet-rich and droplet-poor phase. Normally the density of an oil is lower than that of water, in which case the oil droplets and droplet aggregates rise, a process referred to as creaming. The greater the rate of aggregation, the greater the droplet size and the greater the rate of creaming. The water droplets in a water-in-oil emulsion generally sediment because of their greater density.

The consistency of emulsions varies widely, ranging from easily pourable liquids to semisolid creams. Generally oil-in-water creams are prepared at high temperature, where they are fluid, and cooled to room temperature, whereupon they solidify as a result of solidification of the internal phase. When this is the case, a high internal-phase volume to external-phase volume ratio is not necessary for semisolid character, and, for example, stearic acid creams or vanishing creams are semisolid with as little as 15% internal phase. Any semisolid character with water-in-oil emulsions generally is attributable to a semisolid external phase.

All emulsions require an antimicrobial agent because the aqueous phase is favorable to the growth of microorganisms. The presence of a preservative is particularly critical in oil-in-water emulsions where contamination of the external phase occurs readily. Since fungi and yeasts are found with greater frequency than bacteria, fungistatic as well as bacteriostatic properties are desirable. Bacteria have been shown to degrade nonionic and anionic emulsifying agents, glycerin, and many natural stabilizers such as tragacanth and guar gum.

Complications arise in preserving emulsion systems, as a result of partitioning of the antimicrobial agent out of the aqueous phase where it is most needed, or of complexation with emulsion ingredients that reduce effectiveness. Therefore, the effectiveness of the preservative system should always be tested

in the final product. Preservatives commonly used in emulsions include methyl-, ethyl-, propyl-, and butyl parabens, benzoic acid, and quaternary ammonium compounds.

See also *Creams* and *Ointments*.

EXTRACTS AND FLUIDEXTRACTS

Extracts are concentrated preparations of vegetable or animal drugs obtained by removal of the active constituents of the respective drugs with suitable menstrua, by evaporation of all or nearly all of the solvent, and by adjustment of the residual masses or powders to the prescribed standards.

In the manufacture of most extracts, the drugs are extracted by percolation. The entire percolates are concentrated, generally by distillation under reduced pressure in order to subject the drug principles to as little heat as possible.

Fluidextracts are liquid preparations of vegetable drugs, containing alcohol as a solvent or as a preservative, or both, and so made that, unless otherwise specified in an individual monograph, each mL contains the therapeutic constituents of 1 g of the standard drug that it represents.

A fluidextract that tends to deposit sediment may be aged and filtered or the clear portion decanted, provided the resulting clear liquid conforms to the Pharmacopeial standards.

Fluidextracts may be prepared from suitable extracts.

GELS

Gels (sometimes called Jellies) are semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system (e.g., *Aluminum Hydroxide Gel*). In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes referred to as a magma (e.g., *Bentonite Magma*). Both gels and magmas may be thixotropic, forming semisolids on standing and becoming liquid on agitation. They should be shaken before use to ensure homogeneity and should be labeled to that effect. (See *Suspensions*.)

Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Single-phase gels may be made from synthetic macromolecules (e.g., *Carbomer*) or from natural gums (e.g., *Tragacanth*). The latter preparations are also called mucilages. Although these gels are commonly aqueous, alcohols and oils may be used as the continuous phase. For example, mineral oil can be combined with a polyethylene resin to form an oleaginous ointment base.

Gels can be used to administer drugs topically or into body cavities (e.g., *Phenylephrine Hydrochloride Nasal Jelly*).

IMPLANTS (PELLETS)

Implants or pellets are small sterile solid masses consisting of a highly purified drug (with or without excipients) made by compression or molding. They are intended for implantation in the body (usually subcutaneously) for the purpose of providing continuous release of the drug over long periods of time. Implants are administered by means of a suitable special injector or surgical incision. This dosage form has been used to administer hormones such as testosterone or estradiol. They are packaged individually in sterile vials or foil strips.

INFUSIONS, INTRAMAMMARY

Intramammary infusions are suspensions of drugs in suitable oil vehicles. These preparations are intended for veterinary use only, and are administered by instillation via the teat canals into the udders of milk-producing animals.

INHALATIONS

Inhalations are drugs or solutions or suspensions of one or more drug substances administered by the nasal or oral respiratory route for local or systemic effect.

Solutions of drug substances in sterile water for inhalation or in sodium chloride inhalation solution may be nebulized by use of inert gases. Nebulizers are suitable for the administration of inhalation solutions only if they give droplets sufficiently fine and uniform in size so that the mist reaches the bronchioles. Nebulized solutions may be breathed directly from the nebulizer or the nebulizer may be attached to a plastic face mask, tent, or intermittent positive pressure breathing (IPPB) machine.

Another group of products, also known as metered dose inhalers (MDIs) are propellant driven drug suspensions or solutions in liquified gas propellant with or without a cosolvent and are intended for delivering metered doses of the drug to the respiratory tract. An MDI contains multiple doses, often exceeding several hundred. The most common single dose volumes delivered are from 25 to 100 µL (also expressed as mg) per actuation.

Examples of MDIs containing drug solutions and suspensions in this pharmacopeia are *Epinephrine Inhalation Aerosol* and *Isoproterenol Hydrochloride and Phenylephrine Bitartrate Inhalation Aerosol*, respectively.

Powders may also be administered by mechanical devices that require manually produced pressure or a deep inhalation by the patient (e.g., *Cromolyn Sodium for Inhalation*).

A special class of inhalations termed inhalants consists of drugs or combination of drugs, that by virtue of their high vapor pressure, can be carried by an air current into the nasal passage where they exert their effect. The container from which the inhalant generally is administered is known as an inhaler.

INJECTIONS

An Injection is a preparation intended for parenteral administration or for constituting or diluting a parenteral article prior to administration (see *Injections* ⟨1⟩).

Each container of an Injection is filled with a volume in slight excess of the labeled "size" or that volume that is to be withdrawn. The excess volumes recommended in the accompanying table are usually sufficient to permit withdrawal and administration of the labeled volumes.

	Recommended Excess Volume	
Labeled Size	For Mobile Liquids	For Viscous Liquids
0.5 mL	0.10 mL	0.12 mL
1.0 mL	0.10 mL	0.15 mL
2.0 mL	0.15 mL	0.25 mL
5.0 mL	0.30 mL	0.50 mL
10.0 mL	0.50 mL	0.70 mL
20.0 mL	0.60 mL	0.90 mL
30.0 mL	0.80 mL	1.20 mL
50.0 mL or more	2%	3%

IRRIGATIONS

Irrigations are sterile solutions intended to bathe or flush open wounds or body cavities. They are used topically, never parenterally. They are labeled to indicate that they are not intended for injection.

LOTIONS

See *Solutions* or *Suspensions*.

In-Process Revision

~~LOZENGES~~

~~Lozenges are solid preparations, that are intended to dissolve or disintegrate slowly in the mouth. They contain one or more medicaments, usually in a flavored, sweetened base. They can be prepared by molding (gelatin and/or fused sucrose or sorbitol base) or by compression of sugar-based tablets. Molded lozenges are sometimes referred to as pastilles while compressed lozenges are often referred to as troches. They are usually intended for treatment of local irritation or infections of the mouth or throat but may contain active ingredients intended for systemic absorption after swallowing.~~

~~OINTMENTS~~

~~Ointments are semisolid preparations intended for external application to the skin or mucous membranes.~~

~~Ointment bases recognized for use as vehicles fall into four general classes: the hydrocarbon bases, the absorption bases, the water-removable bases, and the water-soluble bases. Each therapeutic ointment possesses as its base a representative of one of these four general classes.~~

~~Hydrocarbon Bases~~

~~These bases, which are known also as ''oleaginous ointment bases,'' are represented by *White Petrolatum* and *White Ointment*. Only small amounts of an aqueous component can be incorporated into them. They serve to keep medicaments in prolonged contact with the skin and act as occlusive dressings. Hydrocarbon bases are used chiefly for their emollient effects, and are difficult to wash off. They do not ''dry out'' or change noticeably on aging.~~

~~Absorption Bases~~

~~This class of bases may be divided into two groups: the first group consisting of bases that permit the incorporation of aqueous solutions with the formation of a water-in-oil emulsion *(Hydrophilic Petrolatum* and *Lanolin)*, and the second group consisting of water-in-oil emulsions that permit the incorporation of additional quantities of aqueous solutions *(Lanolin)*. Absorption bases are useful also as emollients.~~

~~Water-Removable Bases~~

~~Such bases are oil-in-water emulsions, e.g., *Hydrophilic Ointment*, and are more correctly called ''creams.'' (See *Creams*.) They are also described as ''water washable,'' since they may be readily washed from the skin or clothing with water, an attribute that makes them more acceptable for cosmetic reasons. Some medicaments may be more effective in these bases than in hydrocarbon bases. Other advantages of the water-removable bases are that they may be diluted with water and that they favor the absorption of serous discharges in dermatological conditions.~~

~~Water-Soluble Bases~~

~~This group of so-called ''greaseless ointment bases'' comprises water-soluble constituents. *Polyethylene Glycol Ointment* is the only Pharmacopeial preparation in this group. Bases of this type offer many of the advantages of the water-removable bases and, in addition, contain no water-insoluble substances such as petrolatum, anhydrous lanolin, or waxes. They are more correctly called ''Gels.'' (See *Gels*.)~~

~~**Choice of Base**—The choice of an ointment base depends upon many factors, such as the action desired, the nature of the medicament to be incorporated and its bioavailability and stability, and the requisite shelf life of the finished product. In some cases, it is necessary to use a base that is less than ideal in order to achieve the stability required. Drugs that hydrolyze rapidly, for example, are more stable in hydrocarbon bases than in bases containing water, even though they may be more effective in the latter.~~

~~OPHTHALMIC PREPARATIONS~~

~~Drugs are administered to the eyes in a wide variety of dosage forms, some of which require special consideration. They are discussed in the following paragraphs.~~

~~Ointments~~

~~Ophthalmic ointments are ointments for application to the eye. Special precautions must be taken in the preparation of ophthalmic ointments. They are manufactured from sterilized ingredients under rigidly aseptic conditions and meet the requirements under *Sterility Tests* ⟨71⟩. If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described under *Sterility Tests* ⟨71⟩, along with aseptic manufacture, may be employed. Ophthalmic ointments must contain a suitable substance or mixture of substances to prevent growth of, or to destroy, microorganisms accidentally introduced when the container is opened during use, unless otherwise directed in the individual monograph, or unless the formula itself is bacteriostatic (see *Added Substances* under *Ophthalmic Ointments* ⟨771⟩). The medicinal agent is added to the ointment base either as a solution or as a micronized powder. The finished ointment must be free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* under *Ophthalmic Ointments* ⟨771⟩. The immediate containers for ophthalmic ointments shall be sterile at the time of filling and closing. It is mandatory that the immediate containers for ophthalmic ointments be sealed and tamper-proof so that sterility is assured at time of first use.~~

~~The ointment base that is selected must be nonirritating to the eye, permit diffusion of the drug throughout the secretions bathing the eye, and retain the activity of the medicament for a reasonable period under proper storage conditions.~~

~~Petrolatum is mainly used as a base for ophthalmic drugs. Some absorption bases, water-removable bases, and water-soluble bases may be desirable for water-soluble drugs. Such bases allow for better dispersion of water-soluble medicaments, but they must be nonirritating to the eye.~~

~~Solutions~~

~~Ophthalmic solutions are sterile solutions, essentially free from foreign particles, suitably compounded and packaged for instillation into the eye. Preparation of an ophthalmic solution requires careful consideration of such factors as the inherent toxicity of the drug itself, isotonicity value, the need for buffering agents, the need for a preservative (and, if needed, its selection), sterilization, and proper packaging. Similar considerations are also made for nasal and otic products.~~

~~ISOTONICITY VALUE~~

~~Lacrimal fluid is isotonic with blood, having an isotonicity value corresponding to that of a 0.9% sodium chloride solution. Ideally, an ophthalmic solution should have this isotonicity value; but the eye can tolerate isotonicity values as low as that of a 0.6% sodium chloride solution and as high as that of a 2.0% sodium chloride solution without marked discomfort.~~

~~Some ophthalmic solutions are necessarily hypertonic in order to enhance absorption and provide a concentration of the active ingredient(s) strong enough to exert a prompt and effective action. Where the amount of such solutions used is small, dilution with lacrimal fluid takes place rapidly so that discomfort from the hypertonicity is only temporary. However, any adjust-~~

ment toward isotonicity by dilution with tears is negligible where large volumes of hypertonic solutions are used as collyria to wash the eyes; it is, therefore, important that solutions used for this purpose be approximately isotonic.

BUFFERING

Many drugs, notably alkaloidal salts, are most effective at pH levels that favor the undissociated free bases. At such pH levels, however, the drug may be unstable so that compromise levels must be found and held by means of buffers. One purpose of buffering some ophthalmic solutions is to prevent an increase in pH caused by the slow release of hydroxyl ions by glass. Such a rise in pH can affect both the solubility and the stability of the drug. The decision whether or not buffering agents should be added in preparing an ophthalmic solution must be based on several considerations. Normal tears have a pH of about 7.4 and possess some buffer capacity. The application of a solution to the eye stimulates the flow of tears and the rapid neutralization of any excess hydrogen or hydroxyl ions within the buffer capacity of the tears. Many ophthalmic drugs, such as alkaloidal salts, are weakly acidic and have only weak buffer capacity. Where only 1 or 2 drops of a solution containing them are added to the eye, the buffering action of the tears is usually adequate to raise the pH and prevent marked discomfort. In some cases pH may vary between 3.5 and 8.5. Some drugs, notably pilocarpine hydrochloride and epinephrine bitartrate, are more acid and overtax the buffer capacity of the lacrimal fluid. Ideally, an ophthalmic solution should have the same pH, as well as the same isotonicity value, as lacrimal fluid. This is not usually possible since, at pH 7.4, many drugs are not appreciably soluble in water. Most alkaloidal salts precipitate as the free alkaloid at this pH. Additionally, many drugs are chemically unstable at pH levels approaching 7.4. This instability is more marked at the high temperatures employed in heat sterilization. For this reason, the buffer system should be selected that is nearest to the physiological pH of 7.4 and does not cause precipitation of the drug or its rapid deterioration.

An ophthalmic preparation with a buffer system approaching the physiological pH can be obtained by mixing a sterile solution of the drug with a sterile buffer solution using aseptic technique. Even so, the possibility of a shorter shelf life at the higher pH must be taken into consideration, and attention must be directed toward the attainment and maintenance of sterility throughout the manipulations.

Many drugs, when buffered to a therapeutically acceptable pH, would not be stable in solution for long periods of time. These products are lyophilized and are intended for reconstitution immediately before use (e.g., *Acetylcholine Chloride for Ophthalmic Solution*).

STERILIZATION

The sterility of solutions applied to an injured eye is of the greatest importance. Sterile preparations in special containers for individual use on one patient should be available in every hospital, office, or other installation where accidentally or surgically traumatized eyes are treated. The method of attaining sterility is determined primarily by the character of the particular product (see *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩).

Whenever possible, sterile membrane filtration under aseptic conditions is the preferred method. If it can be shown that product stability is not adversely affected, sterilization by autoclaving in the final container is also a preferred method.

Buffering certain drugs near the physiological pH range makes them quite unstable at high temperature.

Avoiding the use of heat by employing a bacteria retaining filter is a valuable technique, provided caution is exercised in the selection, assembly, and use of the equipment. Single filtration, presterilized disposable units are available and should be utilized wherever possible.

PRESERVATION

Ophthalmic solutions may be packaged in multiple dose containers when intended for the individual use of one patient and where the ocular surfaces are intact. It is mandatory that the immediate containers for ophthalmic solutions be sealed and tamper proof so that sterility is assured at time of first use. Each solution must contain a suitable substance or mixture of substances to prevent the growth of, or to destroy, microorganisms accidentally introduced when the container is opened during use.

Where intended for use in surgical procedures, ophthalmic solutions, although they must be sterile, should not contain antibacterial agents, since they may be irritating to the ocular tissues.

THICKENING AGENT

A pharmaceutical grade of methylcellulose (e.g., 1% if the viscosity is 25 centipoises, or 0.25% if 4000 centipoises) or other suitable thickening agents such as hydroxypropyl methylcellulose or polyvinyl alcohol occasionally are added to ophthalmic solutions to increase the viscosity and prolong contact of the drug with the tissue. The thickened ophthalmic solution must be free from visible particles.

Suspensions

Ophthalmic suspensions are sterile liquid preparations containing solid particles dispersed in a liquid vehicle intended for application to the eye (see *Suspensions*). It is imperative that such suspensions contain the drug in a micronized form to prevent irritation and/or scratching of the cornea. Ophthalmic suspensions should never be dispensed if there is evidence of caking or aggregation.

Strips

Fluorescein sodium solution should be dispensed in a sterile, single use container or in the form of a sterile, impregnated paper strip. The strip releases a sufficient amount of the drug for diagnostic purposes when touched to the eye being examined for a foreign body or a corneal abrasion. Contact of the paper with the eye may be avoided by leaching the drug from the strip onto the eye with the aid of sterile water or sterile sodium chloride solution.

PASTES

Pastes are semisolid dosage forms that contain one or more drug substances intended for topical application. One class is made from a single phase aqueous gel (e.g., *Carboxymethylcellulose Sodium Paste*). The other class, the fatty pastes (e.g., *Zinc Oxide Paste*), consists of thick, stiff ointments that do not ordinarily flow at body temperature, and therefore serve as protective coatings over the areas to which they are applied.

The fatty pastes appear less greasy and more absorptive than ointments by reason of a high proportion of drug substance(s) having an affinity for water. These pastes tend to absorb serous secretions, and are less penetrating and less macerating than ointments, so that they are preferred for acute lesions that have a tendency towards crusting, vesiculation, or oozing.

A dental paste is intended for adhesion to the mucous membrane for local effect (e.g., *Triamcinolone Acetonide Dental Paste*). Some paste preparations intended for administration to animals are applied orally. The paste is squeezed into the mouth of the animal, generally at the back of the tongue, or is spread inside the mouth.

In-Process Revision

PELLETS

See *Implants*.

POWDERS

Powders are intimate mixtures of dry, finely divided drugs and/or chemicals that may be intended for internal (Oral Powders) or external (Topical Powders) use. Because of their greater specific surface area, powders disperse and dissolve more readily than compacted dosage forms. Children and those adults who experience difficulty in swallowing tablets or capsules may find powders more acceptable. Drugs that are too bulky to be formed into tablets or capsules of convenient size may be administered as powders. Immediately prior to use, oral powders are mixed in a beverage or apple sauce.

Often, stability problems encountered in liquid dosage forms are avoided in powdered dosage forms. Drugs that are unstable in aqueous suspensions or solutions may be prepared in the form of granules or powders. These are intended to be constituted by the pharmacist by the addition of a specified quantity of water just prior to dispensing. Because these constituted products have limited stability, they are required to have a specified expiration date after constitution and may require storage in a refrigerator.

Oral powders may be dispensed in doses premeasured by the pharmacist, i.e., divided powders, or in bulk. Traditionally, divided powders have been wrapped in materials such as bond paper and parchment. However, the pharmacist may provide greater protection from the environment by sealing individual doses in small cellophane or polyethylene envelopes.

Granules for veterinary use may be administered by sprinkling the dry powder on animal feed or by mixing it with animal food.

Bulk oral powders are limited to relatively nonpotent drugs such as laxatives, antacids, dietary supplements, and certain analgesics that the patient may safely measure by the teaspoonful or capful. Other bulky powders include douche powders, tooth powders, and dusting powders. Bulk powders are best dispensed in tight, wide mouth glass containers to afford maximum protection from the atmosphere and to prevent the loss of volatile constituents.

Dusting powders are impalpable powders intended for topical application. They may be dispensed in sifter-top containers to facilitate dusting onto the skin. In general, dusting powders should be passed through at least a 100-mesh sieve to assure freedom from grit that could irritate traumatized areas (see *Powder Fineness* ⟨811⟩).

PREMIXES

Premixes are mixtures of one or more drug substances with suitable vehicles. Premixes are intended for admixture to animal feedstuffs before administration. They are used to facilitate dilution of the active drug components with animal feed. Premixes should be as homogeneous as possible. It is essential that materials of suitable fineness be used and that thorough mixing be achieved at all stages of premix preparation. Premixes may be prepared as powder, pellets, or in granulated form. The granulated form is free flowing and free from aggregates.

SOLUTIONS

Solutions are liquid preparations that contain one or more chemical substances dissolved, i.e., molecularly dispersed, in a suitable solvent or mixture of mutually miscible solvents. Since molecules in solutions are uniformly dispersed, the use of solutions as dosage forms generally provides for the assurance of uniform dosage upon administration, and good accuracy when diluting or otherwise mixing solutions.

Substances in solutions, however, are more susceptible to chemical instability than the solid state and dose for dose, generally require more bulk and weight in packaging relative to solid dosage forms. For all solutions, but particularly those containing volatile solvents, tight containers, stored away from excessive heat, should be used. Consideration should also be given to the use of light resistant containers when photolytic chemical degradation is a potential stability problem. Dosage forms categorized as "Solutions" are classified according to route of administration, such as "Oral Solutions" and "Topical Solutions," or by their solute and solvent systems, such as "Spirits," "Tinctures," and "Waters." Solutions intended for parenteral administration are officially entitled "Injections" (see *Injections* ⟨1⟩).

Oral Solutions

Oral Solutions are liquid preparations, intended for oral administration, that contain one or more substances with or without flavoring, sweetening, or coloring agents dissolved in water or cosolvent water mixtures. Oral Solutions may be formulated for direct oral administration to the patient or they may be dispensed in a more concentrated form that must be diluted prior to administration. It is important to recognize that dilution with water of Oral Solutions containing cosolvents, such as alcohol, could lead to precipitation of some ingredients. Hence, great care must be taken in diluting concentrated solutions when cosolvents are present. Preparations dispensed as soluble solids or soluble mixtures of solids, with the intent of dissolving them in a solvent and administering them orally, are designated "for Oral Solution" (e.g., *Potassium Chloride for Oral Solution*).

Oral Solutions containing high concentrations of sucrose or other sugars traditionally have been designated as Syrups. A near saturated solution of sucrose in purified water, for example, is known as Syrup or "Simple Syrup." Through common usage the term, syrup, also has been used to include any other liquid dosage form prepared in a sweet and viscid vehicle, including oral suspensions.

In addition to sucrose and other sugars, certain polyols such as sorbitol or glycerin may be present in Oral Solutions to inhibit crystallization and to modify solubility, taste, mouth feel, and other vehicle properties. Antimicrobial agents to prevent the growth of bacteria, yeasts, and molds are generally also present. Some sugarless Oral Solutions contain sweetening agents such as sorbitol or aspartame, as well as thickening agents such as the cellulose gums. Such viscid sweetened solutions, containing no sugars, are occasionally prepared as vehicles for administration of drugs to diabetic patients.

Many oral solutions, that contain alcohol as a cosolvent, have been traditionally designated as Elixirs. However, many others designated as Oral Solutions also contain significant amounts of alcohol. Since high concentrations of alcohol can produce a pharmacologic effect when administered orally, other cosolvents, such as glycerin and propylene glycol, should be used to minimize the amount of alcohol required. To be designated as an Elixir, however, the solution must contain alcohol.

Topical Solutions

Topical Solutions are solutions, usually aqueous but often containing other solvents, such as alcohol and polyols, intended for topical application to the skin, or as in the case of *Lidocaine Oral Topical Solution,* to the oral mucosal surface. The term "lotion" is applied to solutions or suspensions applied topically.

Otic Solutions

Otic Solutions, intended for instillation in the outer ear, are aqueous, or they are solutions prepared with glycerin or other solvents and dispersing agents (e.g., *Antipyrine and Benzocaine Otic Solution* and *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution*).

Ophthalmic Solutions

See *Ophthalmic Preparations*.

Spirits

Spirits are alcoholic or hydroalcoholic solutions of volatile substances prepared usually by simple solution or by admixture of the ingredients. Some spirits serve as flavoring agents while others have medicinal value. Reduction of the high alcoholic content of spirits by admixture with aqueous preparations often causes turbidity.

Spirits require storage in tight, light resistant containers to prevent loss by evaporation and to limit oxidative changes.

Tinctures

Tinctures are alcoholic or hydroalcoholic solutions prepared from vegetable materials or from chemical substances.

The proportion of drug represented in the different chemical tinctures is not uniform but varies according to the established standards for each. Traditionally, tinctures of potent vegetable drugs essentially represent the activity of 10 g of the drug in each 100 mL of tincture, the potency being adjusted following assay. Most other vegetable tinctures represent 20 g of the respective vegetable material in each 100 mL of tincture.

PROCESS P

Carefully mix the ground drug or mixture of drugs with a sufficient quantity of the prescribed solvent or solvent mixture to render it evenly and distinctly damp, allow it to stand for 15 minutes, transfer it to a suitable percolator, and pack the drug firmly. Pour on enough of the prescribed solvent or solvent mixture to saturate the drug, cover the top of the percolator, and, when the liquid is about to drip from the percolator, close the lower orifice and allow the drug to macerate for 24 hours or for the time specified in the monograph. If no assay is directed, allow the percolation to proceed slowly, or at the specified rate, gradually adding sufficient solvent or solvent mixture to produce 1000 mL of tincture, and mix (for definitions of flow rates, see under *Extracts and Fluidextracts*). If an assay is directed, collect only 950 mL of percolate, mix this, and assay a portion of it as directed. Dilute the remainder with such quantity of the prescribed solvent or solvent mixture as calculation from the assay indicates is necessary to produce a tincture that conforms to the prescribed standard, and mix.

PROCESS M

Macerate the drug with 750 mL of the prescribed solvent or solvent mixture in a container that can be closed, and put in a warm place. Agitate it frequently during 3 days or until the soluble matter is dissolved. Transfer the mixture to a filter, and when most of the liquid has drained away, wash the residue on the filter with a sufficient quantity of the prescribed solvent or solvent mixture, combining the filtrates, to produce 1000 mL of tincture, and mix.

Tinctures require storage in tight, light resistant containers, away from direct sunlight and excessive heat.

Waters, Aromatic

Aromatic waters are clear, saturated aqueous solutions (unless otherwise specified) of volatile oils or other aromatic or volatile substances. Their odors and tastes are similar, respectively, to those of the drugs or volatile substances from which they are prepared, and they are free from empyreumatic and other foreign odors. Aromatic waters may be prepared by distillation or solution of the aromatic substance, with or without the use of a dispersing agent.

Aromatic waters require protection from intense light and excessive heat.

SUPPOSITORIES

Suppositories are solid bodies of various weights and shapes, adapted for introduction into the rectal, vaginal, or urethral orifice of the human body. They usually melt, soften, or dissolve at body temperature. A suppository may act as a protectant or palliative to the local tissues at the point of introduction or as a carrier of therapeutic agents for systemic or local action. Suppository bases usually employed are cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights, and fatty acid esters of polyethylene glycol.

The suppository base employed has a marked influence on the release of the active ingredient incorporated in it. While cocoa butter melts quickly at body temperature, it is immiscible with body fluids and this inhibits the diffusion of fat soluble drugs to the affected sites. Polyethylene glycol is a suitable base for some antiseptics. In cases where systemic action is expected, it is preferable to incorporate the ionized rather than the nonionized form of the drug, in order to maximize bioavailability. Although nonionized drugs partition more readily out of water-miscible bases such as glycerinated gelatin and polyethylene glycol, the bases themselves tend to dissolve very slowly and thus retard release in this manner. Oleaginous vehicles such as cocoa butter are seldom used in vaginal preparations because of the nonabsorbable residue formed, while glycerinated gelatin is seldom used rectally because of its slow dissolution. Cocoa butter and its substitutes (Hard Fat) are superior for allaying irritation, as in preparations intended for treating internal hemorrhoids.

Cocoa Butter Suppositories

Suppositories having cocoa butter as the base may be made by means of incorporating the finely divided medicinal substance into the solid oil at room temperature and suitably shaping the resulting mass, or by working with the oil in the melted state and allowing the resulting suspension to cool in molds. A suitable quantity of hardening agents may be added to counteract the tendency of some medicaments such as chloral hydrate and phenol to soften the base. It is important that the finished suppository melt at body temperature.

The approximate weights of suppositories prepared with cocoa butter are given below. Suppositories prepared from other bases vary in weight and generally are heavier than the weights indicated here.

Rectal Suppositories for adults are tapered at one or both ends and usually weigh about 2 g each.

Vaginal Suppositories are usually globular or oviform and weigh about 5 g each. They are made from water soluble or water miscible vehicles such as polyethylene glycol or glycerinated gelatin.

Suppositories with cocoa butter base require storage in well-closed containers, preferably at a temperature below 30° (controlled room temperature).

Cocoa Butter Substitutes

Fat-type suppository bases can be produced from a variety of vegetable oils, such as coconut or palm kernel, which are modified by esterification, hydrogenation, and fractionation to obtain products of varying composition and melting temperatures (e.g., *Hydrogenated Vegetable Oil* and *Hard Fat*). These products can be so designed as to reduce rancidity. At the same time, desired characteristics such as narrow intervals between melt-

In-Process Revision

ing and solidification temperatures, and melting ranges to accommodate various formulation and climatic conditions, can be built in.

Glycerinated Gelatin Suppositories

Medicinal substances may be incorporated into glycerinated gelatin bases by addition of the prescribed quantities to a vehicle consisting of about 70 parts of glycerin, 20 parts of gelatin, and 10 parts of water.

Glycerinated gelatin suppositories require storage in tight containers, preferably at a temperature below 35°.

Polyethylene Glycol Base Suppositories

Several combinations of polyethylene glycols having melting temperatures that are above body temperature have been used as suppository bases. Inasmuch as release from these bases depends on dissolution rather than on melting, there are significantly fewer problems in preparation and storage than exist with melting type vehicles. However, high concentrations of higher molecular weight polyethylene glycols may lengthen dissolution time, resulting in problems with retention. Labels on polyethylene glycol suppositories should contain directions that they be moistened with water before inserting. Although they can be stored without refrigeration, they should be packaged in tightly closed containers.

Surfactant Suppository Bases

Several nonionic surface active agents closely related chemically to the polyethylene glycols can be used as suppository vehicles. Examples of such surfactants are polyoxyethylene sorbitan fatty acid esters and the polyoxyethylene stearates. These surfactants are used alone or in combination with other suppository vehicles to yield a wide range of melting temperatures and consistencies. One of the major advantages of such vehicles is their water dispersibility. However, care must be taken with the use of surfactants, because they may either increase the rate of drug absorption or interact with drug molecules, causing a decrease in therapeutic activity.

Tableted Suppositories or Inserts

Vaginal suppositories occasionally are prepared by the compression of powdered materials into a suitable shape. They are prepared also by encapsulation in soft gelatin.

SUSPENSIONS

Suspensions are liquid preparations that consist of solid particles dispersed throughout a liquid phase in which the particles are not soluble. Dosage forms officially categorized as "Suspensions" are designated as such if they are not included in other more specific categories of suspensions, such as Oral Suspensions, Topical Suspensions, etc. (see these other categories). Some suspensions are prepared and ready for use, while others are prepared as solid mixtures intended for constitution just before use with an appropriate vehicle. Such products are designated "for Oral Suspension", etc. The term "Milk" is sometimes used for suspensions in aqueous vehicles intended for oral administration (e.g., *Milk of Magnesia*). The term "Magma" is often used to describe suspensions of inorganic solids such as clays in water, where there is a tendency for strong hydration and aggregation of the solid, giving rise to gel-like consistency and thixotropic rheological behavior (e.g., *Bentonite Magma*). The term "Lotion" has been used to categorize many topical suspensions and emulsions intended for application to the skin (e.g., *Calamine Lotion*). Some suspensions are prepared in sterile form and are used as Injectables, as well as for ophthalmic and otic administration. These may be of two types, ready to use or intended for constitution with a prescribed amount of Water for Injection or other suitable diluent before use by the designated route. Suspensions should not be injected intravenously or intrathecally.

Suspensions intended for any route of administration should contain suitable antimicrobial agents to protect against bacteria, yeast, and mold contamination (see *Emulsions* for some consideration of antimicrobial preservative properties that apply also to Suspensions). By its very nature, the particular matter in a suspension may settle or sediment to the bottom of the container upon standing. Such sedimentation may also lead to caking and solidification of the sediment with a resulting difficulty in redispersing the suspension upon agitation. To prevent such problems, suitable ingredients that increase viscosity and the gel state of the suspension, such as clays, surfactants, polyols, polymers, or sugars, should be added. It is important that suspensions always be shaken well before use to ensure uniform distribution of the solid in the vehicle, thereby ensuring uniform and proper dosage. Suspensions require storage in tight containers.

Oral Suspensions

Oral Suspensions are liquid preparations containing solid particles dispersed in a liquid vehicle, with suitable flavoring agents, intended for oral administration. Some suspensions labeled as "Milks" or "Magmas" fall into this category.

Topical Suspensions

Topical Suspensions are liquid preparations containing solid particles dispersed in a liquid vehicle, intended for application to the skin. Some suspensions labeled as "Lotions" fall into this category.

Otic Suspensions

Otic Suspensions are liquid preparations containing micronized particles intended for instillation in the outer ear.

Ophthalmic Suspensions

See *Ophthalmic Preparations*.

SYRUPS

See *Oral Solutions*.

SYSTEMS

In recent years, a number of dosage forms have been developed using modern technology that allows for the uniform release or targeting of drugs to the body. These products are commonly called delivery systems. The most widely used of these are Transdermal Systems.

Transdermal Systems

Transdermal drug delivery systems are self contained, discrete dosage forms that, when applied to intact skin, are designed to deliver the drug(s) through the skin to the systemic circulation. Systems typically comprise an outer covering (barrier), a drug reservoir, which may have a rate controlling membrane, a contact adhesive applied to some or all parts of the system and the system/skin interface, and a protective liner that is removed before applying the system. The activity of

these systems is defined in terms of the release rate of the drug(s) from the system. The total duration of drug release from the system and the system surface area may also be stated.

Transdermal drug delivery systems work by diffusion: the drug diffuses from the drug reservoir, directly or through the rate-controlling membrane and/or contact adhesive if present, and then through the skin into the general circulation. Typically, modified-release systems are designed to provide drug delivery at a constant rate, such that a true steady-state blood concentration is achieved and maintained until the system is removed. At that time, blood concentration declines at a rate consistent with the pharmacokinetics of the drug.

Transdermal drug delivery systems are applied to body areas consistent with the labeling for the product(s). As long as drug concentration at the system/skin interface remains constant, the amount of drug in the dosage form does not influence plasma concentrations. The functional lifetime of the system is defined by the initial amount of drug in the reservoir and the release rate from the reservoir.

NOTE—Drugs for local rather than systemic effect are commonly applied to the skin embedded in glue on a cloth or plastic backing. These products are defined traditionally as plasters or tapes.

Ocular System

Another type of system is the ocular system, which is intended for placement in the lower conjunctival fornix from which the drug diffuses through a membrane at a constant rate (e.g., *Pilocarpine Ocular System*).

Intrauterine System

An intrauterine system, based on a similar principle but intended for release of drug over a much longer period of time, e.g., one year, is also available (e.g., *Progesterone Intrauterine Contraceptive System*).

TABLETS

Tablets are solid dosage forms containing medicinal substances with or without suitable diluents. They may be classed, according to the method of manufacture, as compressed tablets or molded tablets.

The vast majority of all tablets manufactured are made by compression, and compressed tablets are the most widely used dosage form in this country. Compressed tablets are prepared by the application of high pressures, utilizing steel punches and dies, to powders or granulations. Tablets can be produced in a wide variety of sizes, shapes, and surface markings, depending upon the design of the punches and dies. Capsule-shaped tablets are commonly referred to as caplets. Boluses are large tablets intended for veterinary use, usually for large animals.

Molded tablets are prepared by forcing dampened powders under low pressure into die cavities. Solidification depends upon crystal bridges built up during the subsequent drying process, and not upon the compaction force.

Tablet triturates are small, usually cylindrical, molded or compressed tablets. Tablet triturates were traditionally used as dispensing tablets in order to provide a convenient, measured quantity of a potent drug for compounding purposes. Such tablets are rarely used today. Hypodermic tablets are molded tablets made from completely and readily water-soluble ingredients and formerly were intended for use in making preparations for hypodermic injection. They are employed orally, or where rapid drug availability is required such as in the case of *Nitroglycerin Tablets*, sublingually.

Buccal tablets are intended to be inserted in the buccal pouch, and sublingual tablets are intended to be inserted beneath the tongue, where the active ingredient is absorbed directly through the oral mucosa. Few drugs are readily absorbed in this way, but for those that are (such as nitroglycerin and certain steroid hormones), a number of advantages may result.

Soluble, effervescent tablets are prepared by compression and contain, in addition to active ingredients, mixtures of acids (citric acid, tartaric acid) and sodium bicarbonate, which release carbon dioxide when dissolved in water. They are intended to be dissolved or dispersed in water before administration. Effervescent tablets should be stored in tightly closed containers or moisture-proof packs and labeled to indicate that they are not to be swallowed directly.

Chewable Tablets

Chewable tablets are formulated and manufactured so that they may be chewed, producing a pleasant-tasting residue in the oral cavity that is easily swallowed and does not leave a bitter or unpleasant aftertaste. These tablets have been used in tablet formulations for children, especially multivitamin formulations, and for the administration of antacids and selected antibiotics. Chewable tablets are prepared by compression, usually utilizing mannitol, sorbitol, or sucrose as binders and fillers, and containing colors and flavors to enhance their appearance and taste.

Preparation of Molded Tablets

Molded tablets are prepared from mixtures of medicinal substances and a diluent usually consisting of lactose and powdered sucrose in varying proportions. The powders are dampened with solutions containing high percentages of alcohol. The concentration of alcohol depends upon the solubility of the active ingredients and fillers in the solvent system and the desired degree of hardness of the finished tablets. The dampened powders are pressed into molds, removed, and allowed to dry. Molded tablets are quite friable and care must be taken in packaging and dispensing.

Formulation of Compressed Tablets

Most compressed tablets consist of the active ingredient and a diluent (filler), binder, disintegrating agent, and lubricant. Approved FD&C and D&C dyes or lakes (dyes adsorbed onto insoluble aluminum hydroxide), flavors, and sweetening agents may also be present. Diluents are added where the quantity of active ingredient is small or difficult to compress. Common tablet fillers include lactose, starch, dibasic calcium phosphate, and microcrystalline cellulose. Chewable tablets often contain sucrose, mannitol, or sorbitol as a filler. Where the amount of active ingredient is small, the overall tableting properties are in large measure determined by the filler. Because of problems encountered with bioavailability of hydrophobic drugs of low water solubility, water-soluble diluents are used as fillers for these tablets.

Binders give adhesiveness to the powder during the preliminary granulation and to the compressed tablet. They add to the cohesive strength already available in the diluent. While binders may be added dry, they are more effective when added out of solution. Common binders include acacia, gelatin, sucrose, povidone, methylcellulose, carboxymethylcellulose, and hydrolyzed starch pastes. The most effective dry binder is microcrystalline cellulose, which is commonly used for this purpose in tablets prepared by direct compression.

A disintegrating agent serves to assist in the fragmentation of the tablet after administration. The most widely used tablet disintegrating agent is starch. Chemically modified starches and cellulose, alginic acid, microcrystalline cellulose, and cross-linked povidone, are also used for this purpose. Effervescent mixtures are used in soluble tablet systems as disintegrating agents. The concentration of the disintegrating agent, method of addition, and degree of compaction play a role in effectiveness.

Lubricants reduce friction during the compression and ejection cycle. In addition, they aid in preventing adherence of tablet material to the dies and punches. Metallic stearates, stearic acid, hydrogenated vegetable oils, and talc are used as lubricants. Because of the nature of this function, most lubricants are hydrophobic, and as such tend to reduce the rates of tablet disintegration and dissolution. Consequently, excessive concentrations of lubricant should be avoided. Polyethylene glycols and some lauryl sulfate salts have been used as soluble lubricants, but such agents generally do not possess optimal lubricating properties, and comparatively high concentrations are usually required.

Glidants are agents that improve powder fluidity, and they are commonly employed in direct compression where no granulation step is involved. The most effective glidants are the colloidal pyrogenic silicas.

Colorants are often added to tablet formulations for esthetic value or for product identification. Both D&C and FD&C dyes and lakes are used. Most dyes are photosensitive and they fade when exposed to light. The federal Food and Drug Administration regulates the colorants employed in drugs.

Manufacturing Methods

Tablets are prepared by three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression. The purpose of both wet and dry granulation is to improve flow of the mixture and/or to enhance its compressibility.

Dry granulation (slugging) involves the compaction of powders at high pressures into large, often poorly formed tablet compacts. These compacts are then milled and screened to form a granulation of the desired particle size. The advantage of dry granulation is the elimination of both heat and moisture in the processing. Dry granulations can be produced also by extruding powders between hydraulically operated rollers to produce thin cakes which are subsequently screened or milled to give the desired granule size.

Excipients are available that allow production of tablets at high speeds without prior granulation steps. These directly compressible excipients consist of special physical forms of substances such as lactose, sucrose, dextrose, or cellulose, which possess the desirable properties of fluidity and compressibility. The most widely used direct compaction fillers are microcrystalline cellulose, anhydrous lactose, spray-dried lactose, compressible sucrose, and some forms of modified starches. Direct compression avoids many of the problems associated with wet and dry granulations. However, the inherent physical properties of the individual filler materials are highly critical, and minor variations can alter flow and compression characteristics so as to make them unsuitable for direct compression.

Physical evidence of poor tablet quality is discussed under *Stability Considerations in Dispensing Practice* ⟨1191⟩.

WEIGHT VARIATION AND CONTENT UNIFORMITY

Tablets are required to meet a weight variation test (see *Uniformity of Dosage Units* ⟨905⟩) where the active ingredient comprises a major portion of the tablet and where control of weight may be presumed to be an adequate control of drug content uniformity. Weight variation is not an adequate indication of content uniformity where the drug substance comprises a relatively minor portion of the tablet, or where the tablet is sugar-coated. Thus, the Pharmacopeia generally requires that coated tablets and tablets containing 50 mg or less of active ingredient, comprising less than 50% by weight of the dosage form unit, pass a content uniformity test (see *Uniformity of Dosage Units* ⟨905⟩), wherein individual tablets are assayed for actual drug content.

DISINTEGRATION AND DISSOLUTION

Disintegration is an essential attribute of tablets intended for administration by mouth, except for those intended to be chewed before being swallowed and for some types of extended release tablets. A disintegration test is provided (see *Disintegration* ⟨701⟩), and limits on the times in which disintegration is to take place, appropriate for the types of tablets concerned, are given in the individual monographs.

For drugs of limited water solubility, dissolution may be a more meaningful quality attribute than disintegration. A dissolution test (see *Dissolution* ⟨711⟩) is required in a number of monographs on tablets. In many cases, it is possible to correlate dissolution rates with biological availability of the active ingredient. However, such tests are useful mainly as a means of screening preliminary formulations and as a routine quality control procedure.

Coatings

Tablets may be coated for a variety of reasons, including protection of the ingredients from air, moisture, or light, masking of unpleasant tastes and odors, improvement of appearance, and control of the site of drug release in the gastrointestinal tract.

PLAIN COATED TABLETS

Classically, tablets have been coated with sugar applied from aqueous suspensions containing insoluble powders such as starch, calcium carbonate, talc, or titanium dioxide, suspended by means of acacia or gelatin. For purposes of identification and esthetic value, the outside coatings may be colored. The finished coated tablets are polished by application of dilute solutions of wax in solvents such as chloroform or powdered mix. Water-protective coatings consisting of substances such as shellac or cellulose acetate phthalate are often applied out of nonaqueous solvents prior to application of sugar coats. Excessive quantities should be avoided. Drawbacks of sugar coating include the lengthy time necessary for application, the need for waterproofing, which also adversely affects dissolution, and the increased bulk of the finished tablet. These factors have resulted in increased acceptance of film coatings. Film coatings consist of water soluble or dispersible materials such as hydroxypropyl methylcellulose, methylcellulose, hydroxypropylcellulose, carboxymethylcellulose sodium, and mixtures of cellulose acetate phthalate and polyethylene glycols applied out of nonaqueous or aqueous solvents. Evaporation of the solvents leaves a thin film that adheres directly to the tablet and allows it to retain the original shape, including grooves or identification codes.

~~DELAYED-RELEASE TABLETS~~

~~Where the drug may be destroyed or inactivated by the gastric juice or where it may irritate the gastric mucosa, the use of "enteric" coatings is indicated. Such coatings are intended to delay the release of the medication until the tablet has passed through the stomach. The term "delayed release" is used for Pharmacopeial purposes, and the individual monographs include tests and specifications for *Drug release* (see *Drug Release* ⟨724⟩) or *Disintegration* (see *Disintegration* ⟨701⟩).~~

~~EXTENDED-RELEASE TABLETS~~

~~Extended-release tablets are formulated in such manner as to make the contained medicament available over an extended period of time following ingestion. Expressions such as "prolonged action," "repeat action," and "sustained release" have also been used to describe such dosage forms. However, the term "extended release" is used for Pharmacopeial purposes, and requirements for *Drug release* typically are specified in the individual monographs.~~

▪GENERAL CONSIDERATIONS

This chapter provides general descriptions of and definitions for drug products, or dosage forms, commonly used to administer the drug substance [active pharmaceutical ingredient (API)]. It discusses general principles involved in the manufacture or compounding of these dosage forms, and recommendations for proper use and storage. A glossary is provided as a resource on nomenclature.

A dosage form is a combination of drug substances and excipients to facilitate dosing, administration, and delivery of the medicine to the patient. The design and testing of all dosage forms target drug product quality.[1] A testing protocol must consider not only the physical, chemical, and biological properties of the dosage form as appropriate but also the administration route and desired dosing regimen. The interrelationships of dosage forms and routes of administration have been summarized in the compendial taxonomy for pharmaceutical dosage forms (*Figure 1*).[2] The organization of this general information chapter is by the physical attributes of each particular dosage form (*Tier Two*), generally without specific reference to route of administration. Information specific to route of administration is given when needed.

Tests to ensure compliance with pharmacopeial standards for dosage form performance fall into one of the following areas.

[1] In the United States, a drug with a name recognized in *USP–NF* must comply with compendial identity standards or be deemed adulterated, misbranded, or both. To avoid being deemed adulterated, such drugs also must comply with compendial standards for strength, quality, or purity, unless labeled to show all respects in which the drug differs. See the Federal Food, Drug, and Cosmetic Act (FDCA), Sections 501(b) and 502(e)(3)(b), and Food and Drug Administration (FDA) regulations at 21 CFR 299.5. In addition, to avoid being deemed misbranded, drugs recognized in *USP–NF* also must comply with compendial standards for packing and labeling, FDCA Section 502(g). "Quality" is used herein as suitable shorthand for all such compendial requirements. This approach also is consistent with U.S. and FDA participation in the International Conference on Harmonization (ICH). The ICH guideline on specifications, Q6A, notes that "specifications are chosen to confirm the quality of the drug substance and drug product. . ." and defines "quality" as "The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as identity, strength, and purity."

[2] Marshall K, Foster TS, Carlin HS, Williams RL. Development of a compendial taxonomy and glossary for pharmaceutical dosage forms. *Pharm Forum.* 2003;29(5):1742–1752.

Figure 1. Compendial Taxonomy for Pharmaceutical Dosage Forms

Tier One: Route of Administration

Gastro-Intestinal by Injection Mucosal Topical Dermal Inhalation

Tier Two: Dosage Form and Physical Properties

AEROSOLS CAPSULES DRY POWDER INHALER EMULSIONS (CREAMS, LOTIONS)
FOAMS MEDICAL GASES GELS GRANULES MEDICATED GUMS IMPLANTS
INSERTS LIQUIDS LOZENGES OINTMENTS PASTES
TRANSDERMAL SYSTEMS (PATCHES) PELLETS PILLS PLASTERS POWDERS
MEDICATED SOAPS AND SHAMPOOS SOLUTIONS SPRAYS
SUPPOSITORIES SUSPENSIONS TABLETS TAPES

Tier Three: Type of Release Pattern

Immediate Extended Delayed

Tier Four: Comprehensive Testing Strategy

Dose Uniformity (see also *Uniformity of Dosage Units* ⟨905⟩)—Consistency in dosing for a patient or consumer requires that the variation in the drug substance content of each dosage unit be accurately controlled throughout the manufactured batch or compounded lot of drug product. Uniformity of dosage units typically is demonstrated by one of two procedures: content uniformity or weight variation. The procedure for content uniformity requires the assay of drug substance content of individual units, and that for weight variation uses the weight of the individual units to estimate their content. Weight variation may be used where the underlying distribution of drug substance in the blend is presumed to be uniform and well-controlled, as in solutions. In such cases the content of drug substance may be adequately estimated by the net weight. Content uniformity does not rely on the assumption of blend uniformity and can be applied in all cases. Tablets and capsules are assigned a limit below which the weight variation procedure is not applicable. Successful development and manufacture of dosage forms requires careful evaluation of drug substance particle or droplet size, incorporation techniques, and excipient properties.

Stability (see also *Pharmaceutical Stability* ⟨1150⟩)—Drug product stability involves the evaluation of chemical stability, physical stability, and performance over time. The chemical stability of the drug substance in the dose form matrix must support the expiration dating for the commercially prepared dosage forms and a beyond-use date for a compounded dosage form. Test procedures for potency must be stability indicating (see *Validation of Compendial Procedures* ⟨1225⟩). Degradation products should be quantified. In the case of dispersed or emulsified systems, consideration must be given to the potential for settling or separation of the formulation components. Any physical changes to the dosage form must be easily reversed (e.g., by shaking) prior to dosing or administration. In vitro release test procedures such as dissolution and disintegration provide a measure of continuing consistency in performance over time (see *Dissolution* ⟨711⟩, *Disintegration* ⟨701⟩, and *Drug Release* ⟨724⟩).

Bioavailability (see also *In Vitro and In Vivo Evaluation of Dosage Forms* ⟨1088⟩, and *Assessment of Drug Product Performance—Bioavailability, Bioequivalence, and Dissolution* ⟨1090⟩)—Bioavailability is influenced by factors such as the method of manufacture or compounding, particle size, crystal form (polymorph) of the drug substance, the properties of the excipients used to formulate the dosage form, and physical changes as the drug product ages. Assurance of consistency in bioavailability over time (bioequivalence) requires close attention to all aspects of the production (or compounding) and testing of the dosage form. In vitro release (disintegration and dissolution) testing is commonly used as a surrogate to demonstrate consistent availability of the API from the formulated dosage.

Manufacture—Although detailed instructions about the manufacture of any of these dosage forms are beyond the scope of this general information chapter, general manufacturing principles have been included, as well as suggested testing for proper use and storage. Further information relative to extemporaneous compounding of dosage forms can be found in *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩ and *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩.

Route of Administration—The primary routes of administration for pharmaceutical dosage forms can be defined as mucosal, oral, parenteral (by injection), inhalation, and topical/dermal, and each has subcategories as needed. Many tests employed to ensure quality generally are applied across all of the administration routes, but some tests are specific for individual routes. For example, products intended for injection must be evaluated for *Sterility* ⟨71⟩ and *Pyrogen Test* ⟨151⟩, and the manufacturing process (and sterilization technique) employed for parenterals (by injection) should ensure compliance with these tests. Tests for particulate matter may be required for solution dosage forms depending on the route of administration (e.g., by injection—*Particulate Matter in Injections* ⟨788⟩, or mucosal—*Particulate Matter in Ophthalmic Solutions* ⟨789⟩). Additionally, dosage forms intended for the inhalation route of administration must be monitored for particle size and spray pattern (for a metered-dose inhaler or dry-powder inhaler) and droplet size (for nasal sprays). Further information regarding administration routes and suggested testing can be found in the *Guide to General Chapters*, *Charts 4–8* and *10–13*.

An appropriate manufacturing process and testing regimen help ensure that a dosage form can meet the appropriate quality attributes for the intended route of administration.

PRODUCT QUALITY TESTS, GENERAL

ICH Guidance Q6A (available at www.ich.org) recommends specifications (list of tests, references to analytical procedures, and acceptance criteria) to ensure that commercialized drug products are safe and effective at the time of release and over their shelf life. Tests that are universally applied to ensure safety and efficacy (and strength, quality, and purity) include description, identification, assay, and impurities.

Description—According to the ICH guidance a qualitative description (size, shape, color, etc.) of the dosage form should be provided. The acceptance criteria should include the final acceptable appearance. If any of these characteristics change during manufacturing or storage, a quantitative procedure may be appropriate. It specifies the content or the label claim of the article. This parameter is not part of the USP dosage form monograph because it is product specific. USP monographs define the product by specifying the range of acceptable assayed content of the active substance(s) present in the dosage form, together with any additional information about the presence or absence of other components, excipients, or adjuvants.

Identification—Identification tests are discussed in the *General Notices and Requirements*. Identification tests should establish the identity of the drug or drugs present in the drug product and should discriminate between compounds of closely related structure that are likely to be present. Identification tests should be specific for the drug substances. The most conclusive test for identity is the infrared absorption spectrum (see *Spectrophotometry and Light-Scattering* ⟨851⟩ and *Spectrophotometric Identification Tests* ⟨197⟩). If no suitable infrared spectrum can be obtained, other analytical methods can be used. Near-infrared (NIR) or Raman spectrophotometric methods also could be acceptable as the sole identification method of the drug product formulation (see *Near-infrared Spectrophotometry* ⟨1119⟩ and *Raman Spectroscopy* ⟨1120⟩). Identification by a chromatographic retention time from a single procedure is not regarded as specific. The use of retention times from two chromatographic procedures for which the separation is based on different principles or a combination of tests in a single procedure can be acceptable (see *Chromatography* ⟨621⟩ and *Thin-Layer Chromatographic Identification Test* ⟨201⟩).

Assay—A specific and stability-indicating test should be used to determine the strength (API content) of the drug product. Some examples of these procedures are *Antibiotics—Microbial Assays* ⟨81⟩, *Chromatography* ⟨621⟩, or *Assay for Steroids* ⟨351⟩. In cases when the use of a nonspecific assay is justified, e.g., *Titrimetry* ⟨541⟩, other supporting analytical procedures should be used to achieve specificity. When evidence of excipient interference with a nonspecific assay exists, a procedure with demonstrated specificity should be used.

Impurities—Process impurities, synthetic by-products, and other inorganic and organic impurities may be present in the API and excipients used in the manufacture of the drug product. These impurities are evaluated by tests in API and excipients monographs. Impurities arising from degradation of the drug substance or from the drug-product manufacturing process should be monitored. *Residual Solvents* ⟨467⟩ is applied to all products where relevant.

In addition to the universal tests listed above, the following tests may be considered on a case-by-case basis.

Physicochemical Properties—Examples include *pH* ⟨791⟩, *Viscosity* ⟨911⟩, and *Specific Gravity* ⟨841⟩.

Particle Size—For some dosage forms, particle size can have a significant effect on dissolution rates, bioavailability, therapeutic outcome, and stability. Procedures such as *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩, and *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩ could be used.

Uniformity of Dosage Units—See discussion of dose uniformity above.

Water Content—A test for water content is included when appropriate (see *Water Determination* ⟨921⟩).

Microbial Limits—The type of microbial test(s) and acceptance criteria are based on the nature of the drug substance, method of manufacture, and the route of administration (see *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩ and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩).

Antimicrobial Preservative Content—Acceptance criteria for preservative content in multidose products should be established. They are based on the levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* ⟨51⟩).

Antioxidant Preservative Content—If antioxidant preservatives are present in the drug product, tests of their content should be performed.

Sterility—Depending on the route of administration—e.g., ophthalmic preparations, implants, and solutions for injection—sterility of the product is demonstrated as appropriate (see *Sterility Tests* ⟨71⟩).

Dissolution—A test to measure release of the drug substance(s) from the drug product normally is included for dosage forms such as tablets, capsules, suspensions, granules for suspensions, implants, transdermal delivery systems, and medicated chewing gums. Single-point measurements typically are used for immediate-release dosage forms. For modified-release dosage forms, appropriate test conditions and sampling procedures are established as needed (see *Dissolution* ⟨711⟩ and *Drug Release* ⟨724⟩). In some cases, dissolution testing may be replaced by disintegration testing (see *Disintegration* ⟨701⟩).

Hardness and Friability—These parameters are evaluated as in-process controls. Acceptance criteria depend on packaging, supply chain, and intended use (see *Tablet Friability* ⟨1216⟩ and *Tablet Breaking Force* ⟨1217⟩).

Extractables—When evidence exists that extractables from the container-closure systems (e.g., rubber stopper, cap liner, or plastic bottle) have an impact on the safety or efficacy of the drug product, a test is included to evaluate the presence of extractables and leachables.

Depending on the type and composition of the dosage form, other tests such as alcohol content, redispersibility, particle size distribution, rheological properties, reconstitution time, endotoxins/pyrogens, particulate matter, functionality testing of delivery systems, and osmolarity may be necessary.

DOSAGE FORMS

Aerosols

Aerosols are preparations packaged under pressure and contain therapeutic agent(s) and a propellant that are released upon activation of an appropriate valve system. Upon activation of the valve system, the drug substance is released as a plume of fine particles or droplets. Only one dose is released from the preparation upon activation of a metered valve. In the case of topical products, activation of the valve results in a continuous release of the formulation.

In this chapter, the aerosol dosage form refers only to those products packaged under pressure that release a fine mist of particles or droplets when activated (see *Glossary*). Other products that produce dispersions of fine droplets or particles will be covered in subsequent sections (e.g., *Dry Powder Inhalers* and *Sprays*).

TYPICAL COMPONENTS

Typical components of aerosols are the formulation containing one or more drug substances and propellant, the container, the valve, and the actuator. Each component plays a role in determining various characteristics of the emitted plume, such as droplet or particle size distribution, uniformity of delivery of the therapeutic agent, delivery rate, and plume velocity and geometry. The metering valve and actuator act in tandem to generate the plume of droplets or particles. The metering valve allows measure of an accurate volume of the liquid formulation under pressure within the container. The activator directs the metered volume to a small orifice that is open to the atmosphere. Upon activation, the formulation is forced through the opening, forming the fine mist of particles that are directed to the site of administration.

Aerosol preparations may consist of either a two-phase (gas and liquid) or a three-phase (gas, liquid, and solid or liquid) formulation. The two-phase formulation consists of drug(s) dissolved in liquefied propellant. Liquid cosolvents, such as alcohol, propylene glycol, and polyethylene glycols often are added to enhance the solubility of the drug substance(s). Three phase inhalation and nasal aerosol systems consist of a suspension or emulsion of the drug substance(s) [i.e., API(s)] in addition to the vaporizable propellants. The suspension or emulsion of the finely divided drug substance typically is dispersed in the liquid propellant with the aid of suitable biocompatible surfactants or other excipients.

Propellants for aerosol formulations are typically low molecular weight hydrofluorocarbons or hydrocarbons that are liquid when constrained in the container, exhibit a suitable vapor pressure at room temperature, and are biocompatible and nonirritating. Compressed gases do not supply a constant pressure over use and typically are not employed as propellants.

Metal containers can withstand the vapor pressure produced by the propellant and reduce the opportunity that leachable components will enter the formulation. Excess formulation may be added to the container to ensure that the full number of labeled doses can be accurately administered. The container and closure must be able to withstand the pressures anticipated under normal use conditions as well as when the system is exposed to elevated temperatures.

TYPES OF AEROSOL DOSAGE FORMS

Aerosol dosage forms can be delivered via various routes. The design of the container and metering valve, as well as the formulation, are designed to target the site of administration.

Inhalation aerosols are intended to produce fine particles or droplets for inhalation through the mouth and deposition in the pulmonary tree. The design of the delivery system releases one dose with each actuation. These products are commonly known as metered-dose inhalers.

Nasal aerosols produce fine particles or droplets for inhalation through the nasal vestibule and deposition in the nasal cavity. One dose is released with each activation of the valve.

Lingual aerosols are intended to produce fine particles or droplets for deposition in the mouth. The design of the delivery system releases one dose with each actuation.

Topical aerosols produce fine particles or droplets for application to the skin. Formulations that are intended for inhalation, nasal, or lingual administration are typically aqueous based, but topical aerosols may utilize nonaqueous solvents to achieve rapid drying or disinfectant action for abraded skin surfaces.

PACKAGING

The accuracy of a system's delivered dose is demonstrated at the range of pressures likely to be encountered as a result of ambient temperature variations or storage in a refrigerator. As an alternative, the system should include clear instructions for use to ensure the container and contents have been equilibrated to room temperature prior to use.

LABELING FOR PROPER USE

Typical warning statements include:

- Contents under pressure. Do not puncture or incinerate container.
- Do not expose to heat or store at temperatures above 49°.

- Keep out of the reach of children unless otherwise prescribed.
- Use only as directed; intentional misuse by deliberate concentration and inhaling of the contents can be harmful or fatal.

Many experts recommend the addition of a statement indicating that patients and/or consumers should seek advice and instruction from a health care professional about the proper use of the device.

Capsules

Capsules are solid dosage forms in which the API and excipients are enclosed within a soluble container or shell. The shells may be composed of two pieces, a body and a cap, or they may be composed of a single piece. Two-piece capsules are commonly referred to as hard-shell capsules, and one-piece capsules are often referred to as soft-shell capsules. This distinction, although it is imprecise, reflects differing levels of plasticizers in the two compositions and the fact that one-piece capsules typically are more pliable than two-piece capsules.

The shells of capsules usually are made from gelatin. However, they also may be made from cellulose polymers or other suitable material. Most capsules are designed for oral administration.

Two-Piece or Hard-Shell Capsules—Two-piece capsules consist of two telescoping cap and body pieces in a range of standard sizes.

One-Piece or Soft-Shell Capsules—One-piece capsules typically are used to deliver an API as a solution or suspension. Liquid formulations placed into one-piece capsules may offer advantages by comparison with dry-filled capsules and tablets in achieving content uniformity of potent APIs or acceptable dissolution of APIs with poor aqueous solubility. Because the contact between the shell wall and its liquid contents is more intimate than in dry-filled capsules, undesired interactions may be more likely to occur (including gelatin crosslinking and pellicle formation).

Modified-Release Capsules—The release of APIs from capsules can be modified in several ways, including coating the filled capsule shells or the contents in the case of dry-filled capsules.

Delayed-Release Capsules—Capsules sometimes are formulated to include enteric-coated granules to protect acid-labile APIs from the gastric environment or to prevent adverse events such as irritation. Enteric-coated multiparticulate capsule dosage forms may reduce variability in bioavailability associated with gastric emptying times for larger particles (i.e., tablets) and to minimize the likelihood of a therapeutic failure when coating defects occur during manufacturing.

PREPARATION

Two-Piece Capsules—Two-piece gelatin capsules usually are formed from blends of gelatins that have relatively high gel strength in order to optimize shell clarity and toughness or from hypromellose. They also may contain colorants such as D&C and FD&C dyes[3] or various iron oxides, opaquing agents such as titanium dioxide, dispersing agents, and preservatives. Gelatin capsule shells normally contain between 12% and 16% water.

[3] In 1960 Congress enacted the Color Additive Amendments, requiring FDA to regulate dyes, pigments, or other coloring agents in foods, drugs, and cosmetics separately from food additives. Under the law, color additives are deemed unsafe unless they are used in compliance with FDA regulations. The law provides a framework for the listing and certification of color additives. See FDCA section 721; see FDA regulations at 21 CFR Part 70. Colors must also be listed in pertinent FDA regulations for specific uses; the list of color additives for drugs that are exempt from certification is published at 21 CFR Part 73, Subpart B. FDA also conducts a certification program for batches of color additives that are required to be certified before sale; see 21 CFR Part 74 (Subpart B re: drugs). Regulations regarding certification procedures, general specifications, and the listing of certified provisionally listed colors, are at 21 CFR Part 80. FDA maintains a color additives website, with links to various legal and regulatory resources, at: http://www.cfsan.fda.gov/~dms/coltoc.html

The shells are manufactured in one set of operations and later filled in a separate manufacturing process. Two-piece shell capsules are made by a process that involves dipping shaped pins into gelatin or hypromellose solutions, followed by drying, cutting, and joining steps.

Powder formulations for two-piece gelatin capsules generally consist of the API and at least one excipient. Both the formulation and the method of filling can affect release of the API. In the filling operation, the body and cap of the shell are separated before filling. Following the filling operation, the machinery rejoins the body and cap and ensures satisfactory closure of the capsule by exerting appropriate force on the two pieces. The joined capsules can be sealed after filling by a band at the joint of the body and cap or by other suitable means. In compounding prescription practice, two-piece capsules may be hand-filled. This permits the prescriber the choice of selecting either a single API or a combination of APIs at the exact dose level considered best for an individual patient.

One-Piece Capsules—One-piece shell capsules are formed, filled, and sealed in a single process on the same machine and are available in a wide variety of sizes, shapes, and colors. The most common type of one-piece capsule is that produced by a rotary die process that results in a capsule with a seam. The soft gelatin shell is somewhat thicker than that of two-piece capsules and is plasticized by the addition of polyols such as glycerin, sorbitol, or other suitable material. The ratio of the plasticizer to the gelatin can be varied to change the flexibility of the shell depending on the nature of the fill material, its intended usage, or environmental conditions.

In most cases, one-piece capsules are filled with liquids. Typically, APIs are dissolved or suspended in a liquid vehicle. Classically, an oleaginous vehicle such as a vegetable oil was used. However, nonaqueous, water-miscible liquid vehicles such as the lower molecular weight polyethylene glycols now are more common. The physicochemical properties of the vehicle can be chosen to ensure stability of the API as well as to influence the release profile from the capsule shell.

Dry Powder Inhalers

The dry powder inhaler (DPI) consists of a mixture of drug(s) and carrier, and all components exist in a finely divided solid state packaged as a unit dose. The dose is released from the packaging by an appropriate mechanism and is mobilized into a fine mist only upon oral inhalation by the patient.

TYPICAL COMPONENTS

The basic components of the DPI are the formulation consisting of the drug(s) and carrier, both in the dry state; packaging that contains an amount equivalent to a unit dose; and a mechanism designed to open the unit-dose container and permit mobilization of the powders by the patient inhaling through the built-in mouthpiece. Typically, the unit-dose container is either a capsule made of gelatin or other suitable non-animal-derived material (e.g., hypromellose or starch), or the container may consist of a series of unit doses in foil-lined blisters arranged in a strip. When the drug is contained in a capsule, release of the medication takes place when the capsule is pierced. As a consequence of this release mechanism, the device is designed to minimize the generation of capsule fragments that might subsequently be inhaled. When the drug is contained in a blister pack, the mechanism is designed to advance an unused blister to a platform where the foil lining can be peeled back to expose the powder mixture to an air stream created when the patient inhales. To facilitate dosing compliance, some delivery devices incorporate dosing administration information such as number of doses remaining.

PACKAGING

For drug contained in blister-pack strips, the packs must be designed to allow individual blister cavities to be opened without compromising the seal of adjacent cavities. Package components must provide acceptable protection from humidity, light, and/or oxygen as appropriate. Containers for DPIs typically are made of plastic, but metal may be suitable. Packaging for the encapsulated drug must provide protection from humidity extremes to ensure that capsule breakage will occur in the desired fashion.

LABELING AND USE

Typical warning statements include:

- Keep out of the reach of children unless otherwise prescribed.
- Do not attempt to dissemble mechanism. Discard the device after all doses have been administered.
- Keep the device level while in use.
- Do not breathe into the device.

Many experts recommend the addition of a statement indicating that patients and/or consumers should seek advice and instruction from a health care professional about the proper use of the device.

Emulsions (Creams and Lotions)

Creams—Creams are semisolid emulsion dosage forms. They often contain more than 20% water and volatiles and typically contain less than 50% hydrocarbons, waxes, or polyols as the vehicle for the API. Creams generally are intended for external application to the skin or to the mucous membranes. Creams have a relatively soft, spreadable consistency and can be formulated as either a water-in-oil emulsion (e.g., *Cold Cream* or *Fatty Cream* as in the *European Pharmacopoeia*) or as an oil-in-water emulsion (e.g., *Betamethasone Valerate Cream*). Creams generally are described as either nonwashable or washable, reflecting the fact that an emulsion with an aqueous external continuous phase is more easily removed than one with a nonaqueous external phase (water-in-oil emulsion). Where the term ''cream'' is used without qualification, a water-washable product is generally inferred.

Lotions—Lotions are an emulsified liquid dosage form generally intended for external application to the skin. Historically, some topical suspensions such as calamine lotion have been called lotions but that nomenclature is not currently preferred. Lotions share many characteristics with creams. The distinguishing factor is that they are more fluid than semisolid and thus pourable. Due to their fluid character, lotions are more easily applied to large skin surfaces than semisolid preparations. Lotions may contain antimicrobial agents as preservatives.

PREPARATION

Pharmaceutical Compounding—Nonsterile Preparations ⟨795⟩ provides general information regarding the preparation of emulsions.

Creams—Creams may be formulated from a variety of oils, both mineral and vegetable, and from fatty alcohols, fatty acids, and fatty esters. The solid excipients are melted at the time of preparation. Emulsifying agents include nonionic surfactants, detergents, and soaps. Soaps are usually formed from a fatty acid in the oil phase hydrolyzed by a base dissolved in the aqueous phase in situ during the preparation of creams.

Preparation usually involves separating the formula components into two portions: lipid and aqueous. The lipid portion contains all water-insoluble components and the aqueous portion the water-soluble components. Both phases are heated to a temperature above the melting point of the highest melting component. The phases then are mixed and the mixture is stirred until reaching

ambient temperature or the mixture has congealed. Mixing generally is continued during the cooling process to promote uniformity. Traditionally, the aqueous phase is added to the lipid phase, but comparable results have been obtained with the reverse procedure. High-shear homogenation may be employed to reduce particle or droplet size and improve the physical stability of the resultant dosage form.

The API(s) can be added to the phase in which it is soluble at the beginning of the manufacturing process, or it can be added after the cream is prepared by a suitable dispersion process such as levigation or milling with a roller mill. Creams usually require the addition of a preservative(s) unless they are compounded immediately prior to use and intended to be consumed in a relatively short period of time.

Lotions—Lotions usually are prepared by dissolving or dispersing the API into the more appropriate phase (oil or water), adding the appropriate emulsifying or suspending agents, and mixing the oil and water phases to form a uniform fluid emulsion.

LABELING AND PACKAGING

Some products may require labeling directions indicating to shake well prior to application and to avoid freezing. Storage limits must be specifically indicated to prevent melting of semisolid components. Instructions to ensure proper dosing and administration must accompany the product. Tight containers are used for preparation and storage to prevent loss by evaporation.

Feed Additives

Feed additives are preparations used in veterinary medicine to deliver the API(s) via the water or food given to animals. The feed additive may be either a solid or liquid and sometimes is called a premix. Feed additives are further subdivided into three types.

TYPE A MEDICATED ARTICLES

Type A medicated articles are products containing one or more animal APIs, and that are sold to licensed feed mills or producers and are intended to be further diluted by mixing into food or water prior to consumption by the animals. Because these preparations are not actually dosed to animals, they are not considered dosage forms.

TYPE B MEDICATED FEEDS

Type B medicated feeds are products that contain a type A medicated article, or another type B medicated feed, plus a substantial quantity of nutrients (not less than 25% of the total weight). Like type A medicated articles, type B medicated feeds are intended for mixture with food or water and additional nutrients, are not to be fed directly to the animals, and are not considered dosage forms.

TYPE C MEDICATED FEEDS

Type C medicated feeds are made from type A medicated articles or type B medicated feeds and are prepared at concentrations of the API appropriate for administration to animals by mixing in food or water. Administration of type C medicated feeds can be accomplished by blending directly into the feed; top-dressing the preparation onto the animal's normal daily rations; or heating, steaming, and extruding into pellets that are mixed or top-dressed onto the animal's food. Another form of type C medicated feeds is compressed or molded blocks from which animals receive the API or nutrients via licking the block.

PREPARATION

Type A medicated articles that are liquids are produced by mixing the API(s) with a suitable solvent (e.g., water or propylene glycol). The API(s) is usually dissolved to produce a solution, but suspension products also could be produced.

Type A medicated articles that are solids are produced by blending the API with excipients to provide a uniform dosage form when mixed with the animal's feed. Often the API is first mixed with an excipient (e.g., starch or sodium aluminosilicate) that has a similar particle size and can help distribute the API uniformly throughout the final drug product. This pre-blend is then mixed with bulking excipients (e.g., calcium carbonate or soybean hulls). Mineral oil may be added to aid uniform distribution, to prevent particle segregation during shipping, and to minimize formation of airborne API particles during production of type B or C medicated feeds.

Type B or C medicated feeds are produced at licensed feed mills or by farm producers. Type A medicated articles are added to the feeds (e.g., ground corn or oats) during the milling process of making feeds. Liquid type A medicated articles often are sprayed in at set rates, and solid type A medicated articles are added slowly to aid in creating uniform distribution in the feeds. Liquid type A medicated articles can also be mixed in with bulk water sources at prescribed amounts.

LABELING AND PACKAGING

Type A medicated articles or type B medicated feeds include special labeling to indicate that they should be used in the manufacture of animal feeds or added to the drinking water. The labels indicate that they are not to be fed directly to animals. Also included is a statement indicating ''Not for Human Use''. Type A medicated articles or type B medicated feeds are packaged either in paper bags, often with polyethylene liners, for solids and in plastic containers for liquids. Typical sizes are 50-lb bags or several-gallon containers.

Foams

Medicated foams are emulsions containing a dispersed phase of gas bubbles in a liquid continuous phase containing the API. Medicated foams are packaged in pressurized containers or special dispensing devices and are intended for application to the skin or mucous membranes. The medicated foam is formed at the time of application. Surfactants are used to ensure the dispersion of the gas and the two phases. Medicated foams have a fluffy, semisolid consistency and can be formulated to break to a liquid quickly or to remain as foam to ensure prolonged contact.

Medicated foams intended to treat severely injured skin or open wounds must be sterile.

PREPARATION

A foam may contain one or more APIs, surfactants, aqueous or nonaqueous liquids, and the propellants. If the propellant is in the internal (discontinuous) phase (i.e., is of the oil-in-water type), a stable foam is discharged. If the propellant is in the external (continuous) phase (i.e., is of the water-in-oil type), a spray or a quick-breaking foam is discharged. Quick-breaking foams formulated with alcohol create a cooling sensation when applied to the skin and may have disinfectant properties.

LABELING AND USE

Foams formulated with flammable components should be appropriately labeled. Labeling indicates that prior to dispensing, a foam drug product is shaken well to ensure uniformity. The instructions for use must clearly note spe-

cial precautions that are necessary to preserve sterility. In the absence of a metering valve, delivered volume may be variable.

Medical Gases (Inhalation Materials)

Medical gases are products that are administered directly as a gas. A medical gas has a direct pharmacological action or acts as a diluent for another medical gas. Gases employed as excipients for administration of aerosol products, as an adjuvant in packaging, or produced by other dosage forms, are not included in this definition.

Components—Medical gases may be single components or defined mixtures of components. Mixtures also can be extemporaneously prepared at the point of use.

Administration—Medical gases may be administered to the patient via the pulmonary route or via extracorporeal methods. The dose of medical gas typically is metered by a volume rate of flow under ambient temperature and pressure conditions. Administration of a highly compressed gas generally requires a regulator to decrease the pressure, a variable-volume flow controller, and suitable tubing to conduct the gas to the patient. For pulmonary administration, the gas flow will be directed to the nose or mouth by a suitable device or into the trachea through a mechanical ventilator. When medical gases are administered chronically, provision for humidification is common. Care should be exercised to avoid microbial contamination.

STORAGE

Medical gases are stored in a compressed state in cylinders or other suitable containers. The containers must be constructed of materials that can safely withstand the expected pressure and must be impact resistant. In some cases each container holds a single defined dose (e.g., general anesthetics), but in other cases the container holds sufficient gas for extended administration.

SPECIAL CONSIDERATIONS

The container and system fittings should be appropriate for the medical gas. Adaptors should not be used to connect containers to patient-use supply system piping or equipment. Large quantities of gases such as oxygen or nitrogen can be stored in the liquid state in a cryogenic container and converted into a gas, as needed, by evaporation. Additional rules concerning the construction and use of cryogenic containers are promulgated by governmental agencies (e.g., U.S. Department of Commerce).

Containers, tubing, and administration masks employed for gases containing oxygen are free of any compound that would be sensitive to oxidation or that would be irritating to the respiratory tract.

A significant fraction of the dose of a medical gas may be released into the general vicinity of the patient due to incomplete absorption. Adequate ventilation may be necessary to protect health care workers and others from exposure to the gas (e.g., nitrous oxide).

LABELING

Warning statements to be placed on pressurized containers include:

- Contents under pressure.
- Do not puncture or incinerate container.
- Do not expose to heat or store at temperatures above 49°.
- Keep out of the reach of children unless otherwise prescribed.
- Use only as directed; intentional misuse may be harmful or fatal.

If required under the individual monograph, label to indicate method of manufacture (such as oxygen via air liquefaction). When piped directly from the storage container to the point of use, the gas must be labeled for content at each outlet.

When oxygen is in use, a posted warning should indicate the necessity of extinguishing smoking materials and avoiding the use of open flames or other potential ignition sources.

Gels

Gels (sometimes called jellies) are semisolid systems consisting either of suspensions of small inorganic particles or of organic molecules interpenetrated by a liquid. Gels can be classed either as single-phase or two-phase systems.

A two-phase gel consists of a network of small discrete particles (e.g., *Aluminum Hydroxide Gel* or *Psyllium Hemicellulose*). In a two-phase system the gel mass sometimes is referred to as a magma (e.g., *Bentonite Magma*) if the particle size of the suspended material is large. Both gels and magmas may be thixotropic, forming semisolids on standing and becoming liquid on agitation. They should be shaken before use to ensure homogeneity and should be so labeled (see *Suspensions*).

Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Single-phase gels may be made from natural or synthetic macromolecules (e.g., *Carbomer*, *Hydroxypropyl Methylcellulose*, or *Starch*) or natural gums (e.g., *Tragacanth*). The latter preparations are also called mucilages. Although these gels commonly are aqueous, alcohols and oils may be used as the continuous phase. For example, mineral oil can be combined with a polyethylene resin to form an oleaginous ointment base.

Gels can be administered by the topical or mucosal routes. Gels containing antibiotics administered by teat infusion are often the dosage form used in veterinary medicine to treat mastitis.

PREPARATION

See *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩ for general procedures. Also see the information contained under *Dosage Forms, Suspensions* for the formulation and manufacture of gels containing inorganic components or APIs in the solid phase. See *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ for general procedures for the preparation of sterile gels such as *Lidocaine Hydrochloride Jelly*.

Gels formed with large organic molecules may be formed by dispersing the molecule in the continuous phase (e.g., by heating starch), by cross-linking the dispersed molecules by changing the pH (as for *Carbomer Copolymer*), or by reducing the continuous phase (as for jellies formed with sucrose).

Care should be taken to ensure uniformity of the APIs by dispersing them by vigorous mixing or milling or by shaking if the preparation is less viscous.

PACKAGING AND STORAGE

Store in tight containers to prevent water loss. Avoid freezing.

Granules

Granules are solid dosage forms that are composed of agglomerations of smaller particles. These multicomponent compositions are prepared for oral administration and are used to facilitate flexible dosing regimens, address stability challenges, allow taste masking, or facilitate flexibility in administration (for instance, to pediatric patients, geriatric patients, or animals). Granular dosage forms may be formulated for direct oral administration and may facilitate compounding of multiple APIs by allowing compounding pharmacists to blend various granular compositions in the retail or hospital pharmacy. More commonly, granules are reconsti-

tuted to a suspension by the addition of water or a supplied liquid diluent immediately prior to delivery to the patient. Effervescent granules are formulated to liberate gas (carbon dioxide) upon addition of water. Common examples of effervescent granules include antacid and potassium supplementation preparations. Common therapeutic classes formulated as granule dosage forms include antibiotics, certain laxatives (such as senna extract products), electrolytes, and various cough and cold remedies that contain multiple APIs.

Granular dosages also are employed in veterinary medicine when they are often placed on top of or mixed with an animal's food. They are frequently provided with a measuring device to allow addition to feeds. The resultant mix facilitates dosing.

PREPARATION

Granules often are the precursors used in tablet compression or capsule filling. Although this application represents a pharmaceutical intermediate and not a final dosage form, numerous commercial products are based on granules. In the typical manufacture of granules, the API is blended with excipients (processing aids) and wetted with an appropriate pharmaceutical solvent or blend of solvents to promote agglomeration. This composition is dried and sized to yield the desired material properties.

Frequently, granules are used because the API is unstable in aqueous environments and cannot be exposed to water for periods sufficient to accommodate manufacture, storage, and distribution in a suspension. Preparation of the liquid dosage form from the granules immediately prior to dispensing allows acceptable stability for the duration of use. Granules manufactured for this purpose are packaged in quantities sufficient for a limited time period—usually one course of therapy that typically does not exceed 2 weeks. In addition to the API, other ingredients may be added to ensure acceptable stability (e.g., buffers, antioxidants, or chelating agents) or to provide color, sweetness, flavor, and for suspensions, acceptable viscosity to ensure adequate suspension of the particulate to enable uniform dosing.

Effervescent granules typically are formulated from sodium or potassium bicarbonate and an acid such as citric or tartaric acid. To prevent untimely generation of carbon dioxide, manufacturers should take special precautions to limit residual water in the product due to manufacture and to select packaging that protects the product from moisture. The manufacture of effervescent granules can require specialized facilities designed to maintain very low humidity (approximately 10% relative humidity). Effervescent powder mixtures are purposely formed into relatively course granules to reduce the rate of dissolution and provide a more controlled effervescence.

PACKAGING AND STORAGE

Granules for reconstitution may be packaged in unit-of-use containers or in containers with sufficient quantities to accommodate a typical course of therapy (frequently 10 days to 2 weeks with antibiotic products). Packaging should provide suitable protection from moisture. This is particularly true for effervescent granules. Granules may be stored under controlled room temperature conditions unless other conditions are specifically noted.

Many granule products specify refrigerated storage following reconstitution and direct the patient to discard unused contents after a specified date that is based on the stability of the API in the reconstituted preparation.

LABELING AND USE

Effervescent granules (and tablets) are labeled to indicate that they are not to be swallowed directly.

Reconstitution of granules must ensure complete wetting of all ingredients and sufficient time and agitation to allow the soluble components to dissolve. Specific instructions for reconstitution provided by the manufacturer should be carefully followed.

Reconstituted suspensions should be shaken before use to re-suspend the dispersed particulates. This is especially true of suspension preparations dosed from multiple-dose containers. For particularly viscous suspensions prone to air entrapment, instructions may advise the user how to shake the preparation to re-suspend settled particulates while minimizing air entrapment.

SPECIAL CONSIDERATIONS

For granules reconstituted to form suspensions for oral administration, acceptable suspension of the particulate phase depends on the particle size of the dispersed phase as well as the viscosity of the vehicle. Temperature can influence the viscosity, which influences suspension properties and the ease of removal of the dose from the bottle. In addition, temperature cycling can lead to changes in the particle size of the dispersed phase via Ostwald ripening. Thus, clear instructions should be provided regarding the appropriate storage temperature for the product.

Medicated Gums

Medicated gum is a semisolid confection that is designed to be chewed rather than swallowed. Medicated gums release the API(s) into the saliva. Medicated gums can deliver therapeutic agents for local action in the mouth (such as antibiotics to control gum disease) or for systemic absorption via the buccal or gastrointestinal routes (e.g., nicotine or aspirin). Most medicated gums are manufactured using the conventional melting process derived from the confectionary industry or alternatively may be directly compressed from gum powder. Medicated gums are formulated from insoluble synthetic gum bases such as polyisoprene, polyisobutylene, isobutyleneisoprene copolymer, styrene butadiene rubber, polyvinyl acetate, polyethylene, ester gums, or polyterpenes. Plasticizers and softeners such as propylene glycol, glycerin, oleic acid, or processed vegetable oils are added to keep the gum base pliable and to aid incorporation of the API(s), sweeteners, and flavoring agents. Sugars as well as artificial sweeteners and flavorings are incorporated to improve taste, and dyes may be used to enhance appearance. Some medicated gums are coated with magnesium stearate to reduce tackiness and improve handling during packaging. A preservative may be added.

PREPARATION

Melted Gum—The gum base is melted at a temperature of about 115° until it has the viscosity of thick syrup and at that point is filtered through a fine-mesh screen. This molten gum base is transferred to mixing tanks where the sweeteners, plasticizers, and typically the API are added and mixed. Colorings, flavorings, and preservatives are added and mixed while the melted gum is cooling. The cooled mixture is shaped by extrusion or rolling and cutting. Dosage units of the desired shape and potency are packaged individually. Additional coatings such as powder coatings to reduce tackiness or film or sugar coatings may be added to improve taste or facilitate bulk packaging.

Directly Compressed Gum—The gum base is supplied in a free-flowing granular powder form. The powder gum base is then dry blended with sweeteners, flavors, the API, and lubricant. The blend is then processed through a conventional tablet press and tableted into desired shapes. The resulting medicated gum tablets

can be further coated with sugar or sugar-free excipients. These tablets can be packaged in blisters or bottles as needed.

SPECIAL CONSIDERATIONS

Medicated gums are typically dispensed in unit-dose packaging. The patient instructions also may include a caution to avoid excessive heat.

Implants

Implants are long-acting dosage forms that provide continuous release of the API for periods of months to years. They are administered by the parenteral route. For systemic delivery they may be placed subcutaneously, or for local delivery they can be placed in a specific region in the body.

Several types of implants are available. Pellet implants are small, sterile, solid masses composed of an API with or without excipients. They are usually administered by means of a suitable special injector (e.g., trocar) or by surgical incision. Release of the API from pellets typically is controlled by diffusion and dissolution kinetics. The size of the pellets and rate of erosion will influence the release rate, which typically follows first-order kinetics. Drug release from pellets for periods of 6 months or more is possible. Pellet implants have been used to provide extended delivery of hormones such as testosterone or estradiol.

Resorbable microparticles are a type of implants that provide extended release of drug over periods varying from a few weeks to months. They can be administered subcutaneously or intramuscularly for systemic delivery, or they may be deposited in a desired location in the body for site-specific delivery. Injectable resorbable microparticles (or microspheres) generally range from 20 to 100 μm in diameter. They are composed of a drug dispersed within a biocompatible, bioresorbable polymeric excipient (matrix). Poly(lactide-co-glycolide) polymers have been used frequently. These excipients typically resorb by hydrolysis of ester linkages. The microparticles are administered by suspension in an aqueous vehicle followed by injection with a conventional syringe and needle. Release of the drug from the microparticles begins after physiological fluid enters the polymer matrix, dissolving some of the drug that then is released by a diffusion-controlled process. Drug release also can occur as the matrix erodes.

Polymer implants can be formed as a single shaped mass such as a cylinder. The polymer matrix must be biocompatible, but it can be either biodegradable or non-biodegradable. Shaped polymer implants are administered by means of a suitable special injector. Release kinetics typically are not zero-order, but zero-order kinetics are possible. Drug release can be controlled by the diffusion of the API from the bulk polymer matrix or by the properties of a rate-limiting polymeric membrane coating. Polymer implants are used to deliver potent small molecules like steroids (e.g., estradiol for cattle) and large molecules like peptides [e.g., luteinizing hormone-releasing hormone (LHRH)]. Example durations of drug release are 2 and 3 months for biodegradable implants and 1 year for non-biodegradable implants. An advantage of biodegradable implants is that they do not require removal after release of all drug content. Non-biodegradable polymer implants can be removed before or after drug release is complete or may be left in situ. An implant can have a tab with a hole in it to facilitate suturing it in place, e.g., for an intravitreal implant for local ocular delivery. Such implants may provide therapeutic release for periods as long as 2.5 years.

Some implants are designed to form as a mass in situ. These implants are initially prepared as liquid formulations comprising polymer, API, and solvent for the polymer. The polymer solvent can be water or an organic solvent. After administration of the liquid formulation

to a patient by subcutaneous or intramuscular administration, it forms a gel or a solid polymeric matrix that traps the API and extends the API release for days or months. In situ-forming implants also are used for local delivery of the API to treat periodontal disease. The implant is formed within the periodontal pocket.

Another type of implant can be fabricated from a metal such as titanium and plastic components. These implants are administered by means of a suitable injector or by surgical installation. A solution of API inside the implant, like an LHRH solution, is released via an osmotically driven pump inside the implant. Duration of release may be as long as 1 year or more. Release kinetics are zero order. After the drug is delivered, metal-based implants are removed.

Drug-eluting stents combine the mechanical effect of the stent to maintain arterial patency with the prolonged pharmacologic effect of the incorporated API (to reduce restenosis, inhibit clot formation, or combat infection). As an example, a metal stent can be coated with a non-biodegradable or biodegradable polymer-containing API. The resultant coating is a polymeric matrix that controls the extended release of the API.

PREPARATION

Pellet implants are made by API compression or molding. Cylindrical polymeric implants typically are made by melt extrusion of a blend of API and polymer, resulting in a rod that is cut into shorter lengths. Polymer implants also can be made by injection molding. Still other implants are assembled from metal tubes and injection-molded plastic components.

Sterility can be achieved by terminal sterilization or by employing aseptic manufacturing procedures.

PACKAGING AND STORAGE

All implants are individually packaged (typically in their injector or for veterinary use in cartridges that are placed in the injector guns), are sterile (except for some animal health products), and conform to the appropriate standards for injection. Biodegradable implants are protected from moisture so the polymer does not hydrolyze and alter drug release kinetics before use.

Inserts

Inserts are solid dosage forms that are inserted into a body cavity other than the rectum (see *Suppositories*). The API is delivered in inserts for local or systemic action. Inserts applied to the eye, such as *Pilocarpine Ocular System*, typically are sterile. Vaginal inserts for humans are usually globular or oviform and weigh about 5 g each. Vaginal inserts for cattle are T-shaped, are formed of polymer, are removable, and can be used for up to 8 days. One veterinary application is for estrus synchronization. Inserts intended to dissolve in vaginal secretions usually are made from water-soluble or water-miscible vehicles such as polyethylene glycol or glycerinated gelatin. Vaginal inserts such as dinoprostone vaginal insert (e.g., see USP monograph *Dinoprostone Vaginal Suppositories*) are formulated to deliver medication to the cervix and to be removed or recovered once the API has been released. Intrauterine inserts such as *Progesterone Intrauterine Contraceptive System* are used to deliver APIs locally to achieve efficacy while reducing side effects. Some intrauterine inserts are formulated to remain in the uterus for extended periods of time. An intra-urethral insert of alprostadil is available for the treatment of erectile dysfunction.

PREPARATION

For general considerations see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩. Inserts vary considerably in their preparation. Inserts may be molded (using technology similar to that employed to prepare lozenges, suppositories, or plastics), compressed from powders (as in tableting), or formulated as special applications of capsules (soft gelatin capsules and hard gelatin capsules have been employed for extemporaneously compounded preparations). Inserts may be formulated to melt at body temperature or disintegrate upon insertion. Design of the dosage form should take into consideration the fluid volume available at the insertion site and minimize the potential to cause local irritation. Most inserts are formulated to ensure retention at the site of administration.

STORAGE AND LABELING

Appropriate storage conditions must be clearly indicated in the labeling for all inserts, especially for those that are designed to melt at body temperature. Instructions to ensure proper dosing and administration must accompany the product.

Liquids

As a dosage form a liquid consists of a pure chemical in its liquid state. Examples include mineral oil, isoflurane, and ether. This dosage form term is not applied to solutions. In veterinary medicine liquids may be administered topically or diluted via mixing with drinking water or food.

STORAGE AND LABELING

Storage, packaging, and labeling consider the physical properties of the material and are designed to maintain potency and purity.

Lotions (see *Emulsions*)

Lozenges

Lozenges are solid oral dosage forms that are designed to dissolve or disintegrate slowly in the mouth. They contain one or more APIs that are slowly liberated from the flavored and sweetened base. They are frequently intended to provide local action in the oral cavity or the throat but also include those intended for systemic absorption after dissolution. The typical therapeutic categories of APIs delivered in lozenges are antiseptics, analgesics, decongestants, antitussives, and antibiotics. Molded lozenges are called cough drops or pastilles. Molded lozenges mounted on a stick are known as lollipops. Lozenges prepared by compression or by stamping or cutting from a uniform bed of paste sometimes are known as troches. Troches are often produced in a circular shape.

Lozenges can be made using sugars such as sucrose and dextrose or can provide the benefits of a sugar-free formulation that is usually based on sorbitol or mannitol. Polyethylene glycols and hypromellose sometimes are included to slow the rate of dissolution.

MANUFACTURE

Excipients used in molded lozenge manufacture include gelatin, fused sucrose, sorbitol, or another carbohydrate base.

Molded lozenges using a sucrose or sorbitol base containing APIs such as phenol, dextromethorphan, fentanyl, and dyclonine hydrochloride and menthol are

prepared by cooking the sugar (sucrose, corn syrup, and sorbitol) and water at about 150° to reduce the water content to less than 2%. The molten sugar solution is transferred to a cooling belt or cooling table, and medicaments, flavorings, and colorings are added and thoroughly mixed while cooling. Individual dosage units of the desired shape are formed by filling the molten mass into molds. These lozenges are quickly cooled in the molds to trap the base in the glassy state. Once formed, the lozenges are removed from the molds and packaged. Care is taken to avoid excessive moisture during storage to prevent crystallization of the sugar base.

Compressed lozenges are made using excipients that may include a filler, binder, sweetening agent, flavoring agent, and lubricant. Sugars such as sucrose, sorbitol, and mannitol often are included because they can act as filler and binder as well as serve as sweetening agents. Approved FD&C and D&C dyes or lakes (dyes adsorbed onto insoluble aluminum hydroxide) also may be present.

The manufacturing of compressed lozenges is essentially the same as that for conventional tableting, with the exception that a tablet press capable of making larger tablets and exerting greater force to produce harder tablets may be required (see *Tablets*).

The paste used to produce lozenges manufactured by stamping or cutting contains a moistening agent, sucrose, and flavoring and sweetening agents. The homogenous paste is spread as a bed of uniform thickness, and the lozenges are cut or stamped from the bed and are allowed to dry. Some lozenges are prepared by forcing dampened powders under low pressure into mold cavities and then ejecting them onto suitable trays for drying at moderate temperatures.

PACKAGING AND STORAGE

Many lozenges are sensitive to moisture, and typically a monograph indicates that the package or container type is well closed and/or moisture resistant. Storage instructions may include protection from high humidity.

Ointments

Ointments are semisolid preparations intended for external application to the skin or mucous membranes. APIs delivered in ointments are intended for local action or for systemic absorption. Ointments usually contain less than 20% water and volatiles and more than 50% hydrocarbons, waxes, or polyols as the vehicle. Ointment bases recognized for use as vehicles fall into four general classes: hydrocarbon bases, absorption bases, water-removable bases, and water-soluble bases.

Hydrocarbon Bases—Also known as oleaginous ointment bases, they allow the incorporation of only small amounts of an aqueous component. Ointments prepared from hydrocarbon bases act as occlusive dressings and provide prolonged contact of the API with the skin. They are difficult to remove and do not change physical characteristics upon aging.

Absorption Bases—Allow the incorporation of aqueous solutions. Such bases include only anhydrous components (e.g., *Hydrophilic Petrolatum*) or water-in-oil emulsions (e.g., *Lanolin*). Absorption bases are also useful as emollients.

Water-Removable Bases—Oil-in-water emulsions (e.g., *Hydrophilic Ointment*) and are sometimes referred to as creams (see *Emulsions*). They may be readily washed from the skin or clothing with water, making them acceptable for cosmetic reasons. Other advantages of the water-removable bases are that they can be diluted with water and that they favor the absorption of serous discharges in dermatological conditions.

Water-Soluble Bases—Also known as greaseless ointment bases, they are formulated entirely from water-soluble constituents. *Polyethylene Glycol Ointment* is the only official preparation in this group. They offer many of the advantages of the water-removable bases and, in addition, contain no water-insoluble substances such as petrolatum, anhydrous lanolin, or waxes. They are more correctly categorized as gels (see *Gels*).

The choice of an ointment base depends on the action desired, the characteristics of the incorporated API, and the latter's bioavailability if systemic action is desired. The product's stability may require the use of a base that is less than ideal in meeting other quality attributes. APIs that hydrolyze rapidly, for example, are more stable in hydrocarbon bases than in bases that contain water.

Ophthalmic ointments are intended for application directly to the eye or eye-associated structures such as the subconjunctival sac. They are manufactured from sterilized ingredients under aseptic conditions and meet the requirements under *Sterility Tests* ⟨71⟩. Ingredients meeting the requirements described under *Sterility Tests* ⟨71⟩ are used if they are not suitable for sterilization procedures. Ophthalmic ointments in multiple-dose containers contain suitable antimicrobial agents to control microorganisms that might be introduced during use unless otherwise directed in the individual monograph or unless the formula itself is bacteriostatic (see *Ophthalmic Ointments* ⟨771⟩*, Added Substances*). The finished ointment is free from large particles and must meet the requirements for *Leakage* and for *Metal Particles* under *Ophthalmic Ointments* ⟨771⟩. The immediate container for ophthalmic ointments is sterile at the time of filling and closing. The immediate containers for ophthalmic ointments are sealed and made tamper-proof so that sterility is ensured at time of first use.

A suitable ophthalmic ointment base is nonirritating to the eye and permits diffusion of the API throughout the secretions bathing the eye. Petrolatum is most commonly used as a base for ophthalmic drugs. Some absorption bases, water-removable bases, and water-soluble bases may be desirable for water-soluble APIs if the bases are nonirritating.

MANUFACTURE

Ointments typically are prepared by either direct incorporation into a previously prepared ointment base or by fusion (heating during the preparation of the ointment). A levigating agent is often added to facilitate the incorporation of the medicament into the ointment base by the direct incorporation procedure. In the fusion method, the ingredients are heated, often in the range of 60° to 80°. Homogenization is often necessary. The rate of cooling is an important manufacturing detail because rapid cooling can impart increased structure to the product of the fusion method.

PACKAGING AND STORAGE

Protect from moisture. For emulsified systems, temperature extremes can lead to physical instability of the preparation. When this is the case products should be clearly labeled to specify appropriate storage conditions. Ointments typically are packaged either in ointment jars or ointment tubes. Ointment jars are often used for more viscous ointments that do not require sterility. Ointment tubes typically are used for less viscous ointments and those such as ophthalmic ointments that require the maintenance of sterility. The package sizes for ophthalmic preparations are controlled to minimize the likelihood of contamination and loss of sterility.

Pastes

Pastes are semisolid preparations of stiff consistency and contain a high percentage of finely dispersed solids. Pastes are intended for application to the skin, oral cavity, or mucous membranes. In veterinary practice, pastes are used for systemic delivery of APIs.

Pastes ordinarily do not flow at body temperature and thus can serve as occlusive, protective coatings. As a consequence, pastes are more often used for protective action than are ointments.

Fatty pastes that have a high proportion of hydrophilic solids appear less greasy and more absorptive than ointments. They are used to absorb serous secretions and are often preferred for acute lesions that have a tendency toward crusting, vesiculation, or oozing.

Dental pastes may be applied to the teeth, or alternatively they may be indicated for adhesion to the mucous membrane for a local effect (e.g., *Triamcinolone Acetonide Dental Paste*). Some paste preparations intended for animals are administered orally. The paste is squeezed into the mouth of the animal, generally at the back of the tongue, or is spread inside the mouth.

PREPARATION

Pastes can be prepared by direct incorporation or by fusion (the use of heat to soften the base). The solid ingredients often are incorporated following comminution and sieving. If a levigating agent is needed, a portion of the ointment base is often employed rather than a liquid.

LABELING AND STORAGE

Veterinary products should be labeled to ensure they are not administered to humans. Labeling should indicate the need for protection from heat.

Transdermal Systems (Patches)

Transdermal drug delivery systems (TDSs) are discrete dosage forms that are designed to deliver the API(s) through intact skin to the systemic circulation. Typically, a TDS is composed of an outer covering (barrier), a drug reservoir (possibly covered with a rate-controlling membrane), a contact adhesive applied to some or all parts of the system (to attach the TDS to the skin surface), and a protective layer that is removed before the patch is applied. The activity of a TDS is defined in terms of the release rate of the API(s) from the system. The total duration of drug release from the system and the system surface area also may be stated.

Most TDSs can be considered either matrix-type or reservoir-type systems. Matrix-type patches are often further divided into monolithic adhesive matrix or polymer matrix types. Reservoir-type systems include liquid reservoir systems and solid-state reservoir systems. Solid-state reservoir patches also include multilaminate adhesive and multilaminate polymer matrix systems.

Drug delivery from some TDSs is controlled by diffusion kinetics. The API diffuses from the drug reservoir directly or through the rate-controlling membrane and/or contact adhesive and then through the skin into the general circulation. Modified-release systems are generally designed to provide drug delivery at a constant rate so that a true steady-state blood concentration is achieved and maintained until the system is removed. Other TDSs work by active transport of the API. For example, iontophoretic transdermal delivery uses the current between two electrodes to enhance the movement of ionized APIs through the skin.

TDSs are applied to the body areas recommended by the labeling. The API content of the system provides a reservoir that, by design, maintains a constant API concentration at the system-skin interface. The dosing interval of the system is a function of the amount of API in the reservoir and the release rate. Some API concentration

may remain in the reservoir at the end of the dosing interval, in particular for diffusion-controlled delivery mechanisms. [NOTE—Where the API is intended for local action, it may be embedded in adhesive on a cloth or plastic backing. This type of product is more correctly called a plaster or tape (see *Plasters* and *Tapes*).]

PREPARATION

TDSs require a backing, a means of storing the API for delivery to the skin, an adhesive to attach the system to the skin, and a removable release liner to protect the adhesive, API, and excipients before application. The backing has low moisture- and vapor-transmission rates to support product stability. The adhesive layer may contain the API and permeation enhancers in the case of matrix-type systems or multi-laminate reservoir systems for which a priming dose is desired. Adhesive may be applied to the entire patch release surface or merely to the periphery. Liquid reservoir systems are often formed–filled–sealed between the backing and release-controlling materials. For monolithic adhesive matrix systems, the API and excipients are applied as a solution or suspension either to the backing or the release liner, and the solvent is allowed to evaporate.

PACKAGING AND STORAGE

Storage conditions are clearly specified because extreme temperature excursions can influence the performance of some systems.

LABELING

The labeling should clearly indicate any performance limitations of the system (e.g., influence of application site, hydration state, hair, or other variables).

Pellets

Pellets are dosage forms composed of small, solid particles of uniform shape sometimes called beads. Typically, pellets are nearly spherical but this is not required. Pellets may be administered by the oral (gastrointestinal) or by the injection route (see also *Implants*). Pellet formulations may provide several advantages including physical separation for chemically or physically incompatible materials, extended release of the API, or delayed release to protect an acid-labile API from degradation in the stomach or to protect stomach tissues from irritation. Extended-release pellet formulations may be designed with the API dispersed in a matrix, or the pellet may be coated with an appropriate polymer coating that modifies the drug-release characteristics. Alternatively, the pellet design may combine these two approaches. In the case of delayed-release formulations, the coating polymer is chosen to resist dissolution at the lower pH of the gastric environment but to dissolve in the higher pH intestinal environment. Injected or surgically administered pellet preparations (see *Implants*) are often used to provide continuous therapy for periods of months or years.

Pellet dosage forms may be designed as single or multiple entities. Often implanted pellets will contain the desired API content in one or several units. In veterinary practice, 4 to 8 pellets may be implanted in the ears for cattle, depending on animal size. Oral pellets typically are contained within hard gelatin capsules for administration. Although there are no absolute requirements for size, the useful size range of pellets is governed by the practical constraints of the volume of commonly used capsules and the need to include sufficient numbers of pellets in each dose to ensure uniform dosing of the API. As a result, many pellets used for oral administration fall within a size range of 8 to 24 mesh. Pellet formulations sometimes are used to minimize variability associated with larger dosage forms caused by gastric retention upon stomach emptying.

Enteric-coated (delayed-release) pellet formulations and some extended-release formulations are prepared by applying a coating to the formulated particles. The coating must be applied as a continuous film over the entire surface of each particle. Because a small population of imperfectly coated particles may be unavoidable, oral pellets are designed to require the administration of a large number in a single dose to minimize any adverse influence of imperfectly coated pellets on drug delivery.

PREPARATION

The desired performance characteristics determine the manufacturing method chosen. In general, pellet dosage forms are manufactured by wet extrusion processes followed by spheronization, by wet or dry coating processes, or by compression. Manufacture of pellets by wet coating usually involves the application of successive coatings upon nonpareil seeds. This manufacturing process frequently is conducted in fluid-bed processing equipment. Dry powder coating or layering processes often are performed in specialized rotor granulation equipment. The extent of particle growth achievable in wet coating processes is generally more limited than the growth that can be obtained with dry powder layering techniques, but either method allows the formulator to develop and apply multiple layers of coatings to achieve the desired release profile. The manufacture of pellets by compression is largely restricted to the production of material for subcutaneous implantation. This method of manufacture provides the necessary control to ensure dose uniformity and generally is better suited to aseptic processing requirements.

Alternatively, microencapsulation techniques can be used to manufacture pellets. Coacervation coating techniques typically produce coated particles that are much smaller than those made by other techniques.

PACKAGING AND STORAGE

Pellets for oral administration generally are filled into hard gelatin capsules and are placed in bottles or blister packages. The packaging provides suitable protection from moisture to ensure the stability of the pellet formulation as well as to preserve desirable moisture content of the capsule shells. Pellets for implantation are sterile and should be packaged in tight containers suitable for maintaining sterile contents. Pellets may be stored under controlled room temperature conditions unless other conditions are specifically noted.

LABELING AND USE

Pellets for oral administration that are formulated to provide delayed or controlled release must be swallowed intact to ensure preservation of the desired release characteristics. These products should be labeled accordingly to ensure that the material is not crushed or chewed during administration.

Pills

Pills are API-containing small round solid bodies intended for oral administration. At one time pills were the most extensively used oral dosage form, but they have been replaced by compressed tablets and capsules. Pills are distinguished from tablets because pills are manufactured by a wet massing and molding technique, while tablets are formed by compression.

PREPARATION

Excipients are selected on the basis of their ability to produce a mass that is firm and plastic. The API is triturated with powdered excipients in serial dilutions to attain a uniform mixture. Liquid excipients that act to bind and provide plasticity to the mass are subsequently added

to the dry materials. The mass is formed by kneading. The properties of firmness and plasticity are necessary to permit the mass to be worked and retain the shape produced. Cylindrical pill pipes are produced from portions of the mass. The pill pipe is cut into individual lengths corresponding to the intended pill size, and the pills are rolled to form the final shape. Pill-making machines can automate the preparation of the mass, production of pill piping, and the cutting and rolling of pills.

PACKAGING AND LABELING

Labeling and use instructions for pills are similar to those for tablets. Although many pills are resistant to breakage, some pills are friable. Appropriate handling guidelines should be provided in such cases in order to avoid breakage.

Plasters

A plaster is a semisolid substance for external application and usually is supplied on a support material. Plasters are applied for prolonged periods to provide protection, support, or occlusion (maceration).

Plasters consist of an adhesive layer that may contain active substances. This layer is spread uniformly on an appropriate support that is usually made of a rubber base or synthetic resin. Unmedicated plasters are designed to provide protection or mechanical support to the site of application. These plasters are neither irritating nor sensitizing to the skin.

Plasters are available in a range of sizes or cut to size to effectively provide prolonged contact to the site of application. They adhere firmly to the skin but can be peeled off the skin without causing injury.

One example of a plaster currently in use is salicylic acid plasters used for the removal of corns by the keratolytic action of salicylic acid.

PACKAGING AND STORAGE

Plasters are preserved in well-closed containers, preferably at controlled room temperature.

Powders

Powders are defined as a solid or a mixture of solids in a finely divided state intended for internal or external use. Powders used as pharmaceutical dosage forms may contain one or more APIs and can be mixed with water for oral administration or injection. Often pediatric antibiotics utilize a powder dosage form for improved stability. In some areas medicated powders are used for extemporaneous compounding of preparations for simultaneous administration of multiple APIs. Medicated powders also can be inhaled for pulmonary administration (see *Dry Powder Inhalers*). Aerosolized powders for the lungs typically contain processing aids to improve flow and ensure uniformity (see *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩). Powders can also be used topically as a dusting powder.

Externally applied powders should have a particle size of 150 μm or less (typically in the 50- to 100-μm range) in order to prevent a gritty feel on the skin that could further irritate traumatized skin. Powders are grouped according to the following terms: very coarse, coarse, moderately coarse, fine, and very fine (see *Powder Fineness* ⟨811⟩). The performance of powder dosage forms can be affected by the physical characteristics of the powder. Particle size can influence the dissolution rate of the particles and affect bioavailability. For dispersed delivery systems, particle size can influence the mixing and segregation behavior of the particle, which in turn affects the uniformity of the dosage form.

PREPARATION

Powder dosage forms can be produced by the combination of multiple components into a uniform blend. This can also involve particle size reduction, a process referred to as comminution. Mills and pulverizers are used to reduce the particle size of powders when necessary. As the particle size is decreased, the number of particles and the surface area increase, which can increase the dissolution rate and bioavailability of the API.

Blending techniques for powders include those used in compounding pharmacy such as spatulation and trituration (see *Pharmaceutical Compounding Nonsterile Preparations* ⟨795⟩). Industrial processes may employ sifting or tumbling the powders in a rotating container. One of the most common tumble blenders is a V-blender, which is available in a variety of scales suitable for small-scale and large-scale compounding and industrial production.

Powder flow can be influenced by both particle size and shape. Larger particles generally flow more freely than do fine particles. Powder flow is an important attribute that can affect the packaging or dispensing of a medicated powder.

PACKAGING AND STORAGE

Powders for pharmaceutical use can be packaged in multiple- or single-unit containers. Bulk containers have been used for antacid powders and for laxative powders. In these instances the patient dissolves the directed amount in water prior to administration. This type of multiple-unit packaging is acceptable for many APIs but should not be utilized for powders that require exact dosing. Multiple-unit powders for topical application often are packaged in a container with a sifter top.

Potent APIs in a powder dosage form are dispensed in unit-of-use allocations in folded papers, cellophane envelopes, or packets. Powder boxes are often used by the dispensing pharmacist to hold multiple doses of individual folded papers. Hygroscopic powders pose special challenges and typically are dispensed in moisture-resistant packaging.

LABELING

Typical warning statements include:

- External powders must indicate: ''External Use Only''.
- Oral powders should indicate: ''For Oral Use Only''.
- Powders intended for veterinary use must indicate: ''For Veterinary Use Only''.

Individual monographs specify the labeling requirements for powder dosage forms that are listed in *USP–NF*. Oral powders for reconstitution prior to dispensing typically have a limited shelf life (for example, 2 weeks), and the dispensed product should indicate a beyond-use date based on the date of the water addition. Pharmaceutical powders that are compounded indicate a beyond-use date. Compounded preparations typically are intended for immediate use and have short-term storage durations.

Medicated Soaps And Shampoos

Medicated soaps and shampoos are solid or liquid preparations intended for topical application to the skin or scalp followed by subsequent rinsing with water. Soaps and shampoos are emulsions or surface-active compositions that readily form emulsions or foams upon the addition of water followed by rubbing. Incorporation of APIs in soaps and shampoos combines the cleansing/degreasing abilities of the vehicle and facilitates the topical application of the API to affected areas, even large areas, of the body. The surface-active properties of the vehicle facilitate contact of the API with the skin or scalp. Medicated soap and shampoo formulations frequently contain suitable antimicrobial agents to protect against bacteria, yeast, and mold contamination.

PREPARATION

The preparation of medicated soaps and shampoos follows techniques frequently used for the preparation of emulsified systems. To ensure uniformity, the API(s) must be added to the vehicle prior to congealing (in the case of soaps) followed by thorough mixing. If the medication is present as a suspension, the particle size must be controlled to promote uniform distribution of the API and possibly optimize performance. Because soap manufacture frequently involves processing the ingredients at elevated temperature, care must be exercised to avoid excessive degradation of the API during processing.

PACKAGING AND STORAGE

Individual monographs specify the packaging and storage requirements for medicated soaps and shampoos in *USP–NF*.

LABELING AND USE

Medicated soaps and shampoos are clearly labeled to indicate ''For External Use Only''. The preparations also clearly advise the patient to discontinue use and consult a physician/veterinarian if skin irritation or inflammation occurs or persists following application.

Solutions

A solution is a liquid preparation that contains one or more dissolved chemical substances in a suitable solvent or mixture of mutually miscible solvents. Because molecules of a drug substance in solution are uniformly dispersed, the use of solutions as dosage forms generally provides assurance of uniform dosage upon administration and good accuracy when the solution is diluted or otherwise mixed.

Substances in solutions are more susceptible to chemical instability than they are in the solid state and dose-for-dose generally are heavier and more bulky than solid dosage forms. These factors increase the cost of packaging and shipping relative to that of solid dosage forms. Solution dosage forms can be administered by injection; inhalation; and the mucosal, topical/dermal, and gastrointestinal routes. Terminology for solutions in veterinary practice includes spot-ons or pour-ons that refer to solutions that are applied to an animal's skin for systemic absorption, dips that refer to solutions that are used for washing and disinfection (e.g., udders, eggs, and whole animals), and drenches that include solutions that are orally administered to livestock, usually with a dosing device. Solutions administered by injection are officially titled injections (see *Injections* ⟨1⟩).

Solutions intended for oral (gastrointestinal) administration usually contain flavorings and colorants to make the medication more attractive and palatable for the patient or consumer. When needed, they also may contain stabilizers to maintain chemical and physical stability and preservatives to prevent microbial growth.

STORAGE AND USE

Light-resistant containers should be considered when photolytic chemical degradation is a potential issue. To prevent water or solvent loss, solutions are stored in tight containers. Instructions to ensure proper dosing and administration must accompany the product

Sprays (Nasal, Pulmonary, or Solutions For Nebulization)

A spray is a preparation that contains a therapeutic agent(s) in either the liquid or solid state and is intended for administration as a fine mist of small aqueous droplets. The droplets may be generated by means other than the use of a volatile propellant (see *Aerosols*). The mech-

anism for droplet generation and the intended use of the preparation distinguish the various classes of sprays. A spray is composed of a pump, container, valve, actuator, and nozzle in addition to the formulation containing the drug(s), solvents, and excipients. Each component plays a role in determining the critical characteristics of the mist of fine droplets. Droplet size and size distribution, uniformity of delivery of dose, plume geometry, and droplet velocity are critical parameters that influence the efficiency of drug delivery. When the preparation is supplied as a multi-dose container, the addition of a suitable antimicrobial preservative may be necessary.

Spray formulations intended for nasal or pulmonary administration have an aqueous base. Nasal preparations may be solutions, suspensions, or emulsions intended for local or systemic effect. Nasal delivery may be employed for drugs with high hepatic extraction ratios. Pulmonary preparations typically are solutions, although appropriately sized suspension formulations are permissible. Preparations are usually isotonic and may contain excipients to control pH and viscosity.

Metered-dose sprays typically require manually depressing the top of the container to activate a metered valve system. Depending on the design of the formulation and the valve system, the droplets generated may be intended for immediate inhalation through the mouth and deposition in the pulmonary tree or for inhalation into the nose and deposition in the nasal cavity. These preparations are commonly known as metered-dose sprays. The design of the pump, container, valve, actuator, nozzle, and formulation are critical to the performance of the product.

Alternatively, sprays can be generated by package designs that do not accurately control the volume of formulation delivered. These presentations release the formulation as a fine mist of droplets upon physical manipulation of the package by the patient. This generally involves squeezing the sides of the container and expelling the formulation through the nozzle of the container.

Finally, liquid sprays may be generated from solutions by nebulization. This is a method for continuous generation of a fine mist of aqueous droplets from a drug-containing solution by application of the Venturi principle, ultrasonic energy, or other suitable mechanical means. The generated mist is directed to the patient for inhalation, sometimes with the aid of an appropriate tube or face mask. Although formulations for nebulization typically are solutions, they also may be fine suspensions or emulsions.

PACKAGING

Containers typically are made of a rigid plastic, but metal or glass may be suitable.

The nasal spray pump is designed to allow convenient one-handed operation. The nasal spray nozzle is designed so that it fits comfortably into the vestibule of the nasal cavity and allows the plume to be directed toward the appropriate region of the cavity.

LABELING AND USE

Typical warning statements include:

- All inhalation sprays should indicate: ''For Inhalation Administration Only. Keep out of the reach of children unless otherwise prescribed. Avoid spraying into the eyes.''
- All nasal sprays indicate: ''For Intranasal Administration Only''.

The device should contain a statement that patients should seek advice and instruction from a health care professional about the proper use of the device. Guid-

In-Process Revision

ance should be provided about the proper care and cleaning of the device to prevent introduction of microbes into the pulmonary airways.

Suppositories

Suppositories are dosage forms adapted for application into the rectum. They usually melt, soften, or dissolve at body temperature. A suppository may have a local protectant or palliative effect or may deliver an API for systemic or local action.

Suppository bases typically include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights, and fatty acid esters of polyethylene glycol. The suppository base can have a notable influence on the release of the API(s). Although cocoa butter melts quickly at body temperature, it is immiscible with body fluids and this inhibits the diffusion of fat-soluble APIs to the affected sites. Polyethylene glycol is a suitable base for some antiseptics. In cases when systemic action is desired, incorporating the ionized rather than the non-ionized form of the API may help maximize bioavailability. Although non-ionized APIs partition more readily out of water-miscible bases such as glycerinated gelatin and polyethylene glycol, the bases themselves tend to dissolve very slowly, which slows API release. Cocoa butter and its substitutes (e.g, *Hard Fat*) perform better than other bases for allaying irritation in preparations intended for treating internal hemorrhoids. Suppositories for adults are tapered at one or both ends and usually weigh about 2 g each.

PREPARATION

Cocoa butter suppositories have cocoa butter as the base and can be made by incorporating the finely divided API into the solid oil at room temperature and suitably shaping the resulting mass or by working with the oil in the melted state and allowing the resulting suspension to cool in molds. A suitable quantity of hardening agents may be added to counteract the tendency of some APIs (such as chloral hydrate and phenol) to soften the base. The finished suppository melts at body temperature.

A variety of vegetable oils, such as coconut or palm kernel, modified by esterification, hydrogenation, or fractionation, are used as cocoa butter substitutes to obtain products that display varying compositions and melting temperatures (e.g., *Hydrogenated Vegetable Oil* and *Hard Fat*). These products can be designed to reduce rancidity while incorporating desired characteristics such as narrow intervals between melting and solidification temperatures and melting ranges to accommodate formulation and climatic conditions.

APIs can be incorporated into glycerinated gelatin bases by addition of the prescribed quantities to a vehicle consisting of about 70 parts of glycerin, 20 parts of gelatin, and 10 parts of water.

Several combinations of polyethylene glycols that have melting temperatures that are above body temperature are used as suppository bases. Because release from these bases depends on dissolution rather than on melting, there are significantly fewer problems in preparation and storage than is the case for melting-type vehicles. However, high concentrations of higher molecular weight polyethylene glycols may lengthen dissolution time, resulting in problems with retention.

Several non-ionic surface-active agents closely related chemically to the polyethylene glycols can be used as suppository vehicles. Examples include polyoxyethylene sorbitan fatty acid esters and the polyoxyethylene stearates. These surfactants are used alone or in combination with other suppository vehicles to yield a wide range of melting temperatures and consistencies. A notable advantage of such vehicles is their water dispersibility. However, care must be taken with the use of surfactants

because they may either increase the rate of API absorption or interact with the API to reduce therapeutic activity.

Compounding suppositories using a suppository base typically involves melting the suppository base and dissolution or dispersion of the API in the molten base (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩). When compounding suppositories, the manufacturer or compounding professional prepares an excess amount of total formulation to allow the prescribed quantity to be accurately dispensed. In compounding suppositories, avoid caustic or irritating ingredients, carefully select a base that will allow the API to provide the intended effect, and in order to minimize abrasion of the rectal membranes, reduce solid ingredients to the smallest reasonable particle size. A representative number of the compounded suppositories should be weighed to confirm that none is less than 90% or more than 110% of the average weight of all units in the batch.

STORAGE AND USE

Suppositories typically are provided in unit-dose packaging with storage instructions to prevent melting of the suppository base. Suppositories with cocoa butter base require storage in well-closed containers, preferably at a temperature below 30°(controlled room temperature). Glycerinated gelatin suppositories require storage in tight containers, preferably at a temperature below 2°. Although polyethylene glycol suppositories can be stored without refrigeration, they should be packaged in tightly closed containers.

Include instructions about insertion procedures to ensure ease of use and absorption. Labels on polyethylene glycol suppositories should contain directions that they be moistened with water before insertion.

Suspensions

A suspension is a biphasic preparation consisting of solid particles dispersed throughout a liquid phase. Suspension dosage forms may be formulated for specific routes of administration such as oral suspensions, topical suspensions, or suspensions for aerosols (see *Aerosols*). Some suspensions are prepared and ready for use, and others are prepared as solid mixtures intended for reconstitution with an appropriate vehicle just before use. The term ''milk'' is sometimes used for suspensions in aqueous vehicles intended for oral administration (e.g., *Milk of Magnesia*). The term ''magma'' is often used to describe suspensions of inorganic solids, such as clays in water, that display a tendency toward strong hydration and aggregation of the solid, giving rise to gel-like consistency and thixotropic rheological behavior (e.g., *Bentonite Magma*). The term ''lotion'' may refer to a suspension dosage although the liquid phase in these preparations is commonly an emulsion intended for application to the skin (e.g., *Calamine Topical Suspension*; see *Emulsions*). Some suspensions are prepared in sterile form and are used as injectables (see *Injections* ⟨1⟩). Other sterile suspensions are for ophthalmic or otic administration. Suspensions generally are not injected intravenously, epidurally, or intrathecally unless the product labeling clearly specifies these routes of administration.

Limited aqueous solubility of the API(s) is the most common rationale for developing a suspension. Other potential advantages of a suspension include taste masking and improved patient compliance because of the more convenient dosage form. When compared to solutions, suspensions have improved chemical stability. Ideally, a suspension should contain small uniform particles that are readily suspended and easily re-dispersed following settling. Unless the dispersed solid is colloidal, the particulate matter in a suspension likely will settle to the bottom of the container upon standing. Such sedimentation may lead to caking and solidification of the

sediment and difficulty in re-dispersing the suspension upon agitation. To prevent such problems, manufacturers commonly add ingredients to increase viscosity and the gel state of the suspension or flocculation, including clays, surfactants, polyols, polymers, or sugars. Frequently, thixotropic vehicles are employed to counter particle-settling tendencies, but these vehicles must not interfere with pouring or re-dispersal. Additionally, the density of the dispersed phase and continuous phase may be modified to further control settling rate. For topical suspensions, rapid drying upon application is desirable.

The product is both chemically and physically stable throughout its shelf life. Temperature can influence the viscosity (and thus suspension properties and the ease of removing the dose from the bottle), and temperature cycling can lead to changes in the particle size of the dispersed phase via Ostwald ripening. When manufacturers conduct stability studies to establish product shelf life and storage conditions, they should cycle conditions (freeze/thaw) to investigate temperature effects.

All suspensions contain suitable antimicrobial agents to protect against bacterial, yeast, and mold contamination (see *Antimicrobial Effectiveness Testing* ⟨51⟩).

Suspensions for reconstitution are dry powder or granular mixtures that require the addition of water or a supplied formulated diluent before administration. This formulation approach is frequently used when the chemical or physical stability of the API or suspension does not allow sufficient shelf life for a preformulated suspension. Typically, these suspensions are refrigerated after reconstitution to increase their shelf life. For this type of suspension, the powder blend is uniform and the powder readily disperses when reconstituted. Taste of the reconstituted suspension is also an important attribute because many suspensions are used for pediatric populations.

Injectable suspensions generally are intended for either subcutaneous or intramuscular routes of administration and should have a controlled particle size, typically in the range of 5 μm or smaller. The rationale for the development of injectable suspensions includes poor API solubility, improved chemical stability, prolonged duration of action, and avoidance of first-pass metabolism. Care is needed in selecting the sterilization technique because it may affect product stability or alter the physical properties of the material.

PREPARATION

Suspensions are prepared by adding suspending agents or other excipients and purified water or oil to solid APIs and mixing to achieve uniformity. In the preparation of a suspension, the characteristics of both the dispersed phase and the dispersion medium should be considered. During development manufacturers should define an appropriate particle size distribution for the suspended material to minimize the likelihood of particle size changes during storage.

In some instances the dispersed phase has an affinity for the vehicle and is readily wetted upon its addition. For some materials the displacement of air from the solid surface is difficult, and the solid particles may clump together or float on top of the vehicle. In the latter case, a wetting agent is used to facilitate displacement of air from the powder surface. Surfactants, alcohol, glycerin, and other hydrophilic liquids can be employed as wetting agents when an aqueous vehicle will be used as the dispersion phase. These agents function by displacing the air in the crevices of the particles and dispersing the particles. In the large-scale preparation of suspensions, wetting of the dispersed phase may be aided by the use of high-energy mixing equipment such as colloid mills or other rotor–stator mixing devices.

After the powder has been wetted, the dispersion medium (containing the soluble formulation components such as colorants, flavorings, and preservatives) is added in portions to the powder, and the mixture is thoroughly

blended before subsequent additions of the vehicle. A portion of the vehicle is used to wash the mixing equipment free of suspended material, and this portion is used to bring the suspension to final volume and ensure that the suspension contains the desired concentration of solid matter. The final product may be passed through a colloid mill or other blender or mixing device to ensure uniformity. When appropriate, preservatives are included in the formulation of suspensions to protect against bacterial and mold contamination.

Suspensions are shaken before the dose is dispensed. Because of the viscosity of many suspension vehicles, air entrainment may occur during dosing. The formulation process allows evaluation of this possibility; adjustments in vehicle viscosity or the incorporation of low levels of antifoaming agents are common approaches to minimize air entrainment. Alternatively, specific instructions for shaking the formulation may be provided to minimize air incorporation and ensure accurate dosing.

PACKAGING AND STORAGE

Individual monographs specify the packaging and storage requirements for suspension products. Typically, the monograph will indicate a container type such as tight, well-closed, or light-resistant and may indicate storage conditions such as controlled room temperature. For additional information about meeting packaging requirements listed in the individual monographs, refer to *Containers—Glass* ⟨660⟩, *Containers—Plastic* ⟨661⟩, *Containers—Performance Testing* ⟨671⟩, *Good Packaging Practices* ⟨1177⟩, and the *General Notices* for statements about preservation, packaging, storage, and labeling.

Acceptable suspension of the particulate phase depends on the particle size of the dispersed phase as well as the viscosity and density of the vehicle. Clear instruction is provided regarding the appropriate storage temperature for the product because temperature can influence the viscosity and density (that affect suspension properties and the ease of removal of the dose from the bottle), and temperature cycling can lead to changes in particle size of the dispersed phase. Suspensions require storage in tight containers. Avoid freezing.

LABELING AND USE

Instructions to ensure proper dosing and administration must accompany the product. When labeling a suspension, consider any air that might be entrained in the preparation as a result of shaking, and avoid such entrainment. Compounded suspensions should indicate a beyond-use date that is calculated from the time of compounding. Suspensions are shaken well before use to ensure uniform distribution of the solid in the vehicles.

Tablets

Tablets are solid dosage forms in which the API is blended with excipients and compressed into the final dosage. Tablets are the most widely used dosage form in the U.S. Tablet presses use steel punches and dies to prepare compacted tablets by the application of high pressures to powder blends or granulations. Tablets can be produced in a wide variety of sizes, shapes, and surface markings. Capsule-shaped tablets are commonly referred to as caplets. Specialized tablet presses may be used to produce tablets with multiple layers or with specially formulated core tablets placed in the interior of the final dosage form. These specialized tablet presentations can delay or extend the release of the API(s) or physically separate incompatible APIs. Tablets may be coated by a variety of techniques to provide taste masking, protection of photo-labile API(s), prolonged or delayed release, or unique appearance (colors). When no deliberate effort has been made to modify the API release rate, tablets are referred to as immediate-release.

Tablet Triturates—Small, usually cylindrical, molded or compacted tablets. Tablet triturates traditionally were used as dispensing tablets in order to provide a convenient, measured quantity of a potent API for compounding purposes, but they are rarely used today.

Hypodermic Tablets—Molded tablets made from completely and readily water-soluble ingredients; formerly intended for use in making preparations for hypodermic injection. They may be administered orally or sublingually when rapid API availability is required, as in the case of *Nitroglycerin Sublingual Tablets*.

Bolus Tablets—Large, usually elongated, tablets intended for administration to large animals. Conventional tableting processes can be used to manufacture bolus tablets, but due to their size higher compression forces may be necessary.

Buccal Tablets—Intended to be inserted in the buccal pouch, where the API is absorbed directly through the oral mucosa. Few APIs are readily absorbed in this way (examples are nitroglycerin and certain steroid hormones).

Effervescent Tablets—Prepared by compaction and contain, in addition to the API(s), mixtures of acids (e.g., citric acid or tartaric acid) and carbonates and/or hydrogen carbonates. Upon contact with water, these formulations release carbon dioxide, producing the characteristic effervescent action.

Hard and Soft Chewable Tablets—Formulated and manufactured to produce a pleasant-tasting residue in the mouth and to facilitate swallowing. Hard chewable tablets are prepared by compaction, usually utilizing mannitol, sorbitol, or sucrose as binders and fillers, and contain colors and flavors to enhance their appearance and taste. Some chewable tablets may be swallowed without compromising delivery of the API. Chewable tablets are clearly labeled to indicate whether chewing is necessary to ensure reliable release of the API(s). Hard chewable tablets in veterinary medicine often have flavor enhancers like brewers yeast or meat/fish-based flavors. Soft chewable tablets are made by a molding or extrusion process, frequently with more than 10% water to help maintain a pliable, soft product.

Orally Disintegrating Tablets—Intended to disintegrate rapidly within the mouth to provide a fine dispersion before the patient swallows the resulting suspension. Some of these dosage forms have been formulated to facilitate rapid disintegration and are manufactured by conventional means or by using lyophilization or molding processes.

Sublingual Tablets—Intended to be inserted beneath the tongue, where the API is absorbed directly through the oral mucosa. As with buccal tablets, few APIs are extensively absorbed in this way, and much of the API is swallowed and is available for gastrointestinal absorption.

PREPARATION

Most compacted (compressed) tablets consist of the API(s) and a number of excipients. These excipients may include fillers (diluents), binders, disintegrating agents, lubricants, and glidants. Approved FD&C and D&C dyes or lakes, flavors, and sweetening agents also may be present.

Fillers or diluents are added when the quantity of API(s) is too small or the properties of the API do not allow satisfactory compaction in the absence of other ingredients. Binders impart adhesiveness to the powder blend and promote tablet formation and maintenance of API uniformity in the tableting mixture. Disintegrating agents facilitate reduction of the tablet into small particles upon contact with water or biological fluids. Lubricants reduce friction during the compaction and ejection cycles. Glidants improve powder fluidity, powder handling proper-

ties, and tablet weight control. Colorants are often added to tablet formulations for esthetic value or for product identification.

Tablets are prepared from formulations that have been processed by one of three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression.

Wet Granulation—Involves the mixing of dry powders with a granulating liquid to form a moist granular mass that is dried and sized prior to compression. It is particularly useful in achieving uniform blends of low-dose APIs and facilitating the wetting and dissolution of poorly soluble, hydrophobic APIs.

Dry Granulations—Can be produced by passing powders between rollers at elevated pressure (roll compaction). Alternatively, dry granulation also can be carried out by the compaction of powders at high pressures on tablet presses, a process also known as slugging. In either case the compacts are sized before compression. Dry granulation improves the flow and handling properties of the powder formulation without involving moisture in the processing.

Direct Compression—Tablet processing involves dry blending of the API(s) and excipients followed by compression. The simplest manufacturing technique, direct compression is acceptable only when the API and excipients possess acceptable flow and compression properties without prior process steps.

Tablets may be coated to protect the ingredients from air, moisture, or light; to mask unpleasant tastes and odors; to improve tablet appearance; and to reduce dustiness. In addition, coating may be used to protect the API from acidic pH values associated with gastric fluids or to control the rate of drug release in the gastrointestinal tract.

The most common coating in use today is a thin film coating composed of a polymer that is derived from cellulose. Sugar coating is an alternative, less common approach. Sugar-coated tablets have considerably thicker coatings that are primarily sucrose with a number of inorganic diluents. A variety of film-coating polymers are available and enable the development of specialized release profiles. These formulations are employed to protect acid-labile APIs from the acidic stomach environment as well as to prolong the release of the API to reduce dosing frequency (see *Dissolution* ⟨711⟩ or *Disintegration* ⟨701⟩).

PACKAGING, STORAGE, AND LABELING

Individual monographs specify the packaging and storage requirements for tablet products. Typically, the monograph will indicate the container type such as tight, well-closed, or light-resistant. For additional information on meeting USP packaging requirements see *Containers—Glass* ⟨660⟩, *Containers—Plastic* ⟨661⟩ and *Containers—Performance Testing* ⟨671⟩. Effervescent tablets are stored in tightly closed containers or moisture-proof packs and are labeled to indicate that they should not be swallowed directly.

Tapes

A tape is a dosage form suitable for delivering APIs to the skin. It consists of an API(s) impregnated into a durable yet flexible woven fabric or extruded synthetic material that is coated with an adhesive agent. Typically the impregnated API is present in the dry state. The adhesive layer is designed to hold the tape securely in place without the aid of additional bandaging. Unlike transdermal patches, tapes are not designed to control the release rate of the API.

The API content of tapes is expressed as amount per surface area with respect to the tape surface exposed to the skin. The use of an occlusive dressing with the tape

enhances the rate and extent of delivery of the API to deeper layers of the skin and may result in greater systemic absorption of the API.

LABELING, STORAGE, AND USE

Label to indicate ''External Use Only''. Tapes are stored in tight containers protected from light and moisture. To employ the tape, one cuts a patch slightly larger than the area that will be treated. The backing paper is removed from the adhesive side, and the tape is applied to the skin. To ensure optimal adhesion, the tape should not be applied to folds in the skin. To minimize systemic absorption and to ensure good adhesion, tapes should be applied to dry skin.

GLOSSARY

This glossary provides definitions for terms in use in medicine and serves as a source of official names for official articles. Examples of general nomenclature forms for the more frequently encountered categories of dosage forms appear in *Nomenclature* ⟨1121⟩. In an attempt to be comprehensive, this glossary was compiled without the limits imposed by current preferred nomenclature conventions. To clearly identify/distinguish preferred from not preferred terms, entries indicate when a term is not preferred and direct the user to the current preferred term. When a term is described as an attribute of a dosage form, it should not be used in the official name for the dosage form.

AEROSOL: A dosage form consisting of a liquid or solid preparation packaged under pressure and intended for administration as a fine mist. The descriptive term aerosol also refers to the fine mist of small droplets or solid particles that are emitted from the product.

AROMATIC WATER (NOT PREFERRED; see *Solution*): A clear, saturated, aqueous solution of volatile oils or other aromatic or volatile substances.

AURAL (Auricular) (NOT PREFERRED; see *Otic*): For administration into, or by way of, the ear.

BEAD (NOT PREFERRED; see *Pellets*): A solid dosage form in the shape of a small sphere. In most products a unit dose consists of multiple beads.

BLOCKS: A large veterinary product intended to be licked by animals and containing the API(s) and nutrients such as salts, vitamins, and minerals.

BOLUS (NOT PREFERRED; see *Tablet*): A large tablet intended for administration to large animals.

CAPLET (NOT PREFERRED; see *Tablet*): Tablet dosage form in the shape of a capsule.

CAPSULE: A solid dosage form in which the API, with or without other ingredients, is filled into either a hard or soft shell. Most capsule shells are composed mainly of gelatin.

CHEWABLE: Attribute of a solid dosage form that is intended to be chewed before swallowing.

COATED: Attribute of a solid dosage form that is covered by deposition of an outer solid that is different in composition from the core material.

COLLODION (NOT PREFERRED; see *Solution*): A preparation that is a solution dosage form composed of pyroxilin dissolved in a solvent mixture of alcohol and ether and applied externally.

COLLODIAL DISPERSION: A system in which particles of colloidal dimension (i.e., typically between 1 nm and 1 μm) are distributed uniformly throughout a liquid.

CONCENTRATE: A liquid or solid preparation of higher concentration and smaller volume than the final dosage form; usually intended to be diluted prior to administration. The term continues to be used for veterinary preparations but is being phased out of *USP–NF* titles for human applications.

CONVENTIONAL-RELEASE (NOT PREFERRED; see *Immediate-Release*): Descriptive term for a dosage form in which no deliberate effort has been made to modify the release rate of the API. In the case of capsules and tablets, the inclusion or exclusion of a disintegrating agent is not interpreted as a modification.

CREAM: An emulsion dosage form often containing more than 20% water and volatiles or containing less than 50% hydrocarbons, waxes, or polyols as the vehicle for the API. Creams are generally intended for external application to the skin or mucous membranes.

DELAYED-RELEASE: A type of modified-release dosage form. A descriptive term for a dosage form deliberately modified to delay release of the API for some period of time after initial administration. Release of the API is prevented in the gastric environment but promoted in the intestinal environment; this term is synonymous with *Enteric-Coated* or *Gastro-Resistant*.

DENTAL: Descriptive term for a preparation that is applied to the teeth and/or gums for localized action.

DERMAL: Route of administration to the skin surface.

DOSAGE FORM: A formulation of the API(s) and excipients in quantities and physical form designed to allow the accurate and efficient administration of the API to the human or animal patient.

DRY POWDER INHALER: A dosage form consisting of a mixture of the API(s) and carrier; all components exist in a finely divided solid state that is mobilized into a fine mist upon the oral inhalation by the patient.

EFFERVESCENT: Attribute of an oral dosage form, frequently tablets or granules, containing ingredients that, when in contact with water, rapidly release carbon dioxide. The dosage form is dissolved or dispersed in water to initiate the effervescence prior to ingestion.

ELIXIR (NOT PREFERRED; see *Solution*): A preparation that typically is a clear, flavored, sweetened hydroalcoholic solution intended for oral use. The term is no longer used in *USP–NF* but is commonly encountered in compounding pharmacy practice.

EMOLLIENT: Attribute of a cream or ointment indicating an increase in the moisture content of the skin following application of bland, fatty, or oleaginous substances.

EMULSION: A dosage form consisting of a two-phase system composed of at least two immiscible liquids, one of which is dispersed as droplets (internal or dispersed phase) within the other liquid (external or continuous phase), generally stabilized with one or more emulsifying agents. Emulsion is not used as a dosage form term if a more specific term is applicable (e.g., *Cream*, *Lotion*, or *Ointment*).

ENTERIC-COATED (NOT PREFERRED; see *Delayed-Release*): Descriptive term for a solid dosage form in which a polymer coating has been applied to prevent the release of the API in the gastric environment.

EXCIPIENT: An ingredient of a dosage form other than an API.

EXTENDED-RELEASE: Descriptive term for a dosage form that is deliberately modified to protract the release rate of the API compared to that observed for an immediate-release dosage form. The term is synonymous with prolonged- or sustained-release. Many extended-release dosage forms have a pattern of release that begins with a ''burst effect'' that mimics an immediate release followed by a slower release of the remaining API in the dosage form.

FEED ADDITIVE: A preparation used in veterinary medicine that is mixed with an animal's food or water to deliver the API. Three types exist: type A medicated article, type B medicated feed, and type C medicated

feed. Only type C medicated feed preparations contain the API(s) in concentrations appropriate for administration directly to animals.

FILM: A term used to describe a thin, flexible sheet of material, usually composed of a polymer in an amorphous state. Films are applied to solid dosages for taste masking, product identification, and aesthetic purposes. Films also are employed as a means of oral administration of material in a rapidly dissolving form.

FOAM: An emulsion dosage form containing dispersed gas bubbles. When dispensed it has a fluffy, semisolid consistency.

GAS: One of the states of matter having no definite shape or volume and occupying the entire container when confined.

GASTRO-RESISTANT (NOT PREFERRED; see *Delayed-Release*): Descriptive term for a solid dosage form in which a polymer coating has been applied to prevent the release in the gastric environment.

GEL: A dosage form that is a semisolid dispersion of small inorganic particles or a solution of large organic molecules containing a gelling agent to provide stiffness. A gel may contain suspended particles.

GRANULES (NOT PREFERRED): A dosage form composed of dry aggregates of powder particles that may contain one or more APIs, with or without other ingredients. They may be swallowed as such, dispersed in food, or dissolved in water. Granules are frequently compacted into tablets or filled into capsules, with or without additional ingredients.

GUM: A dosage form in which the base consists of a pliable material that, when chewed, releases the API into the oral cavity.

HARD-SHELL CAPSULE (NOT PREFERRED; see *Capsules*): A type of capsule in which one or more APIs, with or without other ingredients, are filled into a two-piece shell. Most hard-shell capsules are composed mainly of gelatin and are fabricated prior to the filling operation.

IMMEDIATE-RELEASE: Descriptive term for a dosage form in which no deliberate effort has been made to modify the API release rate. In the case of capsules and tablets, the inclusion or exclusion of a disintegrating agent is not interpreted as a modification.

IMPLANT: A dosage form that is a solid or semisolid material containing the API, that is inserted into the body. The insertion process is invasive, and the material is intended to reside at the site for a period consistent with the design release kinetics or profile of the API(s).

INHALATION (BY INHALATION): A route of administration for aerosols characterized by dispersion of the API into the airways during inspiration.

BY INJECTION: A route of administration of a liquid or semisolid deposited into a body cavity, fluid, or tissue by use of a needle.

INSERT: A solid dosage form that is inserted into a body cavity other than the rectum. A suppository is an insert intended for application to the rectum (see *Suppository*).

INTRAOCULAR: A route of administration (by injection) for a sterile liquid within the eye.

IRRIGATION: A sterile solution or liquid intended to bathe or flush open wounds or body cavities.

JELLY (NOT PREFERRED; see *Gel*): A semisolid dispersion of small inorganic particles or a solution of large organic molecules containing a gelling agent to promote stiffness.

LIQUID: A dosage form consisting of a pure chemical in its liquid state. This dosage form term should not be applied to solutions. The term is not used in article names. When liquid is used as a descriptive term, it indicates a material that is pourable and conforms to its container at room temperature.

LOTION: An emulsion liquid dosage form applied to the outer surface of the body. Historically, this term has also been applied to suspensions and solutions.

LOZENGE: A solid dosage form intended to disintegrate or dissolve slowly in the mouth.

MODIFIED-RELEASE: A descriptive term for a dosage form with an API release pattern that has been deliberately changed from that observed for the immediate-release dosage form of the same API.

MOLDED TABLET (NOT PREFERRED; see *Tablet*): A tablet that has been formed by dampening the ingredients and pressing into a mold, then removing and drying the resulting solid mass.

MOUTHWASH (NOT PREFERRED; see *Solution*): Term applied to a solution preparation used to rinse the oral cavity.

NASAL: Route of administration (mucosal) characterized by deposition in the nasal cavity for local or systemic effect.

OCULAR (NOT PREFERRED; see *Intraocular*): Route of administration (by injection) indicating deposition of the API within the eye.

OINTMENT: A semisolid dosage form, usually containing less than 20% water and volatiles and more than 50% hydrocarbons, waxes, or polyols as the vehicle. This dosage form generally is for external application to the skin or mucous membranes.

OPHTHALMIC: A route of administration (mucosal) characterized by application of sterile preparation to the external parts of the eye.

ORAL: A route of administration (gastro-intestinal) characterized by deposition of a preparation into the mouth for absorption or action in the digestive tract.

ORALLY DISINTEGRATING: A descriptive term for a solid oral dosage form that disintegrates rapidly in the mouth.

ORO-PHARYNGEAL: A route of administration characterized by deposition of a preparation into the buccal cavity and/or pharyngeal region to exert a local or systemic effect.

OTIC: A route of administration (mucosal) characterized by deposition of a preparation into, or by way of, the ear. Sometimes referred to as *Aural* (*Aural* NOT PREFERRED).

PASTE: A semisolid dosage form containing a high percentage of finely dispersed solids with a stiff consistency. This dosage form is intended for application to the skin, oral cavity, or mucous membranes.

PELLET: A small solid dosage form of uniform, often spherical, shape. Spherical pellets are sometimes referred to as *Beads* (*Beads* NOT PREFERRED).

PILL (NOT PREFERRED but frequently incorrectly used to describe a *Tablet*): A solid spherical pharmaceutical dosage form, usually prepared by a wet massing technique.

PLASTER: A semisolid dosage form supplied on a support material for external application. Plasters are applied for prolonged periods to provide protection, support, or occlusion (for macerating action).

POWDER: A dosage form composed of a solid or mixture of solids reduced to a finely divided state and intended for internal or external use.

PROLONGED-RELEASE: NOT PREFERRED; see *Extended-Release*.

RECTAL: A route of administration (mucosal) characterized by deposition into the rectum to provide local or systemic effect.

RINSE: A liquid preparation used to cleanse by flushing.

SEMISOLID: Attribute of a material characterized by a reduced ability to flow or conform to its container at room temperature. A semisolid does not flow at low shear stress and generally exhibits plastic flow behavior.

SHAMPOO: A solution or suspension dosage form used to clean the hair and scalp. May contain an API intended for topical application to the scalp.

SOAP: The alkali salt(s) of a fatty acid or mixture of fatty acids used to cleanse the skin. Soaps used as dosage forms may contain an API intended for topical application to the skin. Soaps have also been used as liniments and enemas.

SOFT GEL CAPSULE (NOT PREFERRED; see *Capsule*): A specific capsule type characterized by increased levels of plasticizers producing a more pliable and thicker-walled material than hard gelatin capsules. Soft gel capsules are further distinguished because they are single-piece sealed dosages. Frequently used for delivering liquid compositions.

SOLUTION: A clear, homogeneous liquid dosage form that contains one or more chemical substances dissolved in a solvent or mixture of mutually miscible solvents.

SPIRIT (NOT PREFERRED; see *Solution*): A liquid dosage form composed of an alcoholic or hydroalcoholic solution of volatile substances.

SPRAY: Attribute that describes the generation of droplets of a liquid or solution to facilitate application to the intended area.

STENT, DRUG-ELUTING: A specialized form of implant used for extended local delivery of the API to the immediate location of stent placement.

STRIP (NOT PREFERRED; see *Tape*): A dosage form or device in the shape of a long, narrow, thin solid material.

SUBLINGUAL: A route of administration (mucosal) characterized by placement underneath the tongue and for release of the API for absorption in that region.

SUPPOSITORY: A solid dosage form in which one or more APIs are dispersed in a suitable base and molded or otherwise formed into a suitable shape for insertion into the rectum to provide local or systemic effect.

SUSPENSION: A liquid dosage form that consists of solid particles dispersed throughout a liquid phase.

SYRUP (NOT PREFERRED; see *Solution*): A solution containing high concentrations of sucrose or other sugars. This term is commonly used in compounding pharmacy.

TABLET: A solid dosage form prepared from powders or granules by compaction.

TAPE, MEDICATED: A dosage form or device composed of a woven fabric or synthetic material onto which an API is placed, usually with an adhesive on one or both sides to facilitate topical application.

TINCTURE (NOT PREFERRED; see *Solution*): An alcoholic or hydroalcoholic solution prepared from vegetable materials or from chemical substances.

TOPICAL: A route of administration characterized by application to the outer surface of the body.

TROCHE (NOT PREFERRED; see *Lozenge*): A solid dosage form intended to disintegrate or dissolve slowly in the mouth and usually prepared by compaction in a manner similar to that used for tablets.

URETHRAL: A route of administration (mucosal) characterized by deposition into the urethra.

VAGINAL: A route of administration (mucosal) characterized by deposition into the vagina.

VEHICLE: A term commonly encountered in compounding pharmacy that refers to a component for internal or external use that is used as a carrier or diluent in which liquids, semisolids, or solids are dissolved or suspended. Examples include water, syrups, elixirs, oleaginous liquids, solid and semisolid carriers, and proprietary products (see *Excipient*).

VETERINARY: Descriptive term for dosage forms intended for nonhuman use.■2S (*USP33*)

BRIEFING

〈**1231**〉 **Water for Pharmaceutical Purposes,** *USP 32* page 741. Although the majority of water used for production is *Purified Water* and *Water for Injection* that is produced on site (referred to as ''bulk water''), the Pharmaceutical Waters Expert Committee recognizes the need for and the use of commercially available ''packaged'' *Purified Water* and *Water for Injection* in some production environments. In addition, there are sterile waters such as *Sterile Purified Water* and *Sterile Water for Injection* (and *Inhalation* and *Irrigation*). As the tests and limits for these various types of waters have been updated in recent years, some of the terminology regarding ''bulk'', ''sterile'', and ''packaged'' needs to be updated and/or clarified. The proposed remedy is to distinguish between ''bulk'' water, ''sterile'' water, and ''packaged bulk'' water in the relevant monographs and general chapters. The term ''packaged waters'' has been used as a substitute for ''sterile waters'' and as a term to describe commercially available packages of *Purified Water* and *Water for Injection*. The Pharmaceutical Waters Expert Committee proposes that the term ''packaged waters'' be used for the packaged form of bulk *Purified Water* and *Water for Injection* that has been produced elsewhere. Requirements for packaged waters are contained in the *Purified Water* and *Water for Injection* monographs. Sterile waters, although they are also packaged articles, have their own unique monographs and uses. The Expert Committee is discouraging the use of the term ''packaged water'' to mean ''sterile water''.

There are companion changes in *Water Conductivity* 〈645〉 and the monographs for *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*. All changes align the use of these terms.

(PW: A. Hernandez-Cardoso.) RTS—C76228

Change to read:

INTRODUCTION

Water is widely used as a raw material, ingredient, and solvent in the processing, formulation, and manufacture of pharmaceutical products, active pharmaceutical ingredients (APIs) and intermediates, compendial articles, and analytical reagents. This general information chapter provides additional information about water, its quality attributes that are not included within a water monograph, processing techniques that can be used to improve water quality, and a description of minimum water quality standards that should be considered when selecting a water source.

This information chapter is not intended to replace existing regulations or guides that already exist to cover USA and International (ICH or WHO) GMP issues, engineering guides, or other regulatory (FDA, EPA, or WHO) guidances for water. The contents will help users to better understand pharmaceutical water issues and some of the microbiological and chemical concerns unique to water. This chapter is not an all-inclusive writing on pharmaceutical waters. It contains points that are basic information to be considered, when appropriate, for the processing, holding, and use of water. It is the user's responsibility to assure that pharmaceutical water and its production meet applicable governmental regulations, guidances, and the compendial specifications for the types of water used in compendial articles.

Control of the chemical purity of these waters is important and is the main purpose of the monographs in this compendium. Unlike other official articles, the bulk water monographs (*Purified Water* and *Water for Injection*) also limit how the article can be produced because of the belief that the nature and robustness of the purification process is directly related to the resulting purity. The chemical attributes listed in these monographs should be considered as a set of minimum specifications. More stringent specifications may be needed for some applications to ensure suitability for particular uses. Basic guidance on the appropriate applications of these waters is found in the monographs and is further explained in this chapter.

Control of the microbiological quality of water is important for many of its uses. ~~All~~

■Most■2S (*USP33*)

In-Process Revision

packaged forms of water that have monograph standards are required to be sterile because some of their intended uses require this attribute for health and safety reasons. USP has determined that a microbial specification for the bulk monographed waters is inappropriate and has not been included within the monographs for these waters. These waters can be used in a variety of applications, some requiring extreme microbiological control and others requiring none. The needed microbial specification for a given bulk water depends upon its use. A single specification for this difficult-to-control attribute would unnecessarily burden some water users with irrelevant specifications and testing. However, some applications may require even more careful microbial control to avoid the proliferation of microorganisms ubiquitous to water during the purification, storage, and distribution of this substance. A microbial specification would also be inappropriate when related to the ''utility'' or continuous supply nature of this raw material. Microbial specifications are typically assessed by test methods that take at least 48 to 72 hours to generate results. Because pharmaceutical waters are generally produced by continuous processes and used in products and manufacturing processes soon after generation, the water is likely to have been used well before definitive test results are available. Failure to meet a compendial specification would require investigating the impact and making a pass/fail decision on all product lots between the previous sampling's acceptable test result and a subsequent sampling's acceptable test result. The technical and logistical problems created by a delay in the result of such an analysis do not eliminate the user's need for microbial specifications. Therefore, such water systems need to be operated and maintained in a controlled manner that requires that the system be validated to provide assurance of operational stability and that its microbial attributes be quantitatively monitored against established alert and action levels that would provide an early indication of system control. The issues of water system validation and alert/action levels and specifications are included in this chapter.

Change to read:

TYPES OF WATER

There are many different grades of water used for pharmaceutical purposes. Several are described in *USP* monographs that specify uses, acceptable methods of preparation, and quality attributes. These waters can be divided into two general types: bulk waters, which are typically produced on site where they are used; and ~~packaged~~

■sterile■ 2S (*USP33*)
waters, which are produced, packaged, and sterilized to preserve microbial quality throughout their packaged shelf life. There are several specialized types of ~~packaged~~

■sterile■ 2S (*USP33*)
waters, differing in their designated applications, packaging limitations, and other quality attributes.

There are also other types of water for which there are no monographs. These are all bulk waters, with names given for descriptive purposes only. Many of these waters are used in specific analytical methods. The associated text may not specify or imply certain quality attributes or modes of preparation. These nonmonographed waters may not necessarily adhere strictly to the stated or implied modes of preparation or attributes. Waters produced by other means or controlled by other test attributes may equally satisfy the intended uses for these waters. It is the user's responsibility to ensure that such waters, even if produced and controlled exactly as stated, be suitable for their intended use. Wherever the term ''water'' is used within this compendia without other descriptive adjectives or clauses, the intent is that water of no less purity than *Purified Water* be used.

What follows is a brief description of the various types of pharmaceutical waters and their significant uses or attributes. *Figure 1* may also be helpful in understanding some of the various types of waters.

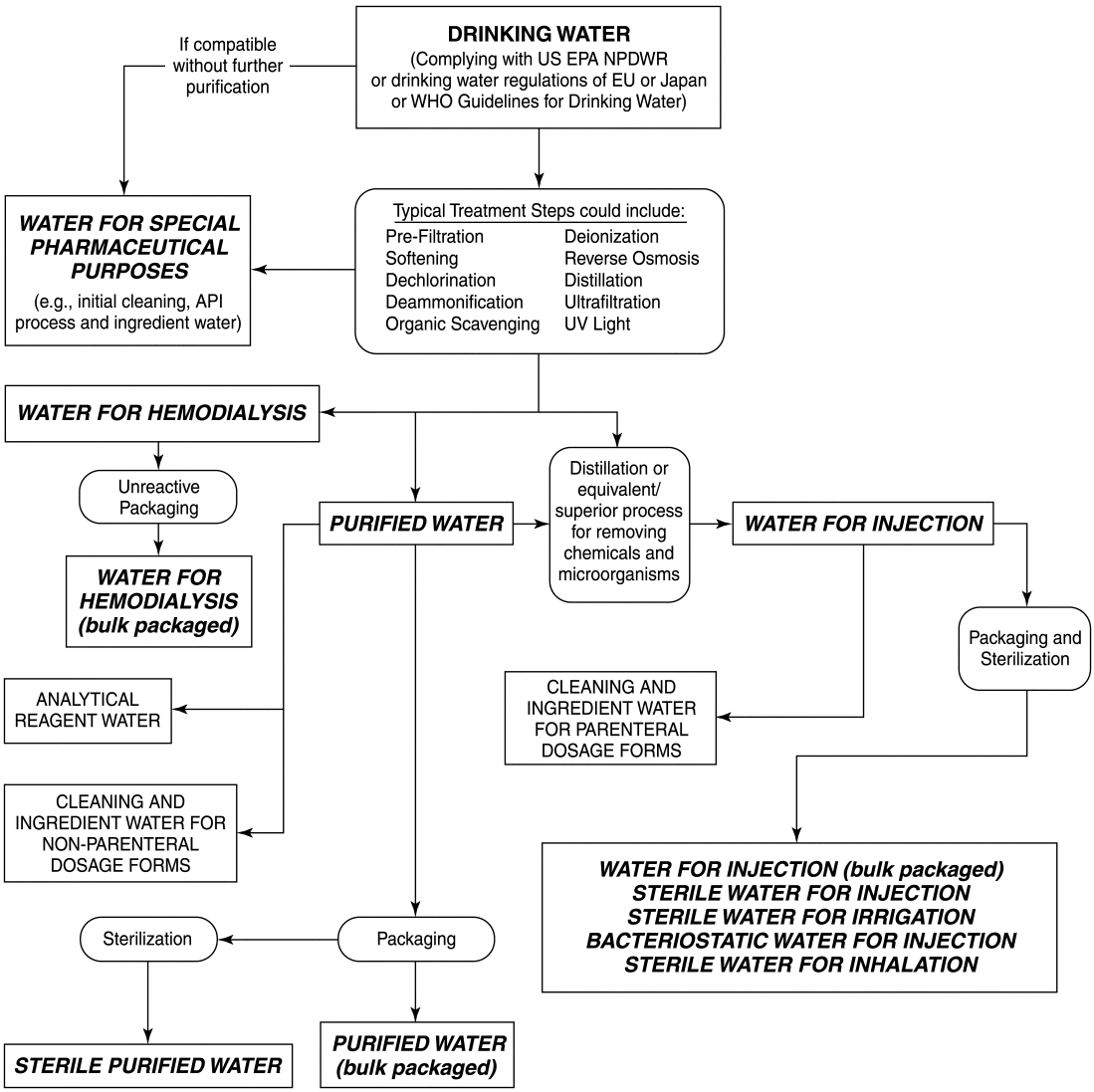


Figure 1. Water for pharmaceutical purposes.

Bulk Monographed Waters and Steam

The following waters are typically produced in large volume by a multiple-unit operation water system and distributed by a piping system for use at the same site. These particular pharmaceutical waters must meet the quality attributes as specified in the related monographs.

Purified Water—*Purified Water* (see the *USP* monograph) is used as an excipient in the production of nonparenteral preparations and in other pharmaceutical applications, such as cleaning of certain equipment and nonparenteral product-contact components. Unless otherwise specified, *Purified Water* is also to be used for all tests and assays for which water is indicated (see *General Notices and Requirements*). *Purified Water* is also referenced throughout the *USP–NF.* Regardless of the font and letter case used in its spelling, water complying with the *Purified Water* monograph is intended. *Purified Water* must meet the requirements for ionic and organic chemical purity and must be protected from microbial contamination. The minimal quality of source or feed water for the production of *Purified Water* is Drinking Water. This source water may be purified using unit operations that include deionization, distillation, ion exchange, reverse osmosis, filtration, or other suitable purification procedures. Purified water systems must be validated to reliably and consistently produce and distribute water of acceptable chemical and microbiological quality. Purified water systems that function under ambient conditions are particularly susceptible to the establishment of tenacious biofilms of microorganisms, which can be the source of undesirable levels of viable microorganisms or endotoxins in the effluent water. These systems require frequent sanitization and microbiological monitoring to ensure water of appropriate microbiological quality at the points of use.

The *Purified Water* monograph also allows bulk packaging for commercial use elsewhere. ~~When this is done, the required specifications are those of the packaged water *Sterile Purified Water,* except for *Sterility* and *Labeling*.~~

■■2S (*USP33*)
There is a potential for the occurrence of microbial contamination and other quality changes in this bulk packaged nonsterile water. Therefore, this form of *Purified Water* should be prepared and stored in a fashion that limits microbial growth and/or simply used in a timely fashion before microbial proliferation renders it unsuitable for its intended use. Also depending on the material used for packaging, there could be extractable compounds leaching into the water from the packaging. Though this article may meet its required chemical attributes, such extractables may render the water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when used in manufacturing, clinical, or analytical applications where the pure bulk form of the water is indicated.

Water for Injection—*Water for Injection* (see the *USP* monograph) is used as an excipient in the production of parenteral and other preparations where product endotoxin content must be controlled, and in other pharmaceutical applications, such as cleaning of certain equipment and parenteral product-contact components. The minimum quality of source or feed water for the generation of *Water for Injection* is Drinking Water as defined by the U.S. EPA, EU, Japan, or WHO. This source water may be pretreated to render it suitable for subsequent distillation (or whatever other validated process is used according to the monograph). The finished water must meet all of the chemical requirements for *Purified Water* as well as an additional bacterial endotoxin specification. Since endotoxins are produced by the kinds of microorganisms that are prone to inhabit water, the equipment and procedures used by the system to purify, store, and distribute *Water for Injection* must be designed to minimize or prevent microbial contamination as well as remove incoming endotoxins from the starting water. *Water for Injection* systems must be validated to reliably and consistently produce and distribute this quality of water.

The *Water for Injection* monograph also allows it to be packed in bulk for commercial use. ~~Required specifications include the test for *Bacterial endotoxins,* and those of the packaged water *Sterile Purified Water,* except for *Labeling*.~~

■■2S (*USP33*)
Bulk packaged *Water for Injection* is required to be sterile, thus eliminating microbial contamination quality changes. However, packaging extractables may render this water an inappropriate choice for some applications. It is the user's responsibility to ensure fitness for use of this packaged article when used in manufacturing, clinical, or analytical applications where the purer bulk form of the water is indicated.

Water for Hemodialysis—*Water for Hemodialysis* (see the *USP* monograph) is used for hemodialysis applications, primarily the dilution of hemodialysis concentrate solutions. It is produced and used on site and is made from EPA Drinking Water which has been further purified to reduce chemical and microbiological components. It may be packaged and stored in unreactive containers that preclude bacterial entry. The term ''unreactive containers'' implies that the container, especially its water contact surfaces, are not changed in any way by the water, such as by leaching of container-related compounds into the water or by any chemical reaction or corrosion caused by the water. The water contains no added antimicrobials and is not intended for injection. Its attributes include specifications for *Water conductivity, Total organic carbon* (or oxidizable substances), *Microbial limits,* and *Bacterial endotoxins.* The water conductivity and total organic carbon attributes are identical to those established for *Purified Water* and *Water for Injection;* however, instead of total organic carbon, the organic content may alternatively be measured by the test for *Oxidizable substances*. The *Microbial limits* attribute for this water is unique among the ''bulk'' water monographs, but is justified on the basis of this water's specific application that has microbial content requirements related to its safe use. The *Bacterial endotoxins* attribute is likewise established at a level related to its safe use.

Pure Steam—*Pure Steam* (see the *USP* monograph) is also sometimes referred to as ''clean steam''. It is used where the steam or its condensate would directly contact official articles or article-contact surfaces, such as during their preparation, sterilization, or cleaning where no subsequent processing step is used to remove any codeposited impurity residues. These *Pure Steam* applications include but are not limited to porous load sterilization processes, product or cleaning solutions heated by direct steam injection, or humidification of processes where steam injection is used to control the humidity inside processing vessels where the official articles or their in-process forms are exposed. The primary intent of using this quality of steam is to ensure that official articles or article-contact surfaces exposed to it are not contaminated by residues within the steam.

Pure Steam is prepared from suitably pretreated source water analogously to either the pretreatment used for *Purified Water* or *Water for Injection*. The water is vaporized with suitable mist elimination, and distributed under pressure. The sources of undesirable contaminants within *Pure Steam* could arise from entrained source water droplets, anticorrosion steam additives, or residues from the steam production and distribution system itself. The attributes in the *Pure Steam* monograph should detect most of the contaminants that could arise from these sources. If the official article exposed to potential *Pure Steam* residues is intended for parenteral use or other applications where the pyrogenic content must be controlled, the *Pure Steam* must additionally meet the specification for *Bacterial Endotoxins* ⟨85⟩.

These purity attributes are measured on the condensate of the article, rather than the article itself. This, of course, imparts great importance to the cleanliness of the *Pure Steam* condensate generation and collection process because it must not adversely impact the quality of the resulting condensed fluid.

Other steam attributes not detailed in the monograph, in particular, the presence of even small quantities of noncondensable gases or the existence of a superheated or dry state, may also be important for applications such as sterilization. The large release of energy (latent heat of condensation) as water changes from the gaseous to the liquid state is the key to

steam's sterilization efficacy and its efficiency, in general, as a heat transfer agent. If this phase change (condensation) is not allowed to happen because the steam is extremely hot and in a persistent superheated, dry state, then its usefulness could be seriously compromised. Noncondensable gases in steam tend to stratify or collect in certain areas of a steam sterilization chamber or its load. These surfaces would thereby be at least partially insulated from the steam condensation phenomenon, preventing them from experiencing the full energy of the sterilizing conditions. Therefore, control of these kinds of steam attributes, in addition to its chemical purity, may also be important for certain *Pure Steam* applications. However, because these additional attributes are use-specific, they are not mentioned in the *Pure Steam* monograph.

Note that less pure ''plant steam'' may be used for steam sterilization of nonproduct contact nonporous loads, for general cleaning of nonproduct contact equipment, as a nonproduct contact heat exchange medium, and in all compatible applications involved in bulk pharmaceutical chemical and API manufacture.

~~**Packaged**~~

▪**Sterile**▪ 2S (*USP33*) **Monographed Waters**

The following monographed waters are packaged forms of either *Purified Water* or *Water for Injection* that have been sterilized to preserve their microbiological properties. These waters may have specific intended uses as indicated by their names and may also have restrictions on packaging configurations related to those uses. In general, these ~~packaged~~

▪▪ 2S (*USP33*)
waters may be used in lieu of the bulk form of water from which they were derived. However, the user should take into consideration that the packaging and sterilization processes used for the articles may leach materials from the packaging material into the water over its shelf life, rendering it less pure than the original water placed into the package. The chemical attributes of these waters are still defined primarily by the wet chemistry methods and specifications similar to those formerly used for the bulk pharmaceutical waters prior to their replacement with water conductivity and total organic carbon (TOC). It is the user's responsibility to ensure fitness for use of this article when used in manufacturing, clinical, or analytical applications where the purer bulk form of the water is indicated.

Sterile Purified Water—*Sterile Purified Water* (see the *USP* monograph) is *Purified Water*, packaged and rendered sterile. It is used in the preparation of nonparenteral compendial dosage forms or in analytical applications requiring *Purified Water* where access to a validated *Purified Water* system is not practical, where only a relatively small quantity is needed, where ~~sterile~~

▪*Sterile*▪ 2S (*USP33*)
Purified Water is required, or where bulk packaged *Purified Water* is not suitably microbiologically controlled.

Sterile Water for Injection—*Sterile Water for Injection* (see the *USP* monograph) is *Water for Injection* packaged and rendered sterile. It is used for extemporaneous prescription compounding and as a sterile diluent for parenteral products. It may also be used for other applications where bulk *Water for Injection* or *Purified Water* is indicated but where ~~assess~~

▪access▪ 2S (*USP33*)
to a validated water system is either not practical or where only a relatively small quantity is needed. *Sterile Water for Injection* is packaged in single-dose containers not larger than 1 L in size.

Bacteriostatic Water for Injection—*Bacteriostatic Water for Injection* (see the *USP* monograph) is sterile *Water for Injection* to which has been added one or more suitable antimicrobial preservatives. It is intended to be used as a diluent in the preparation of parenteral products, most typically for multidose products that require repeated content withdrawals. It may be packaged in single-dose or multiple-dose containers not larger than 30 mL.

Sterile Water for Irrigation—*Sterile Water for Irrigation* (see the *USP* monograph) is *Water for Injection* packaged and sterilized in single-dose containers of larger than 1 L in size that allows rapid delivery of its contents. It need not meet the requirement under small-volume injections in the general test chapter *Particulate Matter in Injections* ⟨788⟩. It may also be used in other applications which do not have particulate matter specifications, where bulk *Water for Injection* or *Purified Water* is indicated but where access to a validated water system is not practical, or where somewhat larger quantities than are provided as *Sterile Water for Injection* are needed.

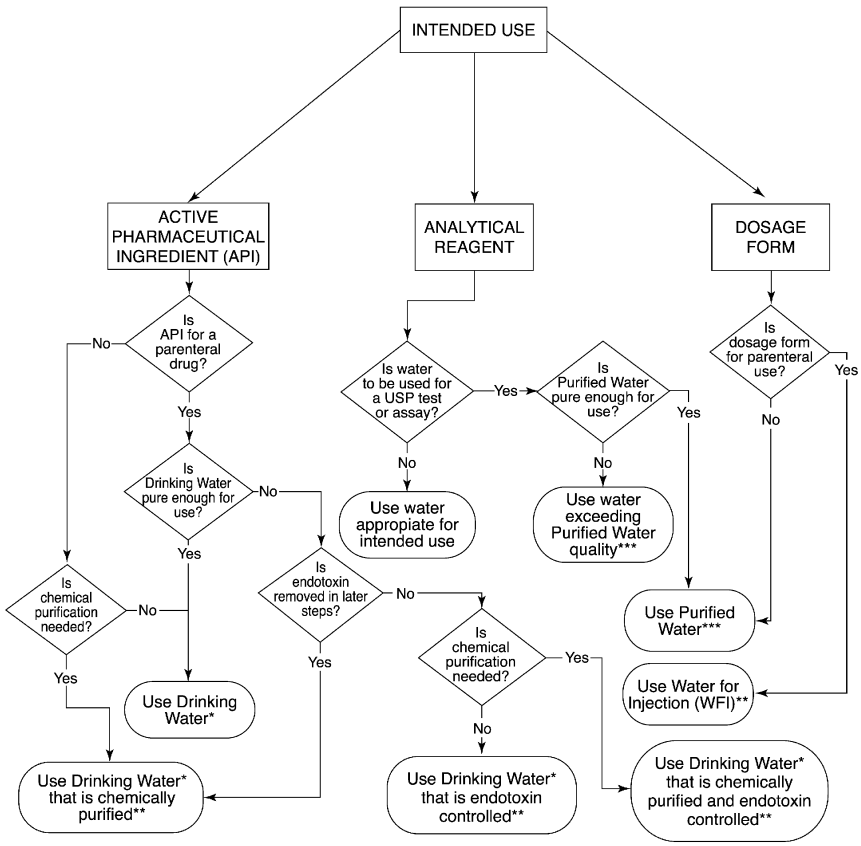
Sterile Water for Inhalation—*Sterile Water for Inhalation* (see the *USP* monograph) is *Water for Injection* that is packaged and rendered sterile and is intended for use in inhalators and in the preparation of inhalation solutions. It carries a less stringent specification for bacterial endotoxins than *Sterile Water for Injection* and therefore is not suitable for parenteral applications.

Nonmonographed Manufacturing Waters

In addition to the bulk monographed waters described above, nonmonographed waters can also be used in pharmaceutical processing steps such as cleaning, synthetic steps, or a starting material for further purification. The following is a description of several of these nonmonographed waters as cited in various locations within this compendia.

Drinking Water—This type of water can be referred to as Potable Water (meaning drinkable or fit to drink), National Primary Drinking Water, Primary Drinking Water, or National Drinking Water. Except where a singular drinking water specification is stated (such as the NPDWR [U.S. Environmental Protection Agency's National Primary Drinking Water Regulations as cited in 40 CFR Part 141]), this water must comply with the quality attributes of either the NPDWR, or the drinking water regulations of the European Union or Japan, or the WHO Drinking Water Guidelines. It may be derived from a variety of sources including a public water utility, a private water supply (e.g., a well), or a combination of these sources. Drinking Water may be used in the early stages of cleaning pharmaceutical manufacturing equipment and product-contact components. Drinking Water is also the minimum quality of water that should be used for the preparation of official substances and other bulk pharmaceutical ingredients. Where compatible with the processes, the allowed contaminant levels in Drinking Water are generally considered safe for use for official substances and other drug substances. Where required by the processing of the materials to achieve their required final purity, higher qualities of water may be needed for these manufacturing steps, perhaps even as pure as *Water for Injection* or *Purified Water*. Such higher purity waters, however, might require only selected attributes to be of higher purity than Drinking Water (see *Figure 2* below). Drinking Water is the prescribed source or feed water for the production of bulk monographed pharmaceutical waters. The use of Drinking Water specifications establishes a reasonable set of maximum allowable levels of chemical and microbiological contaminants with which a water purification system will be challenged. As seasonal variations in the quality attributes of the Drinking Water supply can occur, due consideration to its synthetic and cleaning uses must be given. The processing steps in the production of pharmaceutical waters must be designed to accommodate this variability.

In-Process Revision



* Drinking Water is water complying with US EPA NPDWR or drinking water regulations of EU or Japan or WHO drinking water guidelines.
** Water for sterile API's or dosage forms must first be rendered sterile if there is not a subsequent sterilization step in the process where used.
*** See guidance in this chapter where waters other than Purified Water are required by some USP tests and assays.

Note: All water systems should be validated with whatever microbial control is needed to suit the intended purposes of the water.

Figure 2. Selection of water for pharmaceutical purposes.

Hot Purified Water—This water is used in the preparation instructions for *USP–NF* articles and is clearly intended to be *Purified Water* that has been heated to an unspecified temperature in order to enhance solubilization of other ingredients. There is no upper temperature limit for the water (other than being less than 100°), but for each monograph there is an implied lower limit below which the desired solubilization effect would not occur.

Nonmonographed Analytical Waters

Both *General Notices and Requirements* and the introductory section to *Reagents, Indicators, and Solutions* clearly state that where the term ''water,'' without qualification or other specification, is indicated for use in analyses, the quality of water shall be *Purified Water.* However, numerous such qualifications do exist. Some of these qualifications involve methods of preparation, ranging from specifying the primary purification step to specifying additional purification. Other qualifications call for specific attributes to be met that might otherwise interfere with analytical processes. In most of these latter cases, the required attribute is not specifically tested. Rather, a further ''purification process'' is specified that ostensibly allows the water to adequately meet this required attribute.

However, preparation instructions for many reagents were carried forward from the innovator's laboratories to the originally introduced monograph for a particular *USP–NF* article or general test chapter. The quality of the reagent water described in these tests may reflect the water quality designation of the innovator's laboratory. These specific water designations may have originated without the innovator's awareness of the requirement for *Purified Water* in *USP–NF* tests. Regardless of the original reason for the creation of these numerous special analytical waters, it is possible that the attributes of these special waters could now be met by the basic preparation steps and current specifications of *Purified Water.* In some cases, however, some of the cited post-processing steps are still necessary to reliably achieve the required attributes.

Users are not obligated to employ specific and perhaps archaically generated forms of analytical water where alternatives with equal or better quality, availability, or analytical performance may exist. The consistency and reliability for producing these alternative analytical waters should be verified as producing the desired attributes. In addition, any alternative analytical water must be evaluated on an application-by-application basis by the user to ensure its suitability. Following is a summary of the various types of nonmonographed analytical waters that are cited in the *USP–NF.*

Distilled Water—This water is produced by vaporizing liquid water and condensing it in a purer state. It is used primarily as a solvent for reagent preparation, but it is also specified in the

execution of other aspects of tests, such as for rinsing an analyte, transferring a test material as a slurry, as a calibration standard or analytical blank, and for test apparatus cleaning. It is also cited as the starting water to be used for making *High Purity Water.* Because none of the cited uses of this water imply a need for a particular purity attribute that can only be derived by distillation, water meeting the requirements for *Purified Water* derived by other means of purification could be equally suitable where *Distilled Water* is specified.

Freshly Distilled Water—Also called ''recently distilled water'', it is produced in a similar fashion to *Distilled Water* and should be used shortly after its generation. This implies the need to avoid endotoxin contamination as well as any other adventitious forms of contamination from the air or containers that could arise with prolonged storage. It is used for preparing solutions for subcutaneous test animal injections as well as for a reagent solvent in tests for which there appears to be no particularly high water purity needed that could be ascribable to being ''freshly distilled''. In the ''test-animal'' use, the term ''freshly distilled'' and its testing use imply a chemical, endotoxin, and microbiological purity that could be equally satisfied by *Water for Injection* (though no reference is made to these chemical, endotoxin, or microbial attributes or specific protection from recontamination). For nonanimal uses, water meeting the requirements for *Purified Water* derived by other means of purification and/or storage periods could be equally suitable where ''recently distilled water'' or *Freshly Distilled Water* is specified.

Deionized Water—This water is produced by an ion-exchange process in which the contaminating ions are replaced with either H^+ or OH^- ions. Similarly to *Distilled Water, Deionized Water* is used primarily as a solvent for reagent preparation, but it is also specified in the execution of other aspects of tests, such as for transferring an analyte within a test procedure, as a calibration standard or analytical blank, and for test apparatus cleaning. Also, none of the cited uses of this water imply any needed purity attribute that can only be achieved by deionization. Therefore, water meeting the requirements for *Purified Water* that is derived by other means of purification could be equally suitable where *Deionized Water* is specified.

Freshly Deionized Water—This water is prepared in a similar fashion to *Deionized Water,* though as the name suggests, it is to be used shortly after its production. This implies the need to avoid any adventitious contamination that could occur upon storage. This water is indicated for use as a reagent solvent as well as for cleaning. Due to the nature of the testing, *Purified Water* could be a reasonable alternative for these applications.

Deionized Distilled Water—This water is produced by deionizing (see *Deionized Water*) *Distilled Water.* This water is used as a reagent in a liquid chromatography test that requires a high purity. Because of the importance of this high purity, water that barely meets the requirements for *Purified Water* may not be acceptable. *High Purity Water* (see below) could be a reasonable alternative for this water.

Filtered Distilled or Deionized Water—This water is essentially *Purified Water* produced by distillation or deionization that has been filtered through a 1.2-μm rated membrane. This water is used in particulate matter testing where the presence of particles in the water could bias the test results (see *Particulate Matter in Injections* ⟨788⟩). Because the chemical water purity needed for this test could also be afforded by water purification processes other than distillation or deionization, filtered water meeting the requirements for *Purified Water* but produced by means other than distillation or deionization could be equally suitable.

Filtered Water—This water is *Purified Water* that has been filtered to remove particles that could interfere with the analysis where the water is used. Where used for preparing samples for particulate matter testing (see *Particulate Matter in Injections* ⟨788⟩), though unspecified in monographs, water filtration should be through a 1.2-μm filter to be consistent with the general test chapter. Where used as a chromatography reagent, monograph-specified filter ratings range from 0.5 μm to unspecified.

High Purity Water—The preparation of this water is defined in *Containers—Glass* ⟨660⟩. It is water that is prepared by deionizing previously distilled water, and then filtering it through a 0.45-μm rated membrane. This water must have an in-line conductivity of not greater than 0.15 μS/cm (6.67 Megohm-cm) at 25°. For the sake of purity comparison, the analogous Stage 1 and 2 conductivity requirements for *Purified Water* at the same temperature are 1.3 μS/cm and 2.1 μS/cm, respectively. The preparation specified in *Containers—Glass* ⟨660⟩ uses materials that are highly efficient deionizers and that do not contribute copper ions or organics to the water, assuring a very high quality water. If the water of this purity contacts the atmosphere even briefly as it is being used or drawn from its purification system, its conductivity will immediately degrade, by as much as about 1.0 μS/cm, as atmospheric carbon dioxide dissolves in the water and equilibrates to bicarbonate ions. Therefore, if the analytical use requires that water purity remains as high as possible, its use should be protected from atmospheric exposure. This water is used as a reagent, as a solvent for reagent preparation, and for test apparatus cleaning where less pure waters would not perform acceptably. However, if a user's routinely available purified water is filtered and meets or exceeds the conductivity specifications of *High Purity Water,* it could be used in lieu of *High Purity Water.*

Ammonia-Free Water—Functionally, this water must have a negligible ammonia concentration to avoid interference in tests sensitive to ammonia. It has been equated with *High Purity Water* that has a significantly tighter Stage 1 conductivity specification than *Purified Water* because of the latter's allowance for a minimal level of ammonium among other ions. However, if the user's *Purified Water* were filtered and met or exceeded the conductivity specifications of *High Purity Water,* it would contain negligible ammonia or other ions and could be used in lieu of *High Purity Water.*

Carbon Dioxide-Free Water—The introductory portion of the *Reagents, Indicators, and Solutions* section defines this water as *Purified Water* that has been vigorously boiled for at least 5 minutes, then cooled and protected from absorption of atmospheric carbon dioxide. Because the absorption of carbon dioxide tends to drive down the water pH, most of the uses of *Carbon Dioxide-Free Water* are either associated as a solvent in pH-related or pH-sensitive determinations or as a solvent in carbonate-sensitive reagents or determinations. Another use of this water is for certain optical rotation and color and clarity of solution tests. Though it is possible that this water is indicated for these tests simply because of its purity, it is also possible that the pH effects of carbon dioxide containing water could interfere with the results of these tests. A third plausible reason that this water is indicated is that outgassing air bubbles might interfere with these photometric-type tests. The boiled water preparation approach will also greatly reduce the concentrations of many other dissolved gases along with carbon dioxide. Therefore, in some of the applications for *Carbon Dioxide-Free Water,* it could be the inadvertent deaeration effect that actually renders this water suitable. In addition to boiling, deionization is perhaps an even more efficient process for removing dissolved carbon dioxide (by drawing the dissolved gas equilibrium toward the ionized state with subsequent removal by the ion-exchange resins). If the starting *Purified Water* is prepared by an efficient deionization process and protected after deionization from exposure to atmospheric air, water that is carbon dioxide-free can be effectively made without the application of heat. However this deionization process does not deaerate the water, so if *Purified Water* prepared by deionization is considered as a substitute water in a test requiring *Carbon Dioxide-Free Water,* the user must verify that it is not actually water akin to *Deaerated Water* (discussed below) that is needed for the test. As indicated in *High Purity Water,* even brief contact with the atmosphere can allow small amounts of carbon dioxide to dissolve, ionize, and significantly degrade the conductivity and lower the pH. If the analytical use requires the water to remain as pH-neutral and as carbon dioxide-free as possible, even the analysis should be protected from atmospheric exposure. However, in most applications, atmospheric exposure during testing does not significantly affect its suitability in the test.

Ammonia- and Carbon Dioxide-Free Water—As implied by the name, this water should be prepared by approaches compatible with those mentioned for both *Ammonia-Free Water* and *Carbon Dioxide-Free Water.* Because the carbon dioxide-free attribute requires post-production protection from the atmosphere, it is appropriate to first render the water ammonia-free using the *High Purity Water* process followed by the boiling and carbon dioxide-protected cooling process. The *High Purity Water* deionization process for creating *Ammonia-Free Water* will also remove the ions generated from dissolved carbon dioxide and ultimately, by forced equilibration to the ionized state, all the dissolved carbon dioxide. Therefore, depending on its use, an acceptable procedure for making *Ammonia- and Carbon Dioxide-Free Water* could be to transfer and collect *High Purity Water* in a carbon dioxide intrusion-protected container.

Deaerated Water—This water is *Purified Water* that has been treated to reduce the content of dissolved air by ''suitable means''. In the *Reagents* section, approaches for boiling, cooling (similar to *Carbon Dioxide-Free Water* but without the atmospheric carbon dioxide protection), and sonication are given as applicable for test uses other than dissolution and drug release testing. Though *Deaerated Water* is not mentioned by name in *Dissolution* ⟨711⟩, suggested methods for deaerating dissolution media (which may be water) include warming to 41°, vacuum filtering through a 0.45-μm rated membrane, and vigorously stirring the filtrate while maintaining the vacuum. This chapter specifically indicates that other validated approaches may be used. In other monographs that also do not mention *Deaerated Water* by name, degassing of water and other reagents is accomplished by sparging with helium. *Deaerated Water* is used in both dissolution testing as well as liquid chromatography applications where outgassing could either interfere with the analysis itself or cause erroneous results due to inaccurate volumetric withdrawals. Applications where ambient temperature water is used for reagent preparation, but the tests are performed at elevated temperatures, are candidates for outgassing effects. If outgassing could interfere with test performance, including chromatographic flow, colorimetric or photometric measurements, or volumetric accuracy, then *Deaerated Water* should probably be used, whether called for in the analysis or not. The above deaeration approaches might not render the water ''gas-free''. At best, they reduce the dissolved gas concentrations so that outgassing caused by temperature changes is not likely.

Recently Boiled Water—This water may include recently or freshly boiled water (with or without mention of cooling in the title), but cooling prior to use is clearly intended. Occasionally it is necessary to use when hot. *Recently Boiled Water* is specified because it is used in a pH-related test or carbonate-sensitive reagent, in an oxygen-sensitive test or reagent, or in a test where outgassing could interfere with the analysis, such as specific gravity or an appearance test.

Oxygen-Free Water—The preparation of this water is not specifically described in the compendia. Neither is there an oxygen specification or analysis mentioned. However, all uses involve analyses of materials that could be sensitive to oxidation by atmospheric oxygen. Procedures for the removal of dissolved oxygen from solvents, though not necessarily water, are mentioned in *Polarography* ⟨801⟩ and *Spectrophotometry and Light-Scattering* ⟨851⟩. These procedures involve simple sparging of the liquid with an inert gas such as nitrogen or helium followed by inert gas blanketing to prevent oxygen reabsorption. The sparging times cited range from 5 to 15 minutes to an unspecified period. Some *Purified Water* and *Water for Injection* systems produce water that is maintained in a hot state and that is inert gas blanketed during its preparation and storage and distribution. Though oxygen is poorly soluble in hot water, such water may not be oxygen-free. Whatever procedure used for removing oxygen should be verified as reliably producing water that is fit for use.

LAL Reagent Water—This water is also referred to as endotoxin-free water. This is usually *Water for Injection,* which may have been sterilized. It is free from a level of endotoxin that would yield any detectable reaction or interference with the Limulus amebocyte lysate reagent used in the *Bacterial Endotoxins Test* ⟨85⟩.

Organic-Free Water—This water is defined by *Residual Solvents* ⟨467⟩ as producing no significantly interfering gas chromatography peaks. Referenced monographs specify using this water as the solvent for the preparation of standard and test solutions for the *Residual solvents* test.

Lead-Free Water—This water is used as a transferring diluent for an analyte in a *Lead* ⟨251⟩ test. Though no specific instructions are given for its preparation, it must not contain any detectable lead. *Purified Water* should be a suitable substitute for this water.

Chloride-Free Water—This water is specified as the solvent for use in an assay that contains a reactant that precipitates in the presence of chloride. Though no specific preparation instructions are given for this water, its rather obvious attribute is having a very low chloride level in order to be unreactive with this chloride sensitive reactant. *Purified Water* could be used for this water but should be tested to ensure that it is unreactive.

Hot Water—The uses of this water include solvents for achieving or enhancing reagent solubilization, restoring the original volume of boiled or hot solutions, rinsing insoluble analytes free of hot water soluble impurities, solvents for reagent recrystallization, apparatus cleaning, and as a solubility attribute for various *USP–NF* articles. In only one monograph is the temperature of ''hot'' water specified; so in all the other cases, the water temperature is less important, but should be high enough to achieve the desirable effect. In all cases, the chemical quality of the water is implied to be that of *Purified Water.*

Change to read:

CHEMICAL CONSIDERATIONS

The chemical attributes of *Purified Water* and *Water for Injection*

■in effect prior to *USP 23*■*2S (USP33)*
were specified by a series of chemistry tests for various specific and nonspecific attributes with the intent of detecting chemical species indicative of incomplete or inadequate purification. While these methods could have been considered barely adequate to control the quality of these waters, they nevertheless stood the test of time. This was partly because the operation of water systems was, and still is, based on on-line conductivity measurements and specifications generally thought to preclude the failure of these archaic chemistry attribute tests.

USP moved away from these chemical attribute tests to contemporary analytical technologies for the bulk waters *Purified Water* and *Water for Injection*. The intent was to upgrade the analytical technologies without tightening the quality requirements. The two contemporary analytical technologies employed were TOC and conductivity. The TOC test replaced the test for *Oxidizable substances* that primarily targeted organic contaminants. A multistaged *Conductivity* test which detects ionic (mostly inorganic) contaminants replaced, with the exception of the test for *Heavy metals,* all of the inorganic chemical tests (i.e., *Ammonia, Calcium, Carbon dioxide, Chloride, Sulfate*).

Replacing the heavy metals attribute was considered unnecessary because (a) the source water specifications (found in the NPDWR) for individual *Heavy metals* were tighter than the approximate limit of detection of the *Heavy metals* test for *USP XXII Water for Injection* and *Purified Water* (approximately 0.1 ppm), (b) contemporary water system construction materials do not leach heavy metal contaminants, and (c) test results for this attribute have uniformly been negative—there has not been a confirmed occurrence of a singular test failure (failure of only the *Heavy metals* test with all other attributes passing) since the current heavy metal drinking water standards have been in place. Nevertheless, since the presence of heavy metals

in *Purified Water* or *Water for Injection* could have dire consequences, its absence should at least be documented during new water system commissioning and validation or through prior test results records.

Total solids and *pH* ~~are~~

■were ■2S *(USP33)*
the only tests not covered by conductivity testing. The test for *Total solids* was considered redundant because the nonselective tests of conductivity and TOC could detect most chemical species other than silica, which could remain undetected in its colloidal form. Colloidal silica in *Purified Water* and *Water for Injection* is easily removed by most water pretreatment steps and even if present in the water, constitutes no medical or functional hazard except under extreme and rare situations. In such extreme situations, other attribute extremes are also likely to be detected. It is, however, the user's responsibility to ensure fitness for use. If silica is a significant component in the source water, and the purification unit operations could be operated or fail and selectively allow silica to be released into the finished water (in the absence of co-contaminants detectable by conductivity), then either silica-specific or a total solids type testing should be utilized to monitor and control this rare problem.

The *pH* attribute was eventually recognized to be redundant to the conductivity test (which included *pH* as an aspect of the test and specification); therefore, *pH* was dropped as a separate attribute test.

The rationale used by USP to establish its ~~conductivity specification~~

■*Purified Water* and *Water for Injection* conductivity specifications ■2S *(USP33)*
took into consideration the conductivity contributed by the two least conductive former attributes of *Chloride* and *Ammonia,* thereby precluding their failure had those wet chemistry tests been performed. In essence, the *Stage 3* conductivity specifications (see *Water Conductivity,*
■*Bulk Water* ■2S *(USP33)*
⟨645⟩) were established from the sum of the conductivities of the limit concentrations of chloride ions (from pH 5.0 to 6.2) and ammonia ions (from pH 6.3 to 7.0), plus the unavoidable contribution of other conductivity-contributing ions from water (H^+ and OH^-), dissolved atmospheric CO_2 (as HCO_3^-), and an electro-balancing quantity of either Na^+ ~~of~~

■or ■2S *(USP33)*
Cl^-, depending on the pH-induced ionic imbalance (see *Table 1*). The *Stage 2* conductivity specification is the lowest value on this table, 2.1 μS/cm. The *Stage 1* specifications, designed primarily for on-line measurements, were derived essentially by summing the lowest values in the contributing ion columns for each of a series of tables similar to *Table 1,* created for each 5° increment between 0° and 100°. For example purposes, the italicized values in *Table 1,* the conductivity data table for 25°, were summed to yield a conservative value of 1.3 μS/cm, the *Stage 1* specification for a nontemperature compensated, nonatmosphere equilibrated water sample that actually had a measured temperature of 25° to 29°. Each 5° increment in the table was similarly treated to yield the individual values listed in the table of *Stage 1* specifications (see *Water Conductivity,*
■*Bulk Water* ■2S *(USP33)*
⟨645⟩).

Table 1. Contributing Ion Conductivities of the Chloride–Ammonia Model as a Function of pH
(in atmosphere-equilibrated water at 25°)

	Conductivity (μS/cm)							
pH	H^+	OH^-	HCO_3^-	Cl^-	Na^+	NH_4^+	Combined Conductivities	Stage 3 Limit
5.0	3.49	*0*	*0.02*	*1.01*	*0.19*	*0*	4.71	4.7
5.1	2.77	0	0.02	1.01	0.29	0	4.09	4.1
5.2	2.20	0	0.03	1.01	0.38	0	3.62	3.6
5.3	1.75	0	0.04	1.01	0.46	0	3.26	3.3
5.4	1.39	0	0.05	1.01	0.52	0	2.97	3.0
5.5	1.10	0	0.06	1.01	0.58	0	2.75	2.8
5.6	0.88	0	0.08	1.01	0.63	0	2.60	2.6
5.7	0.70	0	0.10	1.01	0.68	0	2.49	2.5
5.8	0.55	0	0.12	1.01	0.73	0	2.41	2.4
5.9	0.44	0	0.16	1.01	0.78	0	2.39	2.4
6.0	0.35	0	0.20	1.01	0.84	0	2.40	2.4
6.1	0.28	0	0.25	1.01	0.90	0	2.44	2.4
6.2	0.22	0	0.31	1.01	0.99	0	2.53	2.5
6.3	0.18	0	0.39	0.63	0	1.22	2.42	2.4
6.4	0.14	0.01	0.49	0.45	0	1.22	2.31	2.3
6.5	0.11	0.01	0.62	0.22	0	1.22	2.18	2.2
6.6	0.09	0.01	0.78	0	0.04	1.22	2.14	2.1
6.7	0.07	0.01	0.99	0	0.27	1.22	2.56	2.6
6.8	0.06	0.01	1.24	0	0.56	1.22	3.09	3.1
6.9	0.04	0.02	1.56	0	0.93	1.22	3.77	3.8
7.0	*0.03*	0.02	1.97	0	1.39	1.22	4.63	4.6

In-Process Revision

As stated above, this rather radical change to utilizing a conductivity attribute as well as the inclusion of a TOC attribute allowed for on-line measurements. This was a major philosophical change and allowed major savings to be realized by industry. The TOC and conductivity tests can also be performed ''off-line'' in the laboratories using collected samples, though sample collection tends to introduce opportunities for adventitious contamination that can cause false high readings. The collection of on-line data is not, however, without challenges. The continuous readings tend to create voluminous amounts of data where before only a single data point was available. As stated under *Sampling Considerations,* continuous in-process data is excellent for understanding how a water system performs during all of its various usage and maintenance events in real time, but is too much data for QC purposes. Therefore, a justifiable fraction or averaging of the data can be used that is still representative of the overall water quality being used.

~~Packaged~~

■Packaged/sterile■2S (*USP33*)
waters present a particular dilemma relative to the attributes of conductivity and TOC. The package itself is the source of chemicals (inorganics and organics) that leach over time into the water and can easily be detected. The irony of organic leaching from plastic packaging is that when the *Oxidizable substances* test was the only ''organic contaminant'' test for both bulk and ~~packaged~~

■packaged/sterile■2S (*USP33*)
waters, that test's insensitivity to those organic leachables ~~rendered~~

■allowed■2S (*USP33*)
their presence in ~~packaged water at high concentrations (many times the TOC specification for bulk water) virtually undetectable~~

■packaged/sterile water to be quite high (possibly many times the TOC specification for bulk water).■2S (*USP33*)
Similarly, glass containers can also leach inorganics, such as sodium, which are easily detected by conductivity, but ~~are undetected by the wet chemistry tests for water (other than *pH* or *Total solids*)~~

■poorly detected by the former wet chemistry attribute tests.■2S (*USP33*)
Most of these leachables are considered harmless by current perceptions and standards at the rather significant concentrations present. Nevertheless, they effectively degrade the quality of the high-purity waters placed into these packaging ~~system~~

■systems.■2S (*USP33*)
Some packaging materials contain more leachables than others and may not be as suitable for holding water and maintaining its purity.

The attributes of conductivity and TOC tend to reveal more about the packaging leachables than they do about the water's original purity. These

■currently■2S (*USP33*)
''allowed'' leachables could render the ~~packaged~~

■packaged/sterile ■2S (*USP33*)
versions of originally equivalent bulk water essentially unsuitable for many uses where the bulk waters are perfectly adequate.

■Therefore, to better control the ionic packaging leachables, *Water Conductivity* ⟨645⟩ is divided into two sections. The first is titled *Bulk Water*, which applies to *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and *Pure Steam* and includes the three-stage conductivity testing instructions and specifications. The second is titled *Sterile Water*, which applies to *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*. The *Sterile Water* section includes conductivity specifications similar to the *Stage 2* testing approach because it is intended as a laboratory test, and these sterile waters were made from bulk water that already complied with the three-stage conductivity test. In essence, packaging leachables are the primary target ''analytes'' of the conductivity specifications in the *Sterile Water* section of *Water Conductivity* ⟨645⟩. The effect on potential leachables from different container sizes is the rationale for having two different specifications, one for small packages containing nominal volumes of 10 mL or less and another for larger packages. These conductivity specifications are harmonized with the *European Pharmacopoeia* conductivity specifications for *Sterile Water for Injection*. All monographed waters, except *Bacteriostatic Water for Injection*, have a conductivity specification that directs the user to either the *Bulk Water* or the *Sterile Water* section of *Water Conductivity* ⟨645⟩. For the sterile water monographs, this water conductivity specification replaces the redundant wet chemistry limit tests intended for inorganic contaminants that had previously been specified in these monographs.■2S (*USP33*)

BRIEFING

⟨2750⟩ Manufacturing Practices for Dietary Supplements, *USP 32* page 786. FDA published the final rule of *Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements* (cGMP) in June 2007, immediately after the submission to *Pharmacopeial Forum* of the previous revision of this chapter, which had incorporated the *Dietary Supplement and Nonprescription Drug Consumer Protection Act* (AER law). (For the previous revision, see page 1035 of *PF* 33(5) [Sept.–Oct. 2007].) The new cGMP regulations came into force on June 25, 2008, for com-

panies with 500 or more employees, and on June 25, 2009, for companies with 20 to 499 employees. By June 25, 2010, all companies must comply with the new rule.

Even though this chapter has provided stringent quality requirements since its first publication in 1993, updates are necessary in order to clarify its equivalencies to and stringencies against new regulations that provide more general instructions. For instance, the cGMP regulations include specific requirements relating to recordkeeping and documentation; plumbing design, installation, and maintenance; design, suitability, and maintenance of utensils and of automatic, mechanical, or electronic equipment; and control of packaging and labeling operations, including repackaging and relabeling operations.

Therefore, in order to address those differences, this proposed revision is much more explanatory and detailed in its guidance than the current chapter. These additional requirements in the revised chapter will help operators more easily ensure their product quality.

(DS-GC: Y. Tokiwa) RTS—C75199

Delete the following:

~~■As is indicated in the *General Notices* under *Significant Figures and Tolerances*, tolerances are based upon the consideration that the article is produced under recognized principles of good manufacturing practice. Many of the principles in this general information chapter are basic in that they apply equally to various types of products and levels of technology, and that they are derived from the current good manufacturing practices for drugs. However, the practical application of these principles to dietary supplements may be different.~~

~~The principles set forth in this chapter contain recommended minimum current good manufacturing practices for the methods to be used in, and the facilities and controls to be used for, the manufacture of a dietary supplement to assure that such a product meets the requirements of safety, and has the identity and strength and meets the quality and purity characteristics that it is represented to possess.~~

~~Establishments engaged solely in the harvesting, storage, or distribution of one or more ''raw agricultural commodities'' as defined in section 201(r)) of the Dietary Supplements Health and Education Act of 1994, which are ordinarily cleaned, prepared, treated, or otherwise processed before being marketed to the consuming public are excluded from this chapter.~~

~~A glossary of terms used in this general chapter is presented at the end.~~■2S (*USP33*)

Add the following:

■GENERAL PROVISIONS

The principles included in this chapter contain recommended minimum current good manufacturing practices for the methods to be used in, and the facilities and controls to be used for, the manufacture, holding, packaging, labeling, and distribution of dietary ingredients and dietary supplements. These principles are set forth to ensure that such products meet the requirements of safety, have the identity and strength, and meet the quality and purity characteristics that they are represented to possess.

Excluded from this chapter are establishments engaged solely in the harvesting, storage, or distribution of one or more ''raw agricultural commodities'' as defined in Section 201(r) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321(r)), which are ordinarily cleaned, prepared, treated, or otherwise processed before being marketed to the consuming public.

The requirements pertaining to holding dietary ingredients and dietary supplements do not apply to holding those dietary supplements at a retail establishment for the sole purpose of direct retail sale to individual consumers. A retail establishment does not include a warehouse or other storage facility for a retailer or a warehouse or other storage facility that sells directly to individual consumers.

A glossary of terms used in this chapter is presented at the end.■2S (*USP33*)

Change to read:

ORGANIZATION AND PERSONNEL

Responsibilities of a Quality Control Unit

A quality control unit shall be established that has the responsibility and authority to approve or reject all raw materials, product containers, closures, in-process materials, packaging material, labeling, and finished dietary supplements, and the authority to review production records to ensure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality control unit should be responsible for approving or rejecting products manufactured, processed, packed, or held under contract by another company.

Adequate laboratory facilities for the testing and approval (or rejection) of raw materials, product containers, closures, packaging materials, in-process materials,

■dietary ingredients,■2S (*USP33*)
and dietary supplements should be available to the quality control unit.

The quality control unit should have the responsibility for approving or rejecting all procedures or specifications that impact on the identity, strength, quality, and purity of the dietary supplement. All responsibilities and procedures applicable to the quality control unit shall be in writing.

■The designated person within the Quality Control Unit who conducts a material review and makes the disposition decision must, at the time of performance, document that material review and disposition decision.■2S (*USP33*)

Personnel Qualifications

Each person engaged in the manufacture of ~~a dietary supplement~~

■dietary ingredients and dietary supplements■2S (*USP33*) should have the proper education, training, and experience (or any combination thereof) needed to perform the assigned functions. Training should be in the particular operation(s) that the employee performs as they relate to the employee's functions.

Appropriate documentation of training shall be retained by the company.

Each person responsible for supervising the manufacture of ~~a dietary product~~

■a dietary ingredient, a dietary supplement, or both■2S (*USP33*) should have the proper education, training, and experience (or any combination thereof) to perform assigned functions in such a manner as to provide assurance that the product has the safety, identity, strength, quality, and purity that it is represented to possess.

An adequate number of qualified personnel to perform and supervise the manufacture of each ~~dietary product~~

■dietary ingredient, dietary supplement, or both■2S (*USP33*) product should be provided.

Personnel Responsibilities

The company management shall take all reasonable measures and precautions to ensure the following:

1. *Disease control.* Any person who, by medical examination or supervisory observation, is shown to have, or appears to have, an illness, open lesion, including boils, sores, or infected wounds, or any other abnormal source of microbial contamination by which there is a reasonable possibility of an in-process or finished ~~dietary product~~

   ■dietary ingredient or dietary supplement■2S (*USP33*) becoming adulterated, or processing equipment, utensils, or packaging materials becoming contaminated, shall be excluded from any operations which may be expected to result in such adulteration or contamination until the condition is corrected. Personnel shall be instructed to report such health conditions to their supervisors.
2. *Cleanliness.* All persons working in direct contact with raw materials, in-process or finished ~~dietary products~~

   ■dietary ingredients and dietary supplements,■2S (*USP33*) processing equipment, utensils, or packaging materials shall conform to hygienic practices while on duty to the extent necessary to protect against adulteration or contamination of such materials. The methods for maintaining cleanliness include, but are not limited to, the following:

   — Wearing outer garments suitable to the operation in a manner that protects against the adulteration of raw materials or of in-process or finished ~~dietary products~~

   ■dietary ingredients and dietary supplements,■2S (*USP33*) or contamination of processing equipment, utensils, or packaging materials;
   — Maintaining adequate personal cleanliness;
   — Removing cosmetics from parts of the body that may contact raw materials, in-process or finished ~~dietary products~~

   ■dietary ingredients and dietary supplements,■2S (*USP33*) equipment, utensils, or containers;
   — Washing hands thoroughly (and sanitizing if necessary to protect against contamination with undesirable microorganisms) in an adequate hand-washing facility before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated;
   — Removing all unsecured jewelry and other objects that might fall into raw materials, in-process or finished ~~dietary products~~

   ■dietary ingredients and dietary supplements,■2S (*USP33*) equipment, or containers, and removing hand jewelry that cannot be adequately sanitized during periods in which in-process or finished product is manipulated by hand. If such hand jewelry and cosmetics cannot be removed, they may be covered by material that can be maintained in an intact, clean, and sanitary condition and that effectively protects against the adulteration of ~~dietary product~~

   ■dietary ingredients and dietary supplements■2S (*USP33*) or contamination of processing equipment, utensils or packaging materials;
   — Maintaining gloves, if they are used in raw materials or in in-process or finished product handling, in an intact, clean, and sanitary condition. The gloves should be of a material that adequately protects the product from contamination;
   — Wearing, where appropriate, in an effective manner, hair nets, caps, beard covers, or other effective hair restraints;
   — Storing clothing or other personal belongings in areas other than where in-process or finished product is exposed or where processing equipment or utensils are washed;
   — Confining the following actions to areas other than where in-process or finished product may be stored or exposed, or where processing equipment or utensils are washed: eating food, chewing gum, drinking beverages, or using tobacco; and
   — Taking any other necessary precautions to protect against adulteration of raw materials or of in-process or finished product, or contamination of processing equipment, utensils, or packaging materials with microorganisms or foreign substances, including, but not limited to, perspiration, hair, cosmetics, tobacco, chemicals, and medicines applied to the skin.

Change to read:

GROUNDS, BUILDINGS, AND FACILITIES

Grounds

The grounds about a ~~dietary product~~

■dietary ingredient■2S (*USP33*) manufacturing plant

■and a dietary supplement manufacturing plant■2S (*USP33*) under the control of the operator shall be kept in a condition that will protect against the adulteration of ~~dietary products~~

■dietary ingredients and dietary supplements.■2S (*USP33*) The methods for adequate maintenance of grounds include, but are not limited to, the following:

— Properly storing equipment, removing litter and waste, and cutting weeds or grass within the immediate vicinity of the plant building or structures that may constitute an attractant, breeding place, or harborage for pests;
— Maintaining roads, yards, and parking lots so that they do not constitute a source of adulteration in areas where product is exposed;
— Adequately draining areas that may contribute to product adulteration by seepage, foot-borne filth, or providing a breeding place for pests; and
— Operating systems for waste treatment and disposal in an adequate manner so that they do not constitute a source of adulteration in areas where product is exposed. If the plant grounds are bordered by grounds not under the operator's control and not maintained in the manner described above, care shall be exercised in the plant by inspection, extermination, or other means to exclude pests, dirt, and filth that may be a source of product adulteration.

Building Design

Any building or buildings used in the manufacture of ~~a dietary product~~

■a dietary ingredient, a dietary supplement, or both■2S (*USP33*) should be of suitable size and shall be constructed in such a manner that floors, walls, and ceilings may be adequately cleaned and kept clean and in good repair; that drips or condensates from fixtures, ducts, and pipes do not adulterate raw materials or in-process or finished ~~dietary product~~

■dietary ingredients and dietary supplements,■2S (*USP33*) or contaminate product containers, utensils, or packaging materials; and that aisles or working spaces are provided between equipment and walls and are adequately unobstructed and of adequate width to permit employees to perform their duties and to protect against adulterating in-process or finished product, or contaminating processing equipment with clothing or personal contact.

■Adequate screening or other protection against pests and insects should be installed, where necessary.■2S (*USP33*) The building should have adequate space for the orderly placement of equipment and materials to prevent mixups between different raw materials, product containers, closures, labeling, in-process materials, or finished products, and to prevent contamination. The flow of raw materials, product containers, closures, labeling, in-process materials, and products through the building or buildings should be designed to prevent contamination.

Operations should be performed within specifically defined areas of adequate size to prevent contamination or mixups or adulteration of in-process or finished ~~dietary product~~

■dietary ingredients and dietary supplements,■2S (*USP33*) or contamination of processing equipment, utensils, or packaging materials with microorganisms, chemicals, filth, or other extraneous materials. The potential for mixups and product adulteration may be reduced by adequate product safety controls and operating practices or effective design, including the separation of operations in which contamination is likely to occur, by one or more of the following means: location, time, partition, airflow, enclosed systems, or other effective means. There should be separate or defined areas as follows:

1. An area for the receipt, identification, storage, and withholding from use of components, product containers, closures, and labeling, pending the appropriate sampling, testing, or examination by the quality control unit before release for manufacturing or packaging;
2. An area for the storage of released components, product containers, closures, and labeling;
3. An area for storage of in-process materials;
4. An area for manufacturing and processing operations;
5. An area for packaging and labeling operations; and
6. An area for control and laboratory operations.

Any building used in the manufacture of ~~a dietary product~~

■a dietary ingredient or a dietary supplement■2S (*USP33*) shall permit the taking of proper precautions to protect dietary ingredients or dietary supplements in outdoor bulk fermentation vessels by any effective means, including the following:

(i) Using protective coverings,
(ii) Controlling areas over and around the vessels to eliminate harborages for pests,
(iii) Checking on a regular basis for pests and pest infestation, and
(iv) Skimming the fermentation vessels, as necessary.

Lighting

Adequate lighting shall be provided in all areas and should not expose bulk or finished product to adulteration or contamination. Adequate lighting should be provided in hand-washing areas, dressing and locker rooms, and toilet rooms, and in all areas where product is examined, processed, or stored and where equipment or utensils are cleaned; and such lighting should provide safety-type light bulbs, fixtures, skylights, or other glass suspended over exposed product in any step of preparation or otherwise protect against product adulteration in case of glass breakage.

Ventilation, Air Filtration, Air Heating, and Cooling

Adequate ventilation shall be provided, as well as equipment for adequate control over microorganisms, dust, humidity, and temperature when used in the manufacture of ~~a dietary product~~

■a dietary ingredient and a dietary supplement■2S (*USP33*) to minimize odors and vapors (including steam and noxious fumes) in areas where they may adulterate ~~dietary products~~

■dietary ingredients and dietary supplements;■2S (*USP33*) and to locate and operate fans and other air-blowing equipment in a manner that minimizes the potential for adulterating raw materials, in-process or finished ~~dietary products~~

■dietary ingredients and dietary supplements,■2S (*USP33*) or contaminating processing equipment, utensils, or packaging materials.

Plumbing

■The plumbing in the physical plant must be of an adequate size and design and be adequately installed and maintained to:

— Carry sufficient amounts of water to the required locations throughout the physical plant;

— Properly convey sewage and liquid disposable waste from the physical plant; and

— Avoid being a source of contamination to components, raw materials, dietary ingredients, dietary supplements, water supplies, or any contact surface, or creating an unsanitary condition.■2S (*USP33*)

Potable water at a suitable temperature, and under pressure as needed, should be supplied in a plumbing system free of defects that could contribute contamination to any ~~dietary product~~

■dietary ingredients and dietary supplements.■2S (*USP33*)
Potable water should meet the standards prescribed in the Environmental Protection Agency's Primary Drinking Water Regulations (40 CFR Part 141) or any state or local drinking water requirements that are more stringent. Water not meeting such standards should not be permitted in the potable water system for *Purified Water.* If potable water is to be used as a raw material, it should be further purified to satisfy compendial requirements.

Drains should be of adequate size and, where connected directly to a sewer, should have an air break or other mechanical device to prevent back-siphonage.

Sewage and Refuse

Sewage, trash, and other refuse in and from the building and immediate premises shall be disposed of in a safe and sanitary manner.

Washing and Toilet Facilities

Adequate washing facilities shall be provided, including hot and cold water, soap or detergent, air driers or single-service towels, and clean toilet facilities easily accessible to working areas.

General Maintenance and Sanitation

Any building used in the manufacture of ~~a dietary product~~

■a dietary ingredient, a dietary supplement, or both■2S (*USP33*)
should be maintained in a clean and sanitary condition and shall be kept in repair sufficient to prevent raw materials and in-process or finished ~~dietary products~~

■dietary ingredients and dietary supplements■2S (*USP33*)
from becoming adulterated. It shall be free of infestation by rodents, birds, insects, and other vermin. Trash and organic waste matter shall be held and disposed of in a timely and sanitary manner.

Cleaning compounds and sanitizing agents used in cleaning and sanitizing procedures shall be free from undesirable microorganisms and shall be safe and adequate under the conditions of use. Compliance with this requirement may be verified by any effective means, including purchase of these substances under a supplier's guarantee or certification, or examination of these substances for contamination. Only the following toxic materials may be used or stored in a plant where product is processed or exposed:

1. Those required to maintain clean and sanitary conditions;
2. Those necessary for use in laboratory testing procedures;
3. Those necessary for plant and equipment maintenance and operation; and
4. Those necessary for use in the plant's operations.

Written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the building and facilities shall be required.

Toxic cleaning compounds, sanitizing agents, and pesticide chemicals shall be identified, used, held, and stored in a manner that protects against adulteration of raw materials or of in-process or finished product, or contamination of processing equipment or packaging materials. All relevant regulations promulgated by other federal, state, and local government agencies for the application, use or holding of these products should be followed.

No pests shall be allowed in any area of ~~a dietary product~~

■a dietary ingredient■2S (*USP33*)
manufacturing plant

■and a dietary supplement manufacturing plant.■2S (*USP33*)
Effective measures shall be taken to exclude pests from the processing areas and to protect against the adulteration by pests of product on the premises. The use of insecticides or rodenticides is permitted only under precautions and restrictions that will protect against the adulteration of raw materials, in-process or finished product, or contamination of processing equipment, utensils, or packaging materials.

Written procedures are also required for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. These procedures should be designed to prevent the contamination of equipment, raw materials, product containers, closures, packaging, labeling materials, or products. Rodenticides, insecticides, and fungicides should be registered and used in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act.

Sanitation procedures shall apply to work performed by contractors or temporary employees as well as work performed by full-time employees during the ordinary course of operations.

Change to read:

EQUIPMENT

■AND UTENSILS■2S (*USP33*)

Equipment

■and utensils■2S (*USP33*)
used in the manufacture of ~~a dietary supplement~~

■dietary ingredients and dietary supplements■2S (*USP33*)
shall be of appropriate design

■or selection,■2S (*USP33*)
adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance

■and to ensure that the specifications of dietary ingredients and dietary supplements are correct and are met.

Equipment and utensils include, but are not limited to, the following:

— Equipment used to hold or convey;
— Equipment used to measure;
— Equipment using compressed air or gas;
— Equipment used to carry out processes in closed pipes and vessels; and
— Equipment used in automatic, mechanical, or electronic systems.■2S (*USP33*)

Construction

All equipment

■and utensils■2S (*USP33*)
shall be: ~~constructed so that surfaces that contact raw materials, in-process materials, or finished products are not reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the product beyond the established requirements.~~

■— Constructed so that surfaces that contact raw materials, in-process materials, or finished products are not reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the product beyond the established requirements;
— Made of nontoxic materials;
— Designed and constructed to withstand the environment in which they are used; the action of raw materials, in-process materials, dietary ingredients, or dietary supplements; and, if applicable, cleaning compounds and sanitizing agents; and
— Maintained to protect raw materials, in-process materials, dietary ingredients, and dietary supplements from being contaminated by any source.

Equipment and utensils must have seams that are smoothly bonded or maintained to minimize the accumulation of dirt, filth, organic material, particles of raw materials, in-process materials, dietary ingredients, or dietary supplements, or any other extraneous materials or contaminants, in order to minimize the opportunity for growth of microorganisms.

Each freezer, refrigerator, and cold storage compartment used to hold raw materials, in-process materials, dietary ingredients, or dietary supplements

— Must be fitted with an indicating thermometer, temperature-measuring device, or temperature-recording device that shows, indicates, and records, or allows for recording by hand, the temperature accurately within the compartment; and
— Must have an automated device for regulating temperature or an automated alarm system to indicate a significant temperature change in a manual operation.■2S (*USP33*)

The design, construction, and use of equipment and utensils shall preclude the adulteration of raw materials, packaging materials, in-process materials, or finished product with any substances required for operation, such as: ~~lubricants, fuel, or any other contaminants such as metal fragments, contaminated water, etc.~~

■— Lubricants,
— Fuel,
— Coolants,
— Metal or glass fragments,
— Filth or any other extraneous material,
— Contaminated water, or
— Any other contaminants.

Instruments or controls used in the manufacturing, packaging, labeling, or holding of a dietary ingredient, a dietary supplement, or both; and instruments or controls that are used to measure, regulate, or record temperatures, hydrogen-ion concentration (pH), water activity, or other conditions, and to control or prevent the growth of microorganisms or other contamination must be:

— Accurate and precise,
— Adequately maintained, and
— Adequate in number for their designated uses.

For any automated, mechanical, or electronic equipment that is used to manufacture, package, label, or hold a dietary ingredient, a dietary supplement, or both:

— The suitability of the equipment must be determined by ensuring that the equipment is capable of operating satisfactorily within the operating limits required by the process;
— The equipment must be routinely calibrated, inspected, or checked to ensure proper performance. The quality control unit must approve these calibrations, inspections, or checks;
— The appropriate controls for automated, mechanical, and electronic equipment (including software for a computer-controlled process) must be established and used to ensure that any changes to the manufacturing, packaging, labeling, holding, or other operations are approved by the quality control unit and instituted only by authorized personnel; and
— The appropriate controls must be established and used to ensure that the equipment functions in accordance with its intended use. These controls must be approved by the quality control unit.

Compressed air or other gases introduced mechanically into or onto raw materials, in-process materials, dietary ingredients, dietary supplements, or contact surfaces, or that are used to clean any contact surface, must be treated in such a way that the raw material, in-process material, dietary ingredient, dietary supplement, or contact surface is not contaminated.■2S (*USP33*)

Cleaning and Maintenance

Equipment and utensils shall be cleaned, maintained, and sanitized at adequate intervals, between the manufacture of different batches of the same product and between the manufacture of different products, to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the product beyond the established requirements.

■In wet processing during manufacturing, all contact surfaces must be cleaned and sanitized, as necessary, to protect against the introduction of microorganisms into components, dietary ingredients, or dietary supplements. When cleaning and sanitizing is necessary, all contact surfaces must be cleaned and sanitized before use and after any interruption during which the contact surface may have become contaminated.

In a continuous production operation or in back-to-back consecutive operations, which involve different batches of the same dietary ingredient or dietary supplement, the contact surfaces must be adequately cleaned and sanitized.

The surfaces that do not come into direct contact with raw materials, in-process materials, dietary ingredients, or dietary supplements must be cleaned as frequently as necessary to protect against contaminating raw materials, in-process materials, dietary ingredients, and dietary supplements.

Single-service articles (such as utensils intended for one-time use, paper cups, and paper towels) must be:
— Stored in appropriate containers; and
— Handled, dispensed, used, and disposed of in a manner that protects against contamination of raw materials, in-process materials, dietary ingredients, dietary supplements, or any contact surface.

Cleaning compounds and sanitizing agents must be adequate for their intended use and safe under their conditions of use.

The portable equipment and utensils that have contact surfaces must be cleaned, sanitized, and then stored in a location and manner that protects them from contamination.■2S (*USP33*)

Written procedures for cleaning and maintaining equipment, including utensils, used in the manufacture of a product should be established and followed. These procedures should include, but are not necessarily limited to, the following:
— Assignment of responsibility for cleaning and maintaining equipment;
— Maintenance and cleaning schedules, including, where adequate, sanitizing schedules;
— A description in sufficient detail of the methods, equipment, and materials used in cleaning and maintenance operations, and the methods of disassembling and reassembling equipment, as necessary, to ensure proper cleaning and maintenance;
— Removal or obliteration of previous batch identification;
— Identification and protection of clean equipment from contamination before use;
— Inspection of equipment for cleanliness immediately before use;
— Regular calibration and inspection of equipment, or checking machines, to ensure proper performance and function

■must be conducted:

(a) Before first use; and

(b) At frequency specified in writing by supporting references.

— Instruments or controls that cannot be adjusted to agree with the reference standard must be repaired or replaced.■2S (*USP33*)

A written record of calibration, inspection, maintenance of equipment, and major equipment cleaning and use shall be maintained in individual equipment logs that show the date, product, and lot number of each batch processed. The persons performing the cleaning shall record in the log that the work was performed. Entries in the log should be in chronological order.

■The following is specified in order to keep records related to automated, or electric equipment:

— There must be backup file(s) of current software programs (and of outdated software that is necessary to retrieve records that are required to be retained, in accordance with the section *Records and Reports* in this chapter, when current software is not able to retrieve such records) and of data entered into computer systems used to manufacture, package, label, or hold dietary supplements.

(a) A backup file (e.g., a hard copy of data entered, diskettes, tapes, microfilm, or compact disks) must be an exact and complete record of the data entered.

(b) Backup software programs and data must be kept secure from alterations, inadvertent erasures, or loss.■2S (*USP33*)

Change to read:

RAW MATERIALS, PRODUCT CONTAINERS, AND CLOSURES

Written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of raw materials, product containers, and closures should be provided.

Raw materials, product containers, and closures at all times should be handled and stored in a manner to prevent contamination.

Raw agricultural materials that contain soil or other contaminants shall be washed or cleaned as necessary. Water used for washing, rinsing, or conveying raw agricultural materials shall be safe and of adequate sanitary quality. Notwithstanding the general requirement for potable water, water may be reused for washing, rinsing, or conveying raw agricultural materials if it does not increase the level of contamination of such materials.

Bagged or boxed raw materials of product containers or closures should be stored off the floor and suitably spaced to permit cleaning and inspection.

Each lot should be appropriately identified as to its status (i.e., quarantined, approved, or rejected).

Receipt and Storage of Untested Raw Materials, Product Containers, and Closures

Written procedures shall be established and followed describing the receipt, identification, examination, handling, and sampling of raw materials. Upon receipt and before acceptance, each container or grouping of containers of raw materials, product containers, and closures should be examined visually for appropriate labeling as to contents, container damage, or broken seals, and for contamination. They are then stored under quarantine until they have been tested or examined, as appropriate, and released.

Raw materials shall be held in bulk, or in containers designed and constructed so as to protect against adulteration, and shall be held at such temperature and relative humidity and in such a manner as to prevent a dietary ingredient or dietary supplement from becoming adulterated. Frozen raw materials and other ingredients shall be kept frozen. If thawing is required prior to use, it shall be done in a manner that prevents the raw materials and other ingredients from becoming adulterated within the meaning of the Act.

Testing and Approval or Rejection

Each lot of raw materials, product containers, and closures should be sampled, tested, or examined, as appropriate, and released for use by the quality control unit. Based upon adequate process verification, in-process controls and statistical confidence, a skip-lot ~~sampling~~

■testing■2S (*USP33*)
plan is an alternative to

■fully■2S (*USP33*)
testing every batch

■provided that at least one identity test is conducted. ■2S (*USP33*)
An appropriate amount of each lot of raw materials should be reserved for 3 years beyond the shelf life appearing on the label of finished dietary supplements in which the raw materials were used. If adverse event reports are received (see the subsection *Adverse Event Reports*), the reserved raw materials should be kept for 6 years

■(serious events) or 3 years (nonserious events)■2S (*USP33*)
from the date the first report is received.

Representative samples should be collected for testing or examination. Sampling of botanicals should be in compliance with the provisions set in *Articles of Botanical Origin* ⟨561⟩. The number of containers sampled, and the amount of material taken from each container, should be based upon appropriate criteria such as statistical criteria for raw material variability, confidence levels, and degree of precision desired, the past quality history of the supplier, and the quantity needed for analysis and reserve where required. The following procedures should be used to collect the samples:

— The containers of raw materials selected should be cleaned, where necessary, by adequate means.
— The containers should be opened, sampled, and resealed in a manner designed to prevent contamination of their contents and contamination of other raw materials, product containers, or closures.

In-Process Revision

— These containers should be identified so that the following information can be determined: name of the material sampled, the lot number, the container from which the sample was taken, the date on which the sample was taken, and the name of the person who collected the sample.

Use the following procedure to examine and test the samples:

— At least one test should be conducted to verify the identity of each raw material of a product ~~if~~

■even in cases where■2S (*USP33*)
skip-lot testing is used. Such tests may include any appropriate test with established sufficient specificity to determine identity, including chemical and laboratory tests, gross organoleptic analysis, microscopic identification, or analysis of constituent markers.

— Each raw material should be tested for conformity with all appropriate written specifications for purity, strength, and quality. However, a report of analysis may be accepted from the supplier of a raw material, provided that the manufacturer establishes the reliability of the supplier's analyses and provided that at least one identity test is conducted on such raw material by the manufacturer.

— Containers and closures should be tested for conformance with all appropriate written procedures. However, a certificate of testing may be accepted from the supplier, provided that at least a visual identification is conducted on such containers or closures by the manufacturer.

— Each lot of a raw material, rework, product container, or closure that is liable to contamination with filth, insect infestation, or other extraneous adulterant should be examined against established specifications for such contamination and shall comply with any applicable Food and Drug Administration regulations and guidelines. Skip-lot examination should not apply in such cases.

— Each lot of a raw material that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use. Raw materials either shall not contain levels of microorganisms that may produce food poisoning or other disease in humans, or shall be otherwise treated during manufacturing operations so that they no longer contain levels that would cause the product to be adulterated within the meaning of the Act. In lieu of such testing by the manufacturer, a guarantee or certification of analysis may be accepted from the supplier of a component provided that the manufacturer establishes the reliability of the supplier's analysis.

— Raw materials and other ingredients susceptible to adulteration with aflatoxin, other natural toxins, pesticides, or heavy metals shall comply with current Food and Drug Administration regulations, guidelines, and action levels for poisonous or deleterious substances and the requirements in *Articles of Botanical Origin* ⟨561⟩, or in each monograph, before these materials or ingredients are incorporated into a finished dietary ingredient or dietary supplement. Compliance with this requirement may be accomplished by analyzing these materials and ingredients for aflatoxins and other natural toxins; or, in lieu of such testing by the manufacturer, a guarantee or certification of analysis may be accepted from the supplier of a raw material provided that the manufacturer establishes the reliability of the supplier's analysis.

— Any lot of raw material, product container, or closure that meets the appropriate written specifications of identity, strength, quality, and purity and related tests may be approved and released for use. Any lot of such material that does not meet such specifications should be rejected.

Use of Approved Raw Materials, Product Containers, and Closures

Raw materials, product containers, and closures approved for use should be rotated so that the oldest approved stock is used first. Deviation from the requirement is permitted if such deviation is temporary and adequate.

Retesting of Approved Raw Materials, Product Containers, and Closures

Raw materials, product containers, and closures should be retested or reexamined, as appropriate, for identity, strength, quality, and purity and approved or rejected by the quality control unit after a specified time in storage or as necessary, e.g., after exposure to air, heat, or other conditions that might adversely affect the raw material, product container, or closure or after storage of active and inactive ingredients and in-process materials for long periods of time.

Rejected Raw Materials, Product Containers, and Closures

Rejected raw materials, product containers, and closures should be identified and controlled under a quarantine system that prevents their use in manufacturing or processing operations for which they are unsuitable.

Change to read:

PRODUCTION AND PROCESS CONTROLS

Written Procedures

Written procedures should be provided for production and process controls designed to ensure that the

■dietary ingredients and■2S (*USP33*)
dietary supplements have the identity, strength, quality, and purity they are represented to possess. These procedures should be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit. These production and process control procedures should be followed in the execution of the various production and process control functions and should be documented at the time of performance. Any deviation from the written procedures should be recorded and justified.

(1) All operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of ~~dietary products~~

■dietary ingredients and dietary supplements ■2S (*USP33*)
shall be conducted in accordance with adequate sanitation principles.

(2) All reasonable precautions shall be taken to ensure that production procedures do not contribute adulteration from any source. Chemical, microbial, or extraneous-material testing procedures shall be used where necessary to identify sanitation failures or possible product adulteration.

(3) All product that has become contaminated to the extent that it is adulterated within the meaning of the Act shall be rejected, or if permissible, treated or processed to eliminate the contamination.

(4) All product manufacturing, including packaging and storage, shall be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the adulteration of raw materials, in-process materials, and finished product.

(5) Measures taken to destroy microorganisms, reduce the microbial load, or prevent the growth of undesirable microorganisms, particularly those of public health significance, shall be adequate under the conditions of manufacture, handling, and distribution to prevent dietary supplements and ingredients from being adulterated within the meaning of the Act. These measures shall also comply with current regulations affecting dietary supplement products and ingredients.

(6) Work-in-process shall be handled in a manner that protects against adulteration.

■(7) In-process material must be held under appropriate conditions of temperature, humidity, and light.■2S (*USP33*)

(8) Effective measures shall be taken to protect finished dietary ingredients and dietary supplements from adulteration by raw materials, in-process materials, or refuse. When raw materials, in-process materials or refuse are unprotected, they shall not be handled simultaneously in a receiving, loading, or shipping area if that handling could result in adulterated ~~dietary products~~

■dietary ingredients and dietary supplements.■2S (*USP33*)
Dietary ingredients and dietary supplements transported by conveyor shall be protected against adulteration as necessary.

(9) Effective measures shall be taken as necessary to protect against the inclusion of metal or other extraneous material in product. Compliance with this requirement may be accomplished by using sieves, traps, magnets, electronic metal detectors, or other suitable effective means.

(10) Mechanical manufacturing steps such as cutting, sorting, inspecting, shredding, drying, grinding, blending, and sifting shall be performed so as to protect dietary ingredients and dietary supplements against adulteration. Compliance with this requirement may be accomplished by providing adequate physical protection of ~~dietary products~~

■dietary ingredients and dietary supplements■2S (*USP33*)
from contact with adulterants. Protection may be provided by adequate cleaning and sanitizing of all processing equipment between each manufacturing step.

(11) Heat blanching, when required in the preparation of a ~~dietary product~~

■dietary ingredient or a dietary supplement,■2S (*USP33*)
should be effected by heating the product to the required temperature, holding it at this temperature for the required time, and then either rapidly cooling the material or passing it to subsequent manufacturing without delay. Thermophilic growth and contamination in blanchers should be minimized by the use of adequate operating temperatures and by periodic cleaning. Where the blanched product is washed before filling, potable water shall be used.

(12) Intermediate of dehydrated ~~dietary products~~

■dietary ingredients and dietary supplements■2S (*USP33*)
that rely on the control of water (a_w) for preventing the growth of undesirable microorganisms shall be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
(i) Monitoring the water activity (a_w) of the material;
(ii) Controlling the soluble solids–water ratio in finished product; and
(iii) Protecting finished product from moisture pickup, by use of a moisture barrier or by other means, so that the water activity (a_w) of the product does not increase to an unsafe level.

(13) Dietary ingredients and dietary supplements that rely principally on the control of pH for preventing the growth of undesirable microorganisms shall be monitored and maintained at an appropriate pH. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
(i) Monitoring the pH and water activity, if appropriate, of raw materials, in-process material, and finished product; and
(ii) Controlling the amount of acid added to the product.

(14) When ice is used in contact with ~~dietary products~~

■dietary ingredients and dietary supplements,■2S (*USP33*)
it shall be made from potable water, and shall be used only if it has been manufactured in accordance with current good manufacturing practice

■in manufacturing, packing, or holding human food■2S (*USP33*)
as outlined in 21 CFR Part 110.

Charge-In of Raw Materials

Written production and control procedures should include the following, which are designed to ensure that the dietary supplements have the identity, strength, quality, and purity they are represented to possess:

— The batch should be formulated with the intent to provide not less than 100 percent of the labeled or established amount of dietary ingredient.
— Raw materials for product manufacturing should be weighed, measured, or subdivided as appropriate and the appropriate signatures recorded in the batch record.
— Actual yields and percentages of theoretical yield should be determined at appropriate phases of processing.

Material scheduled for rework shall be identified as such.

Equipment Identification

All compounding and storage containers, processing lines, and major equipment used during the production of a batch of a product should be properly identified to indicate their contents and, when necessary, the phase of processing of the batch.

Sampling and Testing of In-Process Materials, ~~and Dietary Products~~

■Dietary Ingredients, and Dietary Supplements■2S (*USP33*)

To ensure batch uniformity and integrity of dietary supplements, written procedures should be established and followed that describe the in-process controls and tests or examinations to be conducted on appropriate samples of in-process materials. Based upon process verification, in-process controls, and statistical confidence, a skip-lot ~~sampling~~

■testing■2S (*USP33*)

In-Process Revision

plan is an alternative to testing every batch

■of finished products provided that at least one representative measure is performed.■2S (*USP33*)
Control procedures should be established to monitor the output of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the finished product. Such control procedures may include, but are not limited to, the following, where appropriate:
— Friability
— Weight variation
— Disintegration time
— Dissolution time
— Clarity, completeness, or pH of solutions

■— Blend uniformity■2S (*USP33*)

In-process specifications for such characteristics should be consistent with finished product specifications. Examination and testing of samples should ensure that the in-process material and dietary supplement conform to the established specifications.

In-process materials should be tested for identity, strength, quality, and purity as adequate, and approved or rejected by the quality control unit during the production process, e.g., at commencement or completion of significant phases or after storage for long periods.

Rejected or adulterated in-process materials should be identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable and to prevent the adulteration of other products.

Change to read:

LABELING AND PACKAGING

Materials Examination and Usage Criteria

Written procedures should be provided describing in sufficient detail the receipt, identification, storage, handling, sampling, examination, or testing of labeling and packaging materials

■or products received for packaging or labeling. Each immediate container or grouping of immediate containers in a shipment of product received for packaging or labeling, or of packaging and labeling materials, must be visually examined for appropriate content label, container damage, or broken seals to determine whether the container condition may have resulted in contamination or deterioration of the received product. The supplier's invoice, guarantee, or certification in a shipment of the received product must be visually examined to ensure that the received product is consistent with the purchase order.■2S (*USP33*)
Labeling and packaging materials

■or products received for packaging or labeling■2S (*USP33*) should be ~~representatively sampled and examined or tested upon receipt and before use in packaging or labeling of a product.~~

■quarantined until:

— Representative samples of each unique shipment, and of each unique lot within each unique shipment, of received product for packaging or labeling, or of packaging and labeling materials, are collected;
— The quality control unit reviews and approves the documentation to determine whether the received product for packaging or labeling, or packaging and labeling materials meet the specifications; and
— The quality control unit approves the received product for packaging or labeling, or packaging and labeling materials, and releases for use from quarantine.■2S (*USP33*)

~~Any labeling or packaging materials meeting appropriate written specifications may be approved and released for use.~~

■■2S (*USP33*)
Those that do not meet such specifications should be

■identified and■2S (*USP33*)
rejected to prevent their use in operations for which they are unsuitable.

A record should be kept of each shipment received of each different labeling and packaging material,

■or each different received product for packaging or labeling,■2S (*USP33*)
indicating receipt, date of examination or testing, and whether accepted or rejected.

■Each unique lot within each unique shipment of received product for packaging or labeling, or of packaging and labeling materials, must be identified in a manner that allows the recipient to trace the lot to the supplier, the date received, the name of the received product, the status of the received product (e.g., quarantined, approved, or rejected), and to the product that was packaged or labeled and distributed.

This unique identifier must be used whenever the disposition of each unique lot within each unique shipment of the received product for packaging or labeling, or of packaging and labeling materials, is recorded.■2S (*USP33*)

Labels and other labeling materials for each different product, strength, product type, or quantity of contents should be stored separately ~~with suitable identification .~~

■under conditions that will protect against contamination and deterioration and avoid mixups.■2S (*USP33*)
Only authorized personnel should have access to the storage area.

▪Packaging and labels must be held under appropriate conditions so that the packaging and labels are not adversely affected (e.g. contamination, deterioration). ▪2S (*USP33*)

Gang printing of labeling to be used for different products or different strengths of the same product (or labeling of the same size and identical or similar format or color schemes) should be minimized. If gang printing is employed, packaging and labeling operations should provide for special control procedures, taking into consideration sheet layout, stacking, cutting, and handling during and after printing.

Printing devices on, or associated with, manufacturing lines used to imprint labeling upon the product unit label or case should be monitored to ensure that all imprinting conforms to the print specified in the batch production record.

Obsolete and outdated labels, labeling, ~~and~~

▪▪2S (*USP33*)
other packaging materials,

▪and products received for packaging or labeling▪2S (*USP33*)
should be destroyed and documented.

Labeling Issuance

Strict control should be exercised over labeling issued for use in product labeling operations. The control procedures employed should be in writing with sufficient detail.

Labeling materials issued for a batch should be carefully examined for identity and conformity to the labeling specified in the master ~~or~~

▪and▪2S (*USP33*)
batch production records.

Procedures should be used to reconcile the quantities of labeling issued, used, and returned, and should require evaluation of discrepancies found. If discrepancies are found between the quantity of product finished and the quantity of labeling issued and are outside preset limits based on historical operating data, such discrepancies should be investigated.

Returned labeling should be maintained and sorted in a manner to prevent mixups and provide proper identification.

All excess labeling bearing lot or control numbers should be destroyed and documented.

Operations

Written procedures designed to ensure that correct labels, labeling, and packaging materials are used for dietary supplements should incorporate the following features:

— Prevention of mixups and cross-contamination by physical or spatial separation from operations on other products;
— Identification of the product with a lot or control number;
— Examination of packaging and labeling materials for suitability and correctness before packaging operations, and documentation of such examination in the batch production record; and
— Inspection of the packaging and labeling facilities immediately before use to ensure that all products have been removed from previous operations. Inspection should also be made to ensure that packaging and labeling materials not suitable for subsequent operations have been removed. Results of the inspection should be documented in the batch production records.

▪Relabeling and Repackaging

— Dietary ingredients and dietary supplements may be repackaged or relabeled only after the quality control unit has approved such repackaging or relabeling.
— A representative sample of each batch of repackaged or relabeled dietary ingredients and dietary supplements must be examined to determine whether the repackaged or relabeled dietary ingredients and dietary supplements meet all established specifications.
— The quality control unit must approve or reject each batch of repackaged or relabeled dietary ingredients and dietary supplements before its release for distribution.▪2S (*USP33*)

Tamper-Resistant Packaging

REQUIREMENTS

Each manufacturer and packer who packages a dietary supplement for retail sale shall package the product in a tamper-resistant package, if this product is accessible to the public while held for sale. A tamper-resistant package is one having an indicator or barrier to entry which, if breached or missing, can reasonably be expected to provide visible evidence to consumers that tampering has occurred. To reduce the likelihood of substitution of a tamper-resistant feature after tampering, the indicator or barrier to entry is required to be distinctive by design or by the use of an identifying characteristic (e.g., a pattern, name, registered trademark, logo, or picture). For purposes of this section, the term ''distinctive by design'' means that the packaging cannot be duplicated with commonly available materials or through commonly available processes. A tamper-resistant package may involve an immediate-container and closure system, or secondary-container or carton system, or any combination of systems intended to provide a visual indication of package integrity. The tamper-resistant feature should be designed to remain intact when handled in a reasonable manner during manufacture, distribution, and retail display.

LABELING

Each retail package of a dietary supplement covered by this section shall bear a statement that is prominently placed so that consumers are alerted to the specific tamper-resistant feature of the package. The labeling statement should be so placed that it will be unaffected if the tamper-resistant feature of the packaging is breached or missing. If the tamper-resistant feature chosen to meet the requirement above is one that uses an identifying characteristic, that characteristic should be referred to in the labeling statement. For example, the labeling statement on a bottle with a shrink band could say ''For your protection, this bottle has an imprinted seal around the neck.''

Dietary Supplement ~~Inspection~~ ■Examination■2S (*USP33*)

Packaged and labeled products should be examined during finishing operations to ensure that containers and packages in the lot have the correct label. A representative sample of units should be collected at the completion of finishing operations and visually examined for correct labeling. Results of these examinations should be recorded in the batch production or control records.

Contact Information

The manufacturer, packer, or distributor of dietary supplements is required to comply with the current labeling requirements in the law that also include a domestic address or phone number through which an adverse event report for a dietary supplement may be received.

Shelf Life

Dietary supplements should bear a date indicative of their shelf life, determined by appropriate testing, to ensure that they meet applicable standards of identity, strength, quality, and purity at or before the labeled shelf-life date.

Shelf life should be related to any storage conditions stated on the labeling.

Change to read:

QUALITY CONTROL OPERATIONS

The establishment of any specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms required by this chapter, including any change in such specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms, shall be drafted by the appropriate organizational unit and reviewed and approved by the quality control unit. The requirements in this section should be followed and documented at the time of performance. Any deviation from the written specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms shall be recorded and justified.

Quality control operations include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to ensure that raw materials, product containers, closures, in-process materials, labeling,

■products received for labeling and packaging operations as dietary supplements,■2S (*USP33*)

and finished products conform to adequate standards of identity, strength, quality, and purity. These controls include the following:

— Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of raw materials, product containers, closures, and labeling used in the manufacture of ~~dietary products~~ ■dietary ingredients and dietary supplements, and of products received for labeling and packaging operations as dietary supplements.■2S (*USP33*)
(The specifications include a description of the sampling and testing procedures used. Samples should be representative and adequately identified. Such procedures also require appropriate retesting of any raw material, product container, or closure that is subject to deterioration.) Based upon adequate process verification, in-process controls, and statistical confidence, a skip-lot ~~sampling~~ ■testing■2S (*USP33*)
plan is an alternative to testing every batch, ■excluding raw materials, which require 100% identity testing.■2S (*USP33*)

— Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. (Such samples should be representative and properly identified.)
— Determination of conformance to written descriptions of sampling procedures and appropriate specifications for finished products. (Such samples should be representative and properly identified.)
— The calibration of instruments, at suitable intervals, in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event that accuracy and/or precision limits are not met. Instruments not meeting established specifications shall not be used until repaired.

Testing and Release for Distribution

There should be appropriate laboratory determination of satisfactory conformance to specifications for the finished product, including the identity and strength prior to release. Based upon adequate process verification, in-process controls, or statistical confidence, a skip-lot or composite ~~sampling~~ ■testing■2S (*USP33*)
plan is an alternative to testing every batch.

There should be appropriate laboratory testing, as necessary, of each batch of dietary supplement required to be free of objectionable microorganisms. The accuracy, linearity, sensitivity, specificity, and precision of test methods employed by the firm, when they differ from compendial methods, should be established and documented.

Written procedures should describe any sampling and testing plans, which should include the method of sampling and the number of units per batch to be tested.

Products failing to meet established standards or specifications and any other relevant quality control criteria should be rejected. Rejected or adulterated ~~dietary products~~ ■dietary ingredients and dietary supplements■2S (*USP33*)
shall be identified, stored, and disposed of in a manner that protects against the adulteration of the other products. Reprocessing may be performed. Prior to acceptance and use, reprocessed material must meet established standards, specifications, and any other relevant criteria. Written procedures shall be established and followed prescribing the method for reprocessing batches or operations start-up materials that do not conform to finished goods standards or specifications. Finished goods manufactured using such materials shall meet all established purity, composition, and quality standards.

Stability Testing

There should be a written protocol designed to assess the stability characteristics of dietary supplements. The results of such testing should be used in determining appropriate storage conditions and shelf life. This protocol should include the following:

— Sample size and test intervals based on statistical criteria for each attribute should be examined to ensure valid estimates of stability;
— Storage conditions for samples retained for testing;
— Reliable, meaningful, and specific test methods should be used; and

— The dietary supplement should be tested in the same type of container–closure system as that in which the dietary supplement is marketed.

An adequate number of batches of each dietary supplement should be tested to determine an adequate shelf life, and a record of these data should be maintained. Accelerated studies combined with basic stability information on the raw materials, dietary supplements, and container-closure systems may be used to support tentative shelf life if full shelf-life studies are not available. Simplified stability testing procedures may be used where data from similar product formulations are available to support a shelf-life estimation of a new product. Where data from accelerated studies are used to project a tentative shelf life date that is beyond a date supported by actual shelf-life studies, stability studies should be conducted, including dietary supplement testing at appropriate intervals, until the tentative shelf life is verified or the adequate shelf life is determined.

Reserve Samples

An appropriately identified reserve sample that is representative of each lot or batch of dietary supplement should be retained and stored under conditions consistent with product labeling until at least 3 years after the shelf life of the product. The reserve sample should be stored in the same immediate container-closure system in which the finished product is marketed or in one that has essentially the same characteristics. The reserve sample consists of at least twice the quantity necessary to perform all the required tests. If an adverse event report is received, the reserve samples of dietary supplements and dietary ingredients from the same lot or batch must be analyzed by an appropriate procedure to confirm their identity and determine any adulteration or contamination. The recovered samples associated with adverse event reports from consumers, distributors, or both should also be analyzed, following the same method used for the reserved samples, if available. The results should be reported with other required information to the federal authority, using the required form. The reserve samples from a particular lot or batch associated with an adverse event report should be held for 6 years

■(serious events) or 3 years (nonserious events)■2S (*USP33*) from the date when the first adverse event report is received by the manufacturer, packer, or distributor.

Change to read:

RECORDS AND REPORTS

Any record for production, control,

■quality control operations,■2S (*USP33*) or distribution that is required to be maintained and is specifically associated with a batch of a product should be retained for at least 3 years after the shelf life of the batch.

Records should be maintained for all raw materials, product containers, closures, and labeling for at least 3 years after the shelf life of the last lot of product incorporating the raw material or using the container, closure, or labeling.

Master Production and Control Records

To ensure uniformity from batch to batch, master production and control records for each product should be prepared, dated, and signed by one person and independently checked, dated, and signed by a second person from the quality control unit.

Master production and control records should include the following:

— The name and strength of the product;

— The name and weight or measure of each dietary ingredient per unit or portion or per unit of weight or measure of the product, and a statement of the total weight or measure of any dosage unit;

— A complete list of raw materials designated by names or codes sufficiently specific to indicate any special quality characteristic;

— An accurate statement of the weight or measure of each raw material, using the same weight system (metric, avoirdupois, or apothecary) for each raw material;

— A statement concerning any calculated excess of raw material;

— A statement of theoretical weight or measure at appropriate phases of processing;

— A statement of theoretical yield, including the maximum and minimum percentages of theoretical yield beyond which investigation is required;

— A description of the product containers, closures, and packaging materials, including a specimen or copy of each label and all other labeling signed and dated by the person or persons responsible for approval of such labeling or, in lieu of specimens or copies of each label or other labeling, a positive identification of all labeling used; and

— Complete manufacturing and control instructions, ~~sampling and testing procedures, specifications,~~

■testing procedures, acceptance limits,■2S (*USP33*) special notations, and precautions to be followed.

■— Specific actions necessary in order to perform and verify points, steps, or stages in the manufacturing process where control is necessary to ensure the quality of dietary ingredients and dietary supplements, and to ensure that dietary ingredients and dietary supplements are packaged and labeled as specified in the master production record.

(a) Such specific actions must include verifying the weight or measure of any component and verifying the addition of any component; and

(b) For manual operations, such specific actions must include:

(i) One person weighing or measuring a component and another person verifying the weight or measure; and

(ii) One person adding the component and another person verifying the addition.

Corrective action plans for use when a specification is not met.■2S (*USP33*)

Batch Production and Control Records

Batch production and control records should be prepared for each batch of product produced and should include complete information relating to the production and control of each batch. These records should be reviewed and signed by a second person from the quality control unit. These records should

In-Process Revision

include accurate reproduction of the appropriate master production or control record and documentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished, including the following:

— Dates;
— Identity of individual major equipment and lines used;
— Specific identification of each batch of raw material or in-process material used;
— Weights and measures of raw materials used in the course of processing;
— In-process and laboratory control results;
— Inspection of the packaging and labeling areas before and after use;
— A statement of the actual yield and a statement of the percentage of theoretical yield at appropriate phases of processing;
— Description of product containers and closures used;
— Complete labeling control records, including: ~~specimens or copies of all labeling used or identification of all labeling used;~~

■(a) The unique identifier assigned to packaging and labels used, the quantity of the packaging and labels used, and, when label reconciliation is required, reconciliation of any discrepancies between issuance and use of labels; and

(b) An actual or representative label, or a cross-reference to the physical location of the actual or representative label specified in the master manufacturing record;■2S (*USP33*)

— Any sampling performed;
— Identification of the persons performing and directly supervising or checking any step in the operation;
— Any investigation made; ~~and~~
— ~~Results of examinations made.~~

■— The results of any tests or examinations conducted on packaged and labeled dietary supplements (including repackaged or relabeled dietary supplements), or a cross-reference to the physical location of such results;

— Documentation at the time of performance that quality control personnel:

(a) Reviewed the batch production record, including:

(i) Review of any required monitoring operation, and

(ii) Review of the results of any tests and examinations, including tests and examinations conducted on components, in-process materials, finished batches of dietary supplements, and packaged and labeled dietary ingredients and dietary supplements;

(b) Approved or rejected any reprocessing or repackaging;

(c) Approved and released, or rejected, the batch for distribution, including any reprocessed batch; and

(d) Approved and released, or rejected, the packaged and labeled dietary supplement, including any repackaged or relabeled dietary supplement;

— Documentation at the time of performance of any required material review and disposition decision; and
— Documentation at the time of performance of any reprocessing.

Records for Raw Materials, Packaging, and Labels and for Product Received for Packaging or Labeling as a Dietary Supplement

The following records must be made and retained:

— Written procedures for fulfilling the requirements for raw materials, packaging, and labels and for product received for packaging or labeling;
— Receiving records (including records such as certificates of analysis, suppliers' invoices, and suppliers' guarantees) for components, packaging, and labels and for products received for packaging or labeling; and
— Documentation that the requirements of *Raw Materials, Labeling and Packaging* were met:

(a) The person who performs the required operation must document, at the time of performance, that the required operation was performed; and

(b) The documentation must include:

(i) The date of receipt of the raw materials, packaging, labels, or products received for packaging or labeling as a dietary supplement;

(ii) The initials of the person performing the required operation;

(iii) The results of any tests or examinations conducted on raw materials, packaging, or labels, and of any visual examination of product received for packaging or labeling as a dietary supplement; and

(iv) Any material review and disposition decision conducted on raw materials, packaging, labels, or products received for packaging or labeling as a dietary supplement.■2S (*USP33*)

Laboratory Records

Laboratory records should include complete data derived from all tests necessary to ensure compliance with established specifications and standards, including examinations and assays, as follows:

— A description of the sample received for testing with identification of source (that is, location from where sample was obtained), quantity, lot number or other distinctive code, and date sample was taken.
— A statement of each method used in the testing of the sample.
— A statement of the weight or measure of sample used for each test, where appropriate.
— A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation, properly identified to show the specific raw material, product container, closure, in-process material, or finished product, and lot tested.
— A record of all calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.
— A statement of the results of tests and how the results compare with established standards of identity, strength, quality, and purity for the raw material, product container, closure, in-process material, or finished product tested.
— The initials or signature of the person who performs each test and the date(s) the tests were performed.

Complete records should be maintained of any modification of an established method employed in testing. Such records should include the reason for the modification and data to verify that the modification produced results that are at least as accurate and reliable for the material being tested as the established method.

Complete records should be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions, the periodic calibration of laboratory instruments, and all stability testing performed. Any deviation should be reviewed and signed by the management of the quality control unit.

▪Quality Control Operation Records

The following records must be made and retained:

— Written procedures for the responsibilities of the quality control operations, including written procedures for conducting a material review and making a disposition decision and written procedures for approving or rejecting any reprocessing;
— Written documentation, at the time of performance, that quality control personnel performed the review, approval, or rejection requirements, by recording the following:
  (i) Date on which the review, approval, or rejection was performed; and
  (ii) Signature of the person performing the review, approval, or rejection; and
— Documentation of any material review and disposition decision and follow-up. Such documentation must be included in the appropriate batch production record and must include:
  (i) Description of the investigation into the cause of the deviation from the specification or the unanticipated occurrence;
  (ii) Evaluation of whether the deviation or unanticipated occurrence has resulted in, or could lead to, a failure to ensure the quality of the dietary supplement or a failure to package and label the dietary supplement as specified in the master manufacturing record;
  (iii) Identification of the action(s) taken to correct, and prevent a recurrence of, the deviation or the unanticipated occurrence;
  (iv) Explanation of the actions taken with the raw material, dietary supplement, packaging, or label;
  (v) A scientifically valid reason for any reprocessing of a dietary supplement that is rejected or any treatment or in-process adjustment of a component that is rejected; and

(vi) The signatures of (1) the individual(s) designated to perform the quality control operation, who have conducted the material review and made the disposition decision; and in addition, (2) each qualified individual who has provided information relevant to that material review and disposition decision.■2S (*USP33*)

Distribution Records

Distribution records should contain the name and strength of the product, name and address of the consignee, date and quantity shipped, and lot or control number of the finished product.

Record Keeping

The manufacturer, packer, or distributor of dietary supplements must keep all required records, as shown in this chapter, for 3 years beyond the shelf life of dietary supplements associated with those records. If adverse event reports are received, those records must be kept for additional 6 years from the date when the first report is received. All records must be accessible by the regulatory authority when requested.

■Records must be kept as original records, as true copies (such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records), or as electronic records.

All electronic records must comply with part 11 of Code of Federal Regulations Title 21 (21 CFR part 11).

If reduction techniques are used, such as microfilming, suitable reader and photocopying equipment must be readily available to auditors and inspectors.■2S (*USP33*)

Complaint Files

Written procedures describing the handling of all written and oral complaints regarding a dietary supplement shall be established and followed. These procedures should include provisions for review by the quality control unit of any complaint involving the possible failure of a product to meet any of its specifications and a determination as to the need for an investigation.

Each complaint should be recorded in a file designed especially for dietary supplement complaints. Written records should be maintained until at least 3 years after the shelf life of the product, or 3 years after the date when the complaint was received, whichever is longer.

The written record should include the following information, where known: the name and strength of the product, lot number, name of complainant, nature of complaint, and reply to complainant.

If an investigation is necessary, the written record should include the findings of the investigation and follow-up.

■The review and investigation of the product complaint by a qualified person, the review by quality control personnel about whether to investigate a product complaint, and the findings and follow-up action of any investigation performed must extend to all relevant batches and records.■2S (*USP33*)

Adverse Event Reports

■Adverse event reports include reports on any health-related adverse event associated with the use of a dietary supplement that is adverse. It includes both nonserious and serious adverse event reports.

The manufacturer, packer, or distributor of a dietary supplement (called the *responsible person*) whose name appears on the label shall be responsible for keeping a report of all nonserious adverse events along with any related records (e.g., records of communications with the person who reported the nonserious event). All such records of nonserious adverse events should be kept for 6 years.■2S (*USP33*)

~~The manufacturer, packer, or distributor of a dietary supplement (responsible person)~~

■The responsible person■2S (*USP33*) whose name appears on the label shall also be responsible for reporting any serious adverse event reported to it, and associated with a dietary supplement that is marketed and used in the same country, to the regulatory authority as soon as appropriate, but no later than 15 business days after receipt of the report, using the appropriate form as defined by the regulation

■(http://www.fda.gov/Food/DietarySupplements/Alerts/ucm111110.htm).■2S (*USP33*)
A serious adverse event is an event that results in

■any of following:■2S (*USP33*)

1. Death,
2. A life-threatening experience,
3. Inpatient hospitalization,
4. A persistent or significant disability or inability,
5. A congenital anomaly or birth defect, or
6. A condition that requires, according to reasonable medical judgment, a medical or surgical intervention to prevent one of the five outcomes listed above.

A retailer whose name appears on the label as a distributor may, by agreement, authorize the manufacturer or packer to submit the required reports to the regulatory authority, as long as the retailer directs all received adverse event reports to the manufacturer or packer. Each serious adverse event report should include a copy of the product's label, the information described in the preceding section *Complaint Files*, and if possible, the contact information of the complainant; daily intake; alcohol consumption and amount; use of prescription medicine

and OTC medicine, including a daily dose; and other medical information. The information associated with personal identification and medical records should be obtained only for the reports and kept safe from disclosure. Any new medical information that is related to an already submitted serious adverse event report that is received within 1 year of the initial report shall be submitted to the regulatory authority as soon as appropriate, but no later than 15 business days after receipt of the information. The records related to each report of ~~an~~

■a serious■2S *(USP33)*
adverse event received by the manufacturer, packer, or retailer should be maintained for 6 years. The authorized person who is designated by the regulatory authority should be permitted access to those records.

Change to read:

RETURNED AND SALVAGED PRODUCTS

Returned Dietary Supplements

Returned products should be identified as such and held. If the conditions under which returned ~~dietary products~~

■dietary ingredients and dietary supplements■2S *(USP33)*
have been held, stored, or shipped before or during their return, or if the condition of the product, its container, carton, or labeling, as a result of storage or shipping, casts doubt on the safety, identity, strength, quality, or purity of the product, the returned product should be destroyed unless examination, testing, or other investigations prove the product meets appropriate standards of safety, identity, strength, quality, or purity. The returned products associated with adverse events must be destroyed after a sufficient sample of products is stored for the purpose of further investigation only. The products related to the adverse event that have been returned should be kept for 6 years

■(serious events) or 3 years (nonserious events)■2S *(USP33)*
from the date when the first report is received. A product may be reprocessed provided that the subsequent product meets adequate standards, specifications, and characteristics. Records of returned products should be maintained and should include the name and label potency of the product, lot number (or control number or batch number), reason for the return, quantity returned, date of disposition, and ultimate disposition of the returned product. If the reason for a product being returned implicates associated batches, an appropriate investigation is necessary.

Dietary Supplement Salvaging

Products that have been involved in adverse events or subjected to improper storage conditions, including extremes in temperature or humidity, smoke, fumes, pressure, age, or radiation due to natural disasters, fires, accidents, or equipment failures should not be salvaged and returned to the marketplace. Whenever there is a question whether products have been subjected to such conditions, salvaging operations may be conducted only if there is (a) evidence from laboratory tests and assays that the products meet all applicable standards of identity, strength, quality, and purity, and (b) evidence that the products and their associated packaging were not subjected to improper storage conditions as a result of the disaster or accident. Organoleptic examinations should be accepted only as supplemental evidence that the dietary supplement meets appropriate standards of identity, strength, quality, and purity. Records including name, lot number, and disposition should be maintained for salvaged products. If the products are involved in adverse events, the instructions described in the preceding section *Records and Reports* should be followed.

Defect Action Levels

Some dietary ingredients and dietary supplements, even when produced under current good manufacturing practice, contain natural or unavoidable defects that at low levels are not hazardous to health. The Food and Drug Administration establishes maximum levels for these defects in ~~dietary products~~

■dietary ingredients and dietary supplements■2S *(USP33)*
produced under current good manufacturing practice and uses these levels in deciding whether to recommend regulatory action.

Defect action levels are established for ~~dietary products~~

■dietary ingredients and dietary supplements■2S *(USP33)*
whenever it is necessary and feasible to do so. These levels are subject to change upon the development of new technology or the availability of new information.

Compliance with defect action levels does not excuse violation of the requirement in section 402(a)(4) of the Act that ~~dietary products~~

■dietary ingredients and dietary supplements■2S *(USP33)*
shall not be prepared, packed, or held under unsanitary conditions or the requirements in this part that ~~dietary product~~ manufacturers, distributors, and holders

■of both dietary ingredients and dietary supplements■2S *(USP33)*
shall observe current good manufacturing practice. Evidence indicating that such a violation exists causes ~~a dietary product~~

■a dietary ingredient and a dietary supplement■2S *(USP33)*
to be adulterated within the meaning of the Act, even though the amounts of natural or unavoidable defects are lower than the currently established defect action levels. The manufacturer, distributor, and holder of ~~a dietary product~~

■a dietary ingredient or a dietary supplement■2S *(USP33)*
shall at all times utilize quality control operations that reduce natural or unavoidable defects to the lowest level currently feasible.

The mixing of a dietary ingredient or dietary supplement containing defects above the current defect action level with another lot of dietary ingredient or dietary supplement is not permitted and renders the final product adulterated within the meaning of the Act, regardless of the defect level of the final product.

A compilation of the current defect action levels for natural or unavoidable defects in ~~dietary products~~

■dietary ingredients and dietary supplements■2S *(USP33)*
that present no health hazard may be obtained upon request from the Industry Activities Staff (HFS-565), Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740-3835.

Change to read:

GLOSSARY

Act means Federal Food, Drug and Cosmetic Act (United States Code [U.S.C.] Title 21, Chapter 9).

Acceptance criteria is the product specifications and acceptance or rejection criteria, such as acceptable quality level and unacceptable quality level, with an associated sampling plan, that are necessary for making a decision to accept or reject a lot or batch (or any other convenient subgroups of manufactured units).

Adequate means that which is needed to accomplish the intended purpose in keeping with good public health practice.

Adverse event means any health-related event that is adverse and that is associated with the use of a dietary supplement.

Adverse event report means a report of an adverse event (see definition above). (See also *Serious adverse event report.)*

Batch is a specific quantity of a finished product or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture.

Blanching means a prepackaging heat treatment of ~~a dietary product~~

■a dietary ingredient and a dietary supplement■2S (*USP33*)

for a sufficient time and at a sufficient temperature to partially or completely inactivate the naturally occurring enzymes and to effect other physical or biochemical changes in the product.

Composition is (1) the identity of a dietary ingredient or dietary supplement, and (2) the concentration of a dietary ingredient (e.g., weight or other unit of use/weight or volume), or the potency or activity of one or more dietary ingredients, as indicated by appropriate procedures.

Dietary ingredient is an ingredient intended for use or used in a dietary supplement that is

— a vitamin;

— a mineral;

— an herb or other botanical;

— an amino acid;

— a dietary substance for use by humans to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract; or

— a combination of any of the foregoing ingredients.

~~*Dietary product* means either a dietary ingredient or dietary supplement as defined in this chapter.~~

■■2S (*USP33*)

Dietary supplement is a product (other than tobacco) that is intended to supplement the diet and that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by humans to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract or combination of these ingredients, that is intended for ingestion in a pill, capsule, tablet or liquid form, that is not represented for use as a conventional food or as the sole item of a meal or diet, and that is labeled as a dietary supplement, and includes products such as new drug, certified antibiotic, or licensed biologic that was marketed as a dietary supplement or food before approval, certification, or license unless a sanitary authority waives this provision.

Inactive ingredient is any raw material other than a dietary ingredient.

In-process material is any material fabricated, compounded, blended, ground, extracted, sifted, sterilized, or processed in any other way that is produced for, and used in, the preparation of the dietary supplement.

Lot is a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits.

Lot number, control number, or *batch number* is any distinctive combination of letters, numbers, or symbols, or any combination of them from which the complete history of the manufacture, processing, packing, holding, and distribution of a batch or lot of finished dietary ingredient, dietary supplement, or other material can be determined.

Manufacture or *manufacturing* includes all operations associated with the production of ~~dietary products~~

■dietary ingredients and dietary supplements,■2S (*USP33*) including packaging and labeling operations, testing, and quality control of a dietary ingredient or dietary supplement.

Microorganisms means yeast, molds, bacteria, and viruses and includes, but is not limited to, species having public health significance. The term ''undesirable microorganisms'' includes those microorganisms that are of public health significance, that subject ~~a dietary product~~

■a dietary ingredient or a dietary supplement■2S (*USP33*) to decomposition, that indicate that a dietary ingredient or dietary supplement is contaminated with filth, or that otherwise may cause ~~a dietary product~~

■a dietary ingredient or a dietary supplement■2S (*USP33*) to be adulterated within the meaning of the Act. Occasionally in these regulations, the adjective ''microbial'' is used instead of an adjectival phrase containing the word ''microorganism.''

Pest refers to any objectionable animals or insects including, but not limited to, bird, rodents, flies, and larvae.

Plant means the building or facility or parts thereof, used for or in connection with the manufacturing, packaging, labeling, or holding of ~~a dietary product~~

■a dietary ingredient and a dietary supplement.■2S (*USP33*)

Process evaluation is a set of tests performed on a process intended to evaluate its capacity to consistently produce the results that it is intended for.

Quality control operation is a planned and systematic procedure for taking all actions necessary to prevent ~~a dietary product~~

■a dietary ingredient and a dietary supplement■2S (*USP33*) from being adulterated.

Quality control unit is any person or organizational element designated by the firm to be responsible for the duties relating to quality control operations.

Raw material is any ingredient intended for use in the manufacture of a dietary ingredient or dietary supplement, including those that may not appear in such finished product. (A dietary ingredient is a raw material when considering the manufacture of a dietary supplement.)

Representative sample is a sample that consists of a number of units that are drawn based on rational criteria such as random sampling and is intended to ensure that the sample accurately portrays the material being sampled.

Rework is a clean, unadulterated material that has been removed from processing for reasons other than unsanitary conditions or that has been successfully reconditioned by reprocessing and that is suitable for use in the manufacture of ~~a dietary product~~

■a dietary ingredient or a dietary supplement.■2S (*USP33*)

Sanitizing is to adequately treat equipment, containers, or utensils by a process that is effective in destroying vegetative cells of microorganisms of public health significance and in substantially reducing other undesirable microorganisms but without affecting the product or its safety for the consumer.

Serious adverse event report means a report of an adverse event that is termed *serious* because it meets certain criteria (see the subsection *Adverse Event Reports*). ~~The new law~~

■The Dietary Supplement and Nonprescription Drug Consumer Protection Act■*2S (USP33)* requires manufacturers and distributors of dietary supplements and OTC drugs to report all serious adverse events to the Secretary of the Food and Drug Administration (FDA). This is an entirely new requirement for dietary supplements.

Shall is used to state requirements that must be met under the provisions of this guideline.

Shelf life is the period of time after manufacturing in which the dietary supplement is ensured to meet applicable standards of identity, strength, quality, and purity.

Shelf-life (*Use by*) *date* is the date beyond which the dietary supplement is no longer ensured to meet applicable standards of identity, strength, quality, and purity.

Should is used to state recommended or advisory procedures or identify recommended equipment.

Skip-lot ~~*sampling*~~ ■*testing (or sampling)*■*2S (USP33)* is a reduced level of ~~sampling or testing~~

■testing (or sampling)■*2S (USP33)* for a particular specified parameter(s) based upon one or more of the following:

— Statistical analysis of an adequate quantity of historical test data;
— Statistical confidence in the capability of the manufacturing process as determined by suitable verification; or
— Ongoing monitoring of the process using recognized statistical process control (SPC) techniques.

Strength means the concentration of the active substance (weight/weight, weight/volume, or unit of use/volume or weight basis); and/or the potency, that is, the activity of the product as indicated by appropriate laboratory tests.

Water activity (a_w) is a measure of the free moisture in a dietary ingredient or dietary supplement and is the quotient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

BRIEFING

Cobalt Chloride, *USP 32* page 816. It is proposed to correct the CAS number of this reagent.

(HDQ: M. Marques.) RTS—C75911

Change to read:

Cobalt Chloride *(Cobaltous Chloride),* $CoCl_2 \cdot 6H_2O$—**237.93** ~~[7646-79-9]~~

■[*7791-13-1*]■2S *(USP33)*
—Use ACS reagent grade.

BRIEFING

Oxygen Certified Standard. It is proposed to add this new reagent used in the monograph for *Medical Air*.

(HDQ: M. Marques.) RTS—C61164

Add the following:

■**Oxygen Certified Standard**—A suitable 99.99% oxygen certified standard is available from most suppliers of specialty gases.■2S *(USP33)*

BRIEFING

Tetramethylbenzidine. It is proposed to add this new reagent used in developing reagent B in the tests for *Limit of Propanolamine* and *Degradation Products* in the *Cyclophosphamide* monograph, which appears elsewhere in this issue of *PF*.

(HDQ: M. Marques) RTS—C73270

Add the following:

■**Tetramethylbenzidine,** *(4-(4-Amino-3,5-dimethylphenyl)-2,6-dimethylaniline; 3,3′,5,5′ Tetramethylbenzidine; 3,3′5,5′-Tetramethyl-[1,1′-biphenyl] 4,4′-diamine)* $C_{16}H_{20}N_2$—**240.34** [*54827-17-7*]—Use a suitable grade.■2S *(USP33)*

Test Solutions

BRIEFING

Lead Subacetate TS, *USP 32* page 866. It is proposed to simplify the preparation of this *Test solution* harmonizing with the text in the *British Pharmacopoeia*.

(HDQ: M. Marques.) RTS—C75914

Change to read:

Lead Subacetate TS~~ Triturate 14 g of lead monoxide to a smooth paste with 10 mL of water, and transfer the mixture to a bottle, using an additional 10 mL of water for rinsing. Dissolve 22 g of lead acetate in 70 mL of water, and add the solution to the lead oxide mixture. Shake it vigorously for 5 minutes, then set it aside, shaking it frequently, during 7 days. Finally filter, and add enough recently boiled water through the filter to make 100 mL.~~

■Dissolve 40.0 g of lead acetate in 90 mL of carbon dioxide-free water. Adjust with 10 M sodium hydroxide to a pH of 7.5, centrifuge, and use the clear supernatant solution. It contains NLT 16.7% (w/w) and NMT 17.4% (w/w) of Pb in a form corresponding to the formula $C_8H_{14}O_{10}Pb_3$. The solution remains clear when stored in a well-closed container.■2S *(USP33)*

BRIEFING

Starch TS, *USP 32* page 869. It is proposed to include the information about using commercially available solutions stabilized with compounds other than mercury salts.

(HDQ: M. Marques.) RTS—C75665

Change to read:

Starch TS—Mix 1 g of soluble starch with 10 mg of red mercuric iodide and sufficient cold water to make a thin paste. Add 200 mL of boiling water, and boil for 1 minute with continuous stirring. Cool, and use only the clear solution. [NOTE—Commercially available, stabilized starch indicator solutions may be used,

■including mercury-free solutions preserved with other compounds such as salicylic acid.]■2S (*USP33*)

Chromatographic Columns (Packings)

BRIEFING

L## (Emtricitabine, Chirobiotic V). It is proposed to add this new column designation used to validate the test for *Organic Impurities* in the USP Pending Monograph *Emtricitabine*.

(HDQ: M. Marques) RTS—C76702

Add the following:

■**L## (Emtricitabine, Chirobiotic V)**—Vancomycin glycopeptide covalently bonded to silica particles, 100 Angstroms, about 5 μm in diameter.■2S (*USP33*)

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 32* page 881, page 4014 of the *First Supplement*, and page 992 of *PF* 35(4) [July–Aug. 2009].

(HDQ) RTS—C34840; C44029; C55687; C61270

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	$T_{▲USP33}$
Add the following:	
■Amlodipine Besylate Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
■Azithromycin Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Balsalazide Disodium Capsules	$T_{■2S\ (USP33)}$
Add the following:	
■Carbidopa and Levodopa Tablets, Extended-Release	W, $LR_{■1S\ (USP33)}$
Add the following:	
■*Crypthecodinium cohnii* Oil Capsules	T, $LR_{■2S\ (USP33)}$

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Change to read:	
■Dantrolene Sodium Capsules	T, ~~LR~~ $_{■2S\ (USP32)}$
Add the following:	
■Fluconazole Tablets	$W_{■2S\ (USP32)}$
Add the following:	
■Granisetron Hydrochloride Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Guggul Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Ketoprofen Capsules, Extended-Release	$T_{■2S\ (USP32)}$
Add the following:	
■Lamivudine and Zidovudine Tablets	W, $LR_{■1S\ (USP33)}$
Add the following:	
■Lisinopril Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	$LR_{■1S\ (USP33)}$
Add the following:	
■Loratadine Orally Disintegrating Tablets	$T_{■1S\ (USP33)}$
Add the following:	
■Losartan Potassium Tablets	$T_{■2S\ (USP32)}$

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Morphine Sulfate Tablets, Extended-Release	T, LR■2S (*USP33*)
Add the following:	
■Mycophenolate Mofetil Capsules	W, LR■2S (*USP33*)
Add the following:	
■Mycophenolate Mofetil Tablets	W, LR■2S (*USP33*)
Add the following:	
■Nateglinide Tablets	T■1S (*USP33*)
Add the following:	
■Olanzapine Tablets	T, LR■1S (*USP33*)
Add the following:	
■Ondansetron Tablets	T, LR■2S (*USP32*)
Add the following:	
■Orlistat Capsules	T■2S (*USP33*)
Add the following:	
▲Oseltamivir Phosphate Capsules	W▲*USP33*
Add the following:	
■Oxcarbazepine Tablets	W■1S (*USP33*)
Add the following:	
■Ramipril Capsules	W■2S (*USP33*)
Add the following:	
■Ribavirin Capsules	W■1S (*USP33*)
Add the following:	
■Riluzole Tablets	W, LR■2S (*USP33*)

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Risedronate Sodium Tablets	W■2S (*USP32*)
Add the following:	
■Schizochytrium Oil Capsules	T, LR■2S (*USP33*)
Add the following:	
■Sumatriptan Tablets	W■2S (*USP33*)
Add the following:	
■Tacrolimus Capsules	T■1S (*USP33*)
Add the following:	
■Tamsulosin Hydrochloride Capsules	T■2S (*USP32*)
Add the following:	
■Telmisartan Tablets	W■1S (*USP33*)
Add the following:	
■ Terazosin Capsules	W, LR■2S (*USP33*)
Add the following:	
■ Terazosin Tablets	W, LR■2S (*USP32*)
Add the following:	
■Ticlopidine Hydrochloride Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Sulfate Tablets	W■1S (*USP33*)
Add the following:	
■Valacyclovir Tablets	T■2S (*USP33*)

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Valganciclovir Tablets	T■2S (*USP32*)
Add the following:	
■Zinc Gluconate Tablets	T, LR■1S (*USP33*)
Add the following:	
■Zolpidem Tartrate Tablets	W■2S (*USP33*)
Add the following:	
■Zolpidem Tartrate Extended-Release Tablets	W■1S (*USP33*)

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 32* page 890, page 4014 of the *First Supplement*, page 817 of *PF* 34(3) [May–June 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], page 188 of *PF* 35(1) [Jan.–Feb. 2009], page 464 of *PF* 35(2) [Mar.–Apr. 2009], page 651 of *PF* 35(3) [May–June 2009], and page 993 of *PF* 35(4) [July–Aug. 2009].

(HDQ) RTS—C42827; C44029; C58037; C61270; C62478; C64233; C65585; C67219; C67224; C68840; C69714; C72216

Add the following:

■**Clenbuterol Hydrochloride:** White or almost white, crystalline powder. Soluble in water and in alcohol, slightly soluble in acetone. It melts with decomposition at 173°.■2S (*USP33*)

Add the following:

■**Diethyl Sebacate:** A colorless to slightly yellow liquid. Miscible with alcohol, with ether, with other organic solvents, and with most fixed oils; insoluble or practically insoluble in water. *NF category*: Flavors and perfumes.■2S (*USP33*)

Add the following:

■**Docetaxel:** White or almost white, crystalline powder. Freely soluble in acetone; soluble in methanol; practically insoluble in water.■2S (*USP33*)

Add the following:

■**Dofetilide:** White to off-white powder. Soluble in 0.1 N sodium hydroxide, in acetone, and in 0.1 N hydrochloric acid; very slightly soluble in water and in isopropyl alcohol.■2S (*USP33*)

Add the following:

■**L-Glutamic Acid Hydrochloride:** A white, crystalline powder. 1 g dissolves in about 3 mL of water. It is almost insoluble in alcohol and in ether. Its solutions are acid to litmus. *NF Category*: Flavors and perfumes.■2S (*USP33*)

Change to read:

Hydrochlorothiazide: White, or practically white, practically odorless, crystalline powder. ~~Slightly soluble in water;~~

■■2S (*USP33*)
Freely soluble in sodium hydroxide solution, in *n*-butylamine, and in dimethylformamide;

■very slightly soluble in water;■2S (*USP33*)
sparingly soluble in methanol; insoluble in ether, in chloroform, and in dilute mineral acids.

Add the following:

■**Levetiracetam:** White to almost white powder. Very soluble in water; soluble in acetonitrile; practically insoluble in hexane.■2S (*USP33*)

Add the following:

▪**Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer:** White or almost white, free-flowing powder. Freely soluble in alcohol, in methanol, and in a 40 g/L solution of sodium hydroxide; soluble in solutions at pH values above pH 5.5 under salt formation; practically insoluble in ethyl acetate and in acidic aqueous solutions. *NF category*: Coating agent; film-forming agent.■2S (*USP33*)

Add the following:

▪**Methylpyrrolidone:** A clear, colorless liquid. Miscible with water and with most organic solvents including alcohol, ketones, and aromatic and chlorinated hydrocarbons. Boiling point: about 202°. Refractive index: about 1.469.■2S (*USP33*)

Add the following:

▪**Orlistat:** White to off-white fine powder or fine powder with lumps. Freely soluble in chloroform; very soluble in methanol and in alcohol; practically insoluble in water.■2S (*USP33*)

Add the following:

▪**Hydrogented Polydextrose:** Off-white to light tan-colored solid. Very soluble in water; soluble in alcohol; slightly soluble in glycerin and in propylene glycol. *NF category*: Bulking agent; coating agent; humectant; tablet binder; suspending and/or viscosity-increasing agent. ■2S (*USP33*)

Add the following:

▪**Riluzole:** White to slightly yellow powder or crystalline powder. Freely soluble in acetonitrile, in alcohol and in methylene chloride; slightly soluble in hexane; very slightly soluble in water.■2S (*USP33*)

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* an *IRA* , or a *Revision Bulletin.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents; Indicators; and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
General Notices (entire *General Notices and Requirements* revised)	34	1	40
USP Monographs			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetaminophen and Tramadol Hydrochloride Tablets (new)	35	1	56
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Medical Air—*Identification* (add); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide, Sulfur Dioxide, Limit of Nitric Acid and Nitrogen Dioxide; Packaging and Storage; Labeling*	35	4	828
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Tablets—*Assay*	31	3	726
Alendronate Sodium Tablets—*Dissolution*	35	1	59
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	35	1	62
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140
Ampicillin Sodium—*Dimethylaniline*	35	1	65

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Articaine Hydrochloride (new)	35	3	544
Aspirin—*Readily carbonizable substances*	34	5	1143
Atenolol—*Identification B*	35	3	545
Atenolol Tablets—*Dissolution*	35	1	66
Atorvastatin Calcium (new)	35	1	66
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Atropine Sulfate—*Identification C* (add); *Organic Impurities—Other Alkaloids* (delete), *Procedure* (add); *Melting Range or Temperature* (delete); *Optical Rotation* (delete); *Optical Rotation* (add); *Acidity* (delete); *Packaging and Storage; USP Reference Standards*	35	4	829
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam for Injection—*Assay*	34	4	906
Balsalazide Disodium (new)	35	4	830
Balsalazide Disodium Capsules (new)	35	4	832
Benzethonium Chloride—*Identification B, C* (delete), *Identification B* (add), *USP Reference Standards* (add)	35	4	833
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Benzoin—*Botanic characteristics, Identification*	35	1	70
Bicalutamide Tablets—*Labeling, Dissolution*	34	5	1147
Bisoctrizole (new)	32	2	309
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Limit of 11-ketobudesonide, Related compounds*	35	3	539
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Capecitabine—*Assay, Organic Impurities*	35	4	834
Capecitabine Tablets—*Assay, Dissolution, Organic Impurities*	35	4	835
Carbidopa—*Specific rotation*	35	1	73
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Carmustine (new)	35	3	546
Carmustine for Injection (new)	35	3	548
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Ciprofloxacin—*Identification B*	35	4	837
Ciprofloxacin Hydrochloride—*Identification B*	35	4	839
Ciprofloxacin Injection—*Identification A*	35	4	840
Ciprofloxacin Ophthalmic Solution—*Identification A*	35	4	842
Ciprofloxacin Tablets—*Identification A, B* (delete)	35	4	843
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Citalopram Tablets—*Identification A, Uniformity of Dosage Units, Organic Impurities*	35	4	844
Anhydrous Citric Acid *(Harmonization)—Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)—Sulfate*	31	3	750
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49
Clarithromycin Tablets—*Dissolution*	35	1	73
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Climbazole (new)	33	5	891

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Deferoxamine Mesylate—*Definition, Identification* (delete), *A* (add), *B* (add), *Assay, Organic Impurities—Procedure* (add), *Packaging and Storage*	35	4	847
Human Acellular Dermal Matrix (new)	35	3	558
Microsized Human Acellular Dermal Matrix (new)	35	3	561
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclazuril (new)	35	1	73
Diclofenac Sodium Delayed-Release Tablets—*Dissolution*	35	2	271
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Disulfiram—*Assay, Inorganic Impurities—Selenium*	35	4	848
Dolasetron Mesylate—*Impurities*	35	2	272
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Dronabinol Capsules—*Definition, Assay, USP Reference Standards*	35	3	549
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Ensulizole—*USP Reference Standards*	35	4	849
Epirubicin Hydrochloride (new)	35	2	273
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158
Esomeprazole Magnesium—*Identification B, Other Components—Content of Magnesium, Optical Rotation* (delete)	35	3	550
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate—*Definition, Assay*	35	2	275
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931
Fluconazole Injection (new)	35	3	552
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766
Fluvestrant (new)	33	5	99
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Labeling* (add), *Dissolution*	34	6	1452
Glimepiride Tablets—*Dissolution*	33	3	411
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Helium—*Identification A* (delete), *B* (delete), *Identification* (add), *Assay, Inorganic Impurities—Carbon Monoxide, Odor, Packaging and Storage, Labeling* (add)	35	4	850
Heparin Sodium (entire monograph revised)	35	2	257
Heparin Sodium Injection (entire monograph revised)	35	2	266
Hydromorphone Hydrochloride Oral Solution (new)	35	4	851
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxypropyl Cellulose Ocular System—*Assay*	35	4	852
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Irbesartan—*Limit of azide*	34	5	1164
Itraconazole (new)	34	4	947
Ketoprofen Extended-Release Capsules (new)	34	4	951
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamivudine and Zidovudine Tablets (new)	35	2	277
Levothyroxine Sodium—*Organic Impurities—Procedure 1, Procedure 2* (add), *Packaging and Storage, Labeling* (add), *USP Reference Standards*	35	3	555
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium Tablets (new)	34	5	1164
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263
Megestrol Acetate Oral Suspension—*Dissolution*	35	1	75
Meloxicam—*Impurities, Procedure 1*	35	2	278
Mesna (new)	34	5	1168
Metformin Hydrochloride Extended-Release Tablets—*Dissolution*	35	1	76
Methylbenzethonium Chloride—*Identification B* (delete), *C* (delete), *D* (delete), *B* (add); *USP Reference Standards* (add)	35	4	853
Methylcellulose (new)—*Stage 6 Harmonization*	35	3	683
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Misoprostol (new)	35	3	564
Mometasone Furoate Cream—*Packaging and storage, Related compounds* (add), *Assay*	35	1	82
Mometasone Furoate Ointment—*Packaging and storage, Related compounds* (add), *Assay*	35	1	84

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Mometasone Furoate Topical Solution—*Packaging and storage, Related compounds* (add), *Assay*	35	1	87
Morantel Tartrate—*pH*	32	6	1735
Morphine Sulfate Extended-Release Capsules—*Assay, Organic Impurities—Procedure, USP Reference Standards*	35	3	565
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Nasal Ointment (new)	34	4	966
Mycophenolate Mofetil—*Identification, Melting range* (delete), *Related compounds, Assay*	35	1	89
Mycophenolate Mofetil Capsules (new)	35	4	854
Mycophenolate Mofetil Tablets (new)	35	4	856
Naratriptan Hydrochloride Oral Suspension (new)	35	1	90
Nefazodone Hydrochloride—*Identification*	35	3	540
Nevirapine Oral Suspension—*Organic Impurities—Procedure*	35	4	857
Nateglinide (new)	34	6	1463
Nateglinide Tablets (new)	35	2	281
Niacinamide—*Readily carbonizable substances*	34	5	1176
Nitrofurantoin—*Packaging and storage*	35	1	92
Nitrofurantoin Capsules—*Packaging and storage*	35	1	92
Nitrofurantoin Oral Suspension—*Packaging and storage*	35	1	92
Nitrofurantoin Tablets—*Packaging and storage*	35	1	92
Nitrous Oxide—*Definition; Identification A, B* (delete), *C* (delete); *Assay; Inorganic Impurities—Ammonia, Nitric Oxide, Nitrogen Dioxide, Halogens, Carbon Monoxide, Carbon Dioxide; Water; Packaging and Storage; Labeling* (add)	35	4	859
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethynodrel (delete entire monograph)	35	1	92
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine Tablets (new)	35	2	282
Olopatadine Hydrochloride (new)	35	3	567
Olopatadine Hydrochloride Ophthalmic Solution (new)	35	3	568
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Oseltamivir Phosphate (new)	34	6	1468
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin Injection (new)	35	2	284
Oxaliplatin for Injection (new)	34	6	1473
Oxazepam Capsules—*Dissolution*	35	2	286
Oxcarbazepine (new)	34	5	1177
Oxcarbazepine Tablets (new)	34	6	1478
Oxybutynin Chloride Tablets—*Dissolution*	35	1	93
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxycodone Hydrochloride Extended-Release Tablets—*Related compounds*	31	4	1104
Oxygen—*Identification—Procedure, B* (delete); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide; Packaging and Storage; Labeling*	35	4	861
Oxygen 93 Percent—*Identification A, B* (delete); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide; Packaging and Storage; Labeling*	35	4	862
Oxymetazoline Hydrochloride Nasal Solution—*pH*	33	5	932
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Pantoprazole Oral Suspension (new)	35	4	863
Paricalcitol—*Identification, Assay*	33	2	252

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Pectin—*Chemical information; Definition; Identification—A, B, C, D* (delete), *Procedure* (add); *Assay—Methoxy Groups* (name change), *Galacturonic Acid, Methoxy Groups* (add); *Impurities—Lead, Procedure 1, Procedure 2* (add), *Procedure 3* (add); *Microbial Enumeration Tests; Packaging and Storage; Labeling; USP Reference Standards* (add)	35	2	287
Penicillamine Capsules—*Dissolution*	31	2	436
Pentamidine Isethionate (new)	35	3	570
Pentobarbital—*Identification B* (delete), *C, Assay, Organic Impurities—Procedure, Melting Range or Temperature* (delete)	35	4	864
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Permethrin (new)	32	4	1100
Permethrin Cream (new)	34	1	103
Petrolatum (new)—*Harmonization*	28	2	569
White Petrolatum (new)—*Harmonization*	28	2	570
Liquefied Phenol—*Identification* (add), *Other requirements*	35	1	93
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Praziquantel Tablets—*Dissolution*	35	2	291
Primidone—*Identification B, C* (delete), *Assay, Organic Impurities—Procedure, Melting Range or Temperature* (delete), *USP Reference Standards*	35	3	571
Primidone Tablets—*Assay, Organic Impurities—Procedure* (add), *USP Reference Standards*	35	3	573
Promethazine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	292
Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution (new)	35	2	295
Promethazine and Phenylephrine Hydrochloride Oral Solution (new)	35	2	298
Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	301
Propafenone Hydrochloride—*USP Reference standards, Chromatographic purity* (delete), *Related compounds* (add)	35	1	94
Propoxyphene Hydrochloride—*Definition, Assay, Organic Impurities—Procedure, Melting Range or Temperature* (delete)	35	4	865
Propoxyphene Hydrochloride Capsules—*Identification B* (delete), *Identification C*	35	3	574
Psyllium Husk—*Impurities—Heavy Metals* (add), *Procedure 3* (add)	35	2	304
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ramipril—*Definition, Assay*	31	3	787
Ramipril Capsules (new)	35	4	867
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Repaglinide Tablets—*Loss on Drying* (delete)	35	2	306

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Ribavirin Capsules (new)	35	3	576
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Risperidone Oral Solution (new)	35	4	870
Ritonavir—*Identification*	35	1	95
Salmeterol Xinafoate (new)	35	2	307
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sennosides—*Content of Sennosides A and B* (add), *USP Reference Standards* (add)	35	2	308
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Sulfate—*Assay*	34	5	1192
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadiazine Tablets—*Dissolution*	35	3	577
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfinpyrazone—*Identification A, B* (add), *Melting Range or Temperature* (delete), *Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	35	3	577
Sumatriptan Tablets (new)	35	4	871
Tacrolimus (new)	35	2	310
Tacrolimus Capsules (new)	35	2	312
Tamsulosin Hydrochloride (new)	35	3	578
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Telmisartan (new)	35	3	580
Telmisartan Tablets (new)	35	3	581
Terazosin Capsules (new)	35	4	872
Terazosin Tablets (new)	35	4	874
Terbinafine Oral Suspension (new)	35	1	96
Terbutaline Oral Suspension (new)	35	1	97
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride Oral Suspension (new)	35	1	98
Ticlopidine Hydrochloride (new)	35	3	582
Ticlopidine Hydrochloride Tablets (new)	35	3	584
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798
Tioconazole—*Assay*	35	4	875
Tizanidine Tablets—*Dissolution*	35	3	585
Topiramate Tablets (new)	34	5	1197
Tramadol Hydrochloride (new)	34	5	1200
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Tranylcypromine Sulfate (new)	35	2	314
Tranylcypromine Tablets (new)	35	3	587
Trenbolone Acetate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Limit of trenbolone acetate 17α-isomer* (delete), *Related compounds* (add), *Assay*	35	1	100
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tromethamine—*Melting Range or Temperature*	35	2	316
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Ursodiol Tablets—*Identification, Assay*	35	4	876
Valacyclovir Hydrochloride (new)	35	3	589
Valacyclovir Tablets (new)	35	4	878
Valganciclovir Tablets (new)	33	1	89
Valproic Acid Capsules—*Disintegration* (delete)	35	3	591
Valrubicin—*Definition, USP Reference standards, Identification, Loss on drying* (delete), *Water* (add), *Limit of residual solvents* (delete), *Related compounds, Assay*	35	1	103
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486
Vancomycin Hydrochloride—*Identification, Inorganic Impurities—Heavy Metals* (add), *Organic Impurities—Procedure: Limit of Monodechlorovancomycin, Sterility Tests* (add), *Bacterial Endotoxins Test* (add), *Composition of Vancomycin, Labeling* (add)	35	4	879
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Identification* (add), *Assay, Uniformity of Dosage Units* (add), *Inorganic Impurities—Heavy Metals* (delete), *pH* (add), *Water Determination* (add), *Composition of Vancomycin, Other Requirements, Labeling* (add)	35	4	881
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Vincristine Sulfate Injection—*Identification*	35	1	106
Vincristine Sulfate for Injection—*Identification*	35	1	106
Pure Steam (new)	31	2	467
Water for Hemodialysis—*Bacterial endotoxins*	31	2	468
Water for Injection—*Definition, Bacterial Endotoxins Test, Water Conductivity, Sterility Tests* (add), *Packaging and Storage* (add), *Labeling* (add)	35	2	316
Purified Water—*Definition, Packaging and Storage* (add), *Labeling* (add)	35	2	317
Sterile Water for Inhalation—*Definition*	35	4	883
Sterile Water for Injection—*Oxidizable substances*	31	3	803
Sterile Water for Irrigation—*Oxidizable substances*	31	3	804
Sterile Purified Water—*Oxidizable substances*	31	3	804
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Ziprasidone Hydrochloride (new)	35	3	592
Zolpidem Tartrate (new)	34	6	1487
Zolpidem Tartrate Extended-Release Tablets (new)	35	3	595
Zolpidem Tartrate Tablets (new)	35	4	883
Zonisamide (new)	34	6	1489
Dietary Supplements Monographs			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
N-Acetyltyrosine (new)	35	1	107
Ashwagandha (new)	35	4	885
Powdered Ashwagandha (new)	35	4	886
Powdered Ashwagandha Extract (new)	35	4	888
Boswellia Serrata (new)	35	4	890
Boswellia Serrata Extract (new)	35	4	891
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Crypthecodinium Cohnii Oil (new)	35	4	892

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Glutamic Acid (new)	34	4	997
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Schizochytrium Oil (new)	35	4	894
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Vitamin A Oral Liquid Preparation (new)	35	3	596
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Gluconate Tablets (new)	35	3	597
USP General Test Chapters			
⟨1⟩ Injections—*Labeling on Ferrules and Cap Overseals* (delayed date to May 1, 2010); *Ingredients, Foreign and Particulate Matter*	35	3	601
⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests (new)	35	3	602
⟨11⟩ USP Reference Standards	31	2	507
	31	6	1680
	32	4	1161
	33	1	95
	33	5	981
	34	2	332
	34	3	680
	34	4	1021
	34	5	1230
	34	6	1531
	35	1	144
	35	2	330
	35	3	612
	35	4	913
⟨11⟩ USP Reference Standards—*Stage 6 Harmonization*	35	4	1022
⟨41⟩ Weights and Balances—*Introduction, Repeatability, Verification of Accuracy, Calibration Check*	35	2	331
⟨63⟩ Mycoplasma Tests (new)	35	1	146
⟨85⟩ Bacterial Endotoxins Test—*Stage 6 Harmonization*	35	3	695
⟨92⟩ Growth Factors and Cytokines Used in Cell Therapy Manufacturing (new)	35	4	915
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨197⟩ Spectrophotometric Identification Tests (entire chapter revised)	35	1	153
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨223⟩ Dimethylaniline—*Chromatographic System, Procedure*	35	1	156
⟨228⟩ Ethylene Oxide and Dioxane (new)	35	4	917
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨331⟩ Amphetamine Assay (delete entire chapter)	35	4	920
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨381⟩ Elastomeric Closures for Injections—*Introduction*	35	3	614
⟨413⟩ Impurities Testing in Medical Gases (new)	35	4	920
⟨415⟩ Medical Gases Assay (new)	35	4	921
⟨429⟩ Light Diffraction Measurement of Particle Size (new)—*Stage 6 Harmonization*	35	3	707
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	35	2	334
⟨525⟩ Sulfur Dioxide—*Method IV* (add), *Method V* (add)	35	2	341
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density (new)—*Stage 6 Harmonization*	35	3	715
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533
⟨696⟩ Characterization of Crystallinity Determination by Solution Calorimetry—*Stage 4 Harmonization*	35	3	675
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨711⟩ Dissolution—*Stage 6 Harmonization*	35	3	719
⟨725⟩ Topical and Transdermal Drug Products—Product Performance Tests (new)	35	3	615

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	35	3	626
⟨741⟩ Melting Range or Temperature—*Introduction; Procedure for Class I, Apparatus II; Procedure for Class Ia, Apparatus II* (add); *Procedure for Class Ib, Apparatus II* (add)	35	4	925
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨788⟩ Particulate Matter in Injections—*Introduction*	35	3	628
⟨795⟩ Pharmaceutical Compounding—Nonsterile Preparations (entire chapter revised)	35	4	926
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨851⟩ Spectrophotometry and Light-Scattering (entire chapter revised)	35	1	157
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨905⟩ Uniformity of Dosage Units—*Stage 6 Harmonization*	35	3	724
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	35	2	346
⟨941⟩ X-Ray Diffraction (new)—*Stage 6 Harmonization*	35	3	731
General Information Chapters			
⟨1024⟩ Bovine Serum (new)	35	3	628
⟨1033⟩ Biological Assay Validation (new)	35	2	349
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1075⟩ Good Compounding Practices (delete entire chapter)	35	4	942
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1097⟩ Bulk Powder Sampling Procedures (new)	35	2	367
⟨1113⟩ Microbial Identification (new)	35	1	167
⟨1117⟩ Microbiological Best Laboratory Practices—*Introduction, Media Preparation and Quality Control, Maintenance of Microbiological Cultures, Maintenance of Laboratory Equipment, Laboratory Layout and Operations, Sample Handling* (add), *Microbiological Media Incubation Times* (add), *Training of Personnel, Laboratory Resources* (add), *Documentation, Maintenance of Laboratory Records, Interpretation of Assay Results*	35	4	945
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1180⟩ Human Plasma (new)	35	2	388
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation* (delete)	35	4	952
⟨1225⟩ Validation of Compendial Procedures—*Validation*	35	2	444
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1235⟩ Vaccines for Human Use—General Considerations (new)	35	4	960
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	35	2	448
⟨1788⟩ Particulate Matter Determination in Parenteral and Ophthalmic Products (new)	34	2	421
Dietary Supplements Chapters			
⟨2040⟩ Disintegration and Dissolution of Dietary Supplements—*Disintegration, Dissolution*	35	4	984
Reagents, Indicators, and Solutions			
Reagents, Indicators, and Solutions—*Introduction*	35	1	176
Alcohol	35	1	177
Ammonium Molybdate	35	1	177

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
t-Butylthiol (new)	35	3	648
Calcium Acetate	35	4	990
Chromotropic Acid	35	1	177
Chromotropic Acid Disodium Salt	35	1	177
Cobalt Nitrate	35	3	648
Diaveridine	35	3	648
1,3-Dicaffeoylquinic Acid (new)	35	4	990
N,N-Dimethyldecylamine (new)	34	4	1041
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Heptyl *p*-Hydroxybenzoate (new)	35	2	460
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Methyl Red	35	4	990
p-Naphtholbenzein	35	3	648
Nitrogen Certified Standard (new)	35	4	990
93.0% Oxygen Certified Standard (new)	35	4	991
Oxygen in Nitrogen Certified Standard (new)	35	4	991
3.0% Oxygen in Nitrogen Certified Standard (new)	35	4	991
21.0% Oxygen in Nitrogen Certified Standard (new)	35	4	991
Oxygen–Helium Certified Standard (delete)	35	4	991
Pectate Lysate (new)	35	2	460
Phosphorous Acid (new)	35	1	178
Potassium Metabisulfite (new)	35	1	178
Potassium Sodium Tartrate	35	1	178
Sodium Acetate	35	2	461
Sodium Biphenyl	35	3	648
Sodium 1-Decanesulfonate	34	5	1319
Stannous Chloride	35	3	649
Sulfuric Acid, Nitrogen Free (new)	35	3	649
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Delta-8-tetrahydrocannabinol (new)	35	4	991
Test Solutions			
Acetic Acid, Glacial, TS	35	1	179
Denatured Alcoholic TS (new)	35	1	179
Cupric Citrate TS 2, Alkaline	35	1	179
Dibasic Sodium Phosphate TS	35	3	649
Volumetric Solutions			
Hydrochloric Acid, Normal (1 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N) in Methanol	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid	35	1	181
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Chromatographc Reagents			
Chromatographic Reagents—*Title, Packings*	35	1	182
Reference Tables			
Container Specifications for Capsules and Tablets	35	4	992
Description and Solubility	29	1	266
	33	5	1053
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
	35	1	188
	35	2	464
	35	3	651
	35	4	993
Description and Solubility—*Stage 6 Harmonization*	35	4	1022
Atomic Weights—*Standard Atomic Weights of the Elements*	35	1	189
Excipients			
USP and *NF* Excipients, Listed by Category	35	4	897
USP and *NF* Excipients, Listed by Category—*Stage 6 Harmonization*	35	4	1017

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
NF General Notices and Requirements—*Title* (delete), *''Official'' and ''Official Articles''* (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
NF Monographs			
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
Alpha-Lactalbumin (new)	34	3	670
Amylene Hydrate—*Identification A, B, C* (delete)	35	4	903
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Benzyl Alcohol—*Stage 6 Harmonization*	35	3	685
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Stage 6 Harmonization*	35	4	1018
Silicified Microcrystalline Cellulose (new)	34	5	1218
Chitosan (new)	35	1	115
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone (new)—*Stage 4 Harmonization*	35	3	671
Cystine (new)	35	1	122
Desoxycholic Acid (new)	34	6	1523
Egg Phospholipids (new)	33	4	703
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity, Coagulum content*	35	1	123
Ethyl Maltol (new)	34	5	1224
Ethylene Glycol and Vinyl Alcohol Graft Copolymer (new)	35	2	324
Ethylparaben—*Harmonization*	34	6	1594
Fumaric Acid—*Identification*	35	3	598
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Hydroxypropyl Cellulose—*Identification*	35	1	124
Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	672
Low-Substituted Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	673
Lactobionic Acid (new)	35	4	904
Anhydrous Lactose—*Stage 4 Harmonization*	35	4	1013
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methacrylic Acid Copolymer—*Title change, Chemical information* (add), *Definition, Identification B, Assay, Organic Impurities—Procedure: Limit of Monomers, Viscosity, Packaging and Storage, Labeling*	35	4	905

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Methacrylic Acid and Ethyl Acrylate Copolymer (new)	35	4	907
Methacrylic Acid and Methyl Methacrylate Copolymer (new)	35	4	909
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Viscosity, Limit of monomers, Coagulum content*	35	1	124
Methyl Alcohol—*Readily carbonizable substances*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Identification; Assay; Inorganic Impurities—Carbon Monoxide, Limit of Oxygen; Odor; Packaging and Storage; Labeling*	35	4	910
Nitrogen 97 Percent—*Definition; Identification; Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide, Sulfur Dioxide, Limit of Nitric Oxide and Nitrogen Dioxide; Packaging and Storage*	35	4	911
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Olive Oil—*CAS number* (add), *Definition, Packaging and storage, Identification* (add), *Fatty acid composition* (add), *Specific gravity* (delete), *Cottonseed oil* (delete), *Peanut oil* (delete), *Sesame oil* (delete), *Teaseed oil* (delete), *Absence of sesame oil* (add), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Specific absorbance* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add)	35	1	126
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete), *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227
Polyethylene Glycol—*Harmonization*	31	3	897
Polyoxyl 15 Hydroxystearate (new)	35	1	128
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polysorbate 80—*Stage 6 Harmonization*	35	4	1019
Polyvinyl Acetate (new)	34	6	1526
Polyvinyl Acetate Dispersion (new)	35	1	134
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylene Glycol Dilaurate—*Chemical information, Identification A, Assay*	35	3	599
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Corn Starch—*Stage 6 Harmonization*	35	3	687
Hydrogenated Starch Hydrolysate (new)	35	1	136
Pea Starch (new)	35	1	140
Potato Starch (new)—*Stage 6 Harmonization*	35	3	689
Rice Starch (new)—*Stage 6 Harmonization*	35	3	690
Wheat Starch (new)—*Stage 6 Harmonization*	35	3	692
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Sucrose Palmitate (new)	35	2	326
Sucrose Stearate (new)	35	2	328
Tagatose (new)	30	5	1672
Tetrafluoroethane (new)	31	6	1672
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	***PF Volume, Issue, and Page Numbers of Canceled Proposals***		
	Vol.	***No.***	***Page(s)***
USP Monographs			
Carvedilol Tablets—*Title* (add), *Definition* (add), *Packaging and storage* (add), *USP Reference standards* (add), *Identification* (add), *Uniformity of dosage units* (add), *Related compounds* (add), *Assay* (add)	33	5	888
Conjugated Estrogens—*Definition*	30	3	840
Desogestrel and Ethinyl Estradiol Tablets—*Related compounds*	30	5	1604
Estradiol Vaginal Inserts—*Dissolution*	31	6	1617
Flavoxate Hydrochloride Tablets—*Dissolution* (add)	33	6	1174
Hydrocodone Bitartrate and Homatropine Methylbromide Tablets—*Dissolution*	30	3	853
Isotretinoin Capsules—*Labeling (add), Dissolution*	34	4	942
Ketoprofen Extended-Release Capsules—*Drug release*	31	5	1378
Leflunomide Tablets—*Dissolution*	31	5	1383
Mirtazapine Orally Disintegrating Tablets—*Water, Method Ia* (add)	33	6	1189
Norethindrone Tablets—*Dissolution* (add)	32	6	1736
Norethindrone Tablets—*Dissolution* (add)	33	6	1193
†Orlistat Capsules (entire submission)	32	6	1739
Piperacillin and Tazobactam for Injection—*Definition* (add), *USP Reference standards* (add), *pH* (add), *Particulate matter* (add), *Assay* (add)	31	2	439
Promethazine Hydrochloride—*USP Reference standards, Related substances*	32	2	365
Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add), *Assay*	32	2	367
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add)	32	4	1107
Terbinafine Hydrochloride—*Melting range*	34	5	1197
Dietary Supplements			
Asian Ginseng Capsules (entire submission)	30	2	571
USP General Test Chapters			
⟨191⟩ Identification Tests—General—*Acetate, Ammonium*	33	4	719
USP General Information Chapters			
⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire submission)	31	2	524
⟨1024⟩ Bovine Serum (entire submission)	34	3	776
⟨1235⟩ Vaccines for Human Use—General Considerations (entire submission)	34	5	1297
NF Monographs			
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
Methacrylic Acid Copolymer (entire submission)	33	6	1251
Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
Sucralose—*Related compounds*	33	6	1255

† New cancellation in *PF* 35(5).

STAGE 4 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF,* this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

MONOGRAPHS (USP)

BRIEFING

Petrolatum. The United States Pharmacopeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Petrolatum monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **OFFICIAL INQUIRY STAGE 4** document, is based on comments from the European and Japanese pharmacopeias. Changes from the existing USP monograph include the following:

1. *Definition*. The term "stabilizer" has been replaced by "antioxidant" at the request of the EP to provide a limitation on a single type of additive.
2. *Identification*. Added an infrared absorption requirement.
3. *Packaging and Storage*. Added the statement, "protected from light" based on comments received.
4. *Labeling*. Added the statement, "Where the labeling indicates the consistency, determine compliance using the test for *Consistency*." The test for *Consistency*, which measures a functionality parameter of the article, is not intended to be a required test in this harmonized monograph. Therefore, the proposed *Labeling* wording is intended to indicate that the test for *Consistency* in the harmonized monograph is to be used to determine consistency values if such values are reported in the labeling for the article.
5. *Specific Gravity*. Deleted based on comments that the wide test limits are not suitable to characterize the article.
6. *Melting Range or Temperature*. On the basis of comments from the EP, the range is widened from 38°–60° to 40°–75° to better reflect the global market.
7. *Polycyclic Aromatic Hydrocarbons*. On the basis of requests from global regulatory authorities, a new test has been added based on the existing EP monograph.

(EM2: Kevin Moore.) RTS—C76301

Add the following:

Petrolatum

DEFINITION

Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum. It may contain a suitable antioxidant.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨**197F**⟩

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION**

  Sample: 2 g

  Analysis: Heat the *Sample* in an open porcelain or platinum dish over a Bunsen flame.

  Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.1% of residue.

Organic Impurities

- **PROCEDURE 1: ORGANIC ACIDS**

  Sample solution: 20.0 g of Petrolatum in 100 mL of neutralized alcohol and water (1:1). Agitate thoroughly, and heat to boiling.

  Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol-water layer.

  Acceptance criteria: NMT 400 µL of 0.1 N sodium hydroxide is required.

- **PROCEDURE 2: POLYCYCLIC AROMATIC HYDROCARBONS**

  Sample solution: 1.0 g of Petrolatum in 50 mL of hexane that has been previously shaken twice with 10 mL of dimethyl sulfoxide

  Reference solution: 6.0 mg/L of USP Napthalene RS in dimethyl sulfoxide

  Analysis: Transfer the *Sample solution* to a 125-mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 mL of dimethyl sulfoxide. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel. Repeat the extraction with a further 20 mL of dimethyl sulfoxide. Shake vigorously the combined lower layers with 20 mL of hexane for 1 min. Allow to stand until two clear layers are formed. Separate the lower layer and dilute with dimethyl sulfoxide to 50.0 mL. Measure the absorbance over the range 260–420 nm using a path length of 4 cm and as compensation liquid the clear lower layer obtained by vigorously shaking 10 mL of dimethyl sulfoxide with 25 mL of hexane for 1 min. Measure the absorbance of the *Reference solution* at the maximum at 278 nm using a path length of 4 cm and dimethyl sulfoxide as compensation liquid.

  Acceptance criteria: At no wavelength in the range of 260–420 nm does the absorbance of the *Sample solution* exceed that of the *Reference solution* at 278 nm, corresponding to NMT 300 ppm.

SPECIFIC TESTS

- **COLOR**

  Sample: 10 g

  Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 15- × 150-mm test tube, keeping the petrolatum melted.

  Acceptance criteria: The petrolatum is not darker than a solution made by mixing 3.8 mL of ferric chloride CS and 1.2 mL of cobaltous chloride CS in a similar tube, the comparison of the two being made in reflected light against a white background, the petrolatum tube being held directly against the background at such an angle that there is no fluorescence.

- **MELTING RANGE OR TEMPERATURE,** *Class III* ⟨**741**⟩**:** 40°–75°
- **CONSISTENCY**

  Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point being truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm. The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

Sample: Petrolatum

Analysis: Place the required number of containers in an oven, and bring them and a quantity of *Sample* to a temperature of 82 ± 2.5°, pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5° over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at 25 ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25 ± 0.5° by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ±3%.

Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.

- **ALKALINITY**

Sample: 35 g

Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hotplate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.

Acceptance criteria: The solution does not acquire a pink color.

- **ACIDITY:** If the addition of phenolphthalein TS in the test for *Alkalinity* produces no pink color, add 0.1 mL of methyl orange TS.

Acceptance criteria: No red or pink color is produced.

- **FIXED OILS, FATS, AND ROSIN**

Sample: 10 g

Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.

Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **LABELING:** Label it to indicate the name and proportion of any added antioxidant. Where the labeling indicates the consistency, determine compliance using the test for *Consistency*.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Napthalene RS

- **DESCRIPTION AND SOLUBILITY**

Petrolatum: Unctuous yellowish to light amber mass, having not more than a slight fluorescence even after being melted. Is transparent in thin layers. Is free or practically free from odor and taste. Freely soluble in benzene, in carbon disulfide, in chloroform, and in turpentine oil; soluble in ether, in solvent hexane, and in most fixed and volatile oils; practically insoluble in cold and hot alcohol and in cold dehydrated alcohol; insoluble in water.

BRIEFING

White Petrolatum. The United States Pharmacopeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the White Petrolatum monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **OFFICIAL INQUIRY STAGE 4** document, is based on comments from the European and Japanese pharmacopeias. Changes from the existing USP monograph include the following:

1. *Definition*. The term "stabilizer" has been replaced by "antioxidant" at the request of the EP to provide a limitation on a single type of additive.
2. *Identification*. Added an infrared absorption requirement.
3. *Packaging and Storage*. Added the statement, "protected from light" based on comments received.
4. *Labeling*. Added the statement, "Where the labeling indicates the consistency, determine compliance using the test for *Consistency*." The test for *Consistency*, which measures a functionality parameter of the article, is not intended to be a required test in this harmonized monograph. Therefore, the proposed *Labeling* wording is intended to indicate that the test for *Consistency* in the harmonized monograph is to be used to determine consistency values if such values are reported in the labeling for the article.
5. *Specific Gravity*. Deleted based on comments that the wide test limits are not suitable to characterize the article.
6. *Melting Range or Temperature*. On the basis of comments from the EP, the range is widened from 38°–60° to 40°–75° to better reflect the global market.
7. *Polycyclic Aromatic Hydrocarbons*. On the basis of requests from global regulatory authorities, a new test has been added based on the existing EP monograph.

(EM2: Kevin Moore.) RTS—C76302

Add the following:

White Petrolatum

DEFINITION

White Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum. It may contain a suitable antioxidant.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨**197F**⟩

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION**

Sample: 2 g

Analysis: Heat the *Sample* in an open porcelain or platinum dish over a Bunsen flame.

Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.1% of residue.

Organic Impurities

- **PROCEDURE 1: ORGANIC ACIDS**

Sample solution: 20.0 g of White Petrolatum in 100 mL of neutralized alcohol and water (1:1). Agitate thoroughly, and heat to boiling.

Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol-water layer.

Acceptance criteria: NMT 400 µL of 0.1 N sodium hydroxide is required.

- **PROCEDURE 2: POLYCYCLIC AROMATIC HYDROCARBONS**

Sample solution: 1.0 g of White Petrolatum in 50 mL of hexane that has been previously shaken twice with 10 mL of dimethyl sulfoxide

Reference solution: 6.0 mg/L of USP Naphthalene RS in dimethyl sulfoxide

Analysis: Transfer the *Sample solution* to a 125-mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 mL of dimethyl sulfoxide. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel. Repeat the extraction with a further 20 mL of dimethyl sulfoxide. Shake vigorously the combined lower layers with 20 mL of hexane for 1 min. Allow to stand until two clear layers are formed. Separate the lower layer and dilute with dimethyl sulfoxide to 50.0 mL. Measure the absorbance over the range of 260–420 nm using a path length of 4 cm and as compensation liquid the clear lower layer obtained by vigorously shaking 10 mL of dimethyl sulfoxide with 25 mL of hexane for 1 min. Measure the absorbance of the *Reference solution* at the maximum at 278 nm using a path length of 4 cm and dimethyl sulfoxide as compensation liquid.

Acceptance criteria: At no wavelength in the range of 260–420 nm does the absorbance of the *Sample solution* exceed that of the *Reference solution* at 278 nm, corresponding to NMT 300 ppm.

SPECIFIC TESTS

- **COLOR**

Sample: 10 g

Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 15- × 150-mm test tube, keeping the White Petrolatum melted.

Acceptance criteria: The White Petrolatum is not darker than a solution made by mixing 1.6 mL of ferric chloride CS and 3.4 mL of water in a similar tube, the comparison of the two being made in reflected light against a white background, the White Petrolatum tube being held directly against the background at such an angle that there is no fluorescence.

- **MELTING RANGE OR TEMPERATURE,** *Class III* ⟨**741**⟩**:** 40°–75°
- **CONSISTENCY**

Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point being truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm. The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

Sample: White Petrolatum

Analysis: Place the required number of containers in an oven, and bring them and a quantity of *Sample* to a temperature of 82 ± 2.5°, pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5° over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at 25 ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25 ± 0.5° by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ±3%.

Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.

- **ALKALINITY**

Sample: 35 g

Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hotplate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the White Petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.

Acceptance criteria: The solution does not acquire a pink color.

- **ACIDITY:** If the addition of phenolphthalein TS in the test for *Alkalinity* produces no pink color, add 0.1 mL of methyl orange TS.

Acceptance criteria: No red or pink color is produced.

- **FIXED OILS, FATS, AND ROSIN**

Sample: 10 g

Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.

Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **LABELING:** Label it to indicate the name and proportion of any added antioxidant. Where the labeling indicates the consistency, determine compliance using the test for *Consistency*.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Napthalene RS

- **DESCRIPTION AND SOLUBILITY**

White Petrolatum: White or faintly yellowish, unctuous mass, transparent in thin layers even after cooling to 0°. Freely soluble in benzene, in carbon disulfide, and in chloroform; soluble in ether, in solvent hexane, and in most fixed and volatile oils; slightly soluble in cold or hot alcohol, and in cold dehydrated alcohol; insoluble in water.

STAGE 6 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). This section includes Stage 6 adopted text which is provided for information. USP cannot incorporate public comments at Stage 6 without consulting PDG partners. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors.*

INSTRUCTIONS TO AUTHORS

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication.

All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current copy of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current copy of *Pharmacopeial Forum* will offer examples of reference formats.

Copyright—Copyright transfer documents will be sent to authors after manuscripts have been accepted for publication.

Contact Person—USP will designate a Scientific Liaison in the Documentary Standards Division as the corresponding author. This ensures that USP receives all comments generated by the *Stimuli* article. Authors should contact the Scientific Liaison if they would like to receive copies of comments generated by their *Stimuli* articles.

Submission Instructions—Manuscripts must be submitted both as an electronic file and as a printed copy of the electronic file. Submit the text in Microsoft® Word or another current word-processing application. The preferred format for graphics submitted electronically is tagged image file format (TIFF). Photocopies are not acceptable. Manuscripts submitted for publication should be addressed to:

Pharmacopeial Forum
Executive Secretariat, USP
12601 Twinbrook Pkwy.
Rockville, MD 20852

General Chapter Management in the 2010–2015 Cycle

USP General Chapter Project Team, Prescription–Nonprescription Stakeholder Forum[a]; Susan de Mars, Darrell R. Abernethy, William F. Koch, Angela G. Long, Anthony J. DeStefano, and Roger L. Williams, *USP*[b]

ABSTRACT This *Stimuli* article summarizes approaches that the United States Pharmacopeial Convention (USP) intends to advance for revision of General Chapters in the 2010 to 2015 and subsequent cycles. The article represents in part the work of USP General Chapter Project Team of the Prescription–Nonprescription Stakeholder Forum and in part USP staff under the direction of the Chair of the Council of Experts. It is designed to inform and guide incoming chairs of the new USP Council of Experts in the 2010–2015 cycle. It is also intended to inform the public at large of the value and complexity of USP's General Chapters, which now number approximately 300. With this *Stimuli* article, USP announces plans for a General Chapter Redesign project that can incorporate some or all of the elements presented in this *Stimuli* article. Comments are welcome.

INTRODUCTION

Information about General Chapters in the *United States Pharmacopeia* (*USP*) appears on the USP Web site at http://www.usp.org/USPNF/submitMonograph/generalChapterWorkplans.html. General Chapters in *USP* support *USP* monographs as well as monographs in the *National Formulary* (*NF*), Pending Monographs (http://www.usp.org/standards/pending/guidelines.html) and Non-US monographs (http://www.usp.org/standards/international/guidelines.html). They also support dietary supplement monographs and thus will be reproduced in their entirely along with such monographs in USP's new *Dietary Supplement Compendium* (http://www.usp.org/products/dietarySupplementsCompendium/). They should conform increasingly with the General Tests and Assays of USP's *Food Chemicals Codex*. The interpretation and application of General Chapters is governed by the *General Notices and Requirements (General Notices)* of *USP* (*1*).

CURRENT STATUS AND OPPORTUNITIES FOR IMPROVEMENT

The basis for many of the General Chapters in *USP* was and to some extent remains to conserve text for print versions of *USP* and *NF* and to allow commonly used procedures to be updated across multiple monographs without the need to individually revise each monograph. The Council of Experts thus might advance a General Chapter when it could be called out in more than a few (e.g., five) monographs. USP's General Chapters are divided roughly into physical and chemical test categories. The numbering system for General Chapters has little underlying logic, although pursuant to the *General Notices*, General Chapters numbered below 1000 generally are considered required and General Chapters above 1000 are considered informative. General Chapters that support only dietary supplements monographs are numbered above 2000. The lack of consistent approaches over time has led to *USP* General Chapters that are not well organized and are not always consistent when viewed singly or collectively. Some attempts in this and the prior cycle were made to advance a more consistent approach. This *Stimuli* article summarizes these approaches and advances more as a means of informing and guiding both the Council of Experts and USP's stakeholders (manufacturers, compounding professionals, conformity assessment bodies, practitioners, and patients) toward a more consistent and logical presentation and understanding of General Chapters. A more structured, comprehensive set of approaches is expected to produce a more uniform and useful set of General Chapters for all of USP's compendia.

CURRENT STRUCTURE AND PRESENTATION OF GENERAL CHAPTERS

General Chapters below 1000—General Chapters numbered below 1000 in *USP* usually are called out in a monograph and thus are considered a requirement for the analyst in executing one or more monograph tests. Even where not specifically referred to in a monograph, these General Chapters may be a requirement under provisions in USP's *General Notices*. In addition, at times an enforcing body such as the US Food and Drug Administration (FDA) may consider these General Chapters a requirement regardless of whether they are referenced in monographs or the *General Notices*.

General Chapters above 1000—As noted above, USP generally intends General Chapters numbered above 1000 not to be required and instead are considered informative. However, if specifically referred to in a monograph or in a required General Chapter, such text may become mandatory. In addition, informative chapters can be made enforceable by being adopted officially by an enforcing body or being included in a manufacturer's

[a] See Appendix for the names of USP General Chapter Project Team of the Prescription–Nonprescription Stakeholder Forum.

[b] Correspondence should be addressed to: Anthony J. DeStefano, PhD, Vice President, General Chapters, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.6303; e-mail ajd@usp.org.

or compounding professional's Standard Operating Procedures. Despite USP's intent that these generally not be required, questions and concerns about their enforceability remain because of their inclusion in *USP* and *NF*, which are in their entirety official compendia of the United States.

In this cycle, as part of the *General Notices* revision made official in *USP 32*, the idea emerged that informative text might be moved out of *USP* into a companion volume that would not be part of an official compendium of the United States. Such text would be elaborated through the USP Council of Experts and would serve analysts in understanding and executing the tests of USP's compendia. No decision has been reached as of the date of this *Stimuli* article about whether to advance a separate volume of informative text, which may be considered in a subsequent revision to the *General Notices*.

Classification/Taxonomy of General Chapters—As a means of bringing more clarity and order to *USP*'s General Chapters, staff working with the Council of Experts created a 13-chart guide to General Chapters. The charts sort chapters by article and topic categories. Within each chart General Chapters use the ICH categories of universal tests (description, identification, assay, and impurities) and specific tests (e.g., physicochemical characterization, equipment, or water content) to group General Chapters. Although the charts may not be specifically useful to an analyst using a single monograph, they do present a number of coherent views of the purpose and utility of General Chapters in *USP*. In this context, the charts are designed to support and inform drug development and drug registration decision-making.

General Chapters by Analytical Procedures/Methods—Although General Chapters may cover a broad array of topics, required General Chapters should speak to one or more tests, procedures, and acceptance criteria. A procedure is the series of steps followed by the analyst in conducting a compendial test. In turn, a procedure can rely on any of several analytical methods. Methods can be grouped as shown in *Table 1* and are closely related to the procedures of a monograph.

GENERAL CHAPTERS PROJECT TEAM

Project Teams are established under the Prescription–Nonprescription Stakeholder Forum in accordance with the Rules and Procedures of the Council of Experts. In 2006, the Chair of the Council of Experts formed the General Chapters Project Team to review General Chapters. The team focused on the chapters assigned to the 10 Expert Committees that make up the 2005–2010 General Chapters Collaborative Group as well as the excipient chapters, resulting in the examination of more than 200 of the 300 existing General Chapters. The purpose of the Project Team was twofold: first to understand which General Chapters or parts thereof should remain or become required; second to determine which General Chapters should be updated. To facilitate decision-making, the Project Team established criteria against which to make decisions for each of the assigned General Chapters. These criteria are presented in *Figure 1.*

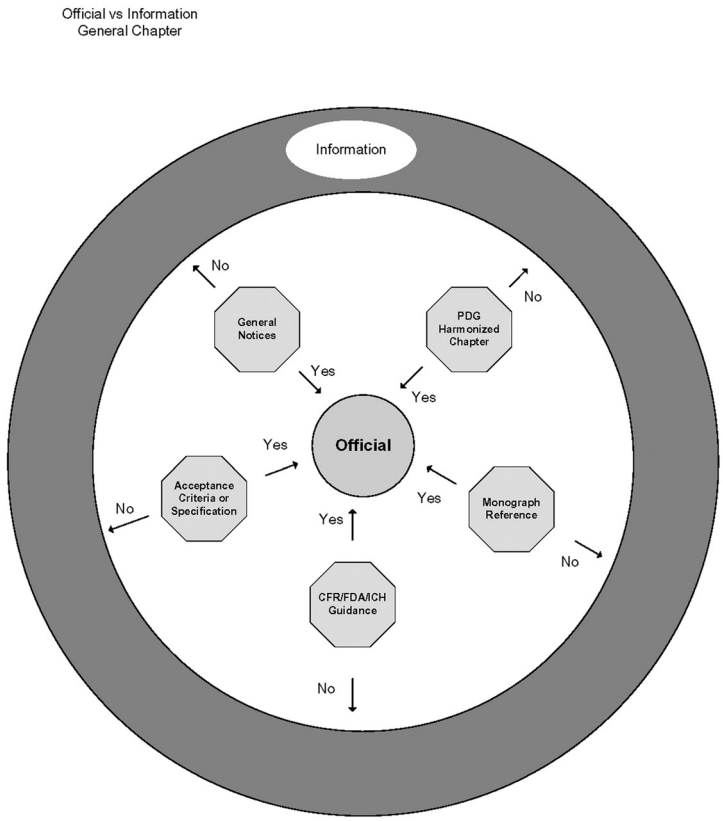


Figure 1. Project Team Decision Wheel for the Assessment of General Chapter Content Categorization.

Project Team members reviewed each of a set of assigned chapters and provided a recommendation about whether the contained standards should be included in the required series or should be considered informative. The team recommended reallocation of text for approximately 25% of the reviewed General Chapters and made recommendations with respect to the state of the technology of many of the chapters. This information will be used in the 2010–2015 cycle as input to revise content in General Chapters, moving text between required and informative General Chapters where needed according to specified criteria and providing guidance to the new Expert Committees with regard to technology considered out of date or no longer useful.

FUTURE OPPORTUNITIES: OBJECTIVES

Overall, USP expects that all General Chapters in USP compendia will be clear, concise, and highly useful to the analyst when considered alone in the performance of a monograph test and also collectively as a general representation of modern measurement science. Words should be kept to a minimum with careful editing for clarity. General Chapters that are informative should provide more general information without specifying procedures and acceptance criteria for official articles. Required and informative chapters can form comprehensive sets of overarching and interrelated text. The interrelationship between General Chapters should be clearly presented and carefully described. Specific opportunities to achieve this desired state through the General Chapter Redesign are presented in this section of the *Stimuli* article.

General Chapters Grouped According to Development/Registration Topics—General Chapters might be grouped by drug development topics according to relevant parts of the ICH Common Technical Document. This grouping could take into account all of the ICH Quality guidance documents. To some extent this has already occurred in current versions of *USP* and *NF*; e.g., the aforementioned charts are usually developed along the lines of the ICH Quality documents, and the concluding Monograph Redesign project provides a framework for *USP*'s monographs directed toward the control elements defined in ICH Module 3.

General Chapters Grouped According to Compendial Topics—*USP*'s General Chapters might be grouped according to compendial topic (*Table 2*).

Required General Chapters—USP intends that required General Chapters will be brief and will focus on procedures and acceptance criteria where needed for tests of a monograph. This may lead to the redesign of current below 1000 General Chapters, with the intent of editing and reformatting without necessarily changing the contained standard.

Informative General Chapters—USP intends that General Chapters above 1000 will be entirely informational and will not contain monograph-related requirements. Topics that might be covered in informational chapters include:

- Background, theory, and future directions/applications
- Areas that need standards, e.g., nanotechnology
- Safety approaches and information
- Guidance chapters for good food and drug practices
- Drug development and registration documents
- Supply chain management documents, including GMP analyses and comparisons
- Comparisons across the USP compendia.

Evaluation of Existing General Chapters—A key objective of the evaluation of a General Chapter is a critical assessment of both the quality of the science and its presentation. Chapter evaluation and follow-up recommendations for the next cycle will involve a series of decisions based on the current state of the chapter and its impact. These evaluations will be done independently by the Project Team, USP Staff, and the appropriate Expert Committees (see *Table 3*).

General Chapter Information Database—USP possesses the following information regarding the history, current status, and impact of each chapter. This information comprises a database that will be made available on the USP Web site.

1. Official status [e.g., Official, Official in *Pharmacopeial Forum* (*PF*), New in *PF*, or In Development]
2. If official, original official date and most recent official revision
3. Most recent publication in *PF*
4. Chapter impact—number of monographs referencing each chapter
5. Associated reference standards
6. Harmonization status
7. Responsible liaison
8. Current Expert Committee assignment.

Templates—USP's monograph redesign approach was based on a standardized template. USP expects this template will be useful for General Chapters as well, particularly for the procedural chapters considered required and thus will be closely allied with monographs. Templates are also expected to be useful in defining groups of General Chapters that speak to a common topic (see *Table 4*). The overall goal will be to provide continuity and consistency in General Chapter texts.

Harmonization—Intensive efforts during the past several decades have emphasized the importance of harmonizing General Chapters with other compendia. Harmonized tests, procedures, and acceptance criteria greatly assist an increasingly global pharmaceutical industry in developing one set of approaches applicable in multiple geographies. This approach also facilitates and leads to an internationally recognized reference measurement system for drugs. This will result in fewer required methods, increased testing efficiency, facilitation of global method transfers, and an advance toward modern measurement science.

These opportunities will require significant time and effort. With strong focus and a commitment from both USP and its stakeholders, the intent is to close the General Chapter Redesign project at the end of the 2015 cycle.

THE COUNCIL OF EXPERTS

The Council of Experts and its Expert Panels in the coming cycle are critical to the success of the General Chapter Redesign project. The methods undergirding the procedures of compendial text will fall primarily to three Expert Committees with focus on physical, chemical, and biological procedures. However, all Expert Committees will necessarily be involved in the overall effort. Careful staff support will be needed in the following areas.

Expert Committee Assignments/Reassignments—The number of Expert Committees has been reduced in the 2010–2015 cycle with the intent of creating topic-specific Expert Panels when needed (www.usp.org/pdf/EN/aboutUSP/expertCommitteeFocusAreas.pdf). Reassignment of work from Expert Committees in the current cycle to Expert Committees in the 2010–2015 cycle appears on the USP Web site at www.usp.org/USPNF/submitMonograph/generalChapterWorkplans.html.

Work Plan Establishment and Tracking—USP staff will provide Expert Committees devoted to consideration of General Chapters with a comprehensive set of information about each General Chapter as a means of defining work plans and to determine the need for and assignments of Expert Panels. Specific steps in the process are suggested in *Table 5*.

CONCLUSION AND NEXT STEPS

USP's General Chapters are key to the scientific progress of USP's compendia. Through recent *Stimuli* articles USP envisions an approach according to which future monographs might contain criteria for good procedures (*2*) with elimination of the step-by-step procedures that characterize current monographs. Acceptable procedures might be declared equivalent or better based on well-designed and -reviewed studies (*3*). The general approach relies on well-characterized reference materials that are certifiable through careful collaborative studies. The approach is in accord with modern principles of metrology (*4*) and also paves the way for further compen-

dial harmonization (*5*). General Chapters are integral to the advancement of this approach and to other scientific advances.

USP offers this *Stimuli* now to solicit comments on the proposed General Chapter Redesign initiative via the *Pharmacopeial Forum* process. As appropriate, comments will be incorporated into the project and used to inform newly elected chairs of the Council of Experts following the USP Convention elections in April 2010. Taken together with the impressive advances of the current cycle, the General Chapter Redesign Project may be considered a capstone in a series of efforts—*General Notices* revision, Monograph Redesign, and allied efforts—that advance USP's documentary and reference material standards to meet challenges of the 21st century to ensure access to good quality medicines for patients and consumers.

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APPENDIX: Members of USP General Chapter Project Team of the Prescription–Nonprescription Stakeholder Forum

(Name, Company Name, Stakeholder Association[a])
Phyllis Walsh, Schering-Plough Corporation, NJPQCA
Matthew K. Anderson, Baxter, PDA
Monica E. Caphart, FDA
Chung Chow Chan, Azopharma Contract Pharmaceutical Services, CVG
Kathleen Cimbala, Baxter, PDA
Sumitra M. Ghate, Eli Lilly, Midwest Compendial Discussion Group
Saul Gylys, Perrigo Company, CHPA
Diana Hickey, EMD Chemicals, IPEC
Mark W. Lehmann, PhD, Fort Dodge Animal Health, PhRMA
F. Scott Lyon, Mylan Pharmaceuticals, GPhA
Mark S. Saulter, Glaxo Smith Kline, Midwest Compendial Discussion Group
Diane Raccasi, FDA
Andrew M. Sopirak, Cephalon, NJPQCA
Ronald J. Tscherne, PhD, Hoffmann–La Roche, Inc., NJPQCA

[a] NJPQCA = New Jersey Pharmaceutical Quality Control Association; PDA = Parenteral Drug Association; CVG = Calibration and Validation Group (Canada); CHPA = Consumer Healthcare Products Association; IPEC = International Pharmaceutical Excipients Council; GPhA = Generic Pharmaceutical Association; and PhRMA = Pharmaceutical Research and Manufacturers Association.

Table 1. Methods in Support of Procedures

Chemical–Structural	Physical	Biological
Titrimetry	Mass	Immunoassay
Mass Spectrometry	Volume	Polymerase Chain Reaction
Ultraviolet Spectrometry	Temperature	Enzymatic Analysis
Infrared Spectrometry	Microscopy	Amino Acid Analysis
Near-infrared Spectrometry	Solubility	Peptide Mapping
Mid-infrared Spectrometry	Particle Count	Isoelectric Focusing
Thermogravimetry	Particle Size	Gel Electrophoresis
Differential Scanning Calorimetry	Structure	Capillary Electrophoresis
X-ray Fluorescence	Melting Point	
Nuclear Magnetic Resonance	Boiling Point	
Colorimetry	Water Content	
Liquid Chromatography	Ash Content	
Gas Chromatography	Hardness	
Thin-layer Chromatography	Reynolds Number	
Ion Chromatography	Viscosity	
pH	Chirality	
Gas Analysis	Polarity	
	Conductance • Electrical • Thermal	

Table 2. Comparison of ECs during the 2005–2010 and 2010–2015 Cycles

2005–2010 EC	2010–2015 EC
Parenteral Products Industrial	Pharmaceutical Dosage Forms
Biopharmaceutics	Pharmaceutical Dosage Forms
Aerosols	Pharmaceutical Dosage Forms
Pharmaceutical Dosage Forms	Pharmaceutical Dosage Forms
Microbiology and Sterility Assurance	Microbiology and Sterility Assurance
Packaging and Storage	Packaging, Storage, and Distribution
Statistics	Statistics
General Toxicology and Medical Devices	Toxicology
Pharmaceutical Waters	Chemical Analysis
General Chapters	Chemical Analysis and Physical Analysis
Biologics GCs	Biological Analysis
Excipient GCs	Chemical Analysis and Physical Analysis
Dietary Supplement GCs	Chemical Analysis
Compounding GCs	Physical Analysis

Table 3. Evaluation Approaches for General Chapters

Type	Technology	Impact[a]	Structure	Recommendation[b]
Below 1000	Current	H, M	Procedural, well written	Ok as is
Below 1000	Current	L	Procedural, well written	Needed? Y: To monograph N: Delete
Below 1000	Current	H, M	Procedural, wordy	Rewrite–redesign
Below 1000	Current	L	Procedural, wordy	Needed? Y: Rewrite–to monograph? N: Delete
Below 1000	Current	H, M	Mix of information and procedure	Rewrite–add above 1000 chapter as needed
Below 1000	Current	L	Mix of information and procedure	Needed? Y: Rewrite–remove info N: Delete
Below 1000	Outdated	H, M	Procedural	Update
Below 1000	Outdated	L	Procedural	Needed? Y: Update–to monograph? N: Delete
Below 1000	Outdated	H, M	Mix of information and procedure	Update–add above 1000 chapter
Below 1000	Outdated	L	Mix of information and procedure	Needed? Y: Update–to monograph? N: Delete
Above 1000	Current	N/A	Information only	Ok as is
Above 1000	Current	N/A	Mix of information and procedure	Rewrite and split if appropriate
Above 1000	Outdated	N/A	Information only	Needed? Y: Update N: Delete
Above 1000	Outdated	N/A	Mix of information and procedure	Needed? Y: Update and split if appropriate N: Delete

[a] H = high; M = medium; L = low; N/A = not applicable.
[b] Y = yes; N = no.

Table 4. Potential General Chapter Groupings

Type of Chapter	Representative Groupings
General	Method-specific (*Chromatography* ⟨621⟩, *Spectroscopy* ⟨851⟩)
	Test-specific (*Identification—General* ⟨191⟩, *Thin-layer Chromatography* ⟨201⟩)
	Procedural (*Loss on Drying* ⟨731⟩, *pH* ⟨791⟩)
Dosage Form–Specific	General (*Injections* ⟨1⟩, *Aerosols* ⟨601⟩)
	Performance (*Uniformity of Dosage Units* ⟨905⟩, *Dissolution* ⟨711⟩)
Monograph/Class/Family Specific Procedures	*Antibiotics* ⟨81⟩, *4-Epianhydrotetracycline* ⟨226⟩, *Methoxy Determination* ⟨431⟩
Biologics—Procedural	Bioassays (*Insulin* ⟨121⟩)
	Product or Product-specific Procedures (*LMWH Molecular Weight Determinations* ⟨207⟩, *Tests for 1,6-Anhydro Derivative for Enoxaparin Sodium* ⟨207⟩)
Biologics—Ancillary and Process Materials	Quality Attributes (*Protein A Quality Attributes* ⟨130⟩)
	Testing for Residuals (*Residual Protein A Testing* ⟨131⟩)

Table 5. Workplan Establishment and Tracking

Role	Task
EC Work Plan Establishment	Chapter basics acquired (existing chapter)
	Chapter evaluated against established criteria (existing chapter)
	Provide suggestions from staff/management
	Solicit input from stakeholders
	Solicit input from ECs and EPs
	Hold conference or workshop as needed
	Decision to add chapter to work plan
	Provide EC with chapter status/impact/priorities
EC Work Plan Management All Chapters	Assign chapters to sub-teams or establish EPs
	Establish work and meeting schedule
	Establish timelines and milestones
	Set up and manage meetings
	Ensure teams have all materials needed
	Track progress against time lines
	Assess progress vs time lines
	Propose corrective action as needed
	Implement corrective action and track progress

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Transfer of Analytical Procedures: A Proposal for a New General Information Chapter

Oscar Quattrocchi,[a] Greg Martin,[a] Dennis Runser,[a] Robert Iser,[b] Fred Xi,[a] Horacio Pappa[c]

ABSTRACT This *Stimuli* article presents the basis of a new General Information Chapter on method transfer. This Chapter continues a series of chapters devoted to providing guidance for the quality of the data-acquisition process (i.e., qualification, validation, and verification). The purpose of the procedure transfer process is to qualify the receiving laboratory to perform an analytical procedure that was developed in another laboratory. This concept is similar to the verification process described in USP General Information Chapter *Verification of Compendial Procedures* ⟨1226⟩. A section in the Chapter describes differences and similarities between verification and transfer. This *Stimuli* article aims to initiate discussion and solicit stakeholder comments.

INTRODUCTION

The testing of a pharmaceutical ingredient, in-process intermediate, or finished product is critical in establishing the quality of the final pharmaceutical product. The transfer of analytical procedures (TAP), also referred to as method transfer, is the documented process that qualifies a laboratory (the receiving unit) to use an analytical test procedure that originates in another laboratory (the transferring unit, also named the sending unit), thus ensuring that the receiving unit has the procedure knowledge and ability to perform the transferred analytical procedure as intended.

The purpose of this article is to summarize the types of transfers that may occur, including the possibility of waiver of any verification, and to outline the components of a transfer protocol. It is not the intent of this article to go into any detail about statistical methods. A summary of approaches for the statistically similar problem of comparison of procedures has been published (*1*). That article may become one or more General Chapters and, if so, would be referenced by any General Chapter that is developed regarding method transfer. Also, this Chapter does not encompass the transfer of microbiological or biological procedures.

TYPES OF TRANSFERS OF ANALYTICAL PROCEDURES

TAP can be performed and demonstrated by several approaches. The most common is comparative testing performed on homogeneous lots of the target material from standard production batches or samples intentionally prepared for the test (e.g., by spiking relevant accurate amounts of known impurities into samples). Other approaches include covalidation between laboratories, the complete or partial validation of the analytical procedures by the receiving unit, and the transfer waiver, which is an appropriately justified omission of the transfer process. It is possible to initiate and even finalize the transfer before the validation process is completed. However, the lack of complete knowledge about the variables of the procedure and particularly its robustness may complicate the transfer process by adding more variables to a system that should be as simple as possible.

Comparative Testing

Comparative testing is the most common method for TAP and requires the analysis of one or more samples of the same lot by both the transferring and the receiving units. Such analysis is based on an agreed and preapproved transfer protocol that provides the details of the procedure, the samples that will be used, and the predetermined acceptance criteria. The data generated by both parties are compared to the acceptance criteria in order to assess the equivalence of results between the transferring and the receiving units.

Covalidation between Two or More Laboratories

The laboratory that performs the validation of an analytical procedure is, by definition, qualified to run that method. Manufacturers can involve the receiving unit in an interlaboratory qualification, including them as a part of the validation team and thereby obtaining data for the assessment of reproducibility. This assessment is made using a preapproved transfer protocol that provides the details of the procedure, the samples to be used, and the predetermined acceptance criteria. In such cases it may not be necessary to perform the complete validation because the method transfer exercise is aimed at evaluating laboratory-to-laboratory capability, not at establishing the primary validation and statistical strength of the analytical method itself. General Information Chapter *Validation of Compendial Procedures* ⟨1225⟩ provides useful guidance about which characteristics are appropriate for testing.

[a] USP General Chapter Expert Committee member.

[b] FDA Liaison. The opinions expressed in this paper do not necessarily reflect the views or opinions of FDA.

[c] Correspondence should be addressed to: Horacio Pappa, PhD, Senior Scientist, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8319; e-mail hp@usp.org.

Method Verification or Revalidation

Method verification or revalidation is another acceptable approach for transfer of a validated procedure. TAP is conceptually similar to ⟨1226⟩, although the Chapters' objectives reflect some differences in the process and documentation. General Information Chapter ⟨1226⟩ deals with the transfer of a pharmacopeial procedure from the compendia to a receiving unit. When successful, TAP demonstrates a similar transfer process between the transferring unit that is responsible for the development and validation of the procedure and the receiving unit. In both cases the procedures to be transferred and the acceptance criteria are closely related to the development and the validation processes. The main difference between them resides in the nature of the sample. In a TAP, the sample typically is the same for all units involved. For the transfer of a compendial procedure (⟨1226⟩) a single sample may be evaluated only within one or more operational units that compare performance against an existing monograph or a single compendial test standard.

After a laboratory has performed a successful verification or validation, the laboratory has gained the skill and knowledge necessary to perform the test. This approach is more time-consuming than comparative testing, and manufacturers also may find it more difficult to observe bias between different sites, operators, and instruments.

Transfer Waiver

The conventional TAP may be omitted under certain circumstances. In those cases, the receiving unit is considered to be qualified to use the analytical test procedures without comparison and generation of interlaboratory comparative data. The following list, though not necessarily exhaustive, gives examples that may justify the waiver of the TAP:

- The receiving unit has already used the procedure for a significant amount of time and has gained the necessary skills and knowledge.
- The new product has a composition comparable to that of an existing product and/or the concentration of active ingredient is similar to that of an existing product and is analyzed by procedures for which the receiving unit already has experience.
- The analytical procedure is the same or closely similar to a procedure already in use.
- The personnel in charge of the development and validation moved to the receiving unit.

ELEMENTS RECOMMENDED FOR THE TRANSFER OF ANALYTICAL PROCEDURES

Several elements, many of which are interrelated, are recommended for a successful TAP using the comparative testing approach. When appropriate and as a part of pre-transfer activities, the transferring unit should provide training to the receiving unit, or the receiving unit should run the procedures and identify any issues that may need to be resolved before signing the transfer protocol. Training should be documented.

The transferring unit, often the development unit, is responsible for providing the analytical procedure, any necessary development, validation reports, and documents, as well as for providing the necessary training and assistance to the receiving unit whenever necessary during the transfer. The receiving unit may be a Quality Control unit, another intracompany facility, another company, or a contract research organization, among others. The receiving unit provides qualified staff or properly trains the staff before the transfer, ensures that the facilities and instrumentation are properly calibrated and qualified as needed, and verifies that the laboratory systems are in compliance with applicable regulations and in-house general laboratory procedures. Both the transferring and receiving units should compare and discuss data and any deviations from the protocol. This discussion addresses any necessary corrections or updates to the final report and the analytical procedure as necessary to reproduce the procedure.

PREAPPROVED PROTOCOL

A well-designed protocol should be discussed, agreed upon, and documented before the implementation of TAP. The document expresses a consensus between the parties, indicating an intended execution strategy, and may include each party's requirements and responsibilities. The protocol should describe the objective; the scope; the responsibilities of the transferring and the receiving units; the materials and instruments to be used; the analytical procedure; the experimental design; and the acceptance criteria for all the tests and/or methods included in the transfer. Based on the validation data and procedural knowledge, the transfer protocol should identify the specific procedure characteristics that will be verified and the analysis that will be used to evaluate acceptable outcomes from the transfer exercise.

The acceptance criteria, also based on validation documents, should include the comparability criteria for results from all study sites. These criteria may be based on the difference between mean values and should be accompanied by an estimation of the variability, e.g., percent relative standard deviation for each site and/or a statistical method for the comparison of the means (e.g., two one-sided *t* tests for intersite similarity) for assay and content uniformity tests. The same parameters, with appropriate adjustment in relation to the specification limit, may be used for impurities and degradation products. Dissolution can be evaluated by a comparison of the dissolution profiles using the similarity factor f_2 or by comparison of data at the specified time points as described previously for the assay.

The laboratories should provide a rationale for any procedure not included as well as any transfer waiver. The materials, standards, samples, and instruments that will be used should be described, along with the associated parameters.

Whenever possible, a set of real samples and aged samples should be evaluated to examine potential problems related with differences in sample preparation equipment.

The documentation section of the transfer protocol may include report forms to ensure consistent recording of results and to improve consistency between laboratories. This section should contain the additional information that will be included with the results, such as chromatograms, spectra, and deviation reports. The protocol also explains how any deviation from the acceptance criteria will be managed.

THE ANALYTICAL PROCEDURE

The procedure is written with sufficient detail and explicit instructions to allow a trained analyst to perform it without difficulty. A pre-transfer meeting between the transferring and the receiving units is helpful to clarify any issues and answer any questions regarding the transfer process. If complete or partial validation data exist, they should be available to the receiving unit, along with any technical details required to perform the test in question. In some cases it may be useful for the individuals who were involved with the initial development or validation to be on-site during the transfer. The number of batches, replicates, and injection sequences in the case of liquid or gas chromatography should be clearly expressed, and, in the case of dissolution testing, the number of individual dosage units should be stipulated.

METHOD TRANSFER REPORT

When the TAP is successfully completed the scientists involved should prepare a Transfer Report that describes the results obtained in relation to the acceptance criteria, along with conclusions that confirm that the receiving unit is now qualified to run the procedure. Failure to meet the acceptance criteria does not constitute an out-of-specification (OOS) result and does not require a formal OOS investigation. However, any result that fails to meet the predetermined acceptance criteria should be properly investigated and documented. Any deviations should be discussed and justified. The issues that contributed to failure to achieve the predicted acceptance criteria should be corrected, including training or further clarification of the analytical procedure. When the acceptance criteria are not met, the procedure cannot be transferred. Then remedial steps must be taken until the acceptance criteria are met.

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Visible Particulates in Injections—A History and a Proposal to Revise *USP* General Chapter *Injections* ⟨1⟩

Russell E. Madsen,[a] Roy T. Cherris,[b] John G. Shabushnig,[a] and Desmond G. Hunt[c,d]

ABSTRACT This *Stimuli* article provides a history of visual inspection practices and requirements for parenteral products in the United States. It includes a sampling plan and test for products that have been 100% inspected as part of the manufacturing process and criteria by which a product can be considered ''essentially free'' from visible particulates. The proposed test alone is insufficient for batch release testing—a complete program for the control and monitoring of particulate matter remains an essential prerequisite. The proposal is generally harmonized with the Particulate Contamination: Visible Particles section of the *European Pharmacopoeia* and the Foreign Insoluble Matter Test for Injections in the *Japanese Pharmacopoeia*. The objectives of this *Stimuli* article are to initiate discussion and to solicit public comments that will be considered by the Ad Hoc Advisory Panel—Visual Inspection of Parenterals and subsequently the Parenteral Products: Industrial Expert Committee. The Expert Committee will consider recommendations from the Advisory Panel regarding the proposed revision.

THE NEED TO INSPECT

Visual inspection of parenteral products is driven by the need to minimize the introduction of unintended particulate matter to patients during the delivery of injectable medications. Such inspection also offers the opportunity to reject nonconforming units, such as those with cracks or incomplete seals, that pose a risk to the sterility of the product. The desire to detect these defects at a very low frequency and the randomness of their occurrence have resulted in the current expectation that each finished unit be inspected (100% inspection).

Human visual performance is critical to the assessment of visible particles. The threshold for human vision is generally accepted to be 50 μm. The detection process is probabilistic; i.e., the probability of detection increases with increasing particle size. Analysis of inspection results pooled from several studies involving different groups of inspectors shows that the probability of detection for a single 50-μm particle in clear solution in a 10-mL vial with diffuse illumination between 2000 and 3000 lux is slightly greater than 0%. This probability increases to approximately 40% for a 100-μm particle and becomes greater than 95% for particles 200 μm and larger (*1*).

Many animal studies have been conducted to determine the fate of intravenous particles of differing size and composition (*1–4*). Most studies have focused on subvisible particles that have a diameter of less than 50 μm. The smallest of these particles (approximately 1 μm in diameter) are often trapped in the liver, lungs, and spleen. Intravenous infusion of particles larger than the internal diameter of capillaries may be clinically significant because the particles may increase the risk of foreign particle embolism (*5*, *6*). Larger particles generally do not migrate far from the injection site. The most common response observed is the formation of emboli and granulomas. Although they help explain the physiological response to particulate matter, the large number of particles employed in these studies (e.g., 10^9 particles/kg/injection) provides little guidance about the risk of delivering small numbers of particles to patients.

Several reviews describe the effect on patients of particles in parenterals (*7–13*). Garvin and Gunner were among the first to express concern about the effects of particles in patients (*14*, *15*). Ethical considerations preclude controlled human studies on the effect of particulates in human patients. Some anecdotal information can be obtained from studies that involve intravenous drug abusers (*16–18*). In these case studies, solid oral dosages often are ground up and injected as a slurry. Pulmonary foreign body emboli and granulomas were observed in these patients. Again, the clinical risks of particles administered in other settings are difficult to infer from these observations because of the large number of foreign particles and the uncontrolled conditions in which they were administered.

Even though an estimated 15 billion injectable doses of medicines are dispensed each year (*19*), no reports of adverse events associated with the injection of individual visible particles have been found. Although zero defects is the desired goal and should drive continuous process improvement, it is not a workable acceptance criterion for visible particulate matter because of current packaging components and processing capability. The US Pharmacopeial Convention (USP) has adopted the terminology of ''essentially free'' to recognize this current state. As we move forward, a more precise definition is desirable to prevent misunderstanding and to aid in communication of this important quality attribute.

[a] Member, USP Parenteral Products—Industrial Expert Committee

[b] Member, USP Ad Hoc Advisory Panel—Visual Inspection of Parenterals

[c] Documentary Standards Division, USP

[d] Correspondence should be addressed to: Desmond G. Hunt, PhD, Scientist, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8341; fax 301.816.8373; e-mail dgh@usp.org.

HISTORY OF INSPECTION STANDARDS

In 1915 *USP IX* described the need for injectable compounds to be true solutions. In 1916, the *National Formulary* (*NF IV*) included six monographs for parenteral products and specified the method of preparation, but neither Compendium provided guidance with respect to solution clarity.

The first appearance of ''solution clarity'' and freedom from contaminants for parenterals occurred in 1936 in *NF VI*. A requirement for clarity in injectable solutions specified: ''Aqueous ampule solutions are to be clear; i.e., when observed over a bright light, they shall be substantially free from precipitate, cloudiness or turbidity, specks or flecks, fibers or cotton hairs, or any undissolved material.''

The requirement for visual clarity of parenteral products began in 1942. This was before USP's acquisition of *NF* and required coordination between USP and the American Pharmaceutical Association, the publisher of *NF* at that time. The two compendia that were official at the time,*NF VII* and *USP XII*, were coordinated in response to the need to define and control the quality of injectable products purchased in support of the military during World War II. Together, the compendia introduced the term ''substantially free'' to describe the need for control of particle contamination. *NF VII* stated: ''Aqueous solutions are to be clear; i.e., when observed over a bright light, they shall be substantially free from precipitate, cloudiness, or turbidity, specks or flecks, fibers or cotton hairs, or any undissolved material. Substantially free shall be construed to mean a preparation which is free from foreign bodies that would be readily discernible by the unaided eye when viewed through a light reflected from a 100-watt Mazda lamp using as a median a ground glass and a background of black and white'' (*20*).

USP XII stated ''Appearance of Solutions or Suspensions—Injections which are solutions of soluble medicaments must be clear, and free of any turbidity or undissolved material which can be detected readily without magnification when the solution is examined against black and white backgrounds with a bright light reflected from a 100-watt Mazda lamp or its equivalent'' (*21*).

Both *USP* and *NF* used the same test procedure; however the *USP* procedure was more rigorous because it omitted the qualifying adverb ''substantially.'' These directives were used by FDA in its role as the Quality Control Office for all pharmaceuticals purchased by the Armed Forces during World War II. On the basis of these requirements, FDA rejected many lots of injectable solutions offered to fulfill government contracts.

In 1947, *USP XIII* published requirements for clarity of solutions (*22*): ''Clarity of Solutions—Water for Injection, pharmacopeial Injections or pharmacopeial Solutions of medicament, intended for parenteral administration, unless exempted by individual monographs, must be substantially free of any turbidity or undissolved material which can be detected readily without accessory magnification (except for such optical correction as may be required to establish normal vision), when the solution is examined against a black background and against a light which at a point ten inches below the source provides an intensity of illumination not less than 100 and not more than 350 foot candles. This intensity of illumination may be obtained from a 100-watt, inside-frosted incandescent lamp operating at rated voltage, or from fluorescent lamps, or from any equivalent source of light.''

Following adverse observations during an FDA inspection of Bristol Laboratories, a finding of particle contamination in ampules was tested in court (*23*). The FDA inspector, guided by the ''clarity'' requirement as described in *USP XIII*, found particle-contaminated ampules in six accepted stocks from Bristol Laboratories. As a result of this inspection, the company was served with an FDA injunction and request for recall.

Bristol Laboratories challenged the results of the test by preparing a blinded test group of 150 ampules containing 1.5 mL sterile saline. This test group included 38 ampules that the FDA inspector had rejected as contaminated with particles. The case came to trial in 1949. At the conclusion of the government's testimony, the court granted the defendant's motion for dismissal. When on the witness stand, the FDA expert witness was asked to replicate the inspection using the test group and passed 36 out of 38 previously rejected containers. The case was dismissed on the grounds ''1) that the standards involved were indefinite and 2) that the evidence was insufficient to show such violation of the Act as would warrant the granting of the relief prayed for (destruction of the ampules)''(*23*).

From 1955 through 1970, *USP XV* through *USP XVIII* provided guidance about visual inspection of injections. For example, *USP XV* noted: ''Every care should be exercised in the preparation of injections to prevent contamination with micro-organisms and foreign material. Good pharmaceutical practice also requires that each Injection, in its final container, be subjected individually to visible inspection.'' *USP XVI* and *USP XVII* said: ''Every care should be exercised in the preparation of injections to prevent contamination with micro-organisms and foreign material. Good pharmaceutical practice also requires that each Injection, in its final container, be subjected individually to visible inspection whenever the nature of the container permits.''

In 1959, Fed. Std. No. 00142, Parenteral Preparations, was issued by the United States Navy Bureau of Medicine and Surgery (BuMed) and became mandatory for all Federal agencies. The standard was applicable to sterile parenteral preparations in final containers intended for human consumption. The standard was superseded in 1966 by Fed. Std. No. 142a. The standard provided requirements for clarity of solutions as well as limits for visible particulate matter as follows:

Section S6.2.1 *Clarity of solutions*. Applicable to type I, class 1; type II, class 1; type II, class 3; and solutions of dry solids (type IV, class 1). Solutions of parenteral preparations shall be clear and free from undissolved or particulate matter within the limits permitted in the classification of defects and the applicable acceptable quality level (AQL), when examined without accessory magnification (except for such optical correction as may be required to establish normal vision) against a black background and against a white background and

illumination from a light which at a point 25.4 centimeters (10 inches) from its source, provides an intensity of illumination of not less than 100 and not more than 350 foot-candles. Some biological products need not be clear and entirely free from turbidity, provided this is characteristic of the product. The clarity standards for such products shall be judged on an item-for-item basis with the characteristic properties of the product considered in each case.

NOTE—This standard was applied as a final test to samples of finished products, not to 100% on-line inspection, and the sampling was in accordance with MIL-STD-105.

For aqueous solutions (type I, class 1), the ''solution not clear'' defect was classified as Major A, Inspection Level II and the AQL (percent defective) as 1.0. Therefore, in a 30,000 unit batch, 315 units would be inspected; if only 7 or fewer contained visible particulate matter, the batch would pass the Clarity of Solution Test. Thus, at the time, agencies of the Federal government, including FDA, would deem this level to be acceptable and in compliance with the meaning of the USP term ''essentially free.''

Fed. Std. No. 142a was amended in 1970. It is not known when this standard was abandoned, but the significance of Fed. Std. No. 142a is that it provided government-endorsed acceptance limits for the presence of ''visible'' particles. Parenteral product quality acceptance levels were based on the limitations of sterile-product manufacturing capability at that time. Solomon Pflag, BuMed director in 1968, noted, ''Within the framework of the technology available on the subject of particulate matter, Military Services have been highly successful in the procurement of quality parenterals'' (*24*).

Fed. Std. No. 142a could serve as a model on which to frame a practical visible particulate matter acceptance level reflecting the improvement in present parenteral manufacturing technology.

USP XIX, Supplement 1, initiated the philosophical requirement for a zero-defect quality standard for foreign matter and particles (*25*): ''Every care should be exercised in the preparation of injections to prevent contamination. Good pharmaceutical practice also requires that each Injection, in its final container, be subjected individually to a physical inspection, whenever the nature of the container permits, and that every container whose contents show evidence of contamination with visible foreign material be rejected.'' This requirement was repeated verbatim in *USP XX* in 1980 (*26*).

In 1995, *USP XXIII* repeated the requirement for a zero-defect quality standard for foreign matter and particles (*27*): ''Every care should be exercised in the preparation of all products intended for injection, to prevent contamination with microorganisms and foreign material.'' This revision returned to the view expressed in *USP XIX Revision 1* that the response to particle contamination in injectable fluids must be a graded one. Only one phrase was changed: The previous use of the term *substantially free* was replaced by the term *essentially free*. In response to various publications and comments since 1980, a graded response to the inspection for visible particles appeared in *USP XXIII*, *Particulate Matter in Injections* ⟨788⟩: ''Particulate matter consists of mobile, randomly sourced, extraneous substances...that cannot be quantitated by chemical analysis due to the small amount of material that it represents and to its heterogeneous composition. Injectable solutions, including solutions constituted from sterile solids intended for parenteral use, should be essentially free from particles that can be observed on visual inspection.'' This requirement remains basically unchanged since the printing of *USP XIV* (*28*).

The requirements regarding ''visible particulates'' in the pharmacopeias of countries that participate in the International Conference on Harmonization are somewhat different, as shown below.

General Chapter ⟨1⟩ in *USP 31* states: ''Each final container of all parenteral preparations shall be inspected to the extent possible for the presence of observable foreign and particulate matter (hereafter termed 'visible particulates') in its contents. The inspection process shall be designed and qualified to ensure that every lot of all parenteral preparations is essentially free from visible particulates'' (*29*). No inspection method is specified.

The *Japanese Pharmacopoeia* states: ''Unless otherwise specified, Injections meet the requirements of the *Foreign Insoluble Matter Test for Injections* ⟨6.06⟩'' (*30*). Two inspection methods are described. Method 1 ''is applied to injections either in solutions, or in solution constituted from sterile drug solids'' and contains the following instructions: ''Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 lux under an incandescent lamp: Injections must be clear and free from readily detectable foreign insoluble matter. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity at approximately 8000 to 10,000 lux, with an incandescent lamp at appropriate distances above and below the container.'' Method 2 ''is applied to injections with constituted solution'' and contains the following instructions: ''Clean the exterior of the containers, and dissolve the contents with constituted solution or with water for injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be clear and free from foreign insoluble matter that is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 lux, right under an incandescent lamp.''

The *European Pharmacopoeia* states ''Solutions for injection, examined under suitable conditions of visibility, are clear and practically free from particles'' (*31*). The inspection is described as follows: ''Gently swirl or invert the container...and observe for about 5 s in front of the white panel. Repeat the procedure in front of the black panel. Record the presence of any particles.''

BASIS FOR THE PROPOSAL

The proposal (see Draft Text for Consideration, below) is based on Fed. Std. No. 142a, which was used successfully for more than a decade to ensure the quality of sterile parenteral products delivered to the US government, and on the results of Parenteral Drug Association (PDA) surveys that assessed current practices in the inspection

of parenteral products. Fed. Std. No. 142a classified the defect as Major A, Inspection Level II and the AQL (percent defective) as 1.0. From the 2008 PDA Survey of Visual Inspection Practices (2), the median value for the AQL for Major defects (most often associated with particulate matter) is 0.65%.

The proposed inspection conditions have been harmonized with those specified in the *European Pharmacopeia*, with a recommendation to use a high-frequency ballast with the fluorescent lamps to reduce flicker and associated inspector fatigue.

The proposal uses a General Inspection Level II sampling plan, as found in American National Standards Institute/American Society for Quality (ANSI/ASQ) Z1.4, as a release test for product that has been 100% inspected during manufacturing and a fixed sample size of 60 units when there is a need to re-evaluate a batch that has been released and is in distribution (*33*). The re-evaluation, or ''field'' sample size is applicable to batch sizes greater than 600 units. The 60-sample plan is similar to an ANSI/ASQ Z1.4 Special Level S-4 inspection with an AQL of 0.65%, covering sample size code letters G (n = 32) for batches between 1201 and 10,000, H (n = 50) for batches between 10,001 and 35,000, or J (n = 80) for batches between 35,001 and 500,000. It has an AQL of 0.60%. A batch with 2.8 defective units per hundred would be accepted 50% of the time. For comparison, an ANSI/ASQ Z1.4 General Level II inspection at a comparable AQL (0.65%) would accept a batch with 1.3 defective units per hundred 50% of the time. The re-evaluation sampling plan does not require abnormally high levels of retained samples, and, with the exception of powders and/or freeze-dried products, it is nondestructive.

DRAFT TEXT FOR CONSIDERATION

Definitions

ESSENTIALLY FREE *[Insert at the end of the Definitions section of* ⟨1⟩ *Injections]*:

Where used in this Chapter, the term essentially free *means that when the batch of Injection is inspected as described herein, no more than the specified number of units may be observed to contain visible particulates.*

Visible Particulates in Injections *[Insert as a subheading under Foreign and Particulate Matter]*:

This test is intended to be applied to product that has been 100% inspected as part of the manufacturing process; it is not sufficient for batch release testing alone, and a complete program for the control and monitoring of particulate matter remains an essential prerequisite. This includes dry sterile solids for injection when reconstituted as directed in the labeling. Other methods that have been demonstrated to achieve the same or better sensitivity for visible particulates may be used as an alternative to the one described below.

Injections shall be clear and free from visible particulates when examined without magnification (except for optical correction as may be required to establish normal vision) against a black background and against a white background with illumination that at the inspection point has an intensity between 2000 and 3750 lux. This may be achieved through the use of two 15-W fluorescent lamps (e.g., F15/T8). The use of a high-frequency ballast to reduce flicker from the fluorescent lamps is recommended. Higher illumination intensity is recommended for examination of product in containers other than those made from clear glass.

Before performing the inspection, remove any adherent labels from the container and wash and dry the outside. The unit to be inspected shall be gently swirled, ensuring that no air bubbles are produced, and inspected for approximately 5 s against each of the backgrounds. The presence of any particles should be recorded.

For batch-release purposes, sample and inspect the batch using ANSI/ASQ Z1.4 General Inspection Level II, single sampling plans for normal inspection, AQL 0.65. Not more than the specified number of units contains visible particulates.

For product in distribution, sample and inspect 60 units. Not more than one unit contains visible particulates.

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NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

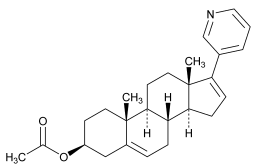
USP Dictionary of USAN and International Drug Names
2009 USP DICTIONARY SUPPLEMENT 3

IMPORTANT—Save this Supplement. This and all supplements appearing in *PF* are needed to keep the 2009 edition of the USP Dictionary (USPD) up-to-date. The cumulative contents of the supplements to the current (2009) edition will be included in the next complete edition of the Dictionary.

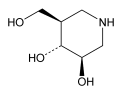
Newly Approved United States Adopted Names (USAN), Released for Publication

The following are newly established United States Adopted Names (USAN). These names will not be listed cumulatively; see preceding and succeeding numbers of *PF* for other new USAN to supplement the Dictionary main volume.

Abiraterone Acetate [*2009*] (a′′ bir a′ ter one ac′ e tate). $C_{26}H_{33}NO_2$. 391.50. [Abiraterone is INN and BAN.] (1) Androsta-5,16-dien-3-ol, 17-(3-pyridinyl)-, acetate (ester), (3β)-; (2) 17-(Pyridin-3-yl)androsta-5,16-dien-3β-yl acetate. *CAS-154229-18-2; CAS-154229-19-3* [abiraterone]. *Castration-resistant prostate cancer.* ✧*CB7630*

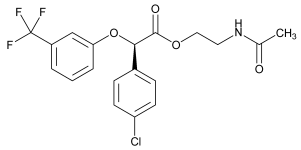


Afegostat [*2008*] (a feg′ oh stat). $C_6H_{13}NO_3$. 147.20. (1) 3,4-Piperidinediol, 5-(hydroxymethyl)-, (3*R*,4*R*,5*R*)-; (2) (3*R*,4*R*,5*R*)-5-(Hydroxymethyl)piperidine-3,4-diol. *CAS-169105-89-9. Treatment of Gaucher disease.*



Afegostat Tartrate [*2009*] (a feg′ oh stat tar′ trate). $C_6H_{13}NO_3.C_4H_6O_6$. 297.30. (1) 3,4-Piperidinediol, 5-(hydroxymethyl)-, (3*R*,4*R*,5*R*)-, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1); (2) (3*R*,4*R*,5*R*)-5-(Hydroxymethyl)piperidine-3,4-diol hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate (salt). *CAS-919364-56-0. Treatment of Gaucher disease.* ✧*AT2101*

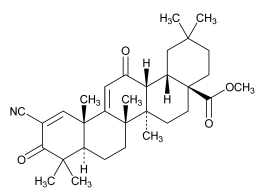
Arhalofenate [*2008*] (ar′′ hal oh fen′ ate). $C_{19}H_{17}ClF_3NO_4$. 415.80. (1) Benzeneacetic acid, 4-chloro-α-[3-(trifluoromethyl)phenoxy]-, 2-(acetylamino)ethyl ester, (–)-; (2) (–)-2-(Acetylamino)ethyl (2*R*)-(4-chlorophenyl)[3-(trifluoromethyl)phenoxy]acetate. *CAS-24136-23-0. Treatment of type 2 diabetes.* ✧*JNJ-39659100; MBX-102*



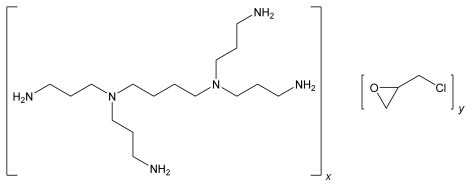
Astuprotimut-R [*2009*] (as′′ too proe′ ti mut). $C_{2243}H_{3465}N_{597}O_{686}S_{16}$. (1) 1-127-Phosphodiesterase, glycerophosphodiester [2-aspartic acid, 3-proline, 19-[*S*-[[(aminocarbonyl)amino]methyl]cysteine]] (*Haemophilus influenza* strain 86-028NP precursor) fusion protein with melanoma-associated antigen MAGE-3 (melanoma-associated antigen-encoding gene 3) [2-aspartic acid, 10-[*S*-[[(aminocarbonyl)amino]-methyl]cysteine], 181-[*S*-[[(aminocarbonyl)amino]methyl]-cysteine], 215-[*S*-[[(aminocarbonyl)amino]methyl]cysteine], 268-[*S*-[[(aminocarbonyl)amino]-methyl]cysteine]] (human) fusion protein with glycylglycyl-L-histidyl-L-histidyl-L-histidyl-L-histidyl-L-histidyl-L-histidyl-L-histidine; (2) 19,137,308,342,395-Penta-[*S*-carbamidomethyl] [2-aspartic acid(K^2>D) 3-proline(L^3>P)]-Glycerophosphoryl diester phosphodiesterase (*Haemophilus influenzae* strain 86-028NP EC 3.1.4.46)(1-127)-peptide fusion protein with [2-aspartic acid(P^2>D)]human melanoma-associated antigen 3 (MAGE-3 antigen, Antigen MZ2-D, Cancer/testis antigen 1.3 or CT1.3) fusion protein with glycylglycylheptahistidine. Molecular weight is approximately 50,280 daltons. *CAS-949885-73-8. Cancer immunotherapeutic.* ✧*MAGE-A3*

Bardoxolone [*2009*] (bar dox′ oh lone). $C_{31}H_{41}NO_4$. 491.70. (1) Oleana-1,9(11)-dien-28-oic acid, 2-cyano-3,12-dioxo-; (2) (+)-2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid. *CAS-218600-44-3. Antineoplastic and anti-inflammatory.* ✧*RTA 401; CDDO*

Bardoxolone Methyl [*2008*] (bar dox′ oh lone meth′ il). $C_{32}H_{43}NO_4$. 505.70. (1) Oleana-1,9(11)-dien-28-oic acid, 2-cyano-3,12-dioxo-, methyl ester; (2) (+)-Methyl 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate. *CAS-218600-53-4. Anti-inflammatory.* ✧*RTA 402; NSC-713200*

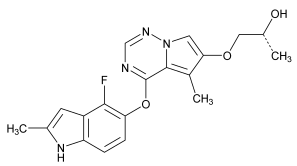


Bixalomer [*2008*] (bix al′ oh mer). $(C_{16}H_{40}N_6)_x.(C_3H_5ClO)_y$. (1) 1,4-Butanediamine, *N*,*N*,*N*′,*N*′-tetrakis(3-aminopropyl)-, polymer with 2-(chloromethyl)oxirane; (2) *N*,*N*,*N*′,*N*′-Tetrakis(3-aminopropyl)butane-1,4-diamine polymer with 2-(chloromethyl)oxirane. *CAS-851373-13-2. Treatment of hyperphosphatemia.* ✧*AMG-223*

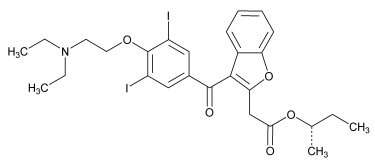


Briakinumab [*2008*] (brye″ a kin′ ue mab). $C_{6376}H_{9874}N_{1722}O_{1992}S_{44}$. Immunoglobulin G1, anti-(human interleukin-12 subunit beta (IL-12 subunit p40, CLMF p40 or NKSF2)); human monoclonal γ1 heavy chain (218-216′)-disulfide with human monoclonal λ light chain, dimer (224-224″:227-227″)-bisdisulfide. Molecular weight is approximately 146,500 daltons. *CAS-339308-60-0. Treatment of autoimmune disorders.* ✧*ABT-874; J695; BSF 415977; LU 415977; WAY-165772; A-796874.0*

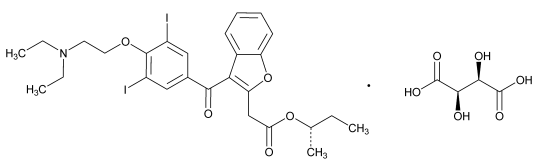
Brivanib [*2008*] (briv′ a nib). $C_{19}H_{19}FN_4O_3$. 370.40. (1) 2-Propanol, 1-[[4-[(4-fluoro-2-methyl-1*H*-indol-5-yl)oxy]-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yl]oxy]-,(2*R*)-; (2) (2*R*)-1-({4-[(4-Fluoro-2-methyl-1*H*-indol-5-yl)oxy]-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yl}oxy)propan-2-ol. *CAS-649735-46-6. Anticancer.* ✧*BMS-540215*



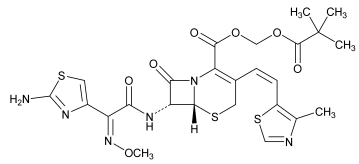
Budiodarone [*2008*] (bue″ di oh′ da rone). $C_{27}H_{31}I_2NO_5$. 703.30. (1) 2-Benzofuranacetic acid, 3-[4-[2-(diethylamino)ethoxy]-3,5-diiodobenzoyl]-, (1*S*)-1-methylpropyl ester; (2) (1*S*)-1-Methylpropyl 2-(3-{4-[2-(diethylamino)ethoxy]-3,5-diiodobenzoyl}benzofuran-2-yl)acetate. *CAS-335148-45-3. Antiarrhythmic.* ✧*ATI-2042*



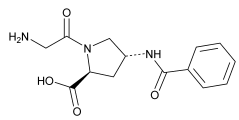
Budiodarone Tartrate [*2008*] (bue″ di oh′ da rone tar′ trate). $C_{27}H_{31}I_2NO_5.C_4H_6O_6$. 853.40. (1) 2-Benzofuranacetic acid, 3-[4-[2-(diethylamino)ethoxy]-3,5-diiodobenzoyl]-, (1*S*)-1-methylpropyl ester, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1); (2) (1*S*)-1-Methylpropyl 2-(3-{4-[2-(diethylamino)ethoxy]-3,5-diiodobenzoyl}benzofuran-2-yl)acetate, hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate. *CAS-478941-93-4. Antiarrhythmic.* ✧*ATI-2042*



Cefditoren Pivoxil [*1999*] (sef″ di tor′ en piv ox′ il). $C_{25}H_{28}N_6O_7S_3$. 620.73. [Cefditoren is INN.] (1) 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[(1*Z*)-2-(4-methyl-5-thiazolyl)ethenyl]-8-oxo-, (2,2-dimethyl-1-oxopropoxy)methyl ester, (6*R*,7*R*)-; (2) (+)-(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-[(*Z*)-2-(4-methyl-5-thiazolyl)vinyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pivaloyloxy methyl ester, 7^2-(*Z*)-(*O*-methyloxime). *CAS-117467-28-4; CAS-104145-95-1* [cefditoren]. JAN. *Anti-infective cephalosporin antibiotic.* Meiact (Meiji Seika Kaisha) ✧*ME1207; CDTR-PI*



Danegaptide [*2008*] (dan″ e gap′ tide). $C_{14}H_{17}N_3O_4$. 291.30. (1) L-Proline, glycyl-4-(benzoylamino)-, (4*R*)-; (2) Glycyl-(4*R*)-4-(benzoylamino)-L-proline; (3) (2*S*,4*R*)-1-(Aminoacetyl)-4-(benzoylamino)pyrrolidine-2-carboxylic acid. *CAS-943134-39-2. Treatment of cardiac arrhythmias.* ✧*GAP-134*

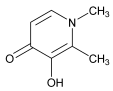


Davalintide [*2008*] (dav″ a lin′ tide). $C_{152}H_{248}N_{50}O_{49}S_2$. 3624.00. (1) L-Tyrosinamide, L-lysyl-L-cysteinyl-L-asparaginyl-L-threonyl-L-alanyl-L-threonyl-L-cysteinyl-L-valyl-L-leucylglycyl-L-arginyl-L-leucyl-L-seryl-L-glutaminyl-L-α-glutamyl-L-leucyl-L-histidyl-L-arginyl-L-leucyl-L-glutaminyl-L-threonyl-L-tyrosyl-L-prolyl-L-arginyl-L-threonyl-L-asparaginyl-L-threonylglycyl-L-seryl-L-asparaginyl-L-threonyl-, cyclic (2→7)-disulfide; (2) L-Lysyl-des-2-L-serine-[4-L-threonine(L>T),5-L-alanine(S>A),11-L-arginine(K>R),18-L-arginine(K>R),30-L-asparagine(G>N),32-L-tyrosine(P>Y)]calcitonin-1 *Oncorhynchus keta* (Chum salmon), calcitonin-1 precursor-(83-114)-peptidamide. *CAS-863919-85-1. Treatment of obesity and obesity-related disorders.* ✧*AC2307*

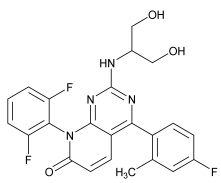
Davalintide Acetate [*2008*] (dav″ a lin′ tide as′ e tate). $C_{152}H_{248}N_{50}O_{49}S_2.xC_2H_4O_2$. 3684.00. (1) L-Tyrosinamide, L-lysyl-L-cysteinyl-L-asparaginyl-L-threonyl-L-alanyl-L-threonyl-L-cysteinyl-L-valyl-L-leucylglycyl-L-arginyl-L-leucyl-L-seryl-L-glutaminyl-L-α-glutamyl-L-leucyl-L-histidyl-L-arginyl-L-leucyl-L-glutaminyl-L-threonyl-L-tyrosyl-L-prolyl-L-arginyl-L-threonyl-L-asparaginyl-L-threonylglycyl-L-seryl-L-asparaginyl-L-threonyl-, cyclic (2→7)-disulfide, acetate (salt); (2) L-Lysyl-des-2-L-serine-[4-L-threonine(L>T),5-L-alanine(S>A),11-L-arginine(K>R),18-L-arginine(K>R),30-L-asparagine(G>N),32-L-tyrosine(P>Y)]calcitonin-1 *Oncorhynchus keta* (Chum salmon), calcitonin-1precursor-(83-114)-peptidamide acetate salt. *CAS-879197-42-9. Treatment for obesity and/or obesity-related metabolic disorders, including diabetes mellitus.* ✧*AC2307*

Nomenclature

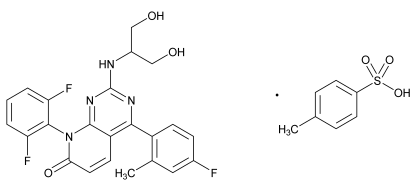
Deferiprone [*2008*] (de fer′ i prone). $C_7H_9NO_2$. 139.15. (1) 4(1*H*)-Pyridinone, 3-hydroxy-1,2-dimethyl-; (2) 3-Hydroxy-1,2-dimethylpyridin-4(1*H*)-one. *CAS-30652-11-0*. INN; BAN. *Treatment of iron overload in patients with excessive body iron stores.* ✧*L1; CP20; APO-66; APO-066; PL1; DN-180-01-AF*



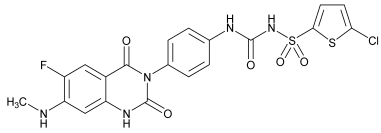
Dilmapimod [*2009*] (dil map′ i mod). $C_{23}H_{19}F_3N_4O_3$. 456.40. (1) Pyrido[2,3-*d*]pyrimidin-7(8*H*)-one, 8-(2,6-difluorophenyl)-4-(4-fluoro-2-methylphenyl)-2-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]-; (2) 8-(2,6-Difluorophenyl)-4-(4-fluoro-2-methylphenyl)-2-{[2-hydroxy-1-(hydroxymethyl)ethyl]amino}pyrido[2,3-*d*]pyrimidin-7(8*H*)-one. *CAS-444606-18-2*. *Chronic obstructive pulmonary disease* (*COPD*), *rheumatoid arthritis, atherosclerosis, neuropathic pain, acute lung injury* (*ALI*), *acute respiratory distress syndrome* (*ARDS*). ✧*SB-681323*



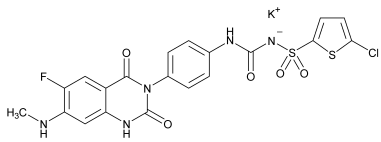
Dilmapimod Tosylate [*2009*] (dil map′ i mod toe′ si late). $C_{23}H_{19}F_3N_4O_3.C_7H_8O_3S$. 628.60. (1) Pyrido[2,3-*d*]pyrimidin-7(8*H*)-one, 8-(2,6-difluorophenyl)-4-(4-fluoro-2-methylphenyl)-2-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]-, 4-methylbenzenesulfonate (1:1); (2) 8-(2,6-Difluorophenyl)-4-(4-fluoro-2-methylphenyl)-2-{[2-hydroxy-1-(hydroxymethyl)ethyl]amino}pyrido[2,3-*d*]pyrimidin-7(8*H*)-one 4-methylbenzenesulfonate. *CAS-937169-00-1*. *Chronic obstructive pulmonary disease* (*COPD*), *rheumatoid arthritis, atherosclerosis, neuropathic pain, acute lung injury* (*ALI*), *acute respiratory distress syndrome* (*ARDS*). ✧*SB-681323-T*



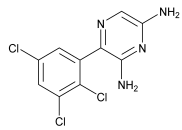
Elinogrel [*2008*] (el in′ oh grel). $C_{20}H_{15}ClFN_5O_5S_2$. 523.90. (1) 2-Thiophenesulfonamide, 5-chloro-*N*-[[[4-[6-fluoro-1,4-dihydro-7-(methylamino)-2,4-dioxo-3(2*H*)-quinazolinyl]phenyl]amino]carbonyl]-; (2) *N*-[(5-Chlorothiophen-2-yl)sulfonyl]-*N*′-{4-[6-fluoro-7-(methylamino)-2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl]phenyl}urea. *CAS-936500-94-6*. *Antithrombotic agent.* ✧*PRT 060128*



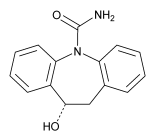
Elinogrel Potassium [*2008*] (el in′ oh grel poe tas′ ee um). $C_{20}H_{14}ClFKN_5O_5S_2$. 562.00. (1) 2-Thiophenesulfonamide, 5-chloro-*N*-[[[4-[6-fluoro-1,4-dihydro-7-(methylamino)-2,4-dioxo-3(2*H*)-quinazolinyl]phenyl]amino]carbonyl]-, potassium salt (1:1); (2) Potassium [(5-chlorothiophen-2-yl)-sulfonyl]({4-[6-fluoro-7-(methylamino)-2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl]phenyl}carbamoyl)azanide. *CAS-936501-01-8*. *Antithrombotic agent.* ✧*PRT060128-potassium*



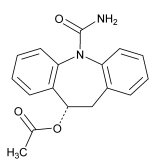
Elpetrigine [*2009*] (el pe′ tri jeen). $C_{10}H_7Cl_3N_4$. 289.50. (1) 2,6-Pyrazinediamine, 3-(2,3,5-trichlorophenyl)-; (2) 3-(2,3,5-Trichlorophenyl)pyrazine-2,6-diamine. *CAS-212778-82-0*. *Treatment of epilepsy and bipolar disorder.* ✧*JZP-4*



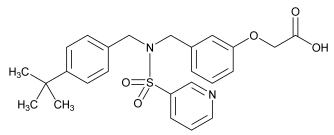
Eslicarbazepine [*2008*] (es′′ li kar baz′ e peen). $C_{15}H_{14}N_2O_2$. 254.28. (1) 5*H*-Dibenz[*b,f*]azepine-5-carboxamide, 10,11-dihydro-10-hydroxy-, (10*S*)-; (2) (10*S*)-10-Hydroxy-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-carboxamide. *CAS-104746-04-5*. INN. *Adjunctive therapy for adults with partial-onset seizures.* Erelib; Pazzul; Stedesa ✧*BIA 2-194*



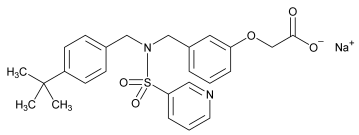
Eslicarbazepine Acetate [*2008*] (es′′ li kar baz′ e peen as′ e tate). $C_{17}H_{16}N_2O_3$. 296.30. (1) 5*H*-Dibenz[*b,f*]azepine-5-carboxamide, 10-(acetyloxy)-10,11-dihydro, (10*S*)-; (2) (10*S*)-5-Carbamoyl-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-10-yl acetate. *CAS-236395-14-5*. *Adjunctive therapy for adults with partial-onset seizures.* Erelib; Pazzul; Stedesa ✧*Sep - 0002093; BIA 2-093*



Evatanepag [*2009*] (ev′′ a tan′ e pag). $C_{25}H_{28}N_2O_5S$. 468.60. (1) Acetic acid, 2-[3-[[[[4-(1,1-dimethylethyl)phenyl]-methyl](3-pyridinylsulfonyl)amino]methyl]phenoxy]-; (2) 2-(3-{[{[4-(1,1-Dimethylethyl)phenyl]methyl}(pyridin-3-yl-sulfonyl)amino]methyl}phenoxy) acetic acid. *CAS-223488-57-1*. *Treatment of fracture.* ✧*CP-533,536*

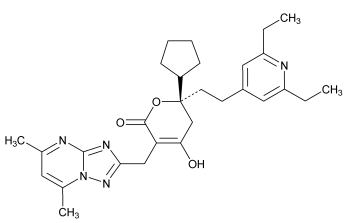


Evatanepag Sodium [*2009*] (ev′′ a tan′ e pag soe′ dee um). $C_{25}H_{27}N_2NaO_5S$. 490.50. (1) Acetic acid, 2-[3-[[[[4-(1,1-dimethylethyl)phenyl]methyl](3-pyridinylsulfonyl)amino]-methyl]phenoxy]-, sodium salt (1:1); (2) Sodium 2-(3-{[{[4-(1,1-dimethylethyl)phenyl]methyl}(pyridin-3-ylsulfo-nyl)amino]methyl}phenoxy)acetate.*CAS-223490-49-1*. *Treatment of fracture.* ✧*C-533,536-02*

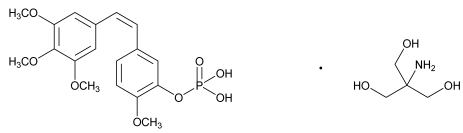


Fezakinumab [*2008*] (fez′′ a kin′ ue mab). $C_{6408}H_{9874}N_{1706}O_{2016}S_{44}$. (1) Immunoglobulin G1, anti-(human interleukin 22) (human monoclonal heavy chain), disulfide with human monoclonal λ-chain, dimer; (2) Immunoglobulin G1, anti-(human/Macaca irus/Rattus/Mus musculus interleukin 22) (human monoclonal heavy chain), disulfide with human monoclonal λ-chain, dimer. Molecular weight is approximately 144,500 daltons. *CAS-1007106-86-6*. *Treatment of inflammatory diseases.* ✧*ILV-094*

Filibuvir [*2008*] (fil ib′ ue vir). $C_{29}H_{37}N_5O_3$. 503.60. (1) 2*H*-Pyran-2-one, 6-cyclopentyl-6-[2-(2,6-diethyl-4-pyridin-yl)ethyl]-3-[(5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)methyl]-5,6-dihydro-4-hydroxy-, (6*R*)-; (2) (6*R*)-6-Cyclopentyl-6-[2-(2,6-diethylpyridin-4-yl)ethyl]-3-[(5,7-di-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)methyl]-4-hydroxy-5,6-dihydro-2*H*-pyran-2-one. *CAS-877130-28-4*. *Treatment of hepatitis C infection.* ✧*PF-00868554*

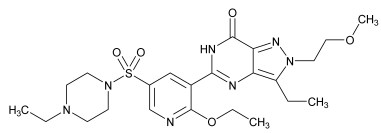


Fosbretabulin Tromethamine [*2008*] (fos′′ bre ta bue′ lin troe meth′ a meen). $C_{18}H_{21}O_8P.C_4H_{11}NO_3$. 517.50. (1) 1,3-Propanediol, 2-amino-2-(hydroxymethyl)-, compd. with 2-methoxy-5-[(1*Z*)-2-(3,4,5-trimethoxyphenyl)ethenyl]-phenyl dihydrogen phosphate (1:1); (2) 1,3-Dihydroxy-2-(hydroxymethyl)propan-2-aminium 2-methoxy-5-[(1*Z*)-2-(3,4,5-trimethoxyphenyl)ethenyl]phenyl hydrogen phos-phate. *CAS-404886-32-4*. *Treatment of cancer.* Zybrestat (Oxigene)

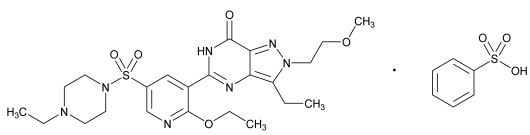


Fresolimumab [*2008*] (fre′′ soe lim′ ue mab). $C_{6392}H_{9926}N_{1698}O_{2026}S_{44}$. (1) Immunoglobulin G4, anti-(transforming growth factor β) (human monoclonal GC-1008 heavy chain), disulfide with human monoclonal GC-1008 light chain, dimer; (2) Immunoglobulin G4, anti-(human transforming growth factors beta-1, beta-2 (G-TSF or cetermin) and beta-3), human monoclonal GC-1008 γ4 heavy chain (134-215′)-disulfide with human monoclonal GC-1008 κ light chain, dimer (226-226′′:229-229′′)-bisdisulfide. Molecular weight is approximately 144,400 daltons (peptide). *CAS-948564-73-6*. *Idiopathic pulmonary fibrosis, focal segmental glomerulosclerosis, and cancer.* ✧*GC1008*

Gisadenafil [*2009*] (jis′′ a den′ a fil). $C_{23}H_{33}N_7O_5S$. 519.60. (1) 7*H*-Pyrazolo[4,3-*d*]pyrimidin-7-one, 5-[2-ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]-3-pyridinyl]-3-ethyl-2,6-dihydro-2-(2-methoxyethyl)-; (2) 5-{2-Ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]pyridin-3-yl}-3-ethyl-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one. *CAS-334826-98-1*. *Treatment of lower urinary tract symptoms associated with benign prostatic hyperplaseia* (*BPH*). ✧*UK-369,003*



Gisadenafil Besylate [*2009*] (jis′′ a den′ a fil bes′ i late). $C_{23}H_{33}N_7O_5S.C_6H_6O_3S$. 677.80. (1) 7*H*-Pyrazolo[4,3-*d*]pyrimidin-7-one, 5-[2-ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]-3-pyridinyl]-3-ethyl-2,6-dihydro-2-(2-methoxyethyl)-, benzenesulfonate (1:1); (2) 5-{2-Ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]pyridin-3-yl}-3-ethyl-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one benzenesulfonate. *CAS-334827-98-4*. *Treatment of lower urinary tract symptoms associated with benign prostatic hyperplaseia* (*BPH*). ✧*UK-369,003-26*



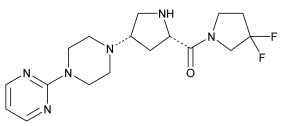
Glucose Oxidase [*2008*] (gloo′ kose ox′ i dase). $C_{5616}H_{8574}N_{1534}O_{1754}S_{28}$. (1) Oxidase, glucose; (2) Glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*. Molecular weight is approximately 126,500 daltons (peptide). *CAS-9001-37-0*. MI. *Treatment or prevention of infections caused by bacteria, fungi or viruses.* ✧*GO; component of E-101*

Nomenclature

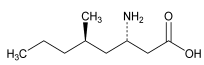
SNGIEASLLT DPKDVSGRTV DYIIAGGGLT GLTTAARLTE NPNISVLVIE
SGSYESDRGP IIEDLNAYGD IFGSSVDHAY ETVELATNNQ TALIRSGNGL
GGSTLVNGGT WTRPHKAQVD SWETVFGNEG WNWDNVAAYS LQAERARAPN
AKQIAAGHYF NASCHGVNGT VHAGPRDTGD DYSPIVKALM SAVEDRGVPT
KKDFGCGDPH GVSMFPNTLH EDQVRSDAAR EWLLPNYQRP NLQVLTGQYV
GKVLLSQNGT TPRAVGVEFG THKGNTHNVY AKHEVLLAAG SAVSPTILEY
SGIGMKSILE PLGIDTVVDL PVGLNLQDQT TATVRSRITS AGAGQGQAAW
FATFNETFGD YSEKAHELLN TKLEQWAEEA VARGGFHNTT ALLIQYENYR
DWIVNHNVAY SELFLDTAGV ASFDVWDLLP FTRGYVHILD KDPYLHHFAY
DPQYFLNELD LLGQAAATQL ARNISNSGAM QTYFAGETIP GDNLAYDADL
SAWTEYIPYH FRPNYHGVGT CSMMPKEMGG VVDNAARVYG VQGLRVIDGS
IPPTQMSSHV MTVFYAMALK ISDAILEDYA SMQ

* glycosylation site

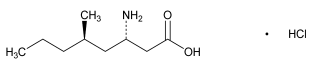
Gosogliptin [*2008*] (goe″ soe glip′ tin). $C_{17}H_{24}F_2N_6O$. 366.40. (1) Methanone, (3,3-difluoro-1-pyrrolidinyl)[(2*S*,4*S*)-4-[4-(2-pyrimidinyl)-1-piperazinyl]-2-pyrrolidinyl]-; (2) (3,3-Difluoropyrrolidin-1-yl){(2*S*,4*S*)-4-[4-(pyrimidin-2-yl)piperazin-1-yl]pyrrolidin-2-yl}methanone. *CAS-869490-23-3*. *Treatment of diabetes.* ✧*PF-734,200*



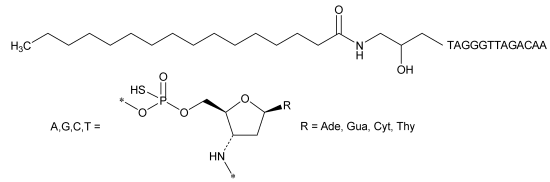
Imagabalin [*2009*] (im″ a gab′ a lin). $C_9H_{19}NO_2$. 173.30. (1) Octanoic acid, 3-amino-5-methyl-, (3*S*,5*R*)-; (2) (3*S*,5*R*)-3-Amino-5-methyl-octanoic acid. *CAS-610300-07-7*. *Treatment of general anxiety disorder.* ✧*PD 0332334; PF-00195889*



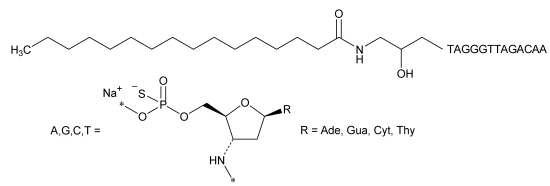
Imagabalin Hydrochloride [*2009*] (im″ a gab′ a lin hye″ droe klor′ ide). $C_9H_{19}NO_2$.HCl. 209.70. (1) Octanoic acid, 3-amino-5-methyl-, hydrochloride, (3*S*,5*R*)-; (2) (3*S*,5*R*)-3-Amino-5-methyl-octanoic acid hydrochloride. *CAS-610300-00-0*. *Treatment of general anxiety disorder.* ✧*PD 0332334-0002* (*PF00195889-01*)



Imetelstat [*2008*] (im″ e tel′ stat). $C_{148}H_{211}N_{68}O_{53}P_{13}S_{13}$. 4610.00. (1) DNA, d(3′-amino-3′-deoxy-*P*-thio)(T-A-G-G-G-T-T-A-G-A-C-A-A), 5′-[*O*-[2-hydroxy-3-[(1-oxohexadecyl)amino]propyl] hydrogen phosphorothioate]; (2) 3′-Amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thiocytidylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenosine 5′-{*O*-[2-hydroxy-3-(hexadecanoylamino)-propyl] hydrogen phosphorothioate}; (3) 5′-[*O*-[2-Hydroxy-3-[(1-oxohexadecyl)amino]propyl] hydrogen phosphorothioate]-d(3′-amino-3′-deoxy-*P*-thio)(T-A-G-G-G-T-T-A-G-A-C-A-A). *CAS-868169-64-6*. *Antineoplastic.* ✧*GRN163L*

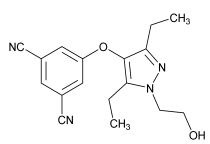


Imetelstat Sodium [*2008*] (im″ e tel′ stat soe′ dee um). $C_{148}H_{198}N_{68}Na_{13}O_{53}P_{13}S_{13}$. 4896.00. (1) DNA, d(3′-amino-3′-deoxy-*P*-thio)(T-A-G-G-G-T-T-A-G-A-C-A-A), 5′-[*O*-[2-hydroxy-3-[(1-oxohexadecyl)amino]propyl] hydrogen phosphorothioate], sodium salt (1:13); (2) 3′-Amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-3′-deoxy-*P*-thiothymidylyl-(3′→5′)-3′-amino-2′,3′-dideoxy- *P*- thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioguanylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thiocytidylyl- (3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenylyl-(3′→5′)-3′-amino-2′,3′-dideoxy-*P*-thioadenosine 5′-{*O*-[2-hydroxy-3-(hexadecanoylamino)-propyl] hydrogen phosphorothioate} tridecasodium salt; (3) 5′-[*O*-[2-Hydroxy-3-[(1-oxohexadecyl)amino]propyl] phosphorothioate]-d(3′-amino-3′-deoxy-*P*-thio)(T-A-G-G-G-T-T-A-G-A-C-A-A), sodium salt (13). *CAS-1007380-51-5*. *Antineoplastic.* ✧*GRN163L*



Intetumumab [*2008*] (in″ te toom′ ue mab). $C_{6468}H_{10008}N_{1744}O_{2006}S_{40}$. Immunoglobulin G1, anti-(human integrin alpha-V (vitronectin receptor subunit alpha or CD51); human monoclonal CNTO 95 γ1 heavy chain (222-215′)-disulfide with human monoclonal CNTO 95 κ light chain dimer (228-228″:231-231″)-bisdisulfide. Molecular weight is approximately 145,600 daltons. *CAS-725735-28-4*. *Treatment of solid tumors.* ✧*CNTO-95; CNTO 95*

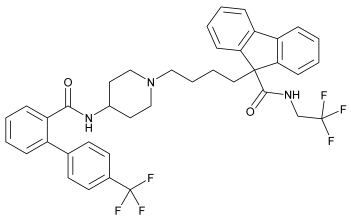
Lersivirine [*2008*] (ler″ si vir′ een). $C_{17}H_{18}N_4O_2$. 310.40. (1) 1,3-Benzenedicarbonitrile, 5-[[3,5-diethyl-1-(2-hydroxyethyl)-1*H*-pyrazol-4-yl]oxy]-; (2) 5-{[3,5-Diethyl-1-(2-hydroxyethyl)-1*H*-pyrazol-4-yl]oxy}benzene-1,3-dicarbonitrile. *CAS-473921-12-9*. *Prevention and progression of AIDS.* ✧*UK-453,061*



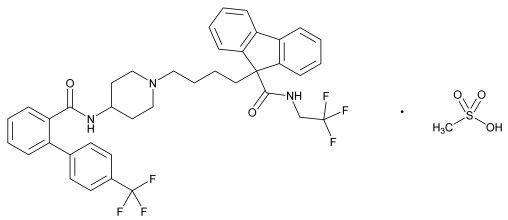
Lomitapide [*2009*] (loe mi′ ta pide). $C_{39}H_{37}F_6N_3O_2$. 693.70. (1) 9*H*-Fluorene-9-carboxamide, *N*-(2,2,2-trifluoroethyl)-9-[4-[4-[[[4′-(trifluoromethyl)[1,1′-biphenyl]-2-yl]carbonyl]ami-

Nomenclature

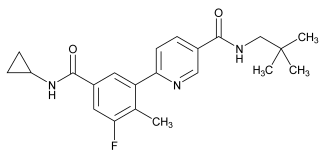
no]-1-piperidinyl]butyl]-; (2) *N*-(2,2,2-Trifluoroethyl)-9-{4-[4-({[4′-(trifluoromethyl)biphenyl-2-yl]carbonyl}amino)piperidin-1-yl]butyl}-9*H*-fluorene-9-carboxamide. *CAS-182431-12-5. Hypercholesterolemia and hypertriglyceridemia.* ✧*AEGR-733; BMS-201038-01*



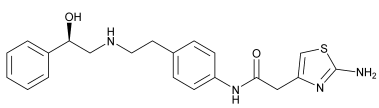
Lomitapide Mesylate [*2009*] (loe mi′ ta pide mes′ i late). $C_{39}H_{37}F_6N_3O_2.CH_4O_3S$. 789.80. (1) 9*H*-Fluorene-9-carboxamide, *N*-(2,2,2-trifluoroethyl)-9-[4-[4-[[[4′-(trifluoromethyl)[1,1′-biphenyl]-2-yl]carbonyl]amino]-1-piperidinyl]butyl]-, methanesulfonate; (2) *N*-(2,2,2-Trifluoroethyl)-9-{4-[4-({[4′-(trifluoromethyl)biphenyl-2-yl]carbonyl}amino)piperidin-1-yl]butyl}-9*H*-fluorene-9-carboxamide methanesulfonate. *CAS-202914-84-9. Hypercholesterolemia and hypertriglyceridemia.* ✧*BMS-201038-04; BMS-201038; AEGR-733*



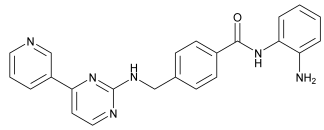
Losmapimod [*2008*] (los map′ i mod). $C_{22}H_{26}FN_3O_2$. 383.50. (1) 3-Pyridinecarboxamide, 6-[5-[(cyclopropylamino)carbonyl]-3-fluoro-2-methylphenyl]-*N*-(2,2-dimethylpropyl)-; (2) 6-[5-(Cyclopropylcarbamoyl)-3-fluoro-2-methylphenyl]-*N*-(2,2-dimethylpropyl)pyridine-3-carboxamide. *CAS-585543-15-3. Treatment of COPD, major depressive disorder, acute coronary syndrome, neuropathic pain.* ✧*GW856553X*



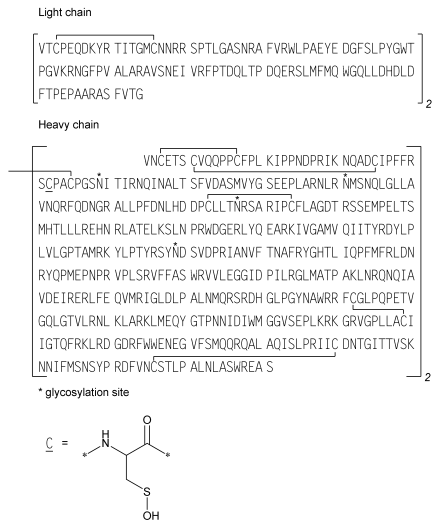
Mirabegron [*2009*] (mir″ a beg′ ron). $C_{21}H_{24}N_4O_2S$. 396.51. (1) 4-Thiazoleacetamide, 2-amino-*N*-[4-[2-[[(2*R*)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl]-; (2) 2-(2-Aminothiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide. *CAS-223673-61-8*. INN. *Overactive bladder with symptoms of urge incontinence.* ✧*YM178*



Mocetinostat [*2008*] (moe″ se tin′ oh stat). $C_{23}H_{20}N_6O$. 396.40. (1) Benzamide, *N*-(2-aminophenyl)-4-[[[4-(3-pyridinyl)-2-pyrimidinyl]amino]methyl]-; (2) *N*-(2-Aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide. *CAS-726169-73-9. Antineoplastic.* ✧*MGCD-0103; MG-0103*



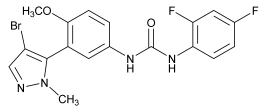
Myeloperoxidase [*2008*] (mye″ el oh per ox′ i dase). $C_{5854}H_{9188}N_{1698}O_{1678}S_{64}$. (1) Peroxidase; (2) Human myeloperoxidase (MPO, EC 1.11.1.7), dimer (155-155′)-disulfide. Molecular weight is approximately 132,300 daltons. *CAS-9003-99-0. Treatment or prevention of infections caused by bacteria, fungi, or viruses.* ✧*MPO; component of E-101*



Narafilcon A [*2007*] (nar″ a fil′ kon). $[C_{24}H_{56}O_8Si_5]_w[C_{16}H_{26}O_7]_v[C_6H_{10}O_3]_z[C_6H_9NO]_y;[C_5H_9NO]_x$. (1) 2-Propenoic acid, 2-methyl-, oxybis(2,1-ethanediyloxy-2,1-ethanediyl) ester, polymer with α-(butyldimethylsilyl)-ω-[[[3-[2-hydroxy-3-[(2-methyl-1-oxo-2-propenyl)oxy]propoxy]propyl]dimethylsilyl]oxy]poly[oxy(dimethylsilylene)],*N*,*N*-dimethyl-2-propenamide, 1-ethenyl-2-pyrrolidinone and 2-hydroxyethyl 2-methyl-2-propenoate; (2) 2,2′-[Oxybis(ethyleneoxy)]diethyl bis(2-methylprop-2-enoate) polymer with *rac*-(2*R*)-3-{3-[ω-butylpoly(dimethylsiloxan)-α-yl]propoxy}-2-hydroxypropyl 2-methylprop-2-enoate, *N*,*N*-dimethylprop-2-enamide, 1-ethenylpyrrolidin-2-one and 2-hydroxyethyl 2-methylprop-2-enoate. *CAS-909294-16-2. Contact lens material* (*hydrophilic*). Vistakon (Johnson & Johnson Vision)

Nelotanserin [*2008*] (nel″ oh tan ser′ in). $C_{18}H_{15}BrF_2N_4O_2$. 437.24. (1) Urea, *N*-[3-(4-bromo-1-methyl-1*H*-pyrazol-5-yl)-4-methoxyphenyl]-*N*′-(2,4-difluorophenyl)-; (2) 1-[3-(4-Bromo-1-methyl-1*H*-pyrazol-5-yl)-4-methoxyphenyl]-3-(2,4-difluorophenyl)urea; (3) 1-[3-(4-Bromo-2-methyl-2*H*-pyrazol-3-yl)-4-methoxyphenyl]-3-(2,4-difluorophenyl)urea. *CAS-839713-36-9. Treatment of insomnia.* ✧*APD125*

Nomenclature

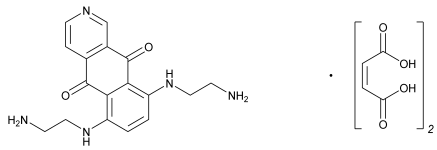


Ofatumumab [*2009*] (oh″ fa toom′ ue mab). $C_{6480}N_{10022}N_{1742}O_{2020}S_{44}$. (1) Immunoglobulin G1, anti-(human CD20 (antigen))(human monoclonal HuMax-CD20 heavy chain), disulfide with human monoclonal HuMax-CD20 κ-chain, dimer; (2) Immunoglobulin G1, anti-(human B-lymphocyte antigen CD20 (Membrane-spanning 4-domains subfamily A member 1, B-lymphocyte surface antigen B1, Leu-16 or Bp35)); human monoclonal HuMax-CD20 γ1 heavy chain (225-214′)-disulfide with human monoclonal HuMax-CD20 κ light chain, dimer (231-231″:234-234″)-bisdisulfide. Molecular weight is approximately 146,100 daltons. *CAS-679818-59-8*. INN. *Treatment of chronic lymphocytic leukemia.* ✧*GSK1841157; HuMax-CD20, 2F2*

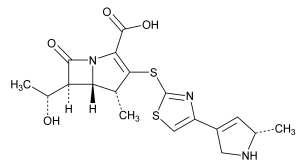
Oprifocon A [*1997*] (oh″ pri foe′ kon). $(C_{11}H_6F_{12}O_4)_u(C_{16}H_{38}O_5Si_4)_v(C_{13}H_{20}O_4)_w(C_{26}H_{58}O_9Si_6)_x(C_4H_6O_2)_y(C_6H_9NO)_z$. (1) Bis[2,2,2-trifluoro-1-(trifluoromethyl)ethyl] methylenebutanedioate polymer with 3-[3,3,3-trimethyl-1,1-bis[(trimethylsilyl)oxy]disiloxanyl]propyl 2-methyl-2-propenoate, 2,2-dimethyl-1,3-propanediyl bis(2-methyl-2-propenoate), [1,1,3,3-tetrakis[(trimethylsilyl)oxy]-1,3-disiloxanediyl]di-3,1-propanediyl bis(2-methyl-2-propenoate), 2-methyl-2-propenoic acid and 1-ethenyl-2-pyrrolidinone; (2) Bis[2,2,2-trifluoro-1-(trifluoromethyl)ethyl] methylenesuccinate polymer with 3-[3,3,3-trimethyl-1,1-bis(trimethylsiloxy)disiloxanyl]propyl methacrylate, 2,2-dimethyltrimethylene dimethacrylate, [tetrakis(trimethylsiloxy)disiloxanylene]bis (trimethylene) dimethacrylate, methacrylic acid and 1-vinyl-2-pyrrolidinone. *CAS-191277-04-0*. *Content lens material (hydrophobic).* Boston Equalens II (Polymer Technology) [NOTE—The water content of the contact lens material is <1.0% at ambient temperature ($23 \pm 2°C$), and the oxygen permeability is $127 \pm 10 \times 10^{-11}$ (cm^2/sec)(mL O_2/mL × mm Hg) at 35°C (Dk value).]

Pemufocon A [*1994*] (pem″ ue foe′ kon). $(C_6H_7F_3O_2)_u(C_{16}H_{38}O_5Si_4)_v(C_5H_8O_2)_w(C_4H_8O_2)_x(C_{10}H_{14}O_4)_y(C_6H_{10}O_3)_z$. (1) 2,2,2-Trifluoroethyl 2-methyl-2-propenoate polymer with 3-[3,3,3-trimethyl-1,1-bis[(trimethylsilyl)oxy]disiloxanyl]-propyl 2-methyl-2-propenoate, methyl 2-methyl-2-propenoate, 2-methyl-2-propenoic acid, 1,2-ethanediyl bis(2-methyl-2-propenoate), and 2-hydroxyethyl 2-methyl-2-propenoate; (2) 2,2,2-Trifluoroethyl methacrylate polymer with 3-[3,3,3-trimethyl-1,1-bis(trimethylsiloxy)disiloxanyl]-propyl methacrylate, methyl methacrylate, methacrylic acid, ethylene dimethacrylate and 2-hydroxyethyl methacrylate. *CAS-155521-84-9*. *Content lens material (hydrophobic).* AccuCon (Innovision) [NOTE—The water content of the contact lens material is 0.75% at ambient temperature (25°C), the purity of 2-hydroxyethyl methacrylate (HEMA) is 99%, and the oxygen permeability is 25×10^{-11} (cm^2/sec)(mL O_2/mL × mm Hg) at 35°C (Dk value).]

Pixantrone Dimaleate [*2009*] (pix′ an trone dye mal′ ee ate). $C_{17}H_{19}N_5O_2.2C_4H_4O_4$. 557.50. (1) Benz[*g*]isoquinoline-5,10-dione, 6,9-bis[(2-aminoethyl)amino]-, (2*Z*)-2-butenedioate (1:2); (2) 6,9-Bis[(2-aminoethyl)amino]benzo[*g*] isoquinoline-5,10-dione (2*Z*)-but-2-enedioate (1:2). *CAS-144675-97-8*. *Treatment of non-Hodgkin's lymphoma (NHL).* ✧*BBR 2778*



Razupenem [*2009*] (raz″ ue pen′ em). $C_{18}H_{21}N_3O_4S_2$. 407.50. (1) 1-Azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, 3-[[4-[(5*S*)-2,5-dihydro-5-methyl-1*H*-pyrrol-3-yl]-2-thiazolyl]thio]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-, (4*R*,5*S*,6*S*)-; (2) (4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-4-methyl-3-({4-[(5*S*)-5-methyl-2,5-dihydro-1*H*-pyrrol-3-yl]thiazol-2-yl}sulfanyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. *CAS-426253-04-5*. *Antibiotic.* ✧*PZ-601; SMP-601; SM-216601*



Rilotumumab [*2008*] (ril″ oh toom′ ue mab). $C_{6464}H_{9932}N_{1708}O_{2010}S_{46}$. (1) Immunoglobulin G2, anti-(human hepatocyte growth factor) (human monoclonal 2.12.1 heavy chain), disulfide with human monoclonal 2.12.1 light chain, dimer; (2) Immunoglobulin G2, anti-(human hepatocyte growth factor (Scatter factor or hepatopoeitin-A)), human monoclonal 2.12.1 γ2 heavy chain (134-215′)-disulfide with human monoclonal 2.12.1 κ light chain dimer (222-222″:223-223″:226-226″:229-229″)-tetrakisdisulfide. Molecular weight is approximately 145,200 daltons. *CAS-872514-65-3*. *Oncology, solid tumors.* ✧*AMG 102*

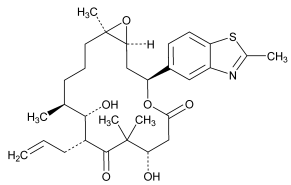
Rontalizumab [*2009*] (ron″ ta liz′ oo mab). $C_{6486}H_{9990}N_{1722}O_{2026}S_{44}$. (1) Immunoglobulin G1, anti-(human interferon α) (human-mouse monoclonal rhuMAb IFNalpha heavy chain), disulfide with human-mouse monoclonal rhuMAb IFNalpha κ-chain, dimer; (2) Immunoglobulin G1, anti-(human interferons alpha type); humanized mouse monoclonal rhuMAB IFNalpha γ1 heavy chain [des-$CH3^{107}$K, $CH1^{97}$R>K-IGHG1*03] (220-218′)-disulfide with humanized mouse monoclonal rhuMAB IFNalpha κ light chain, dimer (226-226″:229-229″)-bisdisulfide. Molecular weight is approximately 145,900 daltons (peptidic part). *CAS-948570-30-7*. *Treatment of systemic lupus erythematosus (SLE).* ✧*RhuMab IFNalpha*

Rozrolimupab [*2008*] (roz″ roe lim′ ue pab). (1) Anti-RhD recombinant human polyclonal antibody comprised of 25 unique IgG1 antibodies; (2) Recombinant human antigen-specific polyclonal antibody (pAb) against RhD antigen expressed in Chinese hamster ovary cells. Molecular weights for individual antibodies are in the range 144,800 to 148,800 daltons. *CAS-909402-77-3*. *Treatment of immune thrombocytopenia purpura (ITP), prevention of isoimmunization in Rh-negative pregnant women.* ✧*Sym001*

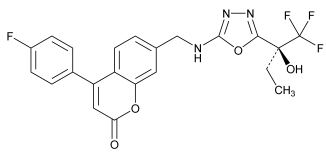
Sagopilone [*2008*] (sa gop′ i lone). $C_{30}H_{41}NO_6S$. 543.71. (1) 4,17-Dioxabicyclo[14.1.0]heptadecane-5,9-dione, 7,11-dihydroxy-8,8,12,16-tetramethyl-3-(2-methyl-5-benzothiazolyl)-10-(2-propenyl)-, (1*S*,3*S*,7*S*,10*R*, 11*S*,12*S*,16*R*)-; (2) (1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,12,16-tetramethyl-3-(2-methyl-1,3-benzothiazol-5-yl)-10-(prop-2-

Nomenclature

enyl)-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione. *CAS-305841-29-6*. INN. *Treatment of advanced solid tumors.* ✧*DE-03757; ZK 219477*

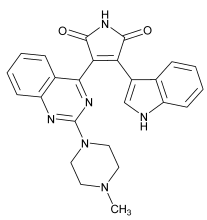


Setileuton [*2008*] (set′′ i loo′ ton). $C_{22}H_{17}F_4N_3O_4$. 463.38. (1) 2*H*-1-Benzopyran-2-one, 4-(4-fluorophenyl)-7-[[[5-[(1*S*)-1-hydroxy-1-(trifluoromethyl)propyl]-1,3,4-oxadiazol-2-yl]amino]methyl]-; (2) 4-(4-Fluorophenyl)-7-[({5-[(1*S*)-1-hydroxy-1-(trifluoromethyl)propyl]-1,3,4-oxadiazol-2-yl}amino)methyl]-2*H*-1-benzopyran-2-one. *CAS-910656-27-8*. *Treatment of asthma.*

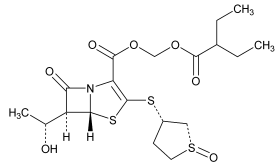


Sifalimumab [*2009*] (sif′′ a lim′ ue mab). $C_{6396}H_{9918}N_{1714}O_{2006}S_{42}$. (1) Immunoglobulin G1, anti-(human interferon α) (human monoclonal MEDI-545 heavy chain), disulfide with human monoclonal MEDI-545 κ-chain, dimer; (2) Immunoglobulin G1, anti-(human interferon alpha subtypes); human monoclonal MEDI-545 γ1 heavy chain (219-215′)-disulfide with human monoclonal MEDI-545 κ light chain, dimer (225-225′′:228-228′′)-bisdisulfide. Molecular weight is approximately 147,000 daltons. *CAS-1006877-41-3*. *Treatment of SLE, dermatomyositis, and polymyositis.* ✧*MEDI-545; MDX-1103*

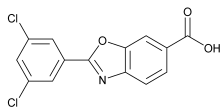
Sotrastaurin [*2008*] (soe′′ tra staw′ rin). $C_{25}H_{22}N_6O_2$. 438.50. (1) 1*H*-Pyrrole-2,5-dione, 3-(1*H*-indol-3-yl)-4-[2-(4-methyl-1-piperazinyl)-4-quinazolinyl]-; (2) 3-(1*H*-Indol-3-yl)-4-[2-(4-methylpiperazin-1-yl)quinazolin-4-yl]-1*H*-pyrrole-2,5-dione. *CAS-425637-18-9*. *Transplantation, T-cell mediated acute or chronic inflammatory diseases or disorders, or autoimmune diseases.* ✧*AEB071*



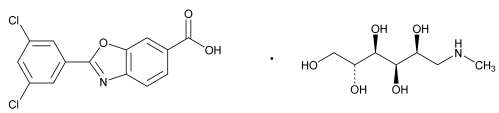
Sulopenem Etzadroxil [*2009*] (sul′′ oh pen′ em et′′ za drox′ il). $C_{19}H_{27}NO_7S_3$. 477.60. (1) 4-Thia-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic acid, 6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-[[(1*R*,3*S*)-tetrahydro-1-oxido-3-thienyl]thio]-, (2-ethyl-1-oxobutoxy)methyl ester, (5*R*,6*S*)-; (2) Methylene 2-ethylbutanoate (5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-{[(1*R*,3*S*)-1-oxotetrahydro-1*H*-1λ⁴-thiophen-3-yl]sulfanyl}-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate. *CAS-1000296-70-7*. *Antibiotic.* ✧*PF-03709270*



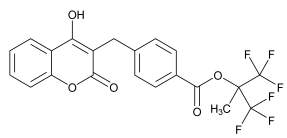
Tafamidis [*2009*] (ta fam′ id is). $C_{14}H_7Cl_2NO_3$. 308.10. (1) 6-Benzoxazolecarboxylic acid, 2-(3,5-dichlorophenyl)-; (2) 2-(3,5-Dichlorophenyl)benzoxazole-6-carboxylic acid. *CAS-594839-88-0*. *Treatment of transthyretin-associated amyloidosis.* ✧*Fx-1006*



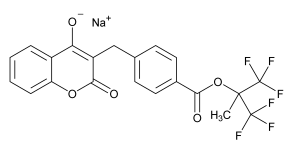
Tafamidis Meglumine [*2009*] (ta fam′ id is me′ gloo meen). $C_{14}H_7Cl_2NO_3.C_7H_{17}NO_5$. 503.34. (1) D-Glucitol, 1-deoxy-1-(methylamino)-, 2-(3,5-dichlorophenyl)-6-benzoxazolecarboxylate (1:1); (2) D-*gluco*-2,3,4,5,6-Pentahydroxy-*N*-methylhexan-1-aminium 2-(3,5-dichlorophenyl)benzoxazole-6-carboxylate. *CAS-951395-08-7*. *Treatment of transthyretin-associated amyloidosis.* ✧*Fx-1006A*



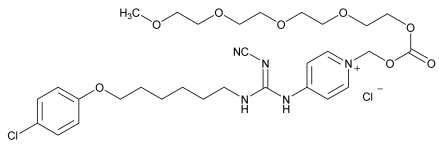
Tecarfarin [*2009*] (te kar′ far in). $C_{21}H_{14}F_6O_5$. 460.30. (1) Benzoic acid, 4-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]-, 2,2,2-trifluoro-1-methyl-1-(trifluoromethyl)ethyl ester; (2) 2,2,2-Trifluoro-1-methyl-1-(trifluoromethyl)ethyl 4-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]benzoate. *CAS-867257-26-9*. *Anticoagulant.* ✧*ATI-5923*



Tecarfarin Sodium [*2009*] (te kar′ far in soe′ dee um). $C_{21}H_{13}F_6NaO_5$. 482.30. (1) Benzoic acid, 4-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]-, 2,2,2-trifluoro-1-methyl-1-(trifluoromethyl)ethyl ester, sodium salt (1:1); (2) Sodium 2-oxo-3-[(4-{[2,2,2-trifluoro-1-methyl-1-(trifluoromethyl)ethoxy]carbonyl}phenyl)methyl]-2*H*-1-benzopyran-4-olate. *CAS-1004551-83-0*. *Anticoagulant.* ✧*ATI-5923; 60 TIS 99*

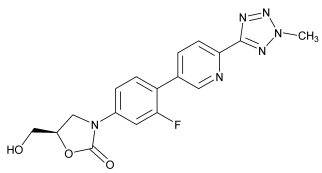


Teglarinad Chloride [*2009*] (teg lar′ i nad klor′ ide). $C_{30}H_{43}Cl_2N_5O_8$. 672.60. (1) Pyridinium, 4-[[[[6-(4-chlorophenoxy)hexyl]amino](cyanoamino)methylene]amino]-1-(3-oxo-2,4,7,10,13,16-hexaoxaheptadec-1-yl)-, chloride; (2) 4-({*N*-[6-(4-Chlorophenoxy)hexyl]-*N*′-cyanocarbamimidoyl}amino)-1-(3-oxo-2,4,7,10,13,16-hexaoxaheptadecan-1-yl)pyridinium chloride. *CAS-432037-57-5*. *Antineoplastic.* ✧*GMX1777; EB1627*

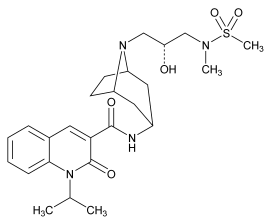


Tiomolibdate Diammonium [*2008*] (tye′′ oh moe lib′ date). $H_8MoN_2S_4$. 260.30. (1) Molybdate(2-), tetrathioxo-, ammonium (1:2), (*T-4*)-; (2) Diammonium tetrathiomolybdate. *CAS-15060-55-6*. *Anticopper drug.* Coprexa (Pipex Therapeutics) ✧*ATTM; TM; NSC-714598*

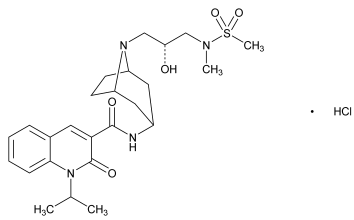
Torezolid [*2009*] (tore ez′ oh lid). $C_{17}H_{15}FN_6O_3$. 370.30. (1) 2-Oxazolidinone, 3-[3-fluoro-4-[6-(2-methyl-2*H*-tetrazol-5-yl)-3-pyridinyl]phenyl]-5-(hydroxymethyl)-, (5*R*)-; (2) (5*R*)-3-{3-Fluoro-4-[6-(2-methyl-2*H*-tetrazol-5-yl)pyridin-3-yl]-phenyl}-5-(hydroxymethyl)oxazolidin-2-one.*CAS-856866-72-3*. *Antibiotic.* ✧*TR-700*



Velusetrag [*2008*] (vel′′ u set′ rag). $C_{25}H_{36}N_4O_5S$. 504.60. (1) 3-Quinolinecarboxamide, 1,2-dihydro-*N*-[(3-*endo*)-8-[(2*R*)-2-hydroxy-3-[methyl(methylsulfonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl]-1-(1-methylethyl)-2-oxo-; (2) *N*-[(1*R*,3*R*,5*S*)-8-{(2*R*)-2-Hydroxy-3-[methyl(methylsulfonyl)amino]propyl}-8-azabicyclo[3.2.1]oct-3-yl]-1-(1-methylethyl)-2-oxo-1,2-dihydroquinoline-3-carboxamide.*CAS-866933-46-2*. *Treatment of constipation.* ✧*TD-5108*



Velusetrag Hydrochloride [*2008*] (vel′′ u set′ rag hye′′ droe klor′ ide). $C_{25}H_{36}N_4O_5S.HCl$. 541.10. (1) 3-Quinolinecarboxamide, 1,2-dihydro-*N*-[(3-*endo*)-8-[(2*R*)-2-hydroxy-3-[methyl(methylsulfonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl]-1-(1-methylethyl)-2-oxo-, monohydrochloride; (2) *N*-[(1*R*,3*R*,5*S*)-8-{(2*R*)-2-Hydroxy-3-[methyl(methylsulfonyl)amino]propyl}-8-azabicyclo[3.2.1]oct-3-yl]-1-(1-methylethyl)-2-oxo-1,2-dihydroquinoline-3-carboxamide hydrochloride. *CAS-866933-51-9*. *Treatment of constipation.*



Zalutumumab [*2009*] (zal′′ ue toom′ ue mab). $C_{6512}H_{10068}N_{1732}O_{2032}S_{46}$. (1) Immunoglobulin G1, anti-(human epidermal growth factor receptor)(human monoclonal 2F8 heavy chain), disulfide with human monoclonal 2F8 κ-chain, dimer; (2) Immunoglobulin G1, anti-(human epidermal growth factor receptor (receptor tyrosine-protein kinase ErbB-1)); human monoclonal 2F8 g1 heavy chain (228-214′)-disulfide with human monoclonal 2F8 κ light chain dimer (234-234′′:237-237′′)-bisdisulfide. Molecular weight is approximately 146,600 daltons. *CAS-667901-13-5*. INN. *Treatment of cancer.* ✧*HuMax-EGFr, 2F8*

Proposed and Recommended International Nonproprietary Names

International Nonproprietary Names (INN) are devised by the World Health Organization (WHO).

Under its charter, WHO is empowered simply to recommend specific actions or procedures to its Member States. This limitation is incorporated into the WHO program concerned with the selection of international nonproprietary names for pharmaceutical substances, in that WHO first publishes the selected names as proposals (''Proposed International Nonproprietary Names''). For a period of four months from the date of publication of *WHO Drug Information,* comments and objections may be submitted by the Member States or other interested parties. In general, an objection reflects a belief that the proposed name may be confused with a preexisting name, perhaps in only a restricted area in which the party has a proprietary interest in the form of trademark rights. In the event that no objection is received, WHO lists and publishes the names as recommendations (''Recommended International Nonproprietary Names''), which the Member States then recognize as the sole or preferred nonproprietary name for use within their respective territories.

Proposed International Nonproprietary Names–List 100

The following 56 names have been selected by WHO as Proposed International Nonproprietary Names. This list, with chemical names, descriptions and molecular formulae, appears in *WHO Drug Information,* Vol. 22, No. 4, 2008.

Suggested INN	Category
Adarotene	*Antineoplastic*
Afamelanotide	*Melanocortine receptor agonist*
Alisporivir	*Antiviral*
Amenamevir	*Antiviral*
Atigliflozin	*Antihyperglycemic*
Balapiravir	*Antiviral*
Beloranib	*Antineoplastic*
Blinatumomab	*Antineoplastic*
Canosimibe	*Antihyperlipidemic*
Cixutumumab	*Antineoplastic*
Coleneuramide	*Neuroprotective agent*
Cositecan	*Antineoplastic*
Cutamesine	*s Receptor ligand*
Davunetide	*Neuroprotective agent*
Delafloxacin	*Antibiotic*
Dirucotide	*Autoimmune disorders*
Dutogliptin	*Antihyperglycemic*
Elotuzumab	*Antineoplastic*
Farletuzumab	*Antineoplastic*
Fidaxomicin	*Antibiotic*
Figitumumab	*Antineoplastic*
Fosbretabulin	*Antineoplastic*
Fostamatinib	*Antineoplastic*
Indeglitazar	*Antihyperglycemic*
Ingenol Mebutate	*Antineoplastic*
Laninamivir	*Antiviral*
Lesogaberan	*GABA receptor agonist*
Limiglidole	*Antihyperglycemic*
Lotilibcin	*Antibiotic*
Macimorelin	*Growth hormone release stimulating peptide*
Namitecan	*Antineoplastic*
Necitumumab	*Antineoplastic*
Oportuzumab Monatox	*Antineoplastic*
Panobacumab	*Antibacterial (against Pseudomonas aeruginosa)*
Pozanicline	*Nicotinic acetylcholine receptor partial agonist/agonist*
Ramucirumab	*Antineoplastic*
Regorafenib	*Antineoplastic*
Riferminogene Pecaplasmid	*Gene therapy product, stimulates angiogenesis*
Robatumumab	*Antineoplastic*
Racotumomab	*Antineoplastic*
Selumetinib	*Antineoplastic*
Serlopitant	*Neurokinin (NK1) receptor antagonist*
Siltuximab	*Antineoplastic*
Sobetirome	*Antihyperlipidemic*
Sofinicline	*Nicotinic acetylcholine receptor partial agonist/agonist*
Solanezumab	*Neuroprotective agent*
Taberminogene Vadenovec	*Gene therapy product, treatment of arterial restenosis*
Tarafenacin	*Muscarinic receptor antagonist*
Telcagepant	*Antimigraine*
Tilivapram	*Phosphodiesterase IV inhibitor*
Toceranib	*Antineoplastic*
Tozasertib	*Antineoplastic*
Vanutide Cridificar	*Neuroprotective agent*
Vedolizumab	*Immunomodulator*
Voreloxin	*Antineoplastic*
Zicronapine	*Antipsychotic*

Recommended International Nonproprietary Names–List 61

The following 68 names have been selected by WHO as Recommended International Nonproprietary Names. This list, with chemical names, descriptions and molecular formulae, appears as prepublication list 61 on the WHO website.

Levomefolic Acid
Aderbasib
Adoprazine
Alipogene Tiparvovec
Apricoxib
Bafetinib
Bederocin
Befiradol
Bevasiranib
Catridecacog
Citatuzumab Bogatox
Conatumumab
Custirsen
Danusertib
Darotropium Bromide
Demiditraz
Denenicokin
Derquantel
Disitertide
Drinabant
Dulanermin
Edoxaban
Elagolix
Elesclomol
Entinostat
Eprotirome
Esreboxetine
Etaracizumab
Foravirumab
Ibipinabant
Intiquinatine
Lancovutide
Larazotide
Lensiprazine
Levomilnacipran
Linagliptin
Lixisenatide
Macitentan
Melogliptin
Mimopezil
Mipomersen
Niraxostat
Olesoxime
Ombrabulin
Otenabant
Palifosfamide
Palovarotene
Radezolid
Rafivirumab
Retaspimycin
Saracatinib
Semagacestat
Semuloparin Sodium
Sivifene
Talarozole
Talmapimod
Tanezumab
Tasimelteon
Tasisulam
Taspoglutide
Tecovirimat
Teneligliptin
Tildipirosin
Tosedostat
Troplasminogen Alfa
Ustekinumab
Vadimezan
Velneperit

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–218; Vol. 35 No. 2, pp. 219-498; Vol. 35 No. 3, pp. 499-784; Vol. 35 No. 4, pp. 785-1050; Vol. 35 No. 5, pp. 1051-1408.*]

MONOGRAPHS

EXCIPIENTS

GENERAL CHAPTERS

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

Test Solutions

Volumetric Solutions

REFERENCE TABLES

GENERAL SUBJECTS

Errata List for *USP 31–NF 26*

Index

CHROMATOGRAPHIC COLUMNS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Columns Used in *USP-NF* and *Pharmacopeial Forum*
Sept.–Oct. 2009

ANASTROZOLE (DSD Mgh #4650)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(2)	L42	HiChrom RPB-250A	Assay and Related Compounds	3.2 mm × 10 cm, 5μm. Manufacturer: HiChrom

AZTREONAM (DSD Mgh #6772)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	G43	OVI-G43	Limit of	Limit of alcohol. 0.53 mm × 30 m, 3 μm. Manufacturer: Supelco

DEFEROXAMINE MESYLATE (DSD Mgh #22400)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	ZORBAX ODS	Assay and Related Compounds	4.6 mm × 7.5 cm, 3.5 μm. Alternative column Zorbax Eclipse XDB C18. Manufacturer: Agilent Technologies

DICLOFENAC SODIUM EXTENDED-RELEASE TABLETS (DSD Mgh #24972)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L7	ZORBAX C8	Organic Impurities	4.6 mm × 25 cm. Manufacturer: Agilent Technologies

DOCETAXEL (DSD Mgh #27795)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	Sunfire C18	Assay and Related Compounds	4.6 mm × 15 cm, 3.5 μm. Manufacturer: Waters Corp.

DOFETILIDE (DSD Mgh #27968)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L7	Nova-Pak C8	Assay	3.9 mm × 15 cm, 4 μm. Manufacturer: Waters Corp.
0(0)	L1	Symmetry C-18	Organic Impurities	3.9 mm × 15 cm, 5 μm. Manufacturer: Waters Corp.

FEXOFENADINE HYDROCHLORIDE TABLETS (DSD Mgh #852)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Spherisorb ODS-2	Dissolution	Dissolution Test 3. 4.6 mm × 10 cm, 5 μm. Manufacturer: Waters Corp.

HYDROGENATED POLYDEXTROSE (DSD Mgh #2982)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L39	Ultrahydrogel 250	Molecular Weight Limit	7.8 mm × 30 cm. Manufacturer: Waters
35(5)	L17	Aminex HPX-87H	Assay and Limit of	Limit of monomers. 7.8 mm × 30 cm. Guard column Cation H cartridge, catalog # 125-0129, Bio-Rad. Manufacturer: BIO-RAD Laboratories

LEVETIRACETAM (DSD Mgh #2646)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	YMC-Pack ODS-AQ	Assay and Related Compounds	4.6 mm × 15 cm, 3 μm. Manufacturer: YMC Co., Inc.
0(0)	L51	Chiralpak AD	Limit of	Limit of Levetiracetam-*R*-enantiomer. 4.6 mm × 25 cm, 10 μm. Manufacturer: Chiral Technologies, Inc.
0(0)	L1	XTerra RP 18	Limit of	Limit of Levetiracetam Related Compound B. 4.6 mm × 25 cm, 5 μm. Manufacturer: Waters Corp.

MEFLOQUINE HYDROCHLORIDE (DSD Mgh #48040)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	LiChrospher 100 RP-18	Assay	4.0mm × 25 cm, 5 μm. Manufacturer: Merck KGaA

ORLISTAT (DSD Mgh #58790)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L7	Superspher 60 RP-Select B	Limit of	Limit of Orlistat Related Compound D. 4.0 mm × 25 cm. 5 μm Manufacturer: Merck KGaA

OXACILLIN SODIUM (DSD Mgh #59030)

PF	LGS#	Reagent Brand	Type of Test	Comments
20(1)	L11	MicroBondapak Phenyl	Assay	4 mm × 30 cm. Manufacturer: Waters Corp

PARTIALLY-NEUTRALIZED METHACRYLIC ACID AND ETHYL ACRYLATE COPOLYMER (DSD Mgh #4428)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	AQUASIL C18	Organic Impurities and Limit of	Limit of methacrylic acid and ethyl acrylate. 4 mm × 12.5 cm, 5 μm. Alternative column: Zorbax SB-AQ, 4.6 mm × 15 cm, 5 μm. Manufacturer: Agilent. Manufacturer: Thermo Scientific

POLYDEXTROSE (DSD Mgh #66235)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L39	Ultrahydrogel 250	Molecular Weight Limit	7.8 mm × 30 cm, 6 μm. Manufacturer: Waters
35(5)	L17	Aminex HPX-87H	Assay and Limit of	Limit of monomers. 7.8 mm × 30 cm. Guard-column Cation H cartridge, manufacturer Bio-Rad, cat # 125-0129 Manufacturer: BIO-RAD Laboratories

RACTOPAMINE HYDROCHLORIDE SUSPENSION (DSD Mgh #1278)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	Supelcosil LC-18-DB	Assay	4.6 mm × 15 cm, 5 μm. Manufacturer: Supelco, Inc.
35(5)	L1	Supelcosil LC-18-DB	Organic Impurities	4.6 mm × 25 cm, 5 μm. Manufacturer: Supelco, Inc.
35(5)	L1	APEX ODS	Diastereoisomer Ratio	4.6 mm × 25 cm, 5 μm. Manufacturer: Phenomenex

TELMISARTAN AND HYDROCHLOROTHIAZIDE TABLETS (DSD Mgh #37985)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	Inertsil ODS	Dissolution	3 mm × 6 cm, 5 μm. Manufacturer: GL Sciences
35(5)	L1	Inertsil ODS	Assay and Organic Impurities	4.0 mm × 12.5 cm, 5 μm. Manufacturer: GL Sciences

TRICLOSAN (DSD Mgh #85150)

PF	LGS#	Reagent Brand	Type of Test	Comments
22(3)	G3	DB-17	Assay and Related Compounds	Alternative column AT-50, 0.53 mm × 15 cm, 1 μm Manufacturer: J & W Scientific

ZIDOVUDINE ORAL SOLUTION (DSD Mgh #89518)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	Hypersil BDS C-18	Organic Impurities	4.6 mm × 10 cm, 3 μm. Manufacturer: Thermo Scientific

ZOLPIDEM TARTRATE TABLETS (DSD Mgh #89990)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(4)	L1	Spherisorb ODS1	Assay and Related Compounds	4.6 mm × 15 cm, 5 μm. Manufacturer: Waters Corp.

Table of Contents*

PHARMACOPEIAL FORUM VOL. 35 NO. 6 **NOV.–DEC. 2009**

* The *USP–NF* (*USP 33–NF 28*), the *Supplement* (*Supp*), or the *Interim Revision Announcement* (*IRA*) for which the revision proposal is targeted is shown in parentheses next to each proposed item.

THE JOURNAL OF STANDARDS DEVELOPMENT AND OFFICIAL COMPENDIA REVISION

Please address comments on *Pharmacopeial Forum* content to
USP Executive Secretariat
12601 Twinbrook Parkway,
Rockville, MD 20852
execsec@usp.org

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

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

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STANDARDS DEVELOPMENT

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

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

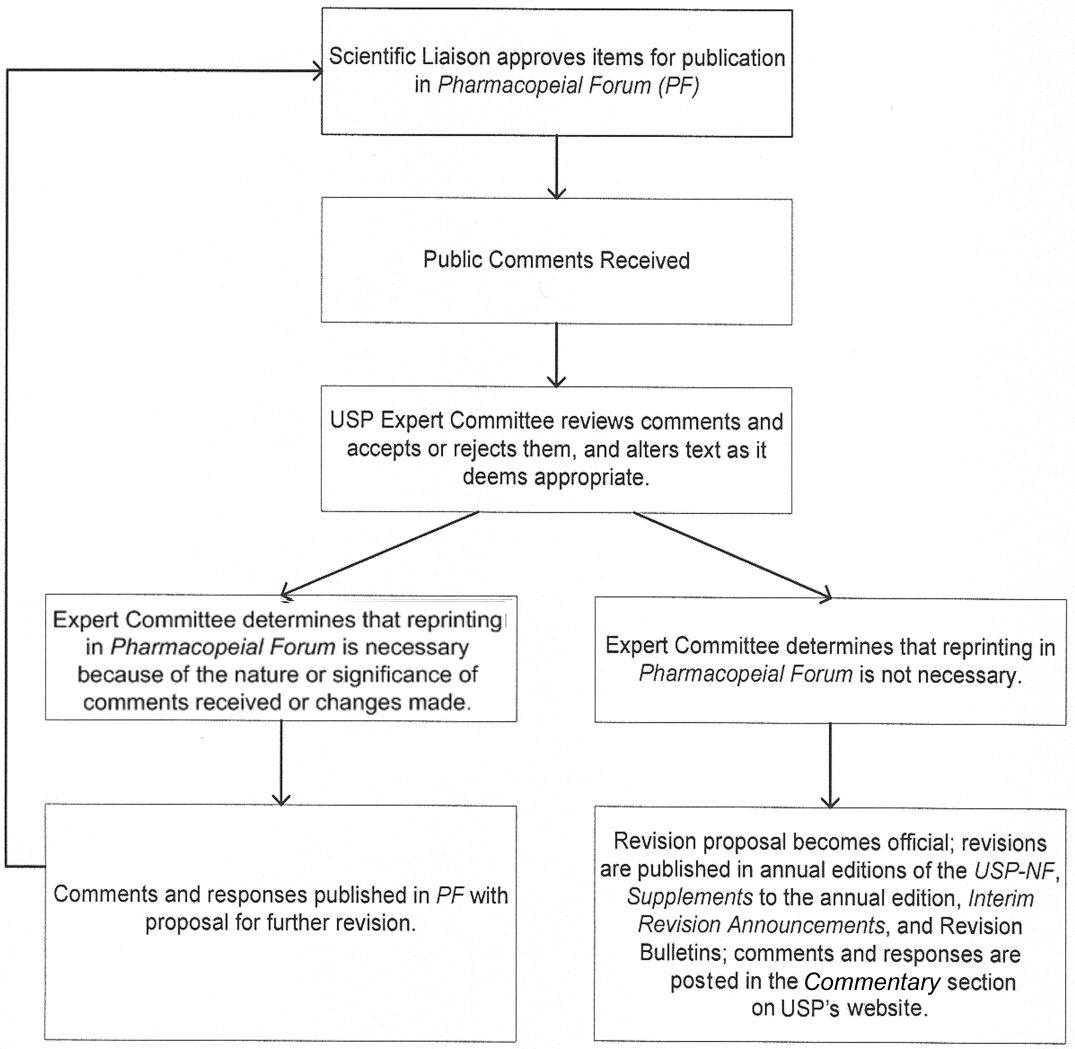
PF includes the following:

1. **Proposed Revisions**—New or revised standards targeted for adoption through USP's typical Revision Process. USP's Revision Process calls for publication of a proposed revision in *PF* for a 90-day notice and comment period. After the comment period and subsequent review of comments and approval by the relevant USP Expert Committee, the official standard is published in the next *USP–NF* or *Supplement*. If comments received are significant, or if the Expert Committee's consideration of comments results in significant additional changes, the Expert Committee may determine that reprinting in *PF* is necessary prior to the revision becoming official. See the *In-Process Revision* section for current proposed revisions.
2. **Accelerated Revisions**—New or revised standards that become official through an accelerated process in accordance with USP's Guideline on Accelerated Revisions (available on the USP website) (e.g., *Interim Revision Announcements*, *Revision Bulletins*, and *Errata*). Accelerated Revisions allow for a revision to become official prior to the next *USP–NF* or *Supplement* and do not always require notice and comment. *Interim Revision Announcements* are first presented for a 60-day public comment period in the *Proposed Interim Revision Announcement* section before becoming official in a later *PF* in the *Interim Revision Announcements* section of the *PF*. Note that *Revision Bulletins* appear only on the USP website.

USP welcomes comments and data on proposed revisions. Comments, along with USP's responses, will be published in the *Commentary* section of the USP website (www.usp.org).

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

Public Review and Comment Process for *USP-NF* Standards Development



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

HOW TO USE *PF*

''How to Use PF'' describes the various parts of *Pharmacopeial Forum*, lists the *Committee Designations*, and includes the *Staff Directory*.

The contents of the various sections of *PF* are briefly described below. A more detailed description of each section is provided at the beginning of that section. A general description of the types and amount of information expected in a *Request for Revision* is available in the *Guideline for Submitting Requests for Revision to the USP–NF* on the USP website (www.usp.org/USPNF/submitMonograph/subGuide.html).

Proposed and Adopted Revisions to the *USP–NF*

Section	Content	How Readers Can Respond
Proposed Interim Revision Announcement Accelerated Revision targeted to become official in an upcoming *PF.*	Proposals for *Interim Revision Announcements (IRAs)* that will be published as official in a future *Pharmacopeial Forum.* BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the USP Scientific Liaison who handled the issue.	Review material and send comments within 60 days of the *PF* publication where the standard was proposed (or per the Briefing). Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing.
Interim Revision Announcement Official Accelerated Revision (on the first day of the second month, unless otherwise indicated).	Standards that have been adopted and will become official on the date that is specified in the section's introductory page or within parentheses following a particular item. New or revised text is marked by the symbols •.	Review material to see whether affected by any of the changes. Note date when standards become official, and ensure compliance.
Errata Accelerated Revision.	Corrections to official standards that will be printed in *USP–NF.*	Review material to see whether affected by any changes.
In-Process Revision Revisions for public review and comment.	Proposals for standards that will be published as official in a future *USP–NF* book or *Supplement*. BRIEFING: Scientific rationale for proposed changes. May include other information useful to the analyst, such as the brand name of the column used in developing the proposed procedure and the appropriate USP Scientific Liaison.	Review material and send comments. Comment deadlines are found at the end of the *Policies and Announcements* section. Direct comments to the USP Scientific Liaison (see the *Staff Directory*) identified at the end of the Briefing. For general inquiries or in cases where a Scientific Liaison is not identified, use the general USP telephone number 301-881-0666 or fax number 301-998-6839.
Previous PF Proposals	Proposals from previous *PF*s that did not advance to official status in an official USP publication. This section is cumulative.	Review material to track pending proposals.
Canceled Proposals	Items that were published in *PF* and were pending, but have since been canceled. Note that canceled proposals may be republished to be considered in the future for adoption in *USP–NF.*	Review material to track canceled proposals.

Proposed and Adopted Revisions to the *USP–NF* *(Continued)*

Section	Content	How Readers Can Respond
Stage 4 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize.	BRIEFING: Scientific rationale for the potential inclusion or change or for the proposed change. Stage 4 is available for comment.	Review material and provide comments to the appropriate Scientific Liaison cited in the Briefing. Individuals who wish to correspond with the European and Japanese Pharmacopoeias concerning monographs in the Official Inquiry and Consensus stages of international harmonization should address their comments to the coordinating pharmacopoeia, with a copy to USP, for a given article. The addresses for the European (PhEur) and Japanese (JP) pharmacopoeias are as follows: PhEur Secretariat Ms. Lynn Kelso-Eleuterio Central Secretariat European Pharmacopoeia Department European Directorate for the Health Care Council of Europe 7, Allée Kastner CS 30026 67081 Strasbourg France Tel: +33 (3) 88 41 31 48 Fax: +33 (3) 88 41 27 71 lynn.kelso@edqm.eu JP Secretariat Dr. Shigenori Harada Quality Expert Pharmaceuticals and Medical Devices Agency (PMDA) Shin-kasumigaseki Building 3-3-2, Kasumigaseki, Chiyoda-ku Tokyo, 100-0013 Japan Phone: +81-3-3506-9431 Fax: +81-3-3506-9440 harada-shigenori@pmda.go.jp
Stage 6 Harmonization Items the Pharmacopeial Discussion Group (PDG) is working to internationally harmonize.	Stage 6 is the final official harmonized standard and is not available for comment. This information is published for informational purposes only. New or revised text to Stage 6 documents is marked with symbols that indicate the publication in which the book or *Supplement* becomes official.	Review material to see whether affected by any changes.

Other Sections

Expert Committee Designations
Names of the Expert Committees (comprising volunteer scientific experts) that work with USP staff on the development of standards

Staff Directory
Names of key USP Standards Division staff members, including Scientific Liaisons, with contact information

Policies and Announcements
- General scientific and policy issues affecting *USP–NF* standards and processes
- Update on standards-related issues being considered by USP
- Guidelines on how to comment
- Publication and comment schedules

Stimuli to the Revision Process
- Articles on standards development issues authored by the USP Council of Experts, USP staff, or other interested parties
- Discussions of issues on which USP desires public input prior to further development

Nomenclature
- Latest adopted United States Adopted Names (USAN) and International Nonproprietary Names (INN) for drugs
- Revisions to existing names as a supplement to the *USP Dictionary of USAN and International Drug Names*
- Suggested, proposed, and recommended USAN and INN
- Information on how nonproprietary drug names are devised
- Articles relevant to compendial nomenclature issues

Index
Cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1)

Chromatographic Columns Used in USP–NF and Pharmacopeial Forum
Update of chromatographic columns based on the proposals published in this issue of *PF*

EXPERT COMMITTEE DESIGNATIONS*

2005–2010

AER	Aerosols
BB BBP	B&B Blood and Blood Products
BB CGT	B&B Cell, Gene, and Tissue Therapies
BB PP	B&B Proteins and Polysaccharides
BB VV	B&B Vaccines and Virology
BPC	Biopharmaceutics
CRX	Compounding Pharmacy
DSB	Dietary Supplements—Botanicals
DS-GC	Dietary Supplements—General Chapters
DSI	Dietary Supplements—Information
DSN	Dietary Supplements—Non-Botanicals
DS-PS	Dietary Supplements—Performance Standards [Formerly Dietary Supplements—Bioavailability (DSB)]
EGC	Excipient General Chapters
EM1	Excipient Monographs 1
EM2	Excipient Monographs 2
FI	Food Ingredients
GC	General Chapters
GTMDB	General Toxicity and Medical Device Biocompatibility
IH	International Health
MSA	Microbiology and Sterility Assurance
MD-ANT	Monograph Development—Antibiotics
MD-AA	Monograph Development—Antivirals and Antimicrobials
MD-CV	Monograph Development—Cardiovascular
MD-CCA	Monograph Development—Cough, Cold, and Analgesics
MD-GRE	Monograph Development—Gastrointestinal, Renal, and Endocrine
MD-OOD	Monograph Development—Ophthalmology, Oncology, and Dermatology
MD-PP	Monograph Development—Psychiatrics and Psychoactives
MD-PS	Monograph Development—Pulmonary and Steroids
NOM	Nomenclature
P&S	Packaging and Storage
PPI	Parenteral Products—Industrial
PDF	Pharmaceutical Dosage Forms
PW	Pharmaceutical Waters
RI	Radiopharmaceutical Information
RMI	Radiopharmaceuticals and Medical Imaging Agents
RS	Reference Standards
SCC	Sterile Compounding
SMU	Safe Medication Use
STAT	Statistics

EXPERT COMMITTEE DESIGNATIONS* *(Continued)*

2005–2010

VET	Veterinary Drugs
VMI	Veterinary Medicine Information

* **HDQ** Indicates USP Headquarters items.

STAFF DIRECTORY

This updated directory reflects assignment changes based on 2005–2010 Expert Committees. The general USP telephone number, (301) 881-0666, may still be used for general inquiries or when a particular Expert Committee is not identified. The fax number is (301) 816-8373.

STAFF	E-MAIL	PHONE	CONTACT FOR
Clydewyn M. Anthony, Ph.D., Senior Scientist	cma@usp.org	(301) 816-8139	Monograph Development—Cough, Cold, and Analgesics (MD-CCA)
Fouad Atouf, Ph.D., Senior Scientific Associate	fa@usp.org	(301) 816-8365	B&B Cell, Gene, and Tissue Therapies (BB CGT)
Shawn C. Becker, M.S., B.S.N., R.N., Director, Patient Safety Initiatives	scb@usp.org	(301) 816-8216	Sterile Compounding
Kristie Bowman, M.S., Senior Scientific Associate	kxb@usp.org	(301) 816-8356	Food Ingredients (FI)
Colleen Brennan, R.Ph., Manager	cyb@usp.org	(301) 816-8548	Safe Medication Use (SMU)
William E. Brown, Senior Scientist	web@usp.org	(301) 816-8380	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF)
Damián A. Cairatti, Senior Scientist	dac@usp.org	(301) 816-8307	*USP–NF* Spanish Edition
Todd L. Cecil, Ph.D., Vice President, Compendial Sciences	tlc@usp.org	(301) 816-8234	
Behnam Davani, Ph.D., Senior Scientist	bd@usp.org	(301) 816-8394	Monograph Development—Antivirals and Antimicrobials (MD-AA)
Natalia Davydova, Ph.D., Scientist	nd@usp.org	(301) 816-8328	Dietary Supplements Performance Standards (DS-PS)
Anthony DeStefano, Ph.D., Vice President, General Chapters	ajd@usp.org	(301) 230-6303	
Susan S. de Mars, J.D., Chief Documentary Standards Officer and General Counsel	sdm@usp.org	(301) 816-8296	
Gabriel I. Giancaspro, Ph.D., Director, Dietary Supplements	gig@usp.org	(301) 816-8343	
Brian D. Gilbert, Ph.D., Scientist	bg@usp.org	(301) 816-8223	
Elena Gonikberg, Ph.D., Senior Scientist	eg@usp.org	(301) 816-8251	Monograph Development—Gastrointestinal, Renal, and Endocrine (MD-GRE); Radiopharmaceuticals and Medical Imaging Agents (RMI); Veterinary Drugs (VET)
James Griffiths, Ph.D., Vice President, Food, Dietary Supplement, and Excipient Standards	jg@usp.org	(301) 998-6811	
Linda Guard, Vice President, Publications	lmg@usp.org	(301) 816-8309	
Antonio Hernandez-Cardoso, M.Sc., Scientist, Latin American Specialist	ahc@usp.org	(301) 816-8308	*USP–NF* Spanish Edition; General Chapters (GC); Pharmaceutical Waters (PW)
Desmond G. Hunt, Ph.D., Scientist	dgh@usp.org	(301) 816-8341	Packaging and Storage (P&S); Parenteral Products—Industrial (PPI)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Alexey Khrenov, Ph.D., Senior Scientific Associate	ak@usp.org	(301) 998-6335	B&B Proteins and Polysaccharides (BB PP)
Randy Kiser, M.S., M.B.A., Program Manager	rwk@usp.org	(301) 816-8324	USP Reference Standards Acquisition
Robert Lafaver, Scientist	rhl@usp.org	(301) 816-8335	Excipient Monographs 1 (EM1); Excipient General Chapters (EGC)
Steven P. Lane, Ph.D., Vice President, Reference Standards Operations	psl@usp.org	(301) 816-8337	
Markus Lipp, Ph.D., Director, Foods Standards	mxl@usp.org	(301) 816-6366	Food Ingredients (FI)
Angela G. Long, Vice President, Volunteer and Organizational Affairs and Executive Secretariat	agl@usp.org	(301) 816-8382	
Feiwen Mao, M.S., Scientist	fm@usp.org	(301) 816-8320	Monograph Development—Ophthalmology, Oncology, and Dermatology (MD-OOD)
Margareth R. C. Marques, Ph.D., Senior Scientist and Latin American Liaison	mrm@usp.org	(301) 816-8106	Biopharmaceutics (BPC); Pharmaceutical Dosage Forms (PDF); Reagents
Kate Meringolo, M.S., M.B.A., Manager, Publication Support	kxm@usp.org	(301) 816-8377	
Jeff Moore, Ph.D., Scientist	jm@usp.org	(301) 816-8288	Food Ingredients (FI)
Kevin Moore, Ph.D., Senior Scientist	ktm@usp.org	(301) 816-8369	Harmonization; Monograph Improvement
Tina S. Morris, Ph.D., Vice President, Biologics and Biotechnology	tsm@usp.org	(301) 816-8397	
Horacio Pappa, Ph.D., Senior Scientist and Latin American Liaison	hp@usp.org	(301) 816-8319	General Chapters (GC); Statistics (STAT)
Curtis S. Phinney, MSPH, Scientist	csp@usp.org	(301) 816-8540	Dietary Supplements—Non-Botanicals (DSN)
Morgan Puderbaugh, Scientific Associate	mxp@usp.org	(301) 998-6833	Small Molecules Monographs
Sujatha Ramakrishna, Ph.D., Scientist	sxr@usp.org	(301) 816-8349	Monograph Development—Cardiovascular (MD-CV)
Hariram Ramanathan, Ph.D., Senior Scientific Associate	hr@usp.org	(301) 816-8313	Small Molecules Monographs
Ravi Ravichandran, Ph.D., Senior Scientist	rr@usp.org	(301) 816-8330	Monograph Development—Psychiatrics and Psychoactives (MD-PP)
Karen A. Russo, Ph.D., Vice President, Small Molecules	kar@usp.org	(301) 816-8379	
Leonel Santos, Ph.D., Senior Scientist	lxs@usp.org	(301) 816-8168	International Health (IH)
Dandapantula Sarma, Ph.D., Senior Scientist	dns@usp.org	(301) 816-8354	Dietary Supplements—Information (DSI)
Rick Schnatz, Pharm.D., Manager, Pharmacy Compounding	rxs@usp.org	(301) 816-8526	Compounding Pharmacy (CRX)

STAFF DIRECTORY *(continued)*

STAFF	E-MAIL	PHONE	CONTACT FOR
Stefan P. Schuber, Ph.D., Director, Scientific Reports	sps@usp.org	(301) 816-8551	
Maged H. M. Sharaf, Ph.D., Senior Scientist	mhs@usp.org	(301) 816-8318	Dietary Supplements—Botanicals (DSB)
Catherine M. Sheehan, M.S., Director, Excipients	cxs@usp.org	(301) 816-8262	Excipients
Tom Sigambris, M.S., Scientist	tzs@usp.org	(301) 998-6789	B&B Vaccines and Virology (BB VV); Proteins and Polysaccharides (BB PP) (small peptides)
Anita Y. Szajek, Ph.D., Senior Scientist	aey@usp.org	(301) 816-8325	B&B Blood and Blood Products (BB BBP)
Radhakrishna S. Tirumalai, Ph.D., Senior Scientist	rst@usp.org	(301) 816-8339	General Toxicity and Medical Device Biocompatibility (GTMDB); Microbiology and Sterility Assurance (MSA)
Yoshiyuki Tokiwa, Ph.D., Senior Scientist	yt@usp.org	(301) 816-8321	Dietary Supplements—General Chapters (DS-GC)
Domenick Vicchio, Ph.D., Senior Scientist	dwv@usp.org	(301) 998-6828	Monograph Development—Pulmonary and Steroids (MD-PS)
Mary ''Jeanie'' Waddell, Scientist	msw@usp.org	(301) 816-8124	Monograph Development—Pulmonary and Steroids (MD-PS)
Hong Wang, Ph.D., Scientist	hw@usp.org	(301) 816-8351	Excipient Monographs 2 (EM2); Excipient General Chapters (EGC)
Lili Wang, Technical Services Scientist	lw@usp.org	(301) 816-8129	USP Reference Standards Evaluation
Andrzej Wilk, Ph.D., Senior Scientist	aw@usp.org	(301) 816-8305	Nomenclature (NOM)
Ahalya Wise, M.S., Scientist	aww@usp.org	(301) 816-8161	Monograph Development—Antibiotics (MD-ANT)
Kahkashan Zaidi, Ph.D., Senior Scientist	kxz@usp.org	(301) 816-8269	Aerosols (AER); General Chapters (GC)

POLICIES AND ANNOUNCEMENTS

This section provides general information resources for *USP–NF* standards and processes. Information resources include announcements on scientific and policy issues currently under consideration, schedules for USP publications, and schedules for comment periods for proposed standards.

CALL FOR CANDIDATES FOR 2010–2015 COUNCIL OF EXPERTS, ITS EXPERT COMMITTEES, AND ITS EXPERT PANELS. APPLICATION DEADLINE FOR COMMITTEE CHAIRS: DECEMBER 18, 2009. In accordance with the Bylaws of the USP Convention, USP is issuing a Call for Candidates for the 2010–2015 Council of Experts (COE). The 2010–2015 COE includes Expert Committees in the areas of Nomenclature, Small Molecules, Biologics and Biotechnology, Excipients, General Chapters, Reference Standards, Compounding, Food Ingredients, and Dietary Supplements. In the 2010–2015 cycle, USP is expanding the number of Expert Panels that report to Expert Committees.

The deadline for applications for the COE (Expert Committee Chairs) is **December 18, 2009**. The deadline for applications for Expert Committee members is **May 15, 2010**. Recruitment for Expert Panel members will begin in July 2010 and will be continuous.

These Expert Committees and Panels are aligned with the new USP Strategic Plan, which focuses on expanding and enhancing USP's core compendial and standards-setting activities. The ability to add Expert Panels according to USP's needs introduces flexibility and scalability into USP's activities. USP plans to continue to attract a global base of experts and therefore encourages any qualified individual to apply. Importantly, this approach also enables USP to closely align its documentary and Reference Standards activities for a more efficient standards-setting process.

Specific Expert Committees and Expert Panels for which USP is seeking candidates are listed at USP's nominations website (www.usp.org/goto/nominate).

For further information, please contact Nelufar Mohajeri, Director, Volunteer Affairs and Compendial Initiatives (nym@usp.org or nominate@usp.org).

USP POSTS COMMENTARY TO INTERIM REVISION ANNOUNCEMENTS ON THE USP WEBSITE. In order to maintain transparency for revisions made to proposed Interim Revision Announcements that become official in the *Pharmacopeial Forum*, USP posts commentary for the proposed *Interim Revision Announcements* on the *Revisions and Commentary* web page on the date that the official standard is released in *Pharmacopeial Forum*. Note that commentary to *In-Process Revisions* is posted on the *Revisions and Commentary* web page under the final book or supplement where the official standard appears. *Commentary* is not part of the official text of the monograph and is not intended to be enforceable by regulatory authorities. Rather, it explains the basis of the Expert Committee's response to public comments. If there is a difference between the contents of the *Commentary* section and the official monograph, the text of the official monograph prevails. In case of a dispute or question of interpretation, the language of the official text, alone and independent of the *Commentary* section, shall prevail.

CUMULATIVE EDITIONS. *USP–NF* is available in print, CD, and online formats. For the CD and online formats, each new edition or *Supplement* integrates content from all previous editions to date.

***PHARMACOPEIAL FORUM* PUBLIC REVIEW and COMMENT PERIOD DEADLINES.** The USP welcomes and encourages interested parties to submit comments and data regarding potential, proposed, or adopted (official) standards. In accordance with the Rules and Procedures of the 2005–2010 Council of Experts, USP has implemented a 90-day comment period by providing a deadline for each issue of *PF* unless otherwise stated in the individual briefing. The listing of comment period deadlines and the targeted official publications appears below.

Pharmacopeial Forum	Comment Deadline	Targeted Official Publication	Release Date	Official Date
PF 35(2)	June 15, 2009	*USP 33–NF 28 1st Supplement*	February 2010	August 1, 2010
PF 35(3)	August 15, 2009			
PF 35(4)	October 15, 2009	*USP 33–NF 28 2nd Supplement*	June 2010	December 1, 2010
PF 35(5)	December 15, 2009			
PF 35(6)	February 15, 2010	*USP 34–NF 29*	November 2010	May 1, 2011
PF 36(1)	March 31, 2010			

All official revisions are published in the annual edition or *Supplements* to *USP–NF* (twice yearly). Between these publications, official revisions are published in *PF* in the *Interim Revision Announcement* section and incorporated in the upcoming *USP–NF* or *Supplement*. They may also be published as *Revision Bulletins* on www.usp.org in the ''New Official Text'' section. The official publication in which an *Interim Revision Announcement* (*IRA*) is incorporated will depend upon publication deadlines. The electronic version of *USP–NF* is updated as each *Supplement* becomes available and therefore contains all official text up to and including the contents of the latest *Supplement*. The table below outlines the publications and their release and official dates, and the *USP–NF* or *Supplement* that supersedes them.

Publication Schedules

Publication	Release Date	Official Date	Superseded by
IRA [*PF* 35(2)]	March 1, 2009	April 1, 2009	*2nd Supplement to USP 32–NF 27*
IRA [*PF* 35(3)]	May 1, 2009	June 1, 2009	*USP 33–NF 28*
2nd Supplement to USP 32–NF 27	June 1, 2009	December 1, 2009	*USP 33–NF 28*
IRA [*PF* 35(4)]	July 1, 2009	August 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(5)]	September 1, 2009	October 1, 2009	*1st Supplement to USP 33–NF 28*
IRA [*PF* 35(6)]	November 1, 2009	December 1, 2009	*2nd Supplement to USP 33–NF 28*
USP 33–NF 28	November 1, 2009	May 1, 2010	*1st Supplement to USP 33–NF 28*
IRA [*PF* 36(1)]	January 1, 2010	February 1, 2010	*2nd Supplement to USP 33–NF 28*
1st Supplement to USP 33–NF 28	February 1, 2010	August 1, 2010	*2nd Supplement to USP 33–NF28*

PRIORITY NEW MONOGRAPH ITEMS. The following list contains monographs USP is seeking for drug substances and drug products that are, or will be, off patent within 5 years. Monographs are marked ''Received'' upon receipt of a monograph proposal. This list has been updated as of June 24, 2009.

Monograph sponsors should consult USP's Guideline for Submitting Requests for Revision to the *USP–NF* at http: // www . usp . org / USPNF / submit Monograph / subGuide.html.

For additional information, contact Randy Kiser, MS, MBA, rwk@usp.org.

Small Molecules (Drug Substances)—As of June 24, 2009

1. Acamprosate Calcium	2. Acrivastine	3. Adapalene
4. Aldesleukin	5. Alemtuzumab	6. Allopurinol Sodium
7. Alosetron Hydrochloride	8. Aminopromazine Fumarate	9. Aminopterin Sodium
10. Amlexanox	11. Amlodipine Maleate	12. Amrinone Lactate
13. Anagrelide Hydrochloride ***(Received)***	14. Artemether	15. Auranofin
16. Azacitidine	17. Azelaic Acid ***(Received)***	18. Azelastine Hydrochloride
19. Bemotrizinol	20. Bentoquatam	21. Benzphetamine Hydrochloride
22. Bepridil Hydrochloride	23. Besifloxacin	24. Bismuth Tribromophenate
25. Bivalirudin ***(Received)***	26. Bromfenac Sodium	27. Butenafine Hydrochloride
28. Caffeine Citrate	29. Calcium Trisodium Pentetate	30. Calfactant
31. Camphorated Metacresol	32. Candesartan Cilexetil ***(Received)***	33. Carbaspirin Calcium
34. Ceftibuten	35. Cerivastatin Sodium	36. Cevimeline Hydrochloride ***(Received)***
37. Chlophedianol Hydrochloride	38. Chlorpheniramine Tannate	39. Cidofovir
40. Cisatracurium Besylate	41. Climbazole	42. Codeine Polistirex
43. Colfosceril	44. Copper Undecylenate	45. Cysteamine Bitartrate
46. Dalfopristin	47. Decitabine	48. Deserpidine
49. Desogestrel	50. Desonide ***(Received)***	51. Diethanolamine Methoxycinnamate
52. Difenoxin Hydrochloride	53. Doconazol	54. Domiphen Bromide
55. Doripenem	56. Entacapone ***(Received)***	57. Epoprostenol Sodium ***(Received)***
58. Esmolol Hydrochloride ***(Received)***	59. Estazolam	60. Estramustine Phosphate Sodium

Small Molecules (Drug Substances)—As of June 24, 2009 *(Continued)*

61. Ethanolamine Oleate	62. Ethyl 4-[Bis(Hydroxypropyl)] Amino-benzoate	63. Etomidate ***(Received)***
64. Etoposide Phosphate	65. Exemestane	66. Famciclovir ***(Received)***
67. Felbamate ***(Received)***	68. Ferric Hexacyanoferrate (Prussian Blue)	69. Ferric Sodium Gluconate
70. Fluoromethane F 18	71. Fomepizole	72. Fosfomycin Tromethamine ***(Received)***
73. Gadobenate Dimeglumine	74. Gadofosveset Trisodium	75. Gadopentetic Acid
76. Gadoxetate Disodium	77. Gallium Nitrate	78. Glyceryl Aminobenzoate
79. Guanidine Hydrochloride	80. Halobetasol Propionate ***(Received)***	81. Haloperidol Decanoate ***(Received)***
82. Hydrocodone Polistirex	83. Ibutilide Fumarate	84. Iloperidone
85. Imipramine Pamoate	86. Imiquimod	87. Iodamide Meglumine
88. Isosulfan Blue	89. Lactulose	90. Latanoprost ***(Received)***
91. Lepirudin	92. Levobetaxolol Hydrochloride	93. Levocetirizine Dihydrochloride
94. Levomethadyl Acetate	95. Lodoxamide Tromethamine	96. Lomustine ***(Received)***
97. Loteprednol Etabonate	98. Loxapine Hydrochloride	99. Lubiprostone
100. Melphalan Hydrochloride	101. Memantine Hydrochloride	102. Mequinol
103. Methotrexate Sodium	104. Methoxycinnamate	105. Methyl Nicotinate
106. Metipranolol Hydrochloride	107. Mibefradil Dihydrochloride	108. Midazolam Hydrochloride
109. Mifepristone	110. Milrinone Lactate	111. Misoprostol Oil
112. Mivacurium Chloride	113. Moexipril Hydrochloride	114. Montelukast Sodium
115. Nabilone	116. Nalbuphine Hydrochloride	117. Nalmefene Hydrochloride
118. Nedocromil Sodium	119. Nefopam	120. Nelarabine
121. Nelfinavir Mesylate	122. Nepafenac	123. Nesiritide Citrate
124. Nevirapine Hemihydrate	125. Nicardipine Hydrochloride ***(Received)***	126. Nilutamide
127. Olsalazine Sodium ***(Received)***	128. Oxiconazole Nitrate	129. Paliperidone
130. Pemirolast Potassium	131. Pemoline	132. Perindopril Erbumine
133. Phenylephrine Tannate	134. Pioglitazone Hydrochloride ***(Received)***	135. Pipecuronium Bromide
136. Piperonyl Butoxide	137. Pirbuterol Acetate	138. Pirbuterol Acetate ***(Received)***
139. Podofilox	140. Poractant Alfa	141. Porfimer Sodium
142. Pramiprexole Dihydrochloride ***(Received)***	143. Prasterone	144. Proguanil Hydrochloride
145. Pyrilamine Tannate	146. Pyrithione Zinc	147. Quetiapine Fumarate ***(Received)***
148. Quinupristin	149. Rabeprazole Sodium	150. Ranitidine
151. Rifapentine	152. Rivastigmine Tartrate ***(Received)***	153. Rizatriptan Benzoate
154. Ropinirole Hydrochloride ***(Received)***	155. Rostaporfin	156. Sertaconazole Nitrate
157. Silodosin	158. Sodium Phenylbutyrate	159. Spirapril Hydrochloride
160. Streptozocin	161. Sulfacytine	162. Sulfosalicylic Acid
163. Tazarotene	164. Tegaserod Maleate	165. Tenofovir Disoproxil Fumarate ***(Received)***
166. Tiludronate Disodium	167. Tiopronin	168. Tolvaptan
169. Toremifene Citrate	170. Trastuzumab	171. Trypan Blue
172. Unithiol	173. Venlafaxine Hydrochloride ***(Received)***	174. Voriconazole ***(Received)***
175. Zafirlukast	176. Zaleplon ***(Received)***	177. Zinc Trisodium Pentetate
178. Zoledronic Acid		

Small Molecules (Drug Products)—As of June 24, 2009

1. Acamprosate Calcium Delayed-Release Tablets	2. Acetaminophen and Tramadol Hydrochloride Tablets	3. Acetaminophen, Butalbital, Caffeine, and Codeine Phosphate Capsules
4. Acetaminophen, Clemastine Fumarate, and Pseudoephedrine Hydrochloride Tablets	5. Acetazolamide Extended-Release Capsules	6. Acetylcysteine Injection
7. Acrivastine and Pseudoephedrine Hydrochloride Capsules	8. Adapalene and Benzoyl Peroxide Gel	9. Adapalene Topical Gel
10. Adapalene Topical Solution	11. Albuterol Extended-Release Tablets	12. Albuterol for Inhalation
13. Albuterol Oral Solution	14. Aldesleukin for Injection	15. Alendronate Sodium Oral Solution
16. Allopurinol for Injection	17. Alosetron Tablets	18. Amantadine Hydrochloride Tablets
19. Aminophylline Extended-Release Tablets	20. Aminopromazine Fumarate and Neomycin Sulfate Tablets	21. Aminopromazine Fumarate Injection
22. Aminopromazine Fumarate Tablets	23. Aminopterin Sodium Tablets	24. Amlexanox Oral Paste
25. Amlodipine and Benazepril Hydrochloride Capsules ***(Received)***	26. Amlodipine Besylate Tablets	27. Amlodipine Maleate Tablets
28. Amoxicillin and Clavulanate Potassium Chewable Tablets	29. Amoxicillin Chewable Tablets	30. Amrinone Lactate Injection
31. Anagrelide Hydrochloride Capsules ***(Received)***	32. Anastrozole Tablets	33. Anecortave Acetate Injectable Suspension
34. Anhydrous Lactose for Inhalation	35. Anisotropine Methylbromide Tablets	36. Artemether and Lumefantrine Tablets
37. Articaine Hydrochloride and Epinephrine Bitartrate Injection	38. Atovaquone and Proguanil Hydrochloride Tablets	39. Atovaquone Tablets
40. Atropine and Pralidoxime Chloride Injection	41. Auranofin Capsules	42. Avobenzone, Ecamsule, and Octocrylene Cream
43. Avobenzone, Ecamsule, Octocrylene, and Titanium Dioxide Cream	44. Azacitidine for Injectable Suspension	45. Azatadine Maleate and Pseudoephedrine Sulfate Extended-Release Tablets
46. Azelastine Hydrochloride Metered-Dose Nasal Solution	47. Azelastine Hydrochloride Ophthalmic Solution	48. Azithromycin for Injection ***(Received)***
49. Azithromycin Tablets ***(Received)***	50. Benazepril Hydrochloride and Hydrochlorothiazide Tablets	51. Bentoquatam Topical Suspension
52. Benzocaine and Cetylpyridinium Chloride Lozenges	53. Benzocaine and Menthol Lotion	54. Benzphetamine Hydrochloride Tablets
55. Bepridil Tablets	56. Besifloxacin Ophthalmic Suspension	57. Betaxolol and Pilocarpine Hydrochloride Ophthalmic Suspension
58. Bismuth Tribromophenate Ointment	59. Bivalirudin for Injection ***(Received)***	60. Bromfenac Sodium Ophthalmic Solution
61. Brompheniramine Maleate, Dextromethorphan Hydrobromide, and Pseudoephedrine Hydrochloride Oral Solution	62. Brompheniramine Maleate, Phenylpropanolamine Hydrochloride, and Codeine Phosphate Syrup	63. Buffered Didanosine for Oral Solution
64. Bupivacaine and Lidocaine Hydrochlorides Injection	65. Buprenorphine Hydrochloride Injection	66. Buspirone Hydrochloride Capsules
67. Busulfan Injection	68. Butalbital and Acetaminophen Capsules	69. Butalbital and Acetaminophen Tablets
70. Butenafine Hydrochloride Cream	71. Cabergoline Tablets	72. Calcitriol Capsules
73. Calcitriol Ointment	74. Calcitriol Oral Solution	75. Calcium Acetate Capsules
76. Calcium Polycarbophil Tablets	77. Calcium Trisodium Pentetate Injection	78. Calfactant Intratracheal Suspension
79. Carbidopa and Levodopa Orally Disintegrating Tablets	80. Carbidopa and Levodopa Tablets for Oral Suspension ***(Received)***	81. Carmustine Implant
82. Cefdinir Tablets	83. Cefepime Injection	84. Cefotaxime Sodium and Dextrose Injection
85. Ceftibuten Capsules	86. Ceftibuten for Oral Suspension	87. Cetirizine Hydrochloride Oral Solution
88. Cetirizine Hydrochloride Tablets ***(Received)***	89. Cevimeline Hydrochloride Capsules	90. Chloramphenicol Ointment
91. Chlorpheniramine and Codeine Polistirexes Extended-Release Oral Suspension	92. Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Tablets	93. Chlorthalidone and Reserpine Tablets
94. Choline Salicylate Oral Solution ***(Received)***	95. Ciclopirox Nail Lacquer	96. Ciclopirox Shampoo
97. Ciclopirox Topical Solution ***(Received)***	98. Cidofovir Injection	99. Cimetidine Oral Solution

Small Molecules (Drug Products)—As of June 24, 2009 *(Continued)*

100. Ciprofloxacin for Injection Concentrate	101. Ciprofloxacin Hydrochloride and Hydrocortisone Otic Suspension	102. Cisatracurium Injection
103. Cisplatin Injection	104. Citric Acid, Gluconolactone, and Magnesium Carbonate Irrigation	105. Cladribine Injection
106. Clemastine Fumarate and Phenylpropanolamine Hydrochloride Extended-Release Tablets	107. Clemastine Fumarate Syrup	108. Clonazepam Orally-Disintegrating Tablets
109. Clorazepate Dipotassium Capsules	110. Clorazepate Dipotassium Extended-Release Tablets	111. Clotrimazole and Betamethasone Dipropionate Lotion
112. Colfosceril and Tyloxapol Suspension	113. Compound Undecylenic Acid Cream	114. Compound Undecylenic Acid Topical Powder
115. Conjugated Estrogens Vaginal Cream	116. Cromolyn Sodium Capsules	117. Cromolyn Sodium Metered-Dose Nasal Solution
118. Cysteamine Bitartrate Capsules	119. Dalfopristin and Quinupristin Injection	120. Dantrolene Sodium Oral Suspension
121. Decitabine for Injection	122. Deserpidine and Hydrochlorothiazide Tablets	123. Deserpidine Tablets
124. Desonide Cream	125. Desonide Foam	126. Dexpanthenol Injection
127. Diazepam Injectable Emulsion	128. Diazepam Rectal Gel	129. Didanosine for Buffered Oral Suspension
130. Diethylpropion Hydrochloride Extended-Release Tablets	131. Dihydroergotamine Mesylate Nasal Solution	132. Diluted Sodium Phosphates Rectal Solution
133. Dinoprostone Vaginal Suppositories	134. Diphenhydramine Citrate and Ibuprofen Tablets	135. Diphenhydramine Hydrochloride Syrup
136. Diphenhydramine Hydrochloride Tablets	137. Divalproex Sodium Delayed-Release Capsules ***(Received)***	138. Docetaxel for Injection
139. Docetaxel Injection	140. Doconazol Cream	141. Doripenem for Injection
142. Dorzolamide and Timolol Ophthalmic Solution	143. Dorzolamide Ophthalmic Solution	144. Doxazosin Extended-Release Tablets
145. Doxepin Hydrochloride Cream	146. Doxorubicin Hydrochloride Pegylated Liposome Injection	147. Doxylamine Succinate Capsules
148. Dried Aluminum Hydroxide and Magnesium Carbonate Gel	149. Econazole Nitrate Cream	150. Edrophonium Chloride and Atropine Sulfate Injection
151. Enalapril Maleate and Diltiazem Malate Extended-Release Tablets	152. Enalapril Maleate and Felodipine Extended-Release Tablets	153. Enalaprilat Injection
154. Entacapone Tablets ***(Received)***	155. Epirubicin Hydrochloride for Injection	156. Epirubicin Hydrochloride Injection
157. Epoprostenol Injection	158. Ergotamine Tartrate, Belladonna Alkaloids, and Phenobarbital Extended-Release Tablets	159. Estazolam Tablets
160. Estramustine Phosphate Sodium Capsules	161. Ethambutol Hydrochloride and Isoniazid Tablets	162. Ethanolamine Oleate Injection
163. Etomidate Injection ***(Received)***	164. Exemestane Tablets	165. Famciclovir Tablets ***(Received)***
166. Felbamate Oral Suspension	167. Felbamate Tablets	168. Fenofibric Acid Delayed-Release Capsules
169. Fentanyl Transdermal System ***(Received)***	170. Ferric Hexacyanoferrate (Prussian Blue) Injection	171. Ferric Sodium Gluconate Injection
172. Ferrous Fumarate and Docusate Sodium Extended- Release Capsules	173. Flunisolide Inhalation Aerosol	174. Flunisolide Nasal Spray
175. Fluorescein Sodium Injection	176. Fluorescein Sodium Ophthalmic Solution	177. Fluorometholone Ointment
178. Fluticasone Propionate Inhalation Powder ***(Received)***	179. Fomepizole for Injection	180. Fomepizole Injection
181. Foscarnet Sodium Injection	182. Fosfomycin for Oral Solution	183. Gadobenate Dimeglumine Injection
184. Gadofosveset Trisodium Injection	185. Gadoxetate Disodium Injection	186. Gallium Nitrate Injection
187. Glipizide Extended-Release Tablets	188. Glycolic Acid Solution	189. Guanidine Hydrochloride Tablets
190. Halobetasol Propionate Cream	191. Halobetasol Propionate Ointment	192. Haloperidol Decanoate Injection
193. Hydralazine Hydrochloride and Hydrochlorothiazide Capsules	194. Hydrochlorothiazide Capsules ***(Received)***	195. Hydrochlorothiazide Oral Solution
196. Hydrocodone Bitartrate and Acetaminophen Oral Solution	197. Hydrocodone Bitartrate and Aspirin Tablets	198. Hydrocodone Bitartrate and Homatropine Methylbromide Syrup

Small Molecules (Drug Products)—As of June 24, 2009 *(Continued)*

199. Hydroflumethiazide and Reserpine Tablets	200. Hydroquinone Lotion	201. Ibuprofen and Pseudoephedrine Hydrochloride Suspension
202. Ibuprofen Capsules	203. Ibuprofen Chewable Tablets	204. Ibutilide Fumarate Injection
205. Idarubicin Hydrochloride Injection	206. Iloperidone Tablets	207. Imipramine Pamoate Capsules
208. Imiquimod Cream	209. Insoluble Prussian Blue Capsules	210. Iodamide Meglumine Injection
211. Iofetamine Hydrochloride I 123 Injection	212. Iohexol Solution	213. Isosulfan Blue Injection
214. Isradipine Extended-Release Tablets	215. Ketoprofen Capsules ***(Received)***	216. Ketoprofen Tablets
217. Ketorolac Tromethamine Ophthalmic Solution	218. Labetalol Hydrochloride and Hydrochlorothiazide Tablets	219. Lactic Acid Lotion
220. Lactose Monohydrate for Inhalation	221. Lamotrigine Chewable Tablets	222. Lamotrigine Injection
223. Lamotrigine Orally Disintegrating Tablets	224. Lamotrigine Tablets ***(Received)***	225. Latanoprost Ophthalmic Solution
226. Lepirudin for Injection	227. Levetiracetam Extended-Release Tablets	228. Levetiracetam Injection
229. Levetiracetam Tablets ***(Received)***	230. Levobetaxolol Ophthalmic Suspension	231. Levocabastine Ophthalmic Suspension
232. Levocetirizine Dihydrochloride Oral Solution	233. Levocetirizine Dihydrochloride Tablets	234. Levofloxacin Injection
235. Levofloxacin Ophthalmic Solution	236. Levofloxacin Tablets	237. Levomethadyl Acetate Hydrochloride Oral Concentrate
238. Lidocaine Oral Transmucosal System	239. Liothyronine Injection	240. Lodoxamide Ophthalmic Solution
241. Lomustine Capsules ***(Received)***	242. Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets	243. Loteprednol Etabonate Ophthalmic Suspension
244. Loxapine Hydrochloride Injection	245. Loxapine Hydrochloride Oral Solution	246. Lubiprostone Capsules
247. Mannitol Irrigation	248. Mefloquine Hydrochloride Tablets	249. Melphalan for Injection
250. Memantine Hydrochloride Tablets	251. Menthol and Methyl Salicylate Transdermal System	252. Mesalamine Suppositories
253. Mesna Injection	254. Mesoridazine Besylate Concentrate	255. Methacholine Chloride for Inhalation Solution
256. Methamphetamine Hydrochloride Extended-Release Tablets	257. Methocarbamol and Aspirin Tablets	258. Methoxsalen Softgels
259. Methyclothiazide and Deserpidine Tablets	260. Metipranolol Ophthalmic Solution	261. Metoprolol Fumarate Extended-Release Tablets
262. Metoprolol Succinate and Hydrochlorothiazide Extended-Release Tablets	263. Mibefradil Dihydrochloride Tablets	264. Midazolam Oral Suspension
265. Milrinone Injection	266. Misoprostol and Diclofenac Sodium Tablets	267. Misoprostol Tablets ***(Received)***
268. Mivacurium Injection	269. Moexipril Hydrochloride and Hydrochlorothiazide Tablets	270. Moexipril Hydrochloride Tablets
271. Molindone Hydrochloride Capsules	272. Molindone Hydrochloride Oral Solution	273. Montelukast Chewable Tablets
274. Montelukast Granules	275. Montelukast Sodium Tablets	276. Mycophenolate Mofetil Oral Solution ***(Received)***
277. Nabilone Capsules	278. Nalbuphine Hydrochloride Injection	279. Nalmefene Hydrochloride Injection
280. Nedocromil Sodium Inhalation Aerosol	281. Nelarabine Injection	282. Nelfinavir Oral Powder
283. Nelfinavir Tablets	284. Neomycin Sulfate Oral Powder	285. Nepafenac Ophthalmic Suspension
286. Nesiritide for Injection	287. Nicardipine Hydrochloride Capsules	288. Nicardipine Hydrochloride Extended-Release Capsules
289. Nicardipine Hydrochloride Injection	290. Nilutamide Tablets	291. Nimodipine Capsules
292. Nitrofurantoin Extended-Release Capsules	293. Olsalazine Sodium Capsules	294. Orphenadrine Citrate Extended-Release Tablets
295. Orphenadrine Citrate, Aspirin, and Caffeine Tablets	296. Oxiconazole Cream	297. Paclitaxel for Suspension
298. Paliperidone Extended-Release Tablets	299. Pargyline Hydrochloride and Methyclothiazide Tablets	300. Polyethylene Glycol 3350 for Oral Solution

Small Molecules (Drug Products)—As of June 24, 2009 *(Continued)*

301. Pemirolast Potassium Ophthalmic Solution	302. Pemoline Tablets	303. Pentamidine Isethionate for Inhalation
304. Pentamidine Isethionate for Injection	305. Pentamidine Isethionate Injection ***(Received)***	306. Pentobarbital Sodium Suppositories
307. Perphenazine Extended-Release Tablets	308. Phendimetrazine Tartrate Extended-Release Capsules	309. Phenobarbital Capsules
310. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Capsules	311. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Extended-Release Tablets	312. Phenylephrine Hydrochloride and Chlorpheniramine Maleate Syrup
313. Phenylephrine Hydrochloride, Chlorpheniramine Maleate, and Acetaminophen Extended-Release Tablets	314. Pilocarpine Hydrochloride Ophthalmic Gel	315. Pilocarpine Hydrochloride Ophthalmic Ointment
316. Pindolol and Hydrochlorothiazide Tablets	317. Pioglitazone Hydrochloride Tablets ***(Received)***	318. Pipecuronium for Injection
319. Piperonyl Butoxide and Pyrethrins Aerosol Foam	320. Pirbuterol Acetate Inhalation Aerosol	321. Podofilox Gel
322. Podofilox Topical Solution	323. Poractant Alfa Suspension	324. Porfimer Sodium for Injection
325. Povacrylate Solution	326. Povacrylate-Iodine Topical Solution	327. Povidone-Iodine Gauze
328. Povidone-Iodine Swabsticks	329. Povidone-Iodine Topical Aerosol Foam	330. Povidone-Iodine Vaginal Suppositories
331. Prasterone Tablets	332. Prazosin Hydrochloride and Polythiazide Capsules	333. Pseudoephedrine Hydrochloride and Brompheniramine Maleate Extended-Release Tablets
334. Pseudoephedrine Hydrochloride and Naproxen Sodium Extended-Release Tablets	335. Pseudoephedrine Sulfate and Dexbrompheniramine Maleate Extended-Release Tablets	336. Pseudoephedrine Sulfate, Dexbrompheniramine Maleate, and Acetaminophen Extended-Release Tablets
337. Pyrilamine Maleate Injection	338. Quinethazone and Reserpine Tablets	339. Quinidine Sulfate Injection
340. Rabeprazole Sodium Delayed-Release Tablets	341. Ranitidine Capsules ***(Received)***	342. Ranitidine Effervescent Granules
343. Ranitidine Effervescent Tablets	344. Rauwolfia Serpentina and Bendroflumethiazide Tablets	345. Reserpine and Polythiazide Tablets
346. Rifampin and Isoniazid Tablets	347. Rifampin Tablets	348. Rifapentine Tablets
349. Rimantadine Hydrochloride Oral Solution	350. Rivastigmine Tartrate Capsules ***(Received)***	351. Rivastigmine Tartrate Oral Solution ***(Received)***
352. Rizatriptan Benzoate Orally-Disintegrating Tablets	353. Rizatriptan Benzoate Tablets	354. Rocuronium Bromide Injection
355. Ropinirole Extended-Release Tablets	356. Salicylic Acid and Sulfur Cleansing Lotion	357. Salicylic Acid and Sulfur Lotion
358. Salicylic Acid and Sulfur Shampoo	359. Salicylic Acid Cream	360. Salicylic Acid Ointment
361. Scopolamine Transdermal System	362. Sertaconazole Nitrate Cream	363. Sibutramine Hydrochloride Capsules
364. Silodosin Capsules	365. Simethicone-Cellulose Suspension	366. Sodium Iodide Injection
367. Sodium Phenylbutyrate Oral Powder	368. Sodium Phenylbutyrate Tablets	369. Sodium Phosphates for Oral Suspension
370. Sodium Phosphates Tablets	371. Sodium Salicylate and Sulfur Shampoo	372. Spirapril Hydrochloride Tablets
373. Sterile Epinephrine Suspension	374. Sterile Estrone and Estrone Potassium Sulfate Suspension	375. Sterile Talc Aerosol
376. Streptozocin for Injection	377. Sucralfate Oral Suspension	378. Sulconazole Nitrate Cream
379. Sulfacetamide Sodium and Fluorometholone Ophthalmic Suspension	380. Sulfacetamide Sodium and Prednisolone Sodium Phosphate Ophthalmic Solution	381. Sulfacytine Tablets
382. Sulfasalazine Oral Suspension	383. Sulisobenzone Lotion	384. Sumatriptan Injection
385. Tazarotene Topical Gel	386. Tegaserod Maleate Tablets	387. Tenofovir Disoproxil Fumarate Tablets ***(Received)***
388. Terazosin Capsules	389. Terazosin Tablets	390. Terbinafine Tablets ***(Received)***
391. Terconazole Vaginal Cream	392. Terconazole Vaginal Suppositories	393. Testosterone Enanthate and Estradiol Valerate Injection
394. Theophylline Elixir	395. Theophylline Extended-Release Tablets	396. Thiabendazole Chewable Tablets
397. Tiludronic Acid Tablets	398. Tinidazole Tablets	399. Tioconazole Vaginal Ointment
400. Tiopronin Tablets	401. Tolnaftate Topical Aerosol Solution	402. Tolvaptan Capsules

Small Molecules (Drug Products)—As of June 24, 2009 *(Continued)*

403. Topiramate Capsules ***(Received)***	404. Toremifene Tablets	405. Torsemide Injection
406. Torsemide Tablets ***(Received)***	407. Trandolapril Tablets ***(Received)***	408. Tranexamic Acid Injection
409. Tranexamic Acid Tablets	410. Trastuzumab Intravenous Injection	411. Trifluridine Ophthalmic Solution
412. Trimeprazine Tartrate Extended-Release Capsules	413. Trimipramine Maleate Capsules	414. Tripelennamine Hydrochloride Extended-Release Tablets
415. Triprolidine and Pseudoephedrine Hydrochlorides and Codeine Phosphate Syrup	416. Triprolidine and Pseudoephedrine Hydrochlorides Extended-Release Capsules	417. Trolamine Salicylate Gel
418. Troleandomycin for Oral Suspension	419. Trovafloxacin Mesylate and Azithromycin Tablets	420. Trypan Blue Ophthalmic Solution
421. Undecylenic Acid Topical Foam Aerosol	422. Urea Cream	423. Valproic Acid Delayed-Release Capsules
424. Vecuronium Bromide for Injection	425. Venlafaxine Extended-Release Capsules ***(Received)***	426. Venlafaxine Tablets ***(Received)***
427. Yttrium Y 90 Chloride Solution	428. Yttrium Y 90 Glass Microspheres	429. Yttrium Y 90 Microspheres Injection
430. Zafirlukast Tablets	431. Zaleplon Capsules ***(Received)***	432. Zidovudine and Lamivudine Tablets ***(Received)***
433. Zileuton Extended-Release Tablets	434. Zileuton Tablets	435. Zinc Acetate Capsules
436. Zinc Trisodium Pentetate Injection	437. Zoledronic Acid for Injection	438. Zonisamide Capsules ***(Received)***

INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:
- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become official before the official date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The official date for these revisions is stated on the next page.)
- Errata

Readers should review this section to determine if they are affected by any of the changes.

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

Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the issue of a given *PF* volume.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements,* that does not accurately reflect the intended official requirements of the Council of Experts. Beginning with *PF* 35(2), Errata will be published both in the *Pharmacopeial Forum* and on the usp.org website. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to *USP 32–NF 27*. The page number indicates where the item is found in *USP–NF.* Errata are updated as necessary in each *Pharmacopeial Forum* issue and monthly on the usp.org website. This information will also be cumulative in future *Supplements,* and will appear in its corrected form in the next annual edition of *USP–NF.* The list of Errata has been relocated to www.usp.org, where updates will be posted monthly.

INTERIM REVISION ANNOUNCEMENT to *USP 32* and to *NF 27*

By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees

Duane M. Kirking, Pharm.D., Ph.D.,
Chair, USP Board of Trustees,
USP Trustee At-Large

Roger L. Williams, M.D., *Chief Executive Officer*
and *Chairman, USP Council of Experts*

Susan de Mars, J.D., *Chief Documentary Standards Officer* and *General Counsel*
William F. Koch, Ph.D., FACB, *Chief Standards Acquisitions* and *Metrology Officer*

Released November 1, 2009 **Official December 1, 2009**

Inquiries regarding *USP–NF* can be addressed to the USP Executive Secretariat, 12601 Twinbrook Parkway, Rockville, MD 20852, USA (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP 32* or *NF 27* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard. Note that the official date is six months after publishing in this PF.

USP *S*-Adenosyl-L-homocysteine RS (March 1, 2010)
USP Fludeoxyglucose Related Compound B RS (May 1, 2010)
USP Alpha Lipoic Acid RS (March 1, 2010)
USP Lypressin RS (March 1, 2010)
USP Propylene Glycol Dilaurate RS (May 1, 2010)
USP Valrubicin RS (March 1, 2010)
USP Vasopressin RS (March 1, 2010)

Unavailable First-Time Official USP Reference Standards

The official dates of any *USP 32* or *NF 27* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of August 10, 2009. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at http://www.uspcatalog.com.

USP Acarbose RS
USP Acarbose System Suitability Mixture RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Powdered *Echinacea pallida* Extract RS
USP Eucatropine Hydrochloride RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Oleyl Oleate RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin Related Compound A RS

ERRATA

Following is a list of errata and corrections to *USP–NF*. The page number indicates where the item is found and in which official or pending official publication of *USP–NF*. If necessary, this list will be updated with every issue of *PF*. This information will also be available as a cumulative table in future *Supplements* and will appear in its corrected form in a future annual edition of *USP–NF*. Errata are considered to be items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirement. USP staff is available to respond to questions regarding the accuracy of a particular requirement by calling 1-800-822-USPC.

***USP32–NF27* Page**	**Title**	**Section**	**Description**
45	⟨11⟩ *USP Reference Standards*	*USP Enzacamene RS*	Change to: ''USP Methyl Benzylidine Camphor RS.'' and place in alphabetical order.
145	⟨381⟩ *Elastomeric Closures for Injections*	*Physicochemical Tests*	Line 1 under *Volatile Sulfides, Requirement*: Change ''Any black stain on the paper produced by *Solution S* is not more intense than that produced by the control solution.'' to: Any black stain on the paper produced by the test solution is not more intense than that produced by the control solution.
500	⟨1056⟩ *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis*	*Characteristics of Polyacrylamide Gels for Protein Electrophoresis*	Line 5 under *Electrophoretic Separation, Sample Buffer 2*: Change ''a final 100 μM DTT concentration.'' to: a final 100 mM DTT concentration.
2257	*Enzacamene*	*USP Reference standards*	Line 1 under *USP Reference standards* ⟨11⟩: Change ''USP Enzacamene RS.'' to: USP Methyl Benzylidene Camphor RS.
		Assay	Line 2 under *Assay, Standard preparation*: Change ''USP Enzacamene RS'' to: USP Methyl Benzylidene Camphor RS Line 7 under *Assay, Procedure*: Change ''USP Enzacamene RS'' to: USP Methyl Benzylidene Camphor RS
2446	*Formoterol Fumarate*	*Specific rotation* ⟨781S⟩	Change ''*Specific Rotation* ⟨781S⟩:'' to: *Optical Rotation, Angular Rotation* ⟨781A⟩:
3334	*Potassium Bromide*	*Limit of iron*	Line 1 under *Citric acid solution*: Change ''Prepare a 200 g citric acid per mL solution.'' to: Prepare a 200 mg citric acid per mL solution.
First Supplement to USP32–NF27			
3934	⟨71⟩ *Sterility Tests*	*Culture Media and Incubation Temperatures*	Third paragraph, line 1: Change ''Mix the L-cystine, sodium chloride,'' to: Mix the L-cystine, agar, sodium chloride,
4050	*Estradiol Vaginal Inserts*	*Microbial enumeration tests* ⟨61⟩ and *Tests for specified microorganisms* ⟨62⟩	Add the following test after *Identification* test *B*: *Microbial enumeration tests* ⟨61⟩ and *Tests for specified microorganisms* ⟨62⟩—The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeasts count does not exceed 10 cfu per g. Inserts meet the requirements of the tests for absence of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans*.

***USP32–NF27* Page**	**Title**	**Section**	**Description**
Second Supplement to USP32–NF27			
4288	*Tylosin Injection*	*Content of tylosins*	Line 1: Change ''*Sodium perchlorate solution*—Prepare a 200 g per L solution. Adjust with 1 N hydrochlorid acid to a pH of 2.5 ± 0.1, and filter. *Mobile phase*—Prepare a mixture of *Sodium perchlorate solution* and acetonitrile (3:2). Degas, and make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).'' to: *Sodium perchlorate solution*—Prepare a 184 g per L solution. *Mobile phase*—Prepare a mixture of *Sodium perchlorate solution* and acetonitrile (3:2). Adjust with 1 N hydrochlorid acid to a pH of 2.5 ± 0.1, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

PROPOSED INTERIM REVISION ANNOUNCEMENTS

This section includes proposals for *Interim Revision Announcements* (*IRAs*) that will be published as official *USP* or *NF* standards. There is a 60-day comment period for these proposals, beginning on the 15th of the first month of this *Pharmacopeial Forum*. The approved official text will be published in a future *Pharmacopeial Forum* and additionally in the ''New Official Text'' section of USP's web site (www.usp.org). Readers should review material in this section and provide comments to the Scientific Liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Expert Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

Proposed IRA

IN-PROCESS REVISION

This section contains proposals for adoption as official *USP* or *NF* standards (either proposed *new* standards or proposed *revisions* of current *USP* or *NF* standards). These may be any of the following: (1) proposed revisions placed directly under *In-Process Revision,* or (2) modifications of revisions previously proposed under *In-Process Revision*. Readers should review material in this section and provide comments to the staff liaison (use the *Staff Directory* to find the contact information). Information on how to comment is found in the *Policies and Announcements* section. It is important to send comments promptly so that the Committee members can consider readers' input as they are deciding whether to advance standards to official status.

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

BRIEFING

Name of Item, citations of the most recent USP publications in which this item appeared. Rationale for the revision. Other relevant information. (For example, if a chromatographic method is being proposed, column specifications and retention times for compounds of interest.) Finally, the Committee designation (see *How to Use PF*), the name of the scientific staff liaison who handled the particular issue, and the USP tracking correspondence number, as shown in the example below:

(DSI: D. Sarma.) RTS—C55678

Symbols Proposed revisions are shown with language proposed for deletion or replacement crossed off. Because of the redesign of monographs, any proposed new text with revisions for *USP 33–NF 28* and beyond will be set off from the current official text by shading where the symbols surround the text changes. Standards that become official as *Interim Revision Announcements (IRAs)* in *Pharmacopeial Forum* will continue to identify changed text in a larger font (print edition only). All *USP–NF* revisions use the following symbols that indicate the final destination of the official text: •new text• if slated for an *IRA*; ▲new text▲ if slated for *USP–NF*; and ■new text■ if slated for a *Supplement* to *USP–NF*. The same symbols *not* set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as ••, or ■■, or ▲▲, it means that text has been deleted and no new text was proposed to replace it.

In all revisions, the closing symbol is accompanied by an identifier that indicates the particular *IRA* or *Supplement* or indicates the *USP* or *NF* as the publication where the revision will appear if approved. For example, •2 indicates that the revision is proposed for the *Interim Revision Announcement* that will appear in issue 2 of a given *PF* volume, ■2S (*USP 32*) indicates that the proposed revision is slated for the *Second Supplement to USP 32*, and ▲*USP33* and ▲*NF28* indicates that the revisions are proposed for *USP 33* and *NF 28*, respectively.

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

USP MONOGRAPHS

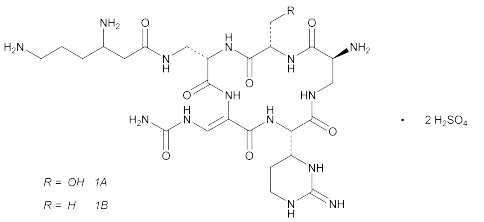
BRIEFING

Capreomycin Sulfate, *USP 32* page 1777. On the basis of comments received, the following revisions are proposed:

- *Identification* test *B*, based on chromatographic retention times, is added.
- The test for *Capreomycin 1 Content* is revised, because the analytical column required for this method is no longer available. The revised test is based on a method validated with the Spherisorb CN brand of L10 column. The typical retention time for capreomycin 1A is 7 min. Relative retention times are provided in the method.

(MD-ANT: A. Wise.) RTS—C68831

Capreomycin Sulfate



Capreomycin, sulfate [1405-37-4].

$C_{25}H_{44}N_{14}O_8$ 668.71
Capreomycin 1A (free base);
3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)- iminohexahydropyrimidin-4-yl] -3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide [37290-35-6].

$C_{25}H_{44}N_{14}O_7$ 652.71
Capreomycin 1B (free base);
3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-11-[(*R*)-iminohexahydropyrimidin-4-yl]-2-methyl-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide [33490-33-4].

DEFINITION

Capreomycin Sulfate is the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*, suitable for parenteral use. It has a potency equivalent to NLT 700 µg and NMT 1050 µg of capreomycin/mg.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨**191**⟩

Add the following:

▲• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.▲*USP34*

ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩ for capreomycin.

Acceptance criteria: 700–1050 µg/mg

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm

SPECIFIC TESTS

Change to read:

- **CAPREOMYCIN 1 CONTENT**

Solution A: ~~0.5 mg/mL~~▲0.4 mg/mL▲*USP34* of ammonium bisulfate in water. Pass through a filter having a porosity of 0.5 µm or less.

Mobile phase: Methanol and *Solution A* ~~(9:11)~~▲(2:3)▲*USP34*

System suitability solution: 0.25 mg/mL of USP Capreomycin Sulfate RS in water

Sample solution: 0.25 mg/mL of Capreomycin Sulfate in water

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: ~~4.6-mm × 15-cm; packing L10 with a 3.5% carbon loading~~▲4.6-mm × 25-cm; 5-µm packing L10
Temperature: 30°▲*USP34*
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Sample: *System suitability solution*
▲[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]▲*USP34*
Suitability requirements
Resolution: NLT 1.5 between capreomycin 1A and capreomycin 1B
Tailing factor: NMT ~~2.5~~▲3.5▲*USP34* for the major peaks (capreomycin 1A and capreomycin 1B)

Analysis
▲[NOTE—Chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]▲*USP34*
Sample: *Sample solution*
Calculate the percentage of capreomycin 1 in the portion of Capreomycin Sulfate taken:

$$\text{Result} = [(r_{1A} + r_{1B})/r_T] \times 100$$

r_{1A} = peak response of capreomycin 1A
r_{1B} = peak response of capreomycin 1B
r_T = total response for all peaks

Acceptance criteria: NLT 90.0% of capreomycin 1

- **pH** ⟨**791**⟩**:** 4.5–7.5
Sample solution: 30 mg/mL
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h: it loses NMT 10.0% of its weight.
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where it is intended for use in preparing injectable dosage forms: NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **OTHER REQUIREMENTS:** Where the label states that Capreomycin Sulfate is sterile, it meets the requirements under *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Capreomycin Sulfate RS
USP Endotoxin RS

In-Process Revision

BRIEFING

Capreomycin for Injection, *USP 32* page 1777. On the basis of comments received, the following revisions are proposed:

- The *Identification* test is deleted from the *Other Requirements* section and included in a new *Identification* section as *Identification* test *A. Identification* test *B*, based on chromatographic retention times, is added.
- The test for *Capreomycin 1 Content* is revised, because the analytical column required for this method is no longer available. The revised test is based on a method validated with the Spherisorb CN brand of L10 column. The typical retention time for capreomycin 1A is 7 min. Relative retention times are provided in the method.

(MD-ANT: A. Wise.) RTS—C68831

Capreomycin for Injection

DEFINITION

Capreomycin for Injection contains an amount of Capreomycin Sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of capreomycin.

IDENTIFICATION

Add the following:

▲• **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨**191**⟩▲*USP34*

Add the following:

▲• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content.*▲*USP34*

ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩ for Capreomycin.
  Acceptance criteria: 90.0%–115.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm

SPECIFIC TESTS

Change to read:

- **CAPREOMYCIN 1 CONTENT**

  Solution A: ~~0.5 mg/mL~~▲0.4 mg/mL▲*USP34* of ammonium bisulfate in water. Pass through a filter having a porosity of 0.5 µm or less.

  Mobile phase: Methanol and *Solution A* ~~(9:11)~~▲(2:3)▲*USP34*

  System suitability solution: 0.25 mg/mL of USP Capreomycin Sulfate RS in water

  Sample solution: 0.25 mg/mL of Capreomycin for Injection in water

  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)

  Mode: LC

  Detector: UV 268 nm

  Column: ~~4.6-mm × 15-cm; packing L10 with a 3.5% carbon loading~~▲4.6-mm × 25-cm; 5-µm packing L10

  Column temperature: 30°▲*USP34*

  Flow rate: 1.5 mL/min

  Injection size: 20 µL

  System suitability

  Sample: *System suitability solution*

  ▲[NOTE—The relative retention times of capreomycin 1A and 1B are 0.85 and 1.0, respectively.]▲*USP34*

  Suitability requirements

  Resolution: NLT 1.5 between capreomycin 1A and capreomycin 1B

  Tailing factor: NMT ~~2.5~~▲3.5▲*USP34* for the major peaks (capreomycin 1A and capreomycin 1B)

  Analysis

  ▲[NOTE—Chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]▲*USP34*

  Sample: *Sample solution*

  Calculate the percentage of capreomycin 1 in the capreomycin sulfate taken:

$$(r_{1A} + r_{1B})/r_T \times 100$$

  r_{1A} = peak area for capreomycin 1A
  r_{1B} = peak area for capreomycin 1B
  r_T = total response for all peaks

  Acceptance criteria: NLT 90.0% of capreomycin 1

- **pH** ⟨**791**⟩**:** 4.5–7.5
  Sample solution: 30 mg/mL
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h: it loses NMT 10.0% of its weight.
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* ⟨1⟩*, Constituted Solutions*.

Change to read:

- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩. ~~and *Identification Tests—General* ⟨191⟩*, Sulfate*~~▲▲*USP34*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Capreomycin Sulfate RS
  USP Endotoxin RS

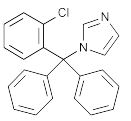
BRIEFING

Clotrimazole, *USP 32* page 1997. On the basis of data received the following revisions are proposed:

1. *Assay* and *Organic Impurities, Procedure 2: Limit of Clotrimazone Related Compound A*: Replace methanol with acetonitrile in the *Mobile phase* in order to increase the resolution between clotrimazole and clotrimazole related compound A while providing cleaner baseline noise and better peak responses. The preparations of the solutions have been revised for ease of use. The procedures are based on analyses performed with the Merck Purospher Star RP 18e brand of L1 column.
2. *Identification:* Replace the TLC *Identification* test with an analysis based on the *Assay* retention time.
3. *Internal standard:* The internal standard has been eliminated from the *Assay* method. The validation data demonstrates that use of an internal standard is not required for this application. This change has the added benefit of alleviating the burden of dealing with testosterone propionate, which is a controlled substance.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C56870

Clotrimazole



$C_{22}H_{17}ClN_2$ 344.84
1*H*-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-;
1-(*o*-Chloro-α,α-diphenylbenzyl)imidazole [23593-75-1].

DEFINITION
Clotrimazole contains NLT 98.0% and NMT 102.0% of $C_{22}H_{17}ClN_2$, calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨197M⟩

Change to read:

• **B. ~~THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST~~ ⟨~~201~~⟩**
~~Sample solution:~~ ~~20 mg/mL in chloroform~~
~~Developing solvent system:~~ ~~Xylene, *n*-propyl alcohol, and ammonium hydroxide (180:20:1)~~
▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲*USP34*

ASSAY

Change to read:

• **~~PROCEDURE~~**
~~Solution A:~~ ~~4.35 mg/mL of dibasic potassium phosphate~~
~~Mobile phase:~~ ~~Methanol and *Solution A* (3:1)~~
~~Pass through a membrane filter having a 0.2-µm or finer porosity. The ratio of volumes may be changed to obtain the required resolution.~~
~~Internal standard solution:~~ ~~Transfer 33 mg of testosterone propionate to a 200-mL volumetric flask, dissolve in 125 mL of methanol, add 50 mL of *Solution A*, and dilute with methanol to volume (165 µg/mL of testosterone propionate).~~
~~Standard stock solution A:~~ ~~Transfer 50 mg of USP Clotrimazole RS to a 50-mL volumetric flask. Add 25 mL of methanol to dissolve, add 12.5 mL of *Solution A*, and dilute with methanol to volume (1 mg/mL of USP Clotrimazole RS).~~
~~Standard solution:~~ ~~Transfer 10.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.~~
~~Standard stock solution B:~~ ~~Transfer 12.5 mg of USP Clotrimazole Related Compound ARS to a 25 mL volumetric flask, add 10 mL of methanol to dissolve, add 6.25 mL of *Solution A*, and dilute with methanol to volume.~~
~~System suitability solution:~~ ~~Transfer 3 mL of *Standard stock solution A* and 5 mL of *Standard stock solution B* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.~~
~~Sample stock solution:~~ ~~Transfer 100 mg of Clotrimazole to a 10 mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Solution A*, and dilute with methanol to volume.~~
~~Sample solution:~~ ~~Transfer 1.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.~~
~~Chromatographic system~~
~~(See *Chromatography* ⟨621⟩, *System Suitability*.)~~
~~Mode:~~ ~~LC~~
~~Detector:~~ ~~UV 254 nm~~
~~Column:~~ ~~4.6-mm × 25-cm; 10 µm packing L1~~
~~Flow rate:~~ ~~1.5 mL/min~~
~~Injection size:~~ ~~20 µL~~
~~System suitability~~
~~Sample:~~ ~~*System suitability solution* and *Standard solution*~~
~~Suitability requirements~~
~~[NOTE—The relative retention times for clotrimazole related compound A, clotrimazole, and testosterone propionate are 0.7, 1.0, and 1.5, respectively.]~~
~~Resolution:~~ ~~NLT 1.9 between clotrimazole and clotrimazole related compound A, *System suitability solution*~~
~~Relative standard deviation:~~ ~~NMT 2.0%, *Standard solution*~~
~~Analysis~~
~~Samples:~~ ~~*Standard solution* and *Sample solution*~~
~~Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Clotrimazole taken:~~

$$\text{~~Result~~} = (R_U/R_S) \times (C_S/C_U) \times 100$$

~~R_U = peak response ratio of clotrimazole to testosterone propionate from the *Sample solution*~~
~~R_S = peak response ratio of clotrimazole to testosterone propionate from the *Standard solution*~~
~~C_S = concentration of the *Standard solution* (mg/mL)~~
~~C_U = concentration of the *Sample solution* (mg/mL)~~
~~Acceptance criteria:~~ ~~98.0%–02.0%~~

▲**Buffer:** 4.35 mg/mL of dibasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (3:1)
Pass through a membrane filter having a 0.2-µm or finer porosity. The ratio of volumes may be changed to obtain the required resolution.
Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol
System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol
Sample stock solution: Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, and dilute with methanol to volume.
Sample solution: 0.5 mg/mL of Clotrimazole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Clotrimazole taken:

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

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Clotrimazole in the *Sample solution* (mg/mL)

In-Process Revision

Acceptance criteria: 98.0%–102.0% on the dried basis▲*USP34*

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Change to read:

Organic Impurities

- **PROCEDURE 1: LIMIT OF IMIDAZOLE**

  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

  Standard solution: 500 µg/mL of USP Imidazole RS in chloroform

  Sample solution: 100 mg/mL of Clotrimazole in chloroform

  Application volume: 5 µL

  Developing solvent system: Methanol and chloroform (3:2)

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Proceed as directed for *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*. After air-drying the plate for 5 min, place it in a closed container with a dish containing 100 g of iodine in a shallow layer, and allow to remain for 60 min. Remove the plate from the container, and observe the chromatogram.

  Acceptance criteria: Any brown spot from the *Sample solution* at an R_F value corresponding to the principal spot from the *Standard solution* is not greater in size or intensity than the principal spot from the *Standard solution*: NMT 0.5% of imidazole.

- **PROCEDURE 2: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**

  ~~**Solution A:**~~ ▲**Buffer,**▲*USP34* **Mobile phase,** ~~**Standard stock solution B,**~~ ▲▲*USP34* **System suitability solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.

  Standard solution: ~~Transfer 5.0 mL of the *Standard stock solution B* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.~~ ▲50 µg/mL of USP Clotrimazole Related Compound A RS prepared by dissolving in methanol using about 75% of the final flask volume. Dilute with *Buffer* to volume.▲*USP34*

  Sample solution: ~~*Sample stock solution* from the *Assay*~~ ▲Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, dilute with methanol to volume, and mix.▲*USP34*

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of clotrimazole related compound A in the portion of Clotrimazole taken:

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

  r_U = peak response for clotrimazole related compound A from the *Sample solution*

  r_S = peak response for clotrimazole related compound A from the *Standard solution*

  C_S = concentration of the *Standard solution* (mg/mL)

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

  Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

  USP Clotrimazole RS

  USP Clotrimazole Related Compound A RS

  USP Imidazole RS

BRIEFING

Clotrimazole Cream, *USP 32* page 1998. On the basis of data received the following revisions are proposed:

1. *Assay*: Replace methanol with acetonitrile in the *Mobile phase* in order to increase the resolution between clotrimazole and clotrimazole related compound A while providing cleaner baseline noise and better peak responses. The preparations of the solutions have been revised for ease of use. The procedure is based on analyses performed with the Merck Purospher Star RP 18e brand of L1 column.
2. *Identification:* Replace the TLC identification test with an analysis based on the *Assay* retention time.
3. *Internal standard*: The internal standard has been eliminated from the *Assay* method. The validation data demonstrates that use of an internal standard is not required for this application. This change has the added benefit of alleviating the burden of dealing with testosterone propionate, which is a controlled substance.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C56871

Clotrimazole Cream

DEFINITION

Clotrimazole Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION

Change to read:

- ~~**THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩~~

  ~~**Standard solution:** 1 mg/mL of USP Clotrimazole RS in chloroform~~

  ~~**Sample solution:** Transfer the equivalent to 5 mg of clotrimazole from Cream to a 50-mL centrifuge tube, add 5 mL of chloroform, and centrifuge to obtain a clear solution.~~

  ~~**Solution A:** 30 mg/mL of bismuth subnitrate and 300 mg/mL of potassium iodide in dilute hydrochloric acid (1 in 4) Dilute 10 mL of this solution with water to 100 mL.~~

  ~~**Chromatographic system**~~

  ~~(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)~~

  ~~**Mode:** TLC~~

  ~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture~~

  ~~**Application volume:** 20 µL~~

  ~~**Spray reagent:** Dilute 10 mL of *Solution A*, and 5 mL of dilute hydrochloric acid (1 in 4) with water to 200 mL.~~

  ~~**Analysis**~~

  ~~**Samples:** *Standard solution* and *Sample solution*~~

  ~~In a suitable chromatographic chamber containing 200 mL of ether, place a beaker containing 25 mL of ammonium hydroxide. Cover the chamber, and allow to equilibrate for 2 h. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light.~~

  ~~**Acceptance criteria:** The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*. Spray the plate evenly with *Spray reagent*: the principal spots from the *Sample solution* and *Standard solution* are orange.~~

  ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲*USP34*

In-Process Revision

ASSAY

Change to read:

- **PROCEDURE**

~~**Solution A:** 4.35 mg/mL of dibasic potassium phosphate~~
~~**Mobile phase:** Methanol and *Solution A* (3:1)~~
~~[NOTE—The ratio of volumes may be changed to obtain the required resolution.]~~
~~**Internal standard solution:** 0.07 mg/mL of testosterone propionate in dehydrated alcohol~~
~~**Standard stock solution A:** 1 mg/mL of USP Clotrimazole RS in dehydrated alcohol~~
~~**Standard stock solution B:** 0.12 mg/mL of USP Clotrimazole Related Compound A RS in dehydrated alcohol~~
~~**Standard solution:** *Standard stock solution A* and *Internal standard solution* (1:1)~~
~~**System suitability solution:** *Standard stock solution A* and *Standard stock solution B* (1:7)~~
~~**Sample solution:** Transfer the equivalent to 10 mg of clotrimazole from Cream to a 50-mL screw-capped centrifuge tube. Add 10.0 mL of *Internal standard solution*, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol-ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a test tube. Add 10.0 mL of dehydrated alcohol to the residue in the centrifuge tube, and repeat the extraction starting with "heat at 50° in a water bath". Transfer the supernatant to the test tube containing the supernatant from the first extraction.~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩, *System Suitability*.)~~
~~**Mode:** LC~~
~~**Detector:** UV 254 nm~~
~~**Guard column:** 2.1-mm × 6-cm; 10 µm packing L7~~
~~**Column:** 3.9-mm × 30-cm; 10 µm packing L1~~
~~**Flow rate:** 1 mL/min~~
~~**Injection size:** 20 µL~~
~~**System suitability**~~
~~**Samples:** *System suitability solution* and *Standard solution*~~
~~[NOTE—The relative retention times for clotrimazole related compound A, clotrimazole, and testosterone propionate are 0.9, 1.0, and 1.5, respectively.]~~
~~**Suitability requirements**~~
~~**Resolution:** NLT 1.2 between clotrimazole related compound A and clotrimazole, *System suitability solution*~~
~~**Relative standard deviation:** NMT 2.0%, *Standard solution*~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Calculate the percentage of $C_{22}H_{17}ClN_2$ in each g of Cream taken:~~

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$~~

~~R_U = peak response ratio of clotrimazole to testosterone propionate from the *Sample solution*~~
~~R_S = peak response ratio of clotrimazole to testosterone propionate from the *Standard solution*~~
~~C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)~~
~~C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)~~
~~**Acceptance criteria:** 90.0%–110.0%~~

▲**Buffer:** 4.35 mg/mL of dibasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (3:1)
[NOTE—The ratio of volumes may be changed to obtain the required resolution.]
Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol
System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol
Sample solution: Transfer the equivalent to 25 mg of clotrimazole from Cream to a 50-mL screw-capped centrifuge tube. Add 25.0 mL of methanol, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol–ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a 50-mL volumetric flask. Add 20.0 mL of methanol to the residue in the centrifuge tube, and repeat the extraction starting with "heat at 50° in a water bath". Transfer the supernatant to the volumetric flask containing the supernatant from the first extraction, dilute with methanol to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Cream taken:

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

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%▲*USP34*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, at a temperature between 2° and 30°.
- **LABELING:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Clotrimazole Vaginal Cream.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clotrimazole RS
  USP Clotrimazole Related Compound A RS

BRIEFING

Dactinomycin for Injection, *USP 32* page 2054. On the basis of comments received, the *Definition* and *Identification* test *B* are updated to current USP convention.

(MD-ANT: A. Wise.) RTS—C68501

In-Process Revision

Dactinomycin for Injection

DEFINITION

Change to read:

Dactinomycin for Injection is a sterile mixture of Dactinomycin and Mannitol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of $C_{62}H_{86}N_{12}O_{16}$. ~~the labeled amount being 0.5 mg in each container.~~ ▲*USP34*

[**CAUTION—**Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

IDENTIFICATION

- **A. PROCEDURE**

Standard solution: 25 µg/mL of USP Dactinomycin RS in methanol
Sample solution: 25 µg/mL of dactinomycin in methanol
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as the *Standard solution*, concomitantly measured.
Ratio: A_{240}/A_{445}, 1.30–1.50

Change to read:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ~~and both chromatograms compare qualitatively.~~ ▲*USP34*

ASSAY

- **PROCEDURE**

[NOTE—Use freshly prepared *Standard solution* and *Sample solution*, protected from light.]
Mobile phase: Acetonitrile and water (3:2)
Standard solution: 250 µg/mL of USP Dactinomycin RS in *Mobile phase*
Sample solution: 250 µg/mL of dactinomycin from Dactinomycin for Injection diluted with *Mobile phase*. Filter, if necessary, to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for dactinomycin is 6 min.]
Suitability requirements
Column efficiency: NLT 1200 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{62}H_{86}N_{12}O_{16}$ taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Dactinomycin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dactinomycin in the *Sample solution* (µg/mL)
Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **pH** ⟨**791**⟩**:** 5.5–7.5, in the solution constituted as directed in the labeling
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 4.0% of its weight.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** It contains NMT 100.0 USP Endotoxin Units/mg of dactinomycin.
- **STERILITY TESTS** ⟨**71**⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined*, *Membrane Filtration*, each container being constituted aseptically by injecting Sterile Water for Injection through the stopper, and the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* ⟨1⟩, *Constituted Solutions*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.
- **LABELING:** Label it to include the statement "Protect from light."
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Dactinomycin RS
USP Endotoxin RS

BRIEFING

Diltiazem Hydrochloride Tablets, *USP 32* page 2164. It is proposed to change the wavelength used in the *Dissolution* test from 240 nm to 237 nm, where the maximum absorbance occurs.

(BPC: M. Marques.) RTS—C78512

Diltiazem Hydrochloride Tablets

DEFINITION

Diltiazem Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).

IDENTIFICATION

- **A. PROCEDURE**

Indicator solution: Transfer 17.4 g of ammonium thiocyanate and 2.8 g of cobalt chloride to a 100-mL volumetric flask, add 50 mL of water, and sonicate for 10 min. Dilute with water to volume.
Analysis: Finely powder 1 Tablet, and transfer to a 15-mL screw-capped test tube. Add 10 mL of 0.1 N hydrochloric acid, shake, and filter. Add 2 mL of *Indicator solution* to 2 mL of the filtrate, and shake. Add 5 mL of chloroform, and shake.
Acceptance criteria: A blue color develops in the chloroform layer.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate, and adjust with 0.1 N sodium hydroxide to a pH of 6.2.
Mobile phase: Acetonitrile, methanol, and *Solution A* (25:25:50)
Standard solution: 1.2 mg/mL of USP Diltiazem Hydrochloride RS in methanol
Sample solution: Transfer to a 500-mL volumetric flask an equivalent to 600 mg of diltiazem hydrochloride from NLT 20

In-Process Revision

finely powdered Tablets. Add 200 mL of methanol, and sonicate for 1 h. Cool, and dilute with methanol to volume. Centrifuge a 25-mL aliquot at 3500 rpm for 15 min, and use the clear supernatant.

System suitability solution: 12 µg/mL each of USP Diltiazem Hydrochloride RS and USP Desacetyl Diltiazem Hydrochloride RS, in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between desacetyl diltiazem and diltiazem, *System suitability solution*

Column efficiency: NLT 1200 theoretical plates for the diltiazem peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{26}N_2O_4S \cdot HCl$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min and 3 h

Detector: UV ~~240~~▲237▲*USP34* nm

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Standard solution: USP Diltiazem Hydrochloride RS in *Medium*

Tolerances: Use the following acceptance criteria for the 30-min time point: at S_1 no unit is more than Q; at S_2 the average value is equal to or less than Q, and no unit is greater than Q + 10%; at S_3 the average value is equal to or less than Q, and NMT 2 units are more than Q + 10%, and no unit is more than Q + 25%. Use the criteria in *Dissolution* ⟨711⟩, *Acceptance Table* for the 3-h time point. NMT 60% (Q) of the labeled amount of $C_{22}H_{26}N_2O_4S \cdot HCl$ is dissolved in 30 min, and NLT 75% (Q) is dissolved in 3 h.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Desacetyl Diltiazem Hydrochloride RS

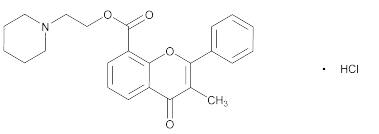
USP Diltiazem Hydrochloride RS

BRIEFING

Flavoxate Hydrochloride, *USP 32* page 2378. On the basis of comments and validated methods received, it is proposed to replace the current *Organic Impurities* test with a more selective, gradient elution HPLC method. The liquid chromatographic procedure is based on analyses performed using an Inertsil ODS brand of L1 column. The typical retention times for flavoxate, flavoxate related compound A, flavoxate related compound B, and flavoxate related compound C are 7.5, 21, 26, and 27.5 min, respectively.

(MD-PP: H. Ramanathan, R. Ravichandran.) RTS—C69182

Flavoxate Hydrochloride



$C_{24}H_{25}NO_4 \cdot HCl$ 427.92

4*H*-1-Benzopyran-8-carboxylic acid, 3-methyl-4-oxo-2-phenyl-, 2-(1-piperidinyl)ethyl ester, hydrochloride;

2-Piperidinoethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate hydrochloride [3717-88-2].

DEFINITION

Flavoxate Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{24}H_{25}NO_4 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** A solution of 10 mg/mL meets the requirements of the silver nitrate precipitate.

ASSAY

- **PROCEDURE**

Solution A: Mixture of 1.0 g of sodium hexanesulfonate in 650 mL of water, 1 mL of triethylamine, and 1.0 mL of phosphoric acid

Mobile phase: Add 350 mL of acetonitrile to *Solution A*.

Standard solution: 50 µg/mL of USP Flavoxate Hydrochloride RS in *Mobile phase*

Sample solution: 50 µg/mL of Flavoxate Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 293 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of flavoxate is about 4.3 min.]
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{25}NO_4 \cdot HCl$ in the portion of Flavoxate Hydrochloride taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flavoxate Hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Change to read:

Organic Impurities
- **PROCEDURE**

~~**Solution A:** Dissolve 1.0 g of sodium hexanesulfonate in a 500-mL volumetric flask with 100 mL of water. Add 1.0 mL of phosphoric acid and 1.0 mL of triethylamine, dilute with water to volume, and filter the solution.~~
~~**Mobile phase:** Acetonitrile and *Solution A* (1:1)~~
~~**Standard solution:** 1 µg/mL of USP Flavoxate Hydrochloride RS and 1 µg/mL of USP Flavoxate Related Compound A RS in *Mobile phase*~~
~~**Sample solution:** 1.0 mg/mL of Flavoxate Hydrochloride in *Mobile phase*. [NOTE—Sonicate briefly if necessary.]~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩*, System Suitability*.)~~
~~**Mode:** LC~~
~~**Detector:** UV 293 nm~~
~~**Column:** 4.6-mm × 15-cm; 5-µm packing L7~~
~~**Flow rate:** 1.2 mL/min~~
~~**Injection size:** 20 µL~~
~~[NOTE—Record chromatograms for about 25 min.]~~
~~**System suitability**~~
~~**Sample:** *Standard solution*~~
~~**Suitability requirements**~~
~~**Resolution:** NLT 6.0 between flavoxate and flavoxate related compound A~~
~~**Column efficiency:** NLT 3000 theoretical plates~~
~~**Tailing factor:** NMT 2.0 for flavoxate related compound A~~
~~**Relative standard deviation:** NMT 2.0% for flavoxate related compound A~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Calculate the percentage of each impurity in the portion of Flavoxate Hydrochloride taken:~~

~~$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/\text{RRF}) \times 100$$~~

~~r_U = peak response of each impurity from the *Sample solution*~~
~~r_S = peak response for flavoxate from the *Standard solution*~~
~~C_S = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (mg/mL)~~
~~C_U = concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)~~
~~RRF = relative response factor (see *Impurity Table 1*)~~
~~**Acceptance criteria**~~
~~**Individual impurities:** See *Impurity Table 1*.~~
~~**Total impurities:** NMT 1.0%~~

~~**Impurity Table 1**~~

~~Name~~	~~Relative Retention Time~~	~~Relative Response Factor~~	~~Acceptance Criteria, NMT (%)~~
~~Flavoxate~~	~~1.0~~	~~—~~	~~—~~
~~Flavoxate Related compound A[a]~~	~~1.55~~	~~1.7~~	~~0.3~~
~~Methyl ester[b]~~	~~3.45~~	~~1.6~~	~~0.1~~
~~Ethyl ester[c]~~	~~4.75~~	~~1.4~~	~~0.1~~
~~Any unspecified impurity~~	~~—~~	~~1.0~~	~~0.1~~

~~[a]3-Methylflavone-8-carboxylic acid.~~
~~[b]3-Methylflavone-8-carboxylic acid methyl ester.~~
~~[c]3-Methylflavone-8-carboxylic acid ethyl ester.~~

▲**Solution A:** 0.05 % (v/v) trifluoroacetic acid in water
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	68	62
15	68	32
25	20	80
35	20	80
36	68	32
41	68	32

Diluent: Methanol and 0.02 % trifluoroacetic acid (1:1)
Standard stock solution 1: 2 mg/mL of USP Flavoxate Hydrochloride RS in *Diluent*
Standard stock solution 2: 0.05 mg/mL of USP Flavoxate Related Compound A RS in *Diluent*
Standard stock solution 3: 0.05 mg/mL of USP Flavoxate Related Compound B RS in methanol
Standard stock solution 4: 0.05 mg/mL of USP Flavoxate Related Compound C prepared as follows: Dissolve USP Flavoxate Related Compound C first in methanol using 10% of final volume, then fill 10% of final volume with 0.02% trifluoroacetic acid. Dilute with *Diluent* to volume.
Standard solution: 1 mg/mL of USP Flavoxate Hydrochloride RS and 5 µg/mL each of USP Flavoxate Related Compound A RS, USP Flavoxate Related Compound B RS, and USP Flavoxate Related Compound C RS in *Diluent* from *Standard stock solutions 1*, *2*, *3*, and *4*, respectively
Sample solution: 1 mg/mL of Flavoxate Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 10 µL
Column temperature: 30°
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between flavoxate related compound B and flavoxate related compound C
Tailing factor: NMT 1.5 for flavoxate related compounds A, B, and C

In-Process Revision

Relative standard deviation: NMT 2.0% for the flavoxate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flavoxate related compounds A, B, and C in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of flavoxate from the *Standard solution*
C_S = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Flavoxate Hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other unspecified degradation product in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_T = sum of responses of all peaks from the *Sample solution*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flavoxate	1.0	—	—
Flavoxate related compound A[a],*	2.8	1.6	0.3
Flavoxate related compound B[b],*	3.5	1.5	0.1
Flavoxate related compound C[c],*	3.7	1.4	0.1
Any single unspecified degradation product	—	1.0	0.1

[a] 3-Methylflavone-8-carboxylic acid.
[b] 3-Methylflavone-8-carboxylic acid methyl ester.
[c] 3-Methylflavone-8-carboxylic acid ethyl ester.
* For identification and system suitability purposes only.

▲*USP34*

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store at room temperature.

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**

USP Flavoxate Hydrochloride RS
USP Flavoxate Related Compound A RS
▲USP Flavoxate Related Compound B RS
3-methylflavone-8-carboxylic acid methyl ester
($C_{18}H_{14}O_4$ 294.30)
USP Flavoxate Related Compound C RS
3-methylflavone-8-carboxylic acid ethyl ester
($C_{19}H_{16}O_4$ 308.33)

▲*USP34*

BRIEFING

Glyburide and Metformin Hydrochloride Tablets, *USP 32* page 2511 and page 1163 of *PF* 34(5) [Sept.–Oct. 2008]. It is proposed to revise the test for *Dissolution* to indicate that the *Sample solution* could be diluted with *Medium*, if necessary.

(BPC: M. Marques.) RTS—C78915

Glyburide and Metformin Hydrochloride Tablets

DEFINITION

Glyburide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glyburide ($C_{23}H_{28}ClN_3O_5S$) and metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. GLYBURIDE:** ~~*Infrared Absorption* ⟨197K⟩—Prepare the test specimen as follows. Grind 5 Tablets to a fine powder. Add 25 mL of water, and shake for 5 minutes. Add 25 mL of chloroform, and shake for an additional 5 minutes. Centrifuge at 2500 rpm for 5 minutes. Transfer the lower chloroform layer to another container, evaporate the solvent under a stream of nitrogen, and dry the extract at 60° for 3 hours: the IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Glyburide RS.~~ ■The retention time of the glyburide peak of the *Sample solution* corresponds to that of the major peak of the *Standard solution*, as obtained in the *Assay* for *Glyburide*.■2S (*USP32*)
- **B. METFORMIN HYDROCHLORIDE:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Metformin Hydrochloride*.

ASSAY

- **GLYBURIDE**

Solution A: 28 mg/mL of monobasic ammonium phosphate

Mobile phase: Acetonitrile and *Solution A* (40:60). Adjust with 1 N sodium hydroxide to a pH of 5.3.

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 0.25 mg/mL of USP Glyburide RS in acetonitrile and water. [NOTE—Dissolve in the acetonitrile and dilute with water to volume. Use volumes of each such that the final ratio of acetonitrile to water is 1:1.]

Standard solution: 0.025 mg/mL of USP Glyburide RS in *Diluent*, from *Standard stock solution*

System suitability solution 1: Prepare a solution containing 0.025 mg/mL of USP Glyburide Related Compound A RS in *Diluent*. Transfer 50 µL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

System suitability solution 2: 5.0 mg/mL of USP Metformin Hydrochloride RS in *System suitability solution 1*

Sample solution: Dissolve NLT 5 Tablets in *Diluent* by stirring with a magnetic stirring bar for a least 1 h. Dilute to obtain a solution containing 0.025 mg/mL of glyburide, based on the label claim. Centrifuge a portion of this solution at 3000 rpm for 10 min and use the clear supernatant. [NOTE—Retain a portion of this solution for the *Assay* for *Metformin Hydrochloride*.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperature: 40°
Flow rate: 1.2 mL/min
Injection size: 100 µL

System suitability
Sample: *System suitability solution 2*
[NOTE—The relative retention time for the peak due to glyburide related compound A is about 0.30 with respect to glyburide.]
Suitability requirements
Capacity factor, k′: NLT 7 for the peak due to glyburide
Column efficiency: NLT 3000 theoretical plates for the peak due to glyburide
Relative standard deviation: NMT 1.5% for the peak due to glyburide; NMT 10% for the peak due to glyburide related compound A

Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for about 1.25 times the retention time of the glyburide peak. Calculate the percentage of $C_{23}H_{28}ClN_3O_5S$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of glyburide label claim

- **METFORMIN HYDROCHLORIDE**

Solution A: Transfer 1.0 g each of sodium heptanesulfonate and sodium chloride to a 2000-mL volumetric flask. Add 1800 mL of water, and adjust with 0.06 M phosphoric acid to a pH of 3.85. Dilute with water to volume.
Mobile phase: Acetonitrile and *Solution A* (10:90)
Diluent: Acetonitrile and water (1:40)
Standard solution: 0.25 mg/mL of USP Metformin Hydrochloride RS in *Diluent*. [NOTE—Sonicate to achieve complete dissolution, if necessary.]
System suitability stock solution: 25 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*
System suitability solution: Transfer 0.5 mL of *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.
Sample solution: Dilute with water a portion of the retained *Sample solution* from the *Assay* for *Glyburide* to obtain 0.25 mg/mL of metformin hydrochloride based on the label claim.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 5 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for metformin related compound B, metformin, and metformin related compound C (this impurity can have a variable retention time) are about 0.86, 1.0, and 2.1–2.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between metformin related compound B and metformin
Tailing factor: 0.8–2.0 for the metformin peak
Relative standard deviation: NMT 1.5% for the metformin peak and NMT 10% for each of the peaks due to metformin related compound B and metformin related compound C

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_4H_{11}N_5 \cdot HCl$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of metformin label claim

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩

Glyburide
Medium: 0.05 M boric acid and 0.05 M potassium chloride solution. Prepare by dissolving 3.09 g of boric acid and 3.73 g of potassium chloride in 250 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.5, and dilute with water to 1 L; 500 mL.
Apparatus 2: 75 rpm
Time: 30 min
Standard solution: Transfer 10 mg of USP Glyburide RS to a 100-mL volumetric flask. Dissolve in 20 mL of acetonitrile, and dilute with *Medium* to volume. Dilute further with *Medium* to obtain a solution having a glyburide concentration, in mg/mL, of L/500 where L is the label claim, in mg, of glyburide.
Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a 0.45-µm polypropylene filter or a 1-µm glass fiber filter. ▲Dilute with *Medium*, if necessary.▲*USP34*
Solution A: 28.7 mg/mL of monobasic ammonium phosphate in water
Mobile phase: *Solution A* and acetonitrile (1:1). Adjust with 1 N sodium hydroxide to a pH of 5.3.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 200 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000
Tailing factor: 0.8–2.0
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of $C_{23}H_{28}ClN_3O_5S$ dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

Tolerances: NLT 85% (Q) of the labeled amount of glyburide is dissolved.

Metformin hydrochloride
Medium: 0.05 M phosphate buffer, pH 6.8. Prepare by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1; 1000 mL.

Apparatus 2: 50 rpm
Time: 30 min
Standard solution: Dissolve a quantity of USP Metformin Hydrochloride RS in *Medium*. Dilute further, if necessary, with *Medium* to obtain a solution having a metformin hydrochloride concentration, in mg/mL, of L/1000 where L is the label claim, in mg, of metformin hydrochloride.
Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a 0.45-µm polypropylene filter or a 1-µm glass fiber filter. ▲Dilute with *Medium*, if necessary.▲*USP34*
Spectrometric conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: UV-Vis
Analytical wavelength: 232 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_4H_{11}N_5 \cdot HCl$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)
Tolerances: NLT 85% (Q) of the labeled amount of metformin hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements for *Weight Variation* for metformin hydrochloride and for *Content Uniformity* for glyburide

IMPURITIES

Organic Impurities

- **PROCEDURE 1: GLYBURIDE**

Solution A, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay* for *Glyburide*.
Standard solution: Dilute 1.0 mL of *Standard solution* from the *Assay* for *Glyburide* with *Diluent* to 100 mL.
Sample solution: Use the *Sample solution* from the *Assay* for *Glyburide*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each glyburide impurity in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)
F = relative response factor, use 0.8 for glyburide related compound A, and use 1.0 for all other impurities
Acceptance criteria
[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]
Glyburide related compound A: NMT 1.0%
Any other individual impurities: NMT 0.2%
Total impurities: NMT 0.50%, excluding glyburide related compound A

- **PROCEDURE 2: METFORMIN HYDROCHLORIDE**

Solution A, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay* for *Metformin Hydrochloride*.
Sample solution: Use the *Sample solution* from the *Assay* for *Metformin Hydrochloride*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of the responses of all peaks from the *Sample solution*
Acceptance criteria
[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]
Individual impurities: NMT 0.1%
Total impurities: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Glyburide RS
USP Glyburide Related Compound A RS
USP Metformin Hydrochloride RS
USP Metformin Related Compound B RS
USP Metformin Related Compound C RS

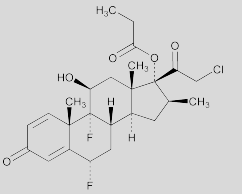
BRIEFING

Halobetasol Propionate. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods of analysis is being proposed. The liquid chromatographic procedures in the *Assay* and test for *Organic Impurities* are based on analyses performed with a Waters Symmetry Shield RP18 brand of L1 column. The typical retention time for halobetasol is about 10.5 min.

(MD-CCA: H. Ramanathan, C. Anthony.) RTS—C49074

Add the following:

▲Halobetasol Propionate



$C_{25}H_{31}ClF_2O_5$ 484.96
Pregna-1,4-diene-3,20-dione, 21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (6α,11β,16β)-;
21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [66852-54-8].

DEFINITION

Halobetasol Propionate contains NLT 98.0% and NMT 102.0% of $C_{25}H_{31}ClF_2O_5$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

In-Process Revision

ASSAY

• **PROCEDURE**

Solution A: Acetonitrile and water (9:11)
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	64.5	35.5
22	64.5	35.5
23	100	0
30	100	0

Standard solution: 0.2 mg/mL of USP Halobetasol Propionate RS in acetonitrile
Sample solution: 0.2 mg/mL of Halobetasol Propionate in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Temperature 40°
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{25}H_{31}ClF_2O_5$ in the portion of Halobetasol Propionate taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.2%
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

• **PROCEDURE**

Mobile phase, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperature: 40°
Flow rate: 0.8 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 22000 theoretical plates
Tailing factor: NLT 0.9 and NMT 1.1 for halobetasol propionate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Halobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_T = sum of responses for all the peaks from the *Sample solution*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
21-Chloro diflorosone[a]	0.75	0.15
21-Acetate 17-Propionate diflorasone[b]	0.88	0.15
11-Propionate 21-chloro diflorasone[c]	0.95	0.15
Halobetasol Propionate	1.0	—
9-Chloro halobetasol propionate[d]	1.12	0.15
6-Chloro halobetasol propionate[e]	1.24	0.15
Any single unspecified degradation product	—	0.10

[a] 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione.
[b] 6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-acetate 17-propionate.
[c] 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 11-propionate.
[d] 9,21-Dichloro-6α,-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.
[e] 6α,21-Dichloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample in a vacuum at 70° for 3 h: it loses NMT 1.0% of its weight.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781**⟩
Sample solution: 10 mg/mL in dioxane
Acceptance criteria Between +87° and +99°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light resistant containers. Store between 2° and 8°
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Halobetasol Propionate RS▲*USP34*

BRIEFING

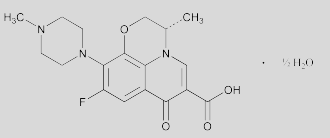
Levofloxacin. Because there is no existing *USP* monograph for this drug substance, a new monograph based on validated methods is proposed. The HPLC procedure used in the *Assay* and test for *Organic Impurities* is based on analyses performed with a GL Science Inertsil ODS-3 or Phenomenex Prodigy ODS (3) column. Typical retention time for levofloxacin is about 20 min.

(MD-AA: M. Puderbaugh, B. Davani.) RTS—C63974

In-Process Revision

Add the following:

▲Levofloxacin



$C_{18}H_{20}FN_3O_4 \cdot {}^1/_2H_2O$ 370.38

7*H*-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-, hydrate (2:1), (*S*)-;

(–)-(*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, hemihydrate [138199-71-0].

Anhydrous [100986-85-41].

DEFINITION

Levofloxacin contains NLT 98.5% and NMT 102.0% of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate, and 1.3 g/L of *L*-isoleucine in water

Mobile phase: Methanol and *Solution A* (3:7)

Standard solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

Sample solution: 1 mg/mL of Levofloxacin in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Temperature: 45°

Flow rate: 0.8 mL/min

Injection size: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing: 0.5–1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{18}H_{20}FN_3O_4$ in the portion of Levofloxacin taken:

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

r_U = peak response of levofloxacin from the *Sample solution*

r_S = peak response of levofloxacin from the *Standard solution*

C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.5%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.2%. Use a platinum crucible.
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities

- **PROCEDURE**

Solution A, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

Sensitivity solution: 0.3 µg/mL of USP Levofloxacin RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 1.0%, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak area response of each impurity

r_S = peak area response for levofloxacin

F = relative response factor for each impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%. [NOTE—Do not include the *D*-Isomer in the calculation for *Total impurities*.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl levofloxacin[a]	0.47	1.0	0.3
Diamine derivative[b]	0.52	0.9	0.3
Levofloxacin *N*-oxide[c]	0.63	1.1	0.3
9-Desfluoro levofloxacin[d]	0.73	1.0	0.3
Levofloxacin	1.0	—	—
D-Isomer[e]	1.23	1.0	0.8
Any unknown impurity	—	1.0	0.1

[a] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid.

[b] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

[c] (*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide.

[d] (*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

[e] (*R*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩

Solvent: Methanol

Sample solution: 5 mg/mL in *Solvent*

Acceptance criteria: –92° to –106°, at 20°

- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** 2.1%–2.7%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Levofloxacin RS▲*USP34*

In-Process Revision

BRIEFING

Lithium Carbonate Tablets, *USP 32* page 2798. It is proposed to add more information on the surfactant and *Surfactant solution* used in the *Dissolution* test.

(BPC: M. Marques.) RTS—C78922

Lithium Carbonate Tablets

DEFINITION
Lithium Carbonate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of Li_2CO_3.

IDENTIFICATION
A portion of the powdered Tablets meets the requirements of the following tests.
- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately causes a white precipitate to form.
- **B.** When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.

ASSAY
- **PROCEDURE**

Standard solution: Transfer 30 mg of USP Lithium Carbonate RS to a 100-mL volumetric flask, and add 20 mL of water and 0.5 mL of hydrochloric acid. Shake until dissolved, and dilute with water to volume. Pipet 20 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of a suitable surfactant solution, and dilute with water to volume.

Sample solution: Powder NLT 20 Tablets. Transfer a portion of powder, nominally equivalent to 600 mg of lithium carbonate, into a 1000-mL volumetric flask. Add water and hydrochloric acid (40:5), shake until the solid is well disintegrated, and dilute with water to volume. Pipet 10 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of the surfactant solution, and dilute with water to volume.

Spectrometric conditions
Mode: Flame photometer
Analytical wavelength: About 671 nm
[NOTE—Adjust the instrument with the surfactant solution.]

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of Li_2CO_3 in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of lithium carbonate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨**711**⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
▲Surfactant solution: Nonoxynol-9 in water, 1 in 100 parts per volume▲*USP34*

Sample solution: Dilute 900 mL of the solution under test with *Medium* to 1000 mL. Pass through a suitable filter. Transfer 20.0 mL of the filtrate to a 1000-mL volumetric flask. Add 500 mL of water, 1 drop of hydrochloric acid, and 20 mL of ~~a suitable surfactant solution, appropriately diluted~~▲*Surfactant solution*.▲*USP34* Dilute with water to volume.

Standard solution: Transfer 30 mg of USP Lithium Carbonate RS to a 100-mL volumetric flask. Add 20 mL of water and 0.5 mL of hydrochloric acid, shake until dissolved, and dilute with water to volume. Pipet 20 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of a suitable surfactant solution, and dilute with water to volume.

Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of Li_2CO_3 dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of Li_2CO_3 is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Lithium Carbonate RS

BRIEFING

Methylphenidate Hydrochloride Extended-Release Tablets, *USP 32* page 2950 and page 1162 of *PF* 35(5) [Sept.–Oct. 2009]. It is proposed to clarify the concentration of the *Standard solution* to be used in the *System suitability* in *Dissolution Test 2*.

(BPC: M. Marques.) RTS—C79246

Methylphenidate Hydrochloride Extended-Release Tablets

DEFINITION
Methylphenidate Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION
- **A. INFRARED ABSORPTION**

Sample specimen: Place a portion of powdered Tablets, equivalent to 100 mg of methylphenidate hydrochloride, in a 100-mL beaker. Add 20 mL of chloroform, stir for 5 min, and filter, collecting the filtrate. Evaporate the filtrate to about 5 mL. Add ethyl ether slowly, with stirring, until crystals form. Filter the crystals, wash with ethyl ether, and dry at 80° for 30 min.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methylphenidate Hydrochloride RS.

Add the following:

■• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.■2S *(USP33)*

ASSAY

• **PROCEDURE**

Acetate buffer: Dissolve 1.64 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0, and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Acetate buffer* (4:3:3)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: 0.2 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*

Standard solution: Transfer 10.0 mL of *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of *Internal standard solution*, and mix.

Sample stock solution: 0.2 mg/mL of methylphenidate hydrochloride from powdered Tablets (NLT 20 Tablets) in *Mobile phase*. [NOTE—Sonicate for 15 min.]

Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable membrane filter, discarding the first portion of the filtrate. [NOTE—Avoid the use of glass filters. Polypropylene filters are suitable for use.]

Sample solution: Transfer 10.0 mL of the clear filtrate to a glass-stoppered, 25-mL conical flask, and add 5.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0% from the peak response ratios of the analyte to the internal standard

Analysis

Samples: *Standard solution and Sample solution*

Calculate the percentage of $C_{14}H_{19}NO_2 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the analyte to the internal standard from the *Sample solution*

R_S = peak response ratio of the analyte to the internal standard from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨**711**⟩

Procedure for a pooled sample

Test 1

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 1, 2, 3.5, 5, and 7 h

Sample solution: Use portions of the solution under test passed through suitable 0.45-µm filter. [NOTE—Do not use glass fiber filters.]

Analysis: Determine the amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.

Tolerances: The percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	25%–45%
2	40%–65%
3.5	55%–80%
5	70%–90%
7	NLT 80

Test 2 (for products labeled for dosing every 24 h)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Acidified water (adjust with phosphoric acid to a pH of 3); 50 mL, at 37 ± 0.5°

Apparatus 7 (see *Drug Release* ⟨724⟩)**:** 30 cycles/min; 2–3 cm amplitude

Use *Sample Preparation A* using a metal coil sample holder (*Figure 4d*). Place 1 Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

Times: 1-h intervals for a duration of 10 h

Determine the percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved by using the following method.

Dilution medium: Mixture of acetonitrile and *Medium* (1:3)

Standard stock solution: 0.3 mg/mL USP Methylphenidate Hydrochloride RS in *Dilution medium*

Standard solutions: Prepare at least six solutions by making serial dilutions of the *Standard stock solution* in *Dilution medium* to bracket the expected drug concentration range.

Solution A: Dissolve 2.0 g of 1-octanesulfonic acid sodium salt in 700 mL of water, mix well, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Mixture of acetonitrile and *Solution A* (3:7)

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.2-mm × 5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Column temperature: 30°

Injection size: 25 µL

System suitability

Sample: ▲Middle range concentration of the▲*USP34* *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Capacity factor: NMT 2

In-Process Revision

Relative standard deviation: NMT 2% from the peak response of the analyte; NMT 2% of the retention time of the analyte

Analysis

Samples: *Standard solutions* and the solution under test

Construct a calibration curve by plotting the peak response versus the concentration of the *Standard solutions*. Determine the amount of $C_{14}H_{19}NO_2 \cdot HCl$ in each interval by linear regression analysis of the standard curve.

Tolerances: The percentages of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	12%–32%
4	40%–60%
10	NLT 85%
Average from 3 to 6 h	9%–15%/h

Calculate the average percentage released from 3 to 6 h:

$$\text{Result} = (Y - X)/3$$

Y = cumulative drug released from 0 to 6 h
X = cumulative drug released from 0 to 3 h

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Add the following:

■Organic Impurities

• **PROCEDURE**

Mobile phase: Dissolve 2 g of 1-octanesulfonic acid in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water. Adjust with phosphoric acid to a pH of 3.

Diluent A: Acetonitrile and *Solution A* (1:3)

Diluent B: Acetonitrile and methanol (1:1)

System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS and 2 µg/mL of USP Methylphenidate Related Compound A RS, in *Diluent A*

Standard solution: 0.2 µg/mL of USP Methylphenidate Hydrochloride RS, 0.5 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS and 1.5 µg/mL of USP Methylphenidate Related Compound A RS, in *Diluent A*

Sample stock solution: Dissolve 10 Tablets in 20% of total volume in *Diluent B*. Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: 0.1 mg/mL of methylphenidate hydrochloride in *Solution A*, from *Sample stock solution*. [NOTE—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm column; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 25 µL

Temperature: 30°

Run time: 2 times the retention time of methylphenidate

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: NMT 2.0 for methylphenidate peak

Resolution: NLT 6.0 between methylphenidate and erythro isomer peaks

Relative standard deviation: NMT 2.0% for methylphenidate peak and NMT 4.0% each for methylphenidate related compound A and erythro isomer peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylphenidate related compound A or erythro isomer in the portion of Tablets taken:

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

r_U = response of methylphenidate related compound A or erythro isomer from the *Sample solution*
r_S = response of methylphenidate related compound A or erythro isomer from the *Standard solution*
C_S = concentration of USP Methylphenidate Related Compound A RS or methylphenidate hydrochloride erythro isomer, in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

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

r_U = response of each impurity from the *Sample solution*
r_S = response of USP Methylphenidate Hydrochloride RS from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 2.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate related compound A[a]	0.47	1.5
Erythro isomer[b]	0.65	0.5
Methylphenidate hydrochloride	1.0	—
Any unspecified degradation product	—	0.2

[a] α-Phenyl-2-piperidineacetic acid.
[b] Methyl (*RS,SR*)-2-phenyl-2-(piperidin-2-yl) acetate.

■2S (*USP33*)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The labeling states the *Dissolution Test* with which the product complies if other than *Test 1*.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Methylphenidate Hydrochloride RS

■USP Methylphenidate Hydrochloride Erythro Isomer Solution RS

USP Methylphenidate Related Compound A RS■2S (*USP33*)

In-Process Revision

BRIEFING

Metolazone Tablets, *USP 32* page 2963. It is proposed to include a *Dissolution* test in the monograph. The chromatographic procedure in this test was validated with the Symmetry C8 brand of L7 packing. With this column, the retention time of the metolazone peak is about 8 min.

(BPC: M. Marques.) RTS—C40224

Metolazone Tablets

DEFINITION

Metolazone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$).

IDENTIFICATION

• **ULTRAVIOLET ABSORPTION** ⟨**197U**⟩

Sample solution: Pipet 3 mL of the *Sample solution* from the *Assay* into a 25-mL volumetric flask, and dilute with methanol to volume.

ASSAY

• **PROCEDURE**

[NOTE—Use low-actinic glassware throughout the *Assay*.]

Solution A: 1.38 g of monobasic potassium phosphate monohydrate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Solution A* (28:7:65)

Standard solution: 5 µg/mL of USP Metolazone RS in methanol

Sample stock solution: Transfer 10 Tablets to a 200-mL volumetric flask. Add 3 mL of water and 100 mL of methanol, and sonicate for 30 min. If disintegration is not complete, sonicate for an additional 30 min. Shake by mechanical means for 30 min. Dilute with methanol to volume.

Sample solution: Nominally equivalent to 5 µg/mL of metolazone from the *Sample stock solution* in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.1 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{16}ClN_3O_3S$ in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metolazone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Add the following:

▲• **DISSOLUTION**

[NOTE—Carry out all tests under subdued light, and use low-actinic glassware.]

Medium: 2% w/v sodium dodecyl sulfate in water; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Buffer solution: 6.8 g/L of anhydrous monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.

Mobile phase: *Buffer solution*, acetonitrile, and methanol (68:27:5)

Standard solution: Transfer 56 mg of USP Metolazone RS to a 200-mL volumetric flask, dissolve in 4.0 mL of methanol, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about L/1000, where L is the Tablet label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm. Dilute with *Medium*, if necessary.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Temperature: 30°

Flow rate: 1.2 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Calculate the percentage of metolazone dissolved.

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = tablet label claim (mg)
V = volume of *Medium* (mL), 900

Tolerances: NLT 55% (Q) of the labeled amount of metolazone is dissolved.▲*USP34*

• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 30°.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Metolazone RS

BRIEFING

Mycophenolate Mofetil for Injection. A new *USP* monograph for this dosage form, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities* are based on analyses performed with the Inertsil-Phenyl, 5-µm brand of L11 column. The typical retention time for mycophenolate mofetil is about 27 min.

(MD-OOD: F. Mao. MSA: R. Tirumalai.) RTS—C38804

In-Process Revision

Add the following:

▲Mycophenolate Mofetil for Injection

DEFINITION

Mycophenolate Mofetil for Injection contains an amount of Mycophenolate Mofetil Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

- The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Protect solutions from light.]

Buffer 1: Transfer 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.

Buffer 2: Transfer 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Solution A: *Buffer 1* and water (4:9)

Diluent: Acetonitrile, *Buffer 2*, and water (7:4:9)

Mobile phase: Acetonitrile and *Solution A* (3:7)

Standard stock solution: Transfer a known quantity of USP Mycophenolate Mofetil RS in a suitable volumetric flask, add acetonitrile equivalent to about 10% of the final volume, and sonicate for about 5 min or until the solid dissolves. Dilute with *Diluent* to volume to obtain a solution containing a known concentration of 1.0 mg/mL of USP Mycophenolate Mofetil RS.

Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, from *Standard stock solution*

Sample solution: Constitute each of the containers of Mycophenolate Mofetil for Injection with 14 mL of 5% Dextrose Injection. Quantitatively transfer the contents of all vials, the combined contents of which are equivalent to about 2 g of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with water to volume. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 249 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 45°

Autosampler temperature: 5°

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil for Injection taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

Organic Impurities

[NOTE—Protect solutions from light.]

- **PROCEDURE**

Mobile phase, Standard solution, Sample solution and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*. [NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respective to mycophenolate mofetil.]

Sensitivity solution: 0.2 µg/mL in *Diluent*, from the *Standard solution*

System suitability

Sample: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*

Signal-to-Noise Ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Sample: *Standard solution* and *Sample solution*. [NOTE—The run time for the *Sample solution* is NLT 1.5 times of the retention time of mycophenolate mofetil peak.]

Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Total impurities: NMT 1.35%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.12	1.4	1.1
Mycophenolate mofetil	1.00	—	—
Any unspecified impurity	—	1.0	0.1

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS ⟨85⟩:** It contains NMT 0.4 USP Endotoxin Unit per mg of mycophenolate mofetil.
- **STERILITY ⟨71⟩:** It meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined, Membrane Filtration*.

In-Process Revision

- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** NMT 1.0%
- **pH** ⟨**791**⟩**:** Between 2.7 and 4.1, in a reconstituted solution
- **PARTICULATE MATTER** ⟨**788**⟩**:** Meets the requirements for small-volume injections
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements under *Injections* ⟨1⟩*, Constituted Solutions*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS
  USP Mycophenolate Mofetil RS
  USP Mycophenolate Mofetil Related Compound A RS
  [2-morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate]
  USP Mycophenolate Mofetil Related Compound B RS
  [(*RS*)-7-hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxotetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuranyl-1-one]▲*USP34*

BRIEFING

Mycophenolate Mofetil for Oral Suspension. A new *USP* monograph for this dosage form, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the *Assay* and in the test for *Organic Impurities* are based on analyses performed with the Inertsil-Phenyl, 5-µm brand of L11 column. The typical retention time for mycophenolate mofetil is about 25 min.

(MD-OOD: F. Mao. BPC: M. Marques.) RTS—C38804

Add the following:

▲Mycophenolate Mofetil for Oral Suspension

DEFINITION
Mycophenolate Mofetil for Oral Suspension is a dry mixure of mycophenolate mofetil and one or more suitable buffers, colors, diluents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION
- The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  [NOTE—Protect solutions from light.]
  Buffer 1: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.
  Buffer 2: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.
  Solution A: *Buffer 1* and water (4:9)
  Extraction solvent: Acetonitrile, *Buffer 2*, and water (13:4:9)
  Diluent: Acetonitrile, *Buffer 2*, and water (7:4:9)
  Mobile phase: Acetonitrile and *Solution A* (3:7)
  Standard stock solution: 4 mg/mL of USP Mycophenolate Mofetil RS in *Extraction solvent*. Sonicate to aid the dissolution.
  Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, from the *Standard stock solution*
  Sample stock solution: Constitute Mycophenolate Mofetil for Oral Suspension as directed on the label. Prepare a composite sample by mixing NLT 4 bottles of the constituted Mycophenolate Mofetil for Oral Suspension. Transfer a volume of the composite sample so obtained, equivalent to 800 mg of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with *Extraction solvent* to volume.
  Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a 45-µm filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 249 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L11
  Column temperature: 45°
  Autosampler temperature: 5°
  Flow rate: 1.5 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil for Oral Suspension taken:

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

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
  C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨**711**⟩
  [NOTE—Prepare all solutions in low-actinic glassware.]
  Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.
  Apparatus 2: 40 rpm
  Time: 20 min
  Standard solution: 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium*.
  Sample solution: Reconstitute Mycophenolate Mofetil for Oral Suspension according to the labeling instructions. Shake well. Use a separate 3-mL syringe for each vessel. Withdraw 2 mL of suspension. Remove air bubbles from the syringe. Adjust the volume to 1.2 mL and accurately weigh the filled syringe. Operate the apparatus, holding the syringe above the surface of the medium, at a location that is halfway between the paddle shaft and the vessel wall. Carefully introduce the sample to the vessel over a 5–10 sec period. Weigh the empty syringe and determine the weight of the sample (g). At the time specified, withdraw an aliquot and immediately pass through a suitable 10-µm filter, discarding the first few mL.
  Spectrometric conditions
  Mode: UV
  Analytical wavelength: 304 nm
  Cell: 0.2 cm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of mycophenolate mofetil dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (V_1/V_2) \times 100$$

  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*

In-Process Revision

C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
L = suspension label claim of mycophenolate mofetil (mg/mL)
V_1 = volume of *Medium*, 900 (mL)
V_2 = volume of sample (mL), weight (g) of the sample divided by the density of the suspension (g/mL)

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements
- **DELIVERABLE VOLUME ⟨798⟩:** Meets the requirements

IMPURITIES

Organic Impurities

[NOTE—Protect solutions from light.]

- **PROCEDURE**

Mobile phase, Standard solution, Sample solution and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*. [NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respect to mycophenolate mofetil.]

Sensitivity solution: 0.2 µg/mL in *Diluent*, from the *Standard solution*

System suitability

Sample: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Sample: *Standard solution* and *Sample solution*

[NOTE—The run time for the*Sample solution* is NLT 1.5 times the retention time of the mycophenolate mofetil peak.]

Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response of each individual impurity from the *Sample solution*
r_S = response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

[NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Total impurities: NMT 3.8%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.12	1.4	3.3
Sorbitol ester of Mycophenolic acid[b]	0.24	0.77	0.2
Mycophenolate mofetil	1.00	—	—
Any unspecified impurity	—	1.0	0.1

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b] sorbitol (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

SPECIFIC TESTS

- **pH ⟨791⟩:** Between 6.0 and 7.0, in the suspension constituted as directed in the labeling
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements under *Injections* ⟨1⟩, *Constituted Solutions*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Mycophenolate Mofetil RS
USP Mycophenolate Mofetil Related Compound A RS [2-morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate]
USP Mycophenolate Mofetil Related Compound B RS [(*RS*)-7-hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxotetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuranyl-1-one]▲*USP34*

BRIEFING

Oxaliplatin, page 973 of *PF* 34(4) [July–Aug. 2008]. On the basis of comments received, the following changes are proposed:

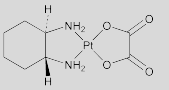
1. Add water as the diluent in the *Sensitivity solution* for clarification under *Organic Impurities, Procedure 1: Limit of Oxalic Acid*.
2. Replace the *Sensitivity* requirement with the *Signal-to-noise ratio* to be more specific, under *Organic Impurities, Procedure 1: Limit of Oxalic Acid*.
3. Correct the formula under *Specific Tests, Content of Platinum*.

(MD-OOD: F. Mao.) RTS—C77193

In-Process Revision

Add the following:

▪Oxaliplatin



$C_8H_{14}N_2O_4Pt$ 397.29

[*SP*-4-2-(1*R*-*trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′)[ethanedioato(2-)-*O*,*O*′]platinum;
cis-[(1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′][oxalato(2-)-*O*,*O*′]platinum [61825-94-3].

DEFINITION

Oxaliplatin contains NLT 98.0% and NMT 102.0% of $C_8H_{14}N_2O_4Pt$, calculated on the dried basis.
[**CAUTION—**Great care should be taken in handling Oxaliplatin, because it is a potentially cytotoxic agent.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Buffer: Weigh 2.72 g of monobasic potassium phosphate (anhydrous) and 1.80 g of 1-pentanesulfonic acid sodium salt into a suitable container. Add 2000 mL of water, and mix well to completely dissolve all solids. Transfer 0.5 mL of triethylamine to the buffer solution, and mix thoroughly. Adjust the pH of the solution to 4.3 ± 0.05 by dropwise addition of phosphoric acid.

Mobile phase: Methanol and *Buffer* (3:17)

Oxaliplatin standard stock solution: 0.5 mg/mL of USP Oxaliplatin RS in water

Oxaliplatin related compound B standard stock solution: Transfer USP Oxaliplatin Related Compound B RS to a suitable volumetric flask, add 25% of the final volume of methanol, and sonicate for approximately 2 min to disperse the solids. Add approximately 65% of the final volume of 0.001 M nitric acid, and sonicate for an additional 30 min to dissolve the solids. Allow to cool if necessary. Dilute with 0.001 M nitric acid to volume, and mix to obtain a solution having a known concentration of about 0.125 mg/mL. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in solution preparation.]

Oxaliplatin related compound C standard stock solution: 0.1 mg/mL of USP Oxaliplatin Related Compound C RS in water

System suitability solution: 2 mg/mL of Oxaliplatin in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 10 mL of this solution, 10 mL of *Oxaliplatin related compound B standard stock solution,* and 5 mL of *Oxaliplatin related compound C standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms the diaquodiaminocyclohexaneplatinum dimer.]

Standard solution: 0.1 mg/mL of Oxaliplatin in water, from *Oxaliplatin standard stock solution*

Sample solution: 0.1 mg/mL of Oxaliplatin in water

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times, measured with respect to oxaliplatin, of oxaliplatin related compounds C and B and diaquodiaminocyclohexaneplatinum dimer are about 0.8, 2.7, and 6, respectively.]
Suitability requirements
Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*
Tailing factor: Between 0.8 and 2.0 for the oxaliplatin peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the oxaliplatin peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_8H_{14}N_2O_4Pt$ in the portion of Oxaliplatin taken:

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

r_U = response from the *Sample solution*
r_S = response from the *Standard solution*
C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **LIMIT OF SILVER**

Sample stock solution: Dissolve 100 mg of Oxaliplatin, weighed, in 50 mL of water to obtain a solution having a concentration of 2 mg/mL.

Sample solution: 1 mg/mL of Oxaliplatin in 0.5 M nitric acid from *Sample stock solution*

Standard stock solution: Dilute a commercially available silver nitrate atomic absorption standard solution containing 1000 ppm of silver in 0.5 M nitric acid quantitatively, and stepwise if necessary, with 0.5 M nitric acid to obtain a 10-ppb solution.

Standard solution 1: Mix 20 µL of the *Sample stock solution* and 8 µL of the *Standard stock solution*, and dilute with 0.5 M nitric acid to 40 µL.

Standard solution 2: Mix 20 µL of the *Sample stock solution* and 16 µL of the *Standard stock solution,* and dilute with 0.5 M nitric acid to 40 µL.

Spectrometric conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Silver emission line of 328.1 nm
Lamp: Silver hollow–cathode
Flame: Graphite furnace
Blank: 0.5 M nitric acid

Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Plot the absorbances of the *Sample solution, Standard solution 1*, and *Standard solution 2* versus their concentrations, in ppb, of silver, and draw the straight line best fitting the three plotted points. The intercept on the x-axis of the extended regression line indicates the silver concentration in the *Sample solution*.
Calculate the silver, in ppm, in the portion of Oxaliplatin taken:

$$\text{Result} = (C/W) \times 100$$

C = absolute value of the intercept, in ppb of silver, on the x-axis

In-Process Revision

W = weight of Oxaliplatin taken for the preparation of the *Sample stock solution* (mg)

Acceptance criteria: NMT 5.0 ppm

• **HEAVY METALS**

Standard stock solution: Transfer 1 mL each of 1000-ppm standard solutions of cadmium, chromium, copper, iron, nickel, and lead (commercially available) to a 100-mL volumetric flask. Add 5 mL of nitric acid, and dilute with water to volume.

Internal standard solution: Transfer 1 mL of a 10,000-ppm standard solution of yttrium (commercially available) to a 100-mL volumetric flask, and dilute with 5% nitric acid to volume.

Standard solutions: Transfer 0.2, 2.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Add 1.0 mL of *Internal standard solution* and 5.0 mL of nitric acid to each flask, and dilute with water to volume. The concentrations of these solutions are 0.02, 0.20, and 2.00 ppm, respectively.

Blank solution: Transfer 1.0 mL of *Internal standard solution* and 5.0 mL of nitric acid to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Weigh 1 g of Oxaliplatin into a 100-mL volumetric flask, and add 80 mL of water. Stir vigorously for several min with a magnetic stirrer until no more sample seems to be dissolving. Add 5 mL of nitric acid, and mix again until the sample is completely dissolved. Remove the stirrer bar from the flask, rinsing it before removal. Add 1.0 mL of the *Internal standard solution,* and dilute with water to volume.

Spectrometric conditions

(See *Plasma Spectrochemistry* ⟨730⟩.)

Measure the responses of the elements cadmium, chromium, copper, iron, nickel, lead, and yttrium (internal standard), using an inductively coupled plasma–atomic optical emission spectrometer (ICP–OES), by measuring the emissions at 226.502, 283.563, 327.395, 259.940, 221.648, 220.353, and 371.029 nm, respectively. Optimize the instrument settings as directed by the manufacturer.

System suitability

Before samples are analyzed, the instrument must pass a suitable performance check. Generate the calibration curve, using the *Blank solution* and the *Standard solutions,* and run these solutions in the following order: the *Blank solution,* then the 0.02-, 0.20-, and 2.00-ppm solutions. The linear regression coefficient is NLT 0.99; the response of the *Blank solution* is between –5.0 and 5.0 ppb for each element; and the responses of yttrium obtained from the *Standard solutions* are drifted by NMT 5.0% of the response obtained from the *Blank solution.* Run the *Standard solution* of 0.20 ppm, and record the responses of each element: the relative standard deviations for replicate runs are NMT 5.0%; and the recovery against the calibration curve is between 95% and 105%. After samples are analyzed, the instrument must pass the same suitable performance check to ensure that the calibration is still valid.

Analysis

Sample: *Sample solution*

Record the responses of each element, and determine the concentration of each element, using the calibration graph. Calculate the content of each element, in ppm, in the portion of Oxaliplatin taken:

$$\text{Result} = (C/W) \times 100$$

C = concentration of each element in the *Sample solution* (ppm)

W = weight of Oxaliplatin taken to prepare the *Sample solution* (g)

Acceptance criteria

Total heavy metals: NMT 20 ppm

Change to read:

Organic Impurities

• **PROCEDURE 1: LIMIT OF OXALIC ACID**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Buffer: Add 1.36 g of potassium dihydrogen phosphate to 10 mL of 10% tetrabutylammonium hydroxide in water, and dilute with water to 1000 mL. Adjust the pH with phosphoric acid to 6.0.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard stock solution: 0.06 mg/mL of USP Oxaliplatin Related Compound A RS in water. [NOTE—USP Oxaliplatin Related Compound A RS is available as oxalic acid dihydrate.]

Standard solution: 15 µg/mL of USP Oxaliplatin Related Compound A RS in water, from *Standard stock solution*

System suitability solution: 0.05 mg/mL of sodium nitrate in water. Transfer 2 mL of this solution and 25 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume

Sensitivity solution: 1.5 µg/mL of USP Oxaliplatin Related Compound A RS ▲in water,▲*USP34* from *Standard solution*

Sample solution: 2 mg/mL of Oxaliplatin in water

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm column; 5-µm packing L1

Flow rate: 2 mL/min

Column temperature: 40°

Injection size: 20 µL

System suitability

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

[NOTE—The elution order is sodium nitrate, followed by oxalic acid.]

Suitability requirements

Resolution: NLT 2.0 between oxalic acid and sodium nitrate, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

~~**Sensitivity**~~▲**Signal-to-noise ratio:**▲*USP34* ~~The peak at approximately the same retention time as that of the *Standard solution* should be visible~~▲NLT 10,▲*USP34* *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = response of oxalic acid from the *Sample solution*

r_S = response of oxalic acid from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)

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

M_{r1} = molecular weight of anhydrous oxalic acid, 90.03

M_{r2} = molecular weight of USP Oxaliplatin Related Compound A RS, 126.07

Acceptance criteria: NMT 0.05%

In-Process Revision

• **PROCEDURE 2: LIMIT OF (*SP*-4-2)-DIAQUA[(1*R*,2*R*)-CYCLOHEXANE-1,2-DIAMINE-*N*,*N*′]PLATINUM, OXALIPLATIN RELATED COMPOUND C, AND UNSPECIFIED IMPURITIES**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase, System suitability solution, Oxaliplatin standard stock solution, Oxaliplatin related compound B standard stock solution, and **Oxaliplatin related compound C standard stock solution:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of oxaliplatin, 0.01 mg/mL of oxaliplatin related compound B, and 0.004 mg/mL of oxaliplatin related compound C in water, from *Oxaliplatin standard stock solution, Oxaliplatin related compound B standard stock solution,* and *Oxaliplatin related compound C standard stock solution*, respectively

Sample solution: 2 mg/mL of Oxaliplatin in water

Chromatographic system: Prepared as directed in the *Assay*.

Suitability requirements

Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*

Tailing factor: Between 0.8 and 2.0 for oxaliplatin peak, *System suitability solution*

Relative standard deviation: NMT 3.0% for oxaliplatin, oxaliplatin related compound B, and oxaliplatin related compound C peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Sample solution*

r_S = response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample* (mg/mL)

M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30

M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28

[NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in solution preparation.]

Calculate the percentage of oxaliplatin related compound C in the portion of Oxaliplatin taken:

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

r_U = response of oxaliplatin related compound C from the *Sample solution*

r_S = response of oxaliplatin related compound C from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound C RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of the unspecified impurity diaquodiaminocyclohexaneplatinum dimer in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = response of diaquodiaminocyclohexaneplatinum dimer from the *Sample solution*

r_S = response of oxaliplatin related compound B from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

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

M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30

M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28

F = relative response factor for diaquodiaminocyclohexaneplatinum dimer, measured with respect to USP Oxaliplatin Related Compound B RS, 2.5

Calculate the percentage of any other unspecified impurity in the portion of Oxaliplatin taken:

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

r_U = response of any other unspecified impurity from the *Sample solution*

r_S = response of oxaliplatin from the *Standard solution*

C_S = concentration of oxaliplatin in the *Standard solution* (mg/mL)

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

Acceptance criteria

(*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum: NMT 0.05%

Oxaliplatin related compound C: NMT 0.03%

Diaquodiaminocyclohexaneplatinum dimer: NMT 0.03%

Any individual unspecified impurity: NMT 0.03%

Total unspecfied impurities: NMT 0.10%. [NOTE—Total unspecfied impurities include diaquodiaminocyclohexaneplatinum dimer and any other unspecified impurities.]

Total impurities: NMT 0.15%. [NOTE—Total impurities include oxalic acid, (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, oxaliplatin related compound C, and the total unspecified impurities.]

• **PROCEDURE 3: LIMIT OF OXALIPLATIN RELATED COMPOUND D**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase: Methanol and ethanol (7:3)

Oxaliplatin related compound D standard stock solution: 0.05 mg/mL of USP Oxaliplatin Related Compound D RS in methanol

Oxaliplatin related compound D standard solution: 15 µg/mL of USP Oxaliplatin Related Compound D RS in methanol, from *Oxaliplatin related compound D standard stock solution*

Oxaliplatin standard stock solution: 0.75 mg/mL of USP Oxaliplatin RS in methanol

Oxaliplatin standard solution: 37.5 µg/mL of USP Oxaliplatin RS in methanol, from *Oxaliplatin standard stock solution*

Standard solutions: Transfer 40 mL of *Oxaliplatin standard stock solution* to separate 50-mL volumetric flasks. Add 1.0, 3.0, and 5.0 mL of *Oxaliplatin related compound D standard solution* to each flask, and dilute with methanol to volume. The concentration of oxaliplatin in these solutions is 0.6 mg/mL. The concentration of oxaliplatin related compound D in these solutions is 0.3, 0.9, and 1.5 µg/mL, respectively.

System suitability solution: Transfer 5.0 mL of *Oxaliplatin standard solution* and 4.0 mL of *Oxaliplatin related compound D standard stock solution* to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: 0.6 mg/mL of Oxaliplatin in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

In-Process Revision

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L70
Flow rate: 0.3 mL/min
Column temperature: 40°
Injection size: 20 µL
Run time: 30 min

System suitability
Samples: *System suitability solution* and the 0.9 µg/mL *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between oxaliplatin and oxaliplatin related compound D, *System suitability solution*
Relative standard deviation: NMT 3.0% for peak height ratio of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D, the 0.9 µg/mL *Standard solution*

Analysis
Samples: *Standard solutions* and *Sample solution*
Plot a calibration curve for the *Standard solutions* with the peak response ratios of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D on the *y*-axis and the concentrations of oxaliplatin related compound D, in µg/mL, on the *x*-axis. Read the concentration of oxaliplatin related compound D, in µg/mL, in the *Sample solution* from the calibration curve obtained.
Calculate the percentage of oxaliplatin related compound D in the portion of Oxaliplatin taken:

$$\text{Result} = (C/W) \times 5$$

C = concentration of oxaliplatin related compound D in the *Sample solution* (mg/mL)
W = weight of Oxaliplatin taken to prepare the *Sample solution* (mg)

Acceptance criteria: NMT 0.10%

SPECIFIC TESTS

- **ACIDITY**
Sample solution: Dissolve 100 mg in 50 mL of carbon dioxide-free water, and add 0.5 mL of phenolphthalein TS.
Acceptance criteria: The solution is colorless, and NMT 0.6 mL of 0.01 M sodium hydroxide is required to change the color to pink.
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 1.0 USP Endotoxin Unit/mg of Oxaliplatin

Change to read:

- **CONTENT OF PLATINUM**
Sample: Ignite an empty porcelain crucible fitted with a lid in a furnace at 800° for 30 min. Cool in a desiccator, and weigh. Add about 200 mg of the Oxaliplatin, weighed, to the crucible, and ignite in a furnace by stepwise increments as follows: introduce into the furnace; and increase the temperature to 200° within 15 min, then to 400° within 15 min, then to 600° within 15 min, then finally to 800° within 15 min. Allow to remain in the furnace at 800° for 30 min. Remove, cool in a desiccator, and reweigh.
Calculate the percentage of platinum in the portion of Oxaliplatin taken:

~~$\text{Result} = (D/W) \times 100$~~

~~D = difference between the net weights before and after the ignition (mg)~~
~~W = weight of oxaliplatin taken for determination (mg)~~

▲

$$\text{Result} = (W_2/W_1) \times 100$$

W_2 = weight of residue after ignition (mg)
W_1 = weight of oxaliplatin before ignition (mg)

▲*USP34*

Acceptance criteria: 48.1%–50.1% of the oxaliplatin taken, on the dried basis
- **LOSS ON DRYING ⟨731⟩:** Dry about 1 g at 100° to 105° for 2 h: it loses NMT 0.4% of its weight.
- **MICROBIAL ENUMERATION TESTS ⟨61⟩:** The total aerobic microbial count does not exceed 20 cfu/g, and the total combined molds and yeast count does not exceed 5 cfu/g.
- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩:** Between +74.5° and +78.0°, measured at 20°
Sample solution: 5 mg/mL, in water

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Endotoxin RS
USP Oxaliplatin RS
USP Oxaliplatin Related Compound A RS
USP Oxaliplatin Related Compound B RS
USP Oxaliplatin Related Compound C RS
USP Oxaliplatin Related Compound D RS■*2S (USP32)*

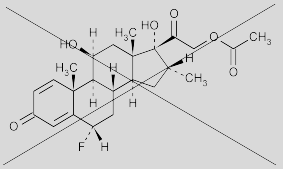
BRIEFING

Paramethasone Acetate, *USP 32* page 3208;
Paramethasone Acetate Tablets, *USP 32* page 3209. It is proposed to omit these monographs from the *USP* for the following reasons.
1. All drug products containing paramethasone acetate have been discontinued in the United States.
2. The drug is currently not used in veterinary medicine in the United States.
3. USP has not been able to secure a viable source of the RS material.

(MD-PS: L. Santos, M. Waddell.) RTS—C77734

Delete the following:

▲~~Paramethasone Acetate~~



~~$C_{24}H_{31}FO_6$~~ ~~434.50~~
~~Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6-fluoro-11,17-dihydroxy-16-methyl-, (6α,11β,16α)-,~~
~~6α-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate [1597-82-6,].~~

~~DEFINITION~~
~~Paramethasone Acetate contains NLT 95.0% and NMT 101.0% of $C_{24}H_{31}FO_6$, calculated on the dried basis.~~

~~IDENTIFICATION~~
- **~~A. INFRARED ABSORPTION ⟨197K⟩~~**
- **~~B. ULTRAVIOLET ABSORPTION ⟨197U⟩~~**
~~Sample solution:~~ ~~20 µg/mL in methanol~~
~~Analytical wavelength:~~ ~~242 nm~~
~~Acceptance criteria:~~ ~~Absorptivities, calculated on the dried basis, do not differ by more than 4.0%.~~

In-Process Revision

• **C. Thin-Layer Chromatography**

Standard solution: 2 mg/mL of USP Paramethasone Acetate RS in chloroform and methanol (1:1)

Sample solution: 2 mg/mL of Paramethasone Acetate in chloroform and methanol (1:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Methylene chloride, nitromethane, and glacial acetic acid (60:40:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent has moved about three-fourths of the length of the plate. Remove the plate, and air-dry for 15 min. Return the plate to the chamber, and develop again, in the same solvent system, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Locate the spots under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution.*

ASSAY

• **Procedure**

Standard solution: Prepare as directed for *Standard Preparation* under *Single-Steroid Assay* ⟨511⟩, using USP Paramethasone Acetate RS to prepare a solution containing 10 mg/mL.

Sample solution: 10 mg/mL of Paramethasone Acetate, previously dried, in alcohol and chloroform (1:1)

Analysis: Proceed as directed for *Procedure* under *Single-Steroid Assay* ⟨511⟩, applying 10 µL each of the *Sample solution* and the *Standard solution* to the chromatographic plate, and using a solvent system of methylene chloride, nitromethane, and glacial acetic acid (60:40:1). Proceed as directed through the fourth sentence of the second paragraph under *Procedure.* Centrifuge the tubes for 5 min, and determine the absorbances of the supernatants in 1-cm cells, at the wavelength of maximum absorbance at 242 nm, against the blank. Calculate the percentage of $C_{24}H_{31}FO_6$ in the portion of Paramethasone Acetate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Paramethasone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Paramethasone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–101.0% on the dried basis

SPECIFIC TESTS

• **X-Ray Diffraction** ⟨941⟩**:** The X-ray diffraction pattern of Paramethasone Acetate conforms to either one pattern or a mixture of the patterns shown in the accompanying table.

Form A		Form B	
d	I/I_1	d	I/I_1
12.09	10	11.62	20
8.42	20	7.80	8
7.78	10	7.13	10
6.41	100	6.50	60
5.65	100	5.98	100
5.50	10	5.63	70
5.18	2	5.30	100
4.59	30	4.85	60
4.41	20	4.65	60
4.24	40	4.43	8
3.93	20	4.30	2
3.64	30b	3.93	60
3.48	15	3.72	6
3.27	20	3.58	4
3.12	30	3.45	4
3.03	8	3.26	10
2.90	2	3.09	10
2.82	8	2.96	10
2.70	10	2.88	10
2.61	8	2.81	6b
2.51	10	2.66	8b
2.35	2	2.55	6
2.29	8	2.48	6b
2.24	6	2.38	10
2.11	4	2.30	6
2.08	2	2.26	8
2.04	4	2.19	6
2.00	4	2.11	10
1.95	1	2.04	8
1.92	4	1.99	10
1.87	2		
1.84	2		
1.82	1		

• **Optical Rotation,** *Specific Rotation* ⟨781S⟩**:** +67° to +77°

Sample solution: 10 mg/mL in chloroform

• **Loss on Drying** ⟨731⟩**:** Dry a sample in a vacuum at 105° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Paramethasone Acetate RS▲*USP34*

Briefing

Paramethasone Acetate Tablets, *USP 32* page 3209—See briefing under *Paramethasone Acetate.*

(MD-PS: L. Santos, M. Waddell.) RTS—C77735

Delete the following:

▲Paramethasone Acetate Tablets

DEFINITION

Paramethasone Acetate Tablets contain NLT 85.0% and NMT 115.0% of the labeled amount of $C_{24}H_{31}FO_6$.

IDENTIFICATION

• The IR absorption spectrum of the *Sample solution*, prepared as directed in the *Assay*, exhibits maxima only at the same wavelengths as that of the *Standard solution*, prepared as directed in the *Assay.*

In-Process Revision

~~**ASSAY**~~
~~• **PROCEDURE**~~
~~**Standard solution:** Transfer 6 mg of USP Paramethasone Acetate RS to a separator containing 15 mL of water, and proceed as directed under *Sample solution*, beginning with "Add 3 drops of hydrochloric acid".~~
~~**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 6 mg of paramethasone acetate, to a separator containing 15 mL of water. Add 3 drops of hydrochloric acid, and heat on a steam bath for 5 min, mixing frequently. Cool the separator to room temperature, add 4 drops of sodium hydroxide solution (1 in 2), and immediately extract with four 25-mL portions of chloroform. Filter the extracts through anhydrous sodium sulfate, collecting the extracts in a beaker.~~
~~[**CAUTION—**Do not allow the filter paper to extend above the top of the funnel.]~~
~~Rinse the filter with several small portions of chloroform, add the rinsings to the beaker, and evaporate the chloroform on a steam bath with the aid of a current of air until about 3 mL remains. Transfer the residual liquid, with the aid of several small portions of chloroform, to a glass-stoppered, 10-mL conical flask, and evaporate on a steam bath with the aid of a current of air to dryness. Add 2.0 mL of chloroform to the flask, insert the stopper, and mix to dissolve the residue. Determine the absorbances of the solutions.~~
~~**Spectrometric conditions**~~
~~**Analytical wavelength:** IR 6.04 µm~~
~~**Cell:** 1 mm~~
~~**Blank:** Chloroform~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Calculate the percentage of $C_{24}H_{31}FO_6$ in the portion of Tablets taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U = absorbance of the *Sample solution*~~
~~A_S = absorbance of the *Standard solution*~~
~~C_S = concentration of USP Paramethasone Acetate RS in the *Standard solution* (mg/mL)~~
~~C_U = nominal concentration of Paramethasone Acetate in the *Sample solution* (mg/mL)~~

~~**PERFORMANCE TESTS**~~
~~• **DISINTEGRATION** ⟨**701**⟩~~
~~[NOTE—The use of disks is being omitted.]~~
~~**Time:** 15 min~~
~~• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements~~
~~**Procedure for content uniformity**~~
~~**Sample solution:** Transfer 1 finely powdered Tablet to a 50-mL volumetric flask. Add 25 mL of chloroform, and shake by mechanical means for 15 min. Dilute with chloroform to volume and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate, if necessary, with chloroform to obtain a solution containing 20 µg/mL of paramethasone acetate.~~
~~**Standard solution:** 20 µg/mL of USP Paramethasone Acetate RS in the same medium. Transfer 10.0 mL each of the *Sample solution* and the *Standard solution* to separate 25-mL volumetric flasks, and transfer 10 mL of chloroform to a third flask to provide the blank. To each flask add 3.0 mL of a 1-in-4000 solution of blue tetrazolium in alcohol and 5.0 mL of a 1-in-20 solution of tetramethylammonium hydroxide TS in alcohol, mixing after each addition. Fifteen min, accurately timed, after the addition of the last reagent, add 1 mL of glacial acetic acid to each flask, and dilute with chloroform to volume. Determine the absorbances of the solutions.~~
~~**Spectrometric conditions**~~
~~**Analytical wavelength:** UV 525 nm~~
~~**Cell:** 1 cm~~
~~**Blank:** Chloroform~~
~~Calculate the percentage of $C_{24}H_{31}FO_6$ in the Tablet taken:~~

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

~~A_U = absorbance of the *Sample solution*~~
~~A_S = absorbance of the *Standard solution*~~
~~C_S = concentration of USP Paramethasone Acetate RS in the *Standard solution* (µg/mL)~~
~~C_U = nominal concentration of Paramethasone Acetate in the *Sample solution* (µg/mL)~~

~~**ADDITIONAL REQUIREMENTS**~~
~~• **PACKAGING AND STORAGE:** Preserve in well-closed containers.~~
~~• **USP REFERENCE STANDARDS** ⟨**11**⟩~~
~~USP Paramethasone Acetate RS▲*USP34*~~

BRIEFING

Pilocarpine Hydrochloride, *USP 32* page 3306 and page 1179 of *PF* 34(5) [Sept.–Oct. 2008]. On the basis of comments received, it is proposed to make the following changes:

1. The *Assay* and *Organic Impurities, Procedure 1* are revised to replace the current methods with a stability-indicating HPLC procedure. The liquid chromatographic procedure in the *Assay* and *Organic Impurities, Procedure 1* were based on analyses performed with the Phenomenex Luna Phenyl Hexyl brand of L11 column. The typical retention time reported for pilocarpine is about 6 min.
2. The *Acceptance criteria* in the *Assay* is revised from 98.5%–101.0% to 98.0%–102.0% to reflect the specification for the marketed, approved product.
3. The test for *Melting Range or Temperature* is deleted. This test was included in the original monograph when there was no selective method to quantify the impurities. The revised monograph contains an HPLC method; therefore, the *Melting Range or Temperature* test contributes no additional value in establishing the quality of the drug substance.
4. The temperature condition is added in *Packaging and Storage* for clarification.

(MD-OOD: F. Mao.) RTS—C73083

Pilocarpine Hydrochloride

$C_{11}H_{16}N_2O_2 \cdot HCl$ 244.72
2(3*H*)-Furanone, 3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, monohydrochloride, (3*S*-*cis*)-;
Pilocarpine monohydrochloride [54-71-7].

DEFINITION

Change to read:

Pilocarpine Hydrochloride contains NLT ~~98.5%~~▲98.0%▲*USP34* and NMT ~~101.0%~~▲102.0%▲*USP34* of $C_{11}H_{16}N_2O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197M**⟩
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements
Sample solution: 50 mg/mL

In-Process Revision

ASSAY

Change to read:

• **PROCEDURE**

~~**Sample solution:** 500 mg of Pilocarpine Hydrochloride in a mixture of 20 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly to effect solution. Cool the solution to room temperature.~~

~~**Analysis:** Titrate the entire *Sample solution* with 0.1 N perchloric acid VS, using 2 drops of crystal violet TS as an indicator. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 24.47 mg of $C_{11}H_{16}N_2O_2 \cdot HCl$.~~

~~**Acceptance criteria:** 98.5%–101.0%~~

▲**Buffer:** 4.4 g/L of potassium phosphate dibasic in water. Adjust with phosphoric acid to a pH of 6.5 ± 0.1.

Mobile phase: Acetonitrile, methanol, and *Buffer* (2:35:63)

Standard solution: 0.5 mg/mL of USP Pilocarpine Hydrochloride RS in water. [NOTE—Sonicate if necessary.]

System suitability solution: Transfer a known amount of USP Pilocarpine Hydrochloride RS in a suitable volumetric flask, and add water, equivalent to 10% of the volume of the flask, to dissolve. [NOTE—Sonicate as needed.] Add 0.1 N sodium hydroxide, equivalent to 10% of the volume of the flask, quench immediately with the same volume of 0.1 N hydrochloric acid, and mix. Dilute with water to volume. [NOTE—The initial concentration of USP Pilocarpine Hydrochloride RS is 0.5 mg/mL. Isopilocarpine is formed in the *System suitability solution* preparation.]

Sample solution: 0.5 mg/mL of Pilocarpine Hydrochloride in water. [NOTE—Sonicate if necessary.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{11}H_{16}N_2O_2 \cdot HCl$ in the portion of Pilocarpine Hydrochloride taken:

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

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis ▲*USP34*

IMPURITIES

Change to read:

Organic Impurities

• ~~**PROCEDURE 1: ORDINARY IMPURITIES** ⟨466⟩~~

~~**Standard solution** and **Sample solution:** Dehydrated alcohol~~

~~**Eluant:** Hexanes, dehydrated alcohol, and ammonium hydroxide (70:30:1)~~

~~**Visualization:** 17~~

~~**Limits:** NMT 1%~~

▲• **PROCEDURE 1: RELATED COMPOUNDS**

Mobile phase, Standard solution, System suitability solution, and **Sample solution:** Proceed as directed under *Assay*.

Sensitivity solution: 0.25 µg/mL of USP Pilocarpine Hydrochloride RS in water, *Standard solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Column temperature: 35°
Flow rate: 1.0 mL/min
Run time: NLT 5 times the retention time of the pilocarpine peak
Injection size: 10 µL

System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*
Signal-to-noise ratio: NLT 10 for the pilocarpine peak, *Sensitivity solution*
Relative standard deviation: NMT 2.0% for the pilocarpine peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pilocarpine Hydrochloride taken:

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

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of pilocarpine from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isopilocarpine[a]	0.94	1.0
Pilocarpine	1.00	—
Pilocarpic acid[b]	1.15	0.5
Isopilocarpic acid[c]	1.19	0.1
Any unspecified impurity	—	0.1

[a] (3*R*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one.
[b] (2*S*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.
[c] (2*R*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.

▲*USP34*

• **PROCEDURE 2: OTHER ALKALOIDS**

Sample solution: 10 mg/mL in water

Analysis: Divide the *Sample solution* into two portions. To one portion add a few drops of 6 N ammonium hydroxide, and to the other, add a few drops of potassium dichromate TS.

Acceptance criteria: No turbidity is produced in either solution.

In-Process Revision

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE** ⟨~~741~~⟩**:** ~~199°–205°, but the range between beginning and end of melting does not exceed 3°~~▲*USP34*

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +88.5° to +91.5°
  Sample solution: 20 mg/mL, in water
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 3.0% of its weight.

Change to read:

- **READILY CARBONIZABLE SUBSTANCES TEST** ⟨**271**⟩
  Sample solution: 50 mg/mL in sulfuric acid ~~TS~~■2S *(USP32)*
  Acceptance criteria: The solution has no more color than *Matching Fluid B*.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at room temperature.▲*USP34*
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Pilocarpine Hydrochloride RS

BRIEFING

Quinidine Sulfate Oral Suspension, *USP 32* page 3465. On the basis of comments received, it is proposed to update the formula in the *Assay* to accommodate the dihydroquinidine contained in the Reference Standard.

(CRX: R. Schnatz.) RTS—C71345

Quinidine Sulfate Oral Suspension

DEFINITION

Quinidine Sulfate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$. Prepare Quinidine Sulfate Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Quinidine Sulfate	1 g
Vehicle: a mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF* (1:1)	A sufficient quantity
To make	100 mL

If using Quinidine Sulfate Tablets, place in a suitable mortar, and comminute into a fine powder, or add quinidine sulfate powder to the mortar. Add 15 mL of the Vehicle, and mix to a uniform paste. Add the Vehicle in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar to the calibrated bottle. Add sufficient Vehicle to volume, and mix well.

ASSAY

Change to read:

- **PROCEDURE**
  Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
  Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.
  Mobile phase: Acetonitrile, *Solution A, Solution B*, and water (10:1:1:40
  Standard solution: 100 µg/mL of USP Quinidine Sulfate RS, in *Mobile phase*
  Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 235 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for quinidine sulfate is about 8.5 min.]
  Suitability requirements
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ in the volume of Oral Suspension taken.

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

~~r_U = peak response from the *Sample solution*~~
~~r_S = peak response from the *Standard solution*~~
~~C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (µg/mL)~~
~~C_U = nominal concentration of quinidine sulfate in the *Sample solution* (µg/mL)~~

▲

$$\text{Result} = 100(C/V)\,(r_{b,U} + r_{d,U})/(r_{b,S} + r_{d,S})$$

C = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)
V = volume of Oral Suspension taken (mL)
$r_{b,U}$ = peak response of quinidine from the *Sample solution*
$r_{b,S}$ = peak response of quinidine from the *Standard solution*
$r_{d,U}$ = peak response of dihydroquinidine from the *Sample solution*
$r_{d,S}$ = peak response of dihydroquinidine from the *Standard solution*

▲*USP34*

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨**791**⟩**:** 3.4–4.4

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature or in a cold place.
- **BEYOND-USE DATE:** 60 days after the day on which it was compounded
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the beyond-use date.

In-Process Revision

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Quinidine Sulfate RS

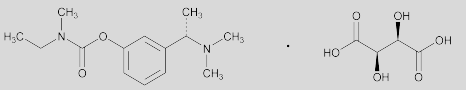
BRIEFING

Rivastigmine Tartrate. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analysis, is being proposed. The liquid chromatographic procedures in the test for *Organic Impurities, Procedure 1* and in the *Assay* are based on analyses performed with the 5-µm Hypersil BDS C8 brand of L7 column. The typical retention times are about 10 min for rivastigmine and about 2 min for the tartrate. The liquid chromatographic procedure in the test for *Enantiomeric Purity* is based on analyses performed with the Chiral AGP brand of L41 column. The typical retention time for the rivastigmine peak is about 8.5 min.

(MD-GRE: E. Gonikberg.) RTS—C54956

Add the following:

▲Rivastigmine Tartrate



$C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ 400.42
Ethylmethylcarbamic acid, 3-[(S)-1-(dimethylamino)ethyl]phenyl ester, (2*R*,3*R*)-2,3-dihydroxybutanedioate;
(*S*)-3-[1-(Dimethylamino)ethyl]phenyl ethylmethylcarbamate, hydrogen tartrate [129101-54-8].
Rivastigmine [123441-03-2].
250.34

DEFINITION
Rivastigmine Tartrate contains NLT 98.0% and NMT 102.0% of the labeled amount of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
• **B. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨**191**⟩**:** A solution containing about 4 mg/mL in methanol meets the requirements of the test for *Tartrate.*
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Buffer: 8.6 mg/mL of monobasic ammonium phosphate. Adjust with ammonia solution to a pH of 7.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (15:15:70)
System suitability solution: 0.05 mg/mL each of USP Rivastigmine Related Compound A RS and USP Rivastigmine Related Compound B RS in *Mobile phase.*
Standard solution: 0.2 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase.*
Sample solution: 0.2 mg/mL of Rivastigmine Tartrate in *Mobile phase.*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5–µm packing L7
Flow rate: 1.2 mL/min
Injection size: 20 µL
[NOTE—The flow rate may be adjusted to 1.5 mL/min, if needed, to achieve a recommended retention time of rivastigmine at about 10 min.]
System suitability
Sample: *System suitability solution* and *Standard solution*
Suitability requirements:
Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, from the *System suitability solution*
Column efficiency: NLT 5,000 theoretical plates, from the *Standard solution*
Tailing factor: NMT 3.0, from the *Standard solution*
Relative standard deviation: NMT 2.0%, from the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ in the portion of Rivastigmine Tartrate taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
Organic Impurities
• **PROCEDURE 1:**
Mobile phase and **System suitability solution:** Proceed as directed in the *Assay.*
Standard solution: 1.0 µg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Rivastigmine Tartrate in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay.* (See *Chromatography* ⟨621⟩*, System Suitability.*)
System suitability
Sample: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, from the *System suitability solution*
Relative standard deviation: NMT 10%, from the *Standard solution*
Analysis [NOTE—The run time is 8 times the retention time of the rivastigmine peak.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Rivastigmine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL).
C_U = concentration of Rivastigmine Tartrate in the *Sample solution* (mg/mL).
F = relative response factor (see *Impurity Table 1*)

In-Process Revision

Acceptance criteria:
Individual impurities See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tartrate	0.18	—	Disregard
Phenol impurity[a]	0.28	1.6	0.3
DPTTA[b]	0.46	0.83	0.15
Nor impurity[c]	0.57	1.2	0.15
Rivastigmine	1.0	1.0	—
Carbamate impurity[d]	4.1	1.3	0.15
Ether impurity[e]	6.5	1.4	0.15
Any other impurity	—	1.0	0.1

[a] (*S*)-*N,N*-Dimethyl-1-[3-(4-nitrophenoxy)phenyl]ethanamine.
[b] 3-Nitrophenyl ethyl(methyl)carbamate.
[c] (*S*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate (rivastigmine related compound B).
[d] (+)-Di-(*p*-toluoyl)-D-tartaric acid (rivastigmine related compound A).
[e] (*S*)-3-[1-(Dimethylamino)ethyl]phenol.

• **PROCEDURE 2: ENANTIOMERIC PURITY**
Buffer: Transfer 1.78 g of dibasic sodium phosphate dihydrate and 1.38 g of monobasic sodium phosphate into a 1000 mL volumetric flask. Dissolve in and dilute with water to volume. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Transfer 20 mL of acetonitrile and 205 µL of *N,N*-dimethyloctylamine to a 1000-mL volumetric flask, and dilute with *Buffer* to volume.
Standard solution: 0.1 µg/mL of USP Rivastigmine Tartrate *R*-Isomer RS in *Mobile phase*
Sensitivity solution: 0.05 µg/mL of USP Rivastigmine Tartrate *R*-Isomer RS in *Mobile phase*, from *Standard solution*
System suitability solution: 100 µg/mL of USP Rivastigmine Tartrate RS and 0.1 µg/mL of USP Rivastigmine Tartrate *R*-Isomer RS in *Mobile phase*
Sample solution: 100 µg/mL of Rivastigmine Tartrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.0-mm × 10-cm; packing L41
Flow rate: 0.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Resolution: NLT 0.8 between the enantiomer peaks, from the *System suitability solution*
[NOTE—The elution order is the *R*-enantiomer, followed by the rivastigmine peak, which is the *S*-enantiomer.]
Signal-to-noise ratio: NLT 10, from the *Sensitivity solution*
Relative standard deviation: NMT 10%, from the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of Rivastigmine Tartrate taken:

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

r_U = peak response for *R*-enantiomer from the *Sample solution*
r_S = peak response for *R*-enantiomer from the *Standard solution*
C_S = concentration of *R*-enantiomer in the *Standard solution* (µg/mL).
C_U = concentration of Rivastigmine Tartrate in the *Sample solution* (µg/mL).
Acceptance criteria: NMT 0.3% of the *R*-enantiomer is found

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method IA* ⟨**921**⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Rivastigmine Tartrate RS
USP Rivastigmine Related Compound A RS
[Di-*p*-toluoyl-D-(+)-tartaric acid monohydrate] ($C_{20}H_{20}O_9$ 404.37)
USP Rivastigmine Related Compound B RS
[*N,N*-Dimethylcarbamic acid-3-[1-(dimethylamino)ethyl]phenyl ester] ($C_{13}H_{20}N_2O_2$ 236.32)
USP Rivastigmine Tartrate *R*-Isomer RS
[(*R*)-3-[1-(Dimethylamino)ethyl]phenyl ethyl(methyl)carbamate, hydrogen tartrate] ($C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ 400.42)▲*USP34*

BRIEFING

Simethicone Emulsion, *USP 32* page 3555 and page 4099 of the *First Supplement*. Difficulties were reported in subtracting the blank toluene spectrum from the spectra for the *Standard solution* and *Sample solution* under *Identification*. On the basis of comments received, it is proposed to replace the current *Infrared Absorption* ⟨197S⟩ method based on the analysis of the toluene solutions with the method employing the attenuated total reflectance technique ⟨197A⟩.

(MD-GRE: E. Gonikberg.) RTS—C78862

Simethicone Emulsion

DEFINITION
Simethicone Emulsion is a water-dispersible form of Simethicone composed of Simethicone, suitable emulsifiers, preservatives, and water. It may contain suitable viscosity-increasing agents. It contains an amount of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) that is NLT 85.0% and NMT 110.0% of the labeled amount of simethicone.

IDENTIFICATION

Delete the following:

▲• ~~**INFRARED ABSORPTION** ⟨**197S**⟩~~
~~[NOTE—Use the blank to set the instrument.]~~
~~**Cell:** 0.5 mm~~
~~**Blank:** Prepare as directed in the *Assay*.~~
~~**Standard solution:** Prepare as directed in the *Assay*.~~
~~**Sample:** Equivalent to 50 mg of simethicone from Emulsion~~
~~**Sample solution:** Prepare as directed in the *Assay*.~~
~~**Acceptance criteria:** The IR absorption spectrum exhibits maxima only at the same wavelengths as that of the *Standard solution*. [NOTE—If necessary, the dilute hydrochloric acid may be omitted to improve separation.]~~▲*USP34*

In-Process Revision

Add the following:

▲• **INFRARED ABSORPTION** ⟨197A⟩
[NOTE—Use the *Blank* to set the instrument.]
Blank, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
Analysis: Place about 5 drops of the *Sample solution* in the sample trough, and dry it with a stream of nitrogen. Examine the spectrum in the range between 1400 cm^{-1} and 700 cm^{-1}.
Acceptance criteria: It exhibits maxima only at the same wavelengths as a similarly obtained spectrum of the *Standard solution.*▲*USP34*

ASSAY

Change to read:

• **PROCEDURE**
■[NOTE—Use glass apparatus only; avoid use of plasticware. Proper mixing of the Emulsion before sampling and testing is crucial.]■1S *(USP32)*
Standard solution: 2 mg/mL of USP Polydimethylsiloxane RS in toluene. [NOTE—Treat a 25.0-mL aliquot in the same manner as the *Blank*, beginning with "add 50 mL of dilute hydrochloric acid (2 in 5)…".]
Sample: Equivalent to 50 mg of simethicone from Emulsion
Sample solution: To the *Sample* add 25.0 mL of toluene, and treat in the same manner as the *Blank*, beginning with "add 50 mL of dilute hydrochloric acid (2 in 5)…".
Spectrometric conditions
Mode: IR spectrophotometry
Cell: 0.5 mm
Analytical wavelength: Maximum absorbance at about 7.9 µm ■(1259 cm^{-1})■1S *(USP32)*
Blank: To 25.0 mL of toluene add 50 mL of dilute hydrochloric acid (2 in 5), close the bottle securely with a cap having an inert liner, ■vigorously shake by hand for 5 s, and then shake for 5–20 min on a suitable shaker. [NOTE—The following shakers were found suitable: (1) a reciprocating shaker at a rate of about 200 oscillations/min and a stroke of 38 ± 2 mm or (2) a wrist-action shaker with a radius of 13.3 ± 0.4 cm (measured from center of shaft to center of bottle) using an arc of 10° at a frequency of 300 ± 30 strokes/min. The recommended shaking time is about 20 min, which can be adjusted as needed.] Centrifuge the mixture. [NOTE—The following centrifuging conditions were found suitable: at 1500 × g for 30–40 min.] Remove from the centrifuge, and immediately transfer about 5 mL of the upper organic (toluene) layer to a 15-mL screw-capped test tube, containing about 1 g of anhydrous sodium sulfate.■1S *(USP32)* Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained. ■[NOTE—The following centrifuging conditions were found suitable: at 1500 × g for about 10 min.]■1S *(USP32)*
Analysis
■[NOTE—Wash the cell with copious amounts of toluene, to ensure that there is no carryover or residue.]■1S *(USP32)*
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of $[–(CH_3)_2SiO–]_n$ in the Emulsion:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Polydimethylsiloxane RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of simethicone in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–110.0%

IMPURITIES
Inorganic Impurities
• **HEAVY METALS** ⟨231⟩
Standard solution: To 20 mL of chloroform add 1.0 mL of a freshly prepared 0.002% solution of dithizone in chloroform, 0.5 mL of *Standard Lead Solution* (see *Heavy Metals* ⟨231⟩) (containing 10 µg of lead/mL), and 0.5 mL of a mixture of 1 volume of ammonia TS and 9 volumes of a 0.2% solution of hydroxylamine hydrochloride.
Sample solution: 1.0 g of simethicone from Emulsion in 10 mL of chloroform. Dilute with the same solvent to 20 mL. Add 1.0 mL of a freshly prepared 0.002% solution of dithizone in chloroform, 0.5 mL of water, and 0.5 mL of a mixture of 1 volume of ammonia TS and 9 volumes of a 0.2% solution of hydroxylamine hydrochloride.
Analysis: Immediately shake both solutions vigorously for 1 min.
Acceptance criteria: NMT 5 ppm. Any red color in the *Sample solution* is not more intense than that in the *Standard solution*.

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 100 cfu/g.
• **DEFOAMING ACTIVITY**
Foaming solution: 10 mg/mL of octoxynol 9 in water
Sample solution: Transfer a quantity of Emulsion, equivalent to 300 mg of simethicone, to a 60-mL bottle. Dilute with water to 30 g, cap the bottle, and shake vigorously.
Analysis: [NOTE—For each test, use a clean, unused, 250-mL glass jar.] Add, dropwise, 500 µL of the *Sample solution* to a clean, unused, cylindrical 250-mL glass jar, fitted with a 50-mm cap, containing 100 mL of the *Foaming solution*. Cap the jar, and clamp it in an upright position on a wrist-action shaker. Using a radius of 13.3 ± 0.4 cm (measured from the center of the shaft to the center of the bottle), shake for 10 s through an arc of 10° at a frequency of 300 ± 30 strokes/min. Record the time required for the foam to collapse. The time, in s, for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period.
Acceptance criteria: The defoaming activity time does not exceed 15 s.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Polydimethylsiloxane RS

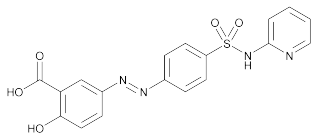
BRIEFING

Sulfasalazine, *USP 32* page 3632 and page 4100 of the *First Supplement;* **Sulfasalazine Tablets,** *USP 32* page 3632 and page 4100 of the *First Supplement*. As part of monograph modernization, it is proposed to replace dimethylformamide (DMF), a possible carcinogenic solvent used in *Identification* test *B*, with the solvent mixture used for the *Sample solution* in the *Assay*. It is also proposed to use more diluted *Standard* and *Sample solutions* in *Identification* test *B* to minimize potential saturation of the spectra.

(MD-AA: B. Davani.) RTS—C77265

In-Process Revision

Sulfasalazine



$C_{18}H_{14}N_4O_5S$ 398.39
Benzoic acid, 2-hydroxy-5-[[4-[(2-pyridinyl-amino)sulfonyl]phenyl]azo]-;
5-[[*p*-(2-Pyridylsulfamoyl)phenyl]azo]salicylic acid [599-79-1].

DEFINITION
Sulfasalazine contains NLT 97.0% and NMT 101.5% of $C_{18}H_{14}N_4O_5S$, calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨**197K**⟩

Change to read:

• **B. ■PROCEDURE**
Standard solution: ~~12 mg/mL USP Sulfasalazine RS in dimethylformamide~~▲Use the *Standard solution*, prepared as directed in the *Assay*.▲*USP34*
Sample solution: ~~12 mg/mL sulfasalazine in dimethylformamide~~▲Use the *Sample solution*, prepared as directed in the *Assay*.▲*USP34*
Acceptance criteria:■1S *(USP32)* The visible absorption spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Sample stock solution: 1.5 mg/mL of Sulfasalazine in 0.1 N sodium hydroxide
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 1000-mL volumetric flask containing 750 mL of water, mix, add 20.0 mL of 0.1 N acetic acid, and dilute with water to volume.
Standard solution: 7.5 µg/mL of USP Sulfasalazine RS in the same medium as the *Sample solution*
Spectrometric conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: UV-Vis
Analytical wavelength: Maximum at about 359 nm
Blank: Water
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Samples*. Calculate the percentage of $C_{18}H_{14}N_4O_5S$ in the portion of Sulfasalazine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (µg/mL)
C_U = concentration of Sulfasalazine in the *Sample solution* (µg/mL)
Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.5%
• **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩
Analysis: Digest 2.0 g of Sulfasalazine with 100 mL of water at 70° for 5 min. Cool immediately to room temperature, and filter. Transfer a 25-mL portion of the filtrate to a 50-mL beaker (retain the remainder of this filtrate for the *Sulfate* test). Add 1 mL of nitric acid, mix, and allow to stand for 5 min. Pass through a fine texture, retentive filter paper (Whatman No. 42 or equivalent).
Acceptance criteria: The filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:**
Analysis: Transfer a 25-mL portion of the filtrate from the *Chloride* test to a 50-mL beaker. Add 1 mL of 3 N hydrochloric acid, mix, and allow to stand for 5 min. Pass through a fine texture, retentive filter paper (Whatman No. 42 or equivalent).
Acceptance criteria: The filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
Organic Impurities
• **PROCEDURE**
Standard stock solution: 10 mg/mL of USP Sulfasalazine RS in a mixture of alcohol and 2 M ammonium hydroxide (4:1)
Standard solutions: Dilute aliquots of the *Standard stock solution* stepwise with the same medium to obtain solutions having concentrations of 200, 150, 100, and 20 µg/mL, corresponding to 2.0%, 1.5%, 1.0%, and 0.2%, respectively (*Standard solutions A, B, C,* and *D*).
Sample solution: 10 mg/mL of Sulfasalazine in a mixture of alcohol and 2 M ammonium hydroxide (4:1)
Chromatographic system
(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and formic acid (12:6:1)
Analysis
Samples: *Standard stock solution, Standard solutions,* and *Sample solution*
Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatogram in an unequilibrated chamber until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, dry with the aid of a current of hot air, and examine the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*. No spots, other than the principal spot, in the chromatogram of the *Sample solution* are larger or more intense than the principal spot of *Standard solution A* (2%), and the sum of the intensities of any secondary spots detected does not exceed 4%.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Sulfasalazine RS

BRIEFING

Sulfasalazine Tablets, *USP 32* page 3632 and page 4100 of the *First Supplement.* See briefing under *Sulfasalazine*.

(MD-AA: B. Davani.) RTS—C79016

In-Process Revision

Sulfasalazine Tablets

DEFINITION

Sulfasalazine Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of sulfasalazine ($C_{18}H_{14}N_4O_5S$).

IDENTIFICATION

Change to read:

• **■PROCEDURE**

Standard solution: ~~12 mg/mL USP Sulfasalazine RS in dimethylformamide~~

▲Use the *Standard solution*, prepared as directed in the *Assay*.▲*USP34*

Sample solution: ~~Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 600 mg of sulfasalazine, to a 50-mL beaker. Add 30 mL of dimethylformamide, break up any lumps with a glass rod, stir, and transfer the mixture to a 50-mL volumetric flask. Dilute with dimethylformamide to volume, mix, filter, and use the filtrate.~~

▲Use the *Sample solution*, prepared as directed in the *Assay*.▲*USP34*

Acceptance criteria:■1S *(USP32)* The visible absorption spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as prepared in the *Assay*.

ASSAY

• **PROCEDURE**

Sample stock solution: 1.5 mg/mL of sulfasalazine, from NLT 20 finely powdered Tablets in 0.1 N sodium hydroxide

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 1000-mL volumetric flask containing 750 mL of water, mix, add 20.0 mL of 0.1 N acetic acid, and dilute with water to volume.

Standard solution: 7.5 µg/mL of USP Sulfasalazine RS in the same medium

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: UV-Vis

Analytical wavelength: Maximum at about 359 nm

Blank: Water

Analysis

Samples: *Standard solution*,, *Sample solution*, and *Blank*

Concomitantly determine the absorbances of the *Samples*. Calculate the percentage of $C_{18}H_{14}N_4O_5S$ in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of sulfasalazine in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩**

Medium: pH 7.5 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Detector: UV 358 nm

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Standard solution: USP Sulfasalazine RS in *Medium*

Tolerances: NLT 85% (Q) of the labeled amount of $C_{18}H_{14}N_4O_5S$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS ⟨11⟩**

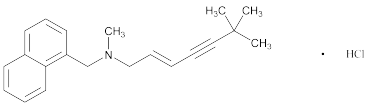
USP Sulfasalazine RS

BRIEFING

Terbinafine Hydrochloride, *USP* 32 Page 3677. On the basis of supporting validation data, it is proposed to replace the non-selective titration-based method in the *Assay* with a selective HPLC method. The liquid chromatographic procedures in the *Assay* are based on analyses performed with an Inertsil ODS-2 brand of L1 column. The typical retention time for terbinafine is 15.6 min. It is also proposed to revise the acceptance criteria in the *Assay* to reflect the change in the analytical technique and the revised limit for approved products. In the test for *Organic Impurities* it is proposed to use the USP Reference Standard for the *System suitability solution*. In addition, minor editorial changes consistent with USP monograph redesign format are proposed.

(MD-AA: H. Ramanathan, B. Davani.) RTS—C74084

Terbinafine Hydrochloride



$C_{21}H_{25}N \cdot HCl$ 327.90

1-Naphthalenemethanamine, *N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-, (*E*)-, hydrochloride;

(*E*)-*N*-(6,6-Dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalenemethylamine, hydrochloride;

(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride [78628-80-5].

DEFINITION

Change to read:

Terbinafine Hydrochloride contains ~~NLT 99.0% and NMT 101.0%~~ ▲NLT 98.0% and NMT 102.0%▲*USP34* of $C_{21}H_{25}N \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION ⟨197K⟩**

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements of the test when using dehydrated alcohol as a solvent

ASSAY

Change to read:

• **PROCEDURE**

~~**Sample solution:** Dissolve 250 mg of Terbinafine Hydrochloride in 50 mL of alcohol.~~

~~**Analysis:** To the *Sample solution* add 5 mL of 0.01 N hydrochloric acid VS, and titrate with 0.1 N sodium hydroxide VS. Read the volume added between the two points of inflexion. 1 mL of 0.1 N sodium hydroxide is equivalent to 32.79 mg of $C_{21}H_{25}N \cdot HCl$.~~

In-Process Revision

~~**Acceptance criteria:** 99.0%–101.0% on the dried basis~~

▲[NOTE—Protect all solutions containing Terbinafine Hydrochloride from light.]

Buffer, Solution A, Solution B, Solution C, Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the test for *Organic Impurities*.

Standard solution: 0.5 mg/mL of USP Terbinafine Hydrochloride RS in *Diluent*

System suitability solution: 1 mg/mL of USP Terbinafine Hydrochloride RS in *Diluent*. Expose to UV light at 254 nm for 1 h.

Sample solution: 0.5 mg/mL of terbinafine hydrochloride in *Diluent*

System suitability

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

[NOTE—The relative retention times for *cis*-terbinafine and terbinafine are 0.94 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between *cis*-terbinafine and terbinafine, *System suitability solution*

Tailing factor: NLT 0.8 and NMT 1.5 for terbinafine, *Standard solution*

Relative standard deviation: NMT 2.0% for terbinafine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{25}N \cdot HCl$. in the portion of Terbinafine Hydrochloride taken:

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

r_U = peak response of terbinafine from the *Sample solution*

r_S = peak response of terbinafine from the *Standard solution*

C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of terbinafine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲*USP34*

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%

Change to read:

Organic Impurities

• **PROCEDURE**

[NOTE—Protect all solutions containing Terbinafine Hydrochloride from light.]

Buffer: Prepare a solution in water containing 2.0 mL of triethylamine/L. Adjust with diluted acetic acid to a pH of 7.5.

Solution A: *Solution C* and *Buffer* (7:3)

Solution B: *Solution C* and *Buffer* (95:5)

Solution C: Methanol and acetonitrile (3:2)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
25	0	100
30	0	100
30.1	100	0
38	100	0

Diluent: Acetonitrile and water (1:1)

Standard solution: 0.5 µg/mL of USP Terbinafine Hydrochloride RS in *Diluent*

Sample solution: 0.5 mg/mL of Terbinafine Hydrochloride in *Diluent*

System suitability solution: 1 mg/mL of ~~terbinafine hydrochloride~~▲USP Terbinafine Hydrochloride RS▲*USP34* in *Diluent*. Expose to UV light at 254 nm for 1 h.

Sensitivity solution: 0.25 µg/mL of terbinafine hydrochloride in *Diluent* from the *Standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection size: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between *cis*-terbinafine and terbinafine, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Calculate the signal-to-noise ratio:

$$\text{Result} = (2H)/h$$

H = measured height of the terbinafine peak

h = amplitude of the average measured baseline noise

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the peaks based on their relative retention times as given in *Impurity Table 1*.

Calculate the percentage of each impurity in the portion of Terbinafine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of terbinafine from the *Standard solution*

C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Terbinafine Hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Impurity Table 1*)

[NOTE—Disregard any peak observed in the blank, and any peak less than 0.05%.]

▲**Acceptance criteria**

Individual impurities: NMT 0.3%

Total impurities: See *Impurity Table 1*.▲*USP34*

In-Process Revision

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Methyl-*C*-(naphthalen-1-yl)methanamine	0.1	1.7	0.1
trans-Isoterbinafine[a]	0.92	1.0	0.1
cis-Terbinafine[b]	0.94	1.0	0.1
Terbinafine	1.0	—	—
4-Methylterbinafine[c]	1.1	1.0	0.1
Terbinafine dimer[d]	1.7	2.5	0.05
Any other individual impurity	—	1.0	0.1
~~Total impurities~~	~~n/a~~	~~n/a~~	~~0.3~~ ▲USP34

[a] (2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine.
[b] (2*Z*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine.
[c] (2*E*)-*N*,6,6-Trimethyl-*N*-[(4-methylnaphthalen-1-yl)methyl]hept-2-en-4-yn-1-amine.
[d] (2*E*,4*E*)-4-(4,4-Dimethylpent-2-ynylidene)-N^1,N^5-dimethyl-N^1,N^5-bis(naphthalen-1-ylmethyl)pent-2-ene-1,5-diamine.

SPECIFIC TESTS

Delete the following:

•• **MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** 204°–208° •(RB 1-Apr-2009)

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Terbinafine Hydrochloride RS

BRIEFING

Venlafaxine Tablets. Because there is no existing *USP* monograph for this drug product, a new monograph, based on validated methods of analyses, is being proposed. The liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with an Inertsil C8-3, brand of L7 column. The typical retention times for venlafaxine related compound A and venlafaxine are 14 and 15 min respectively. The liquid chromatographic procedure for the *Assay* is based on analyses performed with an ACE C18, brand of L1 column. The typical retention time for the venlafaxine peak is 5 min.

(MD-PP: H. Ramanathan, R. Ravichandran. BPC: M. Marques.)
RTS—C55365

Add the following:

▲Venlafaxine Tablets

DEFINITION

Venlafaxine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
Diluent: Methanol and 0.1 N hydrochloric acid (4:1)
Sample solution: Dissolve a quantity of finely powdered Tablets, equivalent to 75 mg of venlafaxine, with 200 mL of *Diluent*, then sonicate for 30 min. Dilute with *Diluent* to 250 mL. Centrifuge a portion at 4000 rpm for 10 min. Dilute a portion of the filtrate with *Diluent* to make an approximate 1-in-2.5 solution. Pass a portion through a suitable 0.45-µm membrane filter.
Wavelength range: 250–350 nm

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY

• **PROCEDURE**
Buffer: 3.4 g of potassium dihydrogen phosphate in 700 mL of water. Add 5 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.
Diluent: Methanol and 0.1 N hydrochloric acid (4:1)
Mobile phase: Acetonitrile and *Buffer* (3:7)
Standard solution: 0.34 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Sample solution: 0.3 mg/mL of venlafaxine in *Diluent* (from NLT 20 finely powdered Tablets). [NOTE—Sonicate for 30 min.] Centrifuge a portion at 4000 rpm for 10 min. Pass a portion of the supernatant through a 0.45-µm membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 10 µL
Run time: 5 times the retention time of the venlafaxine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of label claim of $C_{17}H_{27}NO_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨**711**⟩
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.95 mg/mL of USP Venlafaxine Hydrochloride RS in methanol
Standard solution: 28.5 µg/mL of USP Venlafaxine Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Detector: UV 275 nm
Path length: 1 cm
Blank: *Medium*
Calculate the percentage of venlafaxine dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

In-Process Revision

A_U = absorbance obtained from the *Sample solution*
A_S = absorbance obtained from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg)
V = 900 mL
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Tolerances: NLT 80% (Q) of the labeled amount of venlafaxine

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE**

Buffer: Proceed as directed in the *Assay*.
Solution A: Acetonitrile and *Buffer* (1:4)
Solution B: Acetonitrile and *Buffer* (7:13)
Diluent: Acetonitrile and *Buffer* (3:7)
Standard stock solution: 0.6 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Standard solution: 1.8 µg/mL of USP Venlafaxine Hydrochloride RS in *Diluent* from the *Standard stock solution*
System suitability solution: 1.8 µg/mL of USP Venlafaxine Related Compound A RS in the *Standard stock solution*
Sample solution: Equivalent to 0.6 mg/mL of venlafaxine (from NLT 20 finely powdered Tablets) in the *Diluent*. [NOTE—Sonicate, if necessary.] Centrifuge a portion at 4000 rpm for 10 min. Pass a portion through a suitable 0.45-µm membrane filter.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
25	0	100
30	0	100
32	100	0
45	100	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between venlafaxine related compound A and the venlafaxine peaks, *System suitability solution*
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response for the individual impurity obtained from the *Sample solution*
r_S = peak response of venlafaxine obtained from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Descyclohexanol venlafaxine[a]	0.4	0.6	0.20
Venlafaxine related compound A[b]	0.9	—	—
Venlafaxine	1.0	—	—
Any unspecified degradation product	—	1.0	0.20

[a] 2-(4-Methoxyphenyl)-*N,N*-dimethylethylamine.
[b] 1-(1-(4-Methoxyphenyl)-2-(methylamino)ethyl)cyclohexanol.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**
USP Venlafaxine Hydrochloride RS
USP Venlafaxine Related Compound A RS
[1-(1-(4-methoxyphenyl)-2-(methylamino)ethyl)cyclohexanol] ($C_{16}H_{25}NO_2$ 263.38)▲*USP34*

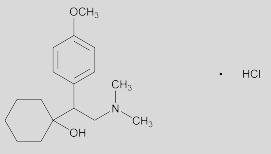
BRIEFING

Venlafaxine Hydrochloride. Because there is no existing *USP* monograph for this drug substance, a new monograph, based on validated methods of analyses is being proposed. The proposed liquid chromatographic procedure in the test for *Organic Impurities* is based on analyses performed with an Inertsil C8, brand of L7 column. The typical retention time for venlafaxine and venlafaxine related compound A is 7.6 and 8.2 min, respectively. The liquid chromatographic procedure for the *Assay* is based on analyses performed with an Kromasil 100 C8, brand of L7 column. The typical retention time under these conditions for venlafaxine is 5.3 min.

(MD-PP: H. Ramanathan, R. Ravichandran.) RTS—C67356

Add the following:

▲Venlafaxine Hydrochloride



$C_{17}H_{27}NO_2 \cdot HCl$ 313.86
Cyclohexanol, 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]-, hydrochloride;
(±)-1-[α-[(Dimethylamino)methyl]-*p*-methoxybenzyl]cyclohexanol hydrochloride [99300-78-4].

In-Process Revision

DEFINITION

Venlafaxine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{17}H_{27}NO_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B.** The retention time of the major peak on the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Phosphoric acid and water (1:10)
Buffer: 3.4 g of monobasic potassium phosphate in 700 mL of water. Add 5 mL of triethylamine, and adjust with *Solution A* to a pH of 3.0.
Diluent: Acetonitrile and water (1:1)
Mobile phase: Acetonitrile and *Buffer* (3:7)
Standard solution: 0.04 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*.
Sample solution: 0.04 mg/mL of Venlafaxine Hydrochloride in *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Temperature 30 ± 2°
Injection size: 20 µL
Run time: 2 times the retention time of venlafaxine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{27}NO_2 \cdot HCl$ in the portion of Venlafaxine Hydrochloride taken:

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

r_U = peak response of venlafaxine from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Venlafaxine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 0.5 mg/mL of USP Venlafaxine Hydrochloride RS and 1.5 µg/mL of USP Venlafaxine Related Compound A RS, in *Mobile phase*
Standard solution: 1 µg/mL of USP Venlafaxine Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Venlafaxine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperature: 30 ± 2°
Flow rate: 0.7 mL/min
Injection size: 10 µL
Run time: 7 times the retention time of venlafaxine peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between venlafaxine and venlafaxine related compound A
Relative standard deviation: NMT 2.0% for the venlafaxine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Venlafaxine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of individual impurity from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Venlafaxine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity peak, from *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Descyclohexanol venlafaxine[a]	0.6	0.8	0.15
Didesmethyl venlafaxine[b]	0.8	1.0	0.15
Venlafaxine related compound A[c]	0.9	1.0	0.15
Venlafaxine	1.0	—	—
Deoxy venlafaxine[d]	3.1	0.75	0.15
Any unknown individual impurity	—	1.0	0.10

[a] 2-(4-Methoxyphenyl)-*N,N*-dimethylethylamine.
[b] 1-[2-Amino-1-(4-methoxyphenyl)ethyl]cyclohexanol.
[c] 1-(1-(4-Methoxyphenyl)-2-(methylamino)ethyl)cyclohexanol.
[d] 2-Cyclohexyl-2-(4-methoxyphenyl)-*N,N*-dimethylethylamine.

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry a sample in vacuum at 105° for 3 h: it loses NMT 0.5% of its weight

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Venlafaxine Hydrochloride RS
USP Venlafaxine Related Compound A RS
[1-(1-(4-methoxyphenyl)-2-(methylamino)ethyl)cyclohexanol] ($C_{16}H_{25}NO_2$ 263.38)▲*USP34*

In-Process Revision

BRIEFING

Water for Hemodialysis, *USP 32* page 3872. The AAMI guidelines for the quality of water used in hemodialysis operations have been commented by FDA for having high microbial and endotoxin specifications (200 cfu/mL and 2 EU/mL, respectively) that may not adequately protect hemodialysis patients. FDA has suggested the levels of 100 cfu/mL and 1 EU/mL as more appropriate. Though the USP monograph for *Water for Hemodialysis* has always included the tighter 100 cfu/mL microbial specification, it has an AAMI-consistent endotoxin specification. This proposed monograph revision decreases the endotoxin specification to be consistent with FDA opinions, thereby offering safer protection for hemodialysis patients. Because this water could be subject to microbial contamination from objectionable microorganisms that could endanger the health of acutely ill hemodialysis patients, a requirement for the absence of the opportunistic pathogen *Pseudomonas aeruginosa* is an appropriate requirement for this water. The deletion of the *Oxidizable Substances* test, as an alternative to *Total Organic Carbon* ⟨643⟩ testing, is also proposed, consistent with other bulk waters.

(PW: A. Hernandez-Cardoso.) RTS—C79382

Water for Hemodialysis

DEFINITION

Water for Hemodialysis is water that complies with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations and that has been subjected to further treatment, using a suitable process, to reduce chemical and microbiological components. It is produced and used onsite under the direction of qualified personnel. It contains no added antimicrobials and is not intended for injection.

SPECIFIC TESTS

Change to read:

[NOTE—See *Water for ~~Health~~▲Hemodialysis▲USP34 Applications* ⟨1230⟩ for guidelines on microbial and chemical testing.]

[NOTE—~~Perform the test for *Oxidizable Substances* or *Total Organic Carbon*.~~]▲▲USP34

Delete the following:

▲~~• **OXIDIZABLE SUBSTANCES**~~
~~**Sample:** 100 mL~~
~~**Analysis:** Add 10 mL of 2 N sulfuric acid, and heat to boiling. Add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min.~~
~~**Acceptance criteria:** The pink color does not completely disappear.~~▲USP34

- **TOTAL ORGANIC CARBON ⟨643⟩:** Meets the requirements

Change to read:

- **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** ~~The total viable count does not exceed 100 cfu/mL~~▲The total aerobic microbial count does not exceed 100 cfu/mL. It meets the requirements of the test for absence of *Pseudomonas aeruginosa*.▲USP34
- **WATER CONDUCTIVITY,** *Bulk Water* **⟨645⟩:** Meets the requirements

Change to read:

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains less than ~~2~~ ▲1▲USP34 USP Endotoxin Unit/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in unreactive storage containers that are designed to prevent bacterial entry. Store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Endotoxin RS
  USP 1,4-Benzoquinone RS
  USP Sucrose RS

DIETARY SUPPLEMENTS—MONOGRAPHS

BRIEFING

Calcium with Vitamin D Tablets, *USP 32* page 968. In order to clarify an interpretation of acceptance criteria for dissolution testing, it is proposed to eliminate the symbol Q, as the amount of dissolved active ingredient expressed as a percentage of the labeled content of the dosage unit, from the *Tolerances* section. The Q value is applicable for interpretation of the dissolution tests, which are performed as directed under *Dissolution* ⟨711⟩.

(DS-PS: Natalia Davydova.) RTS—C78870

Calcium with Vitamin D Tablets

DEFINITION

Calcium with Vitamin D Tablets contain NLT 90.0% and NMT 125.0% of the labeled amount of calcium (Ca), derived from substances generally recognized as safe, and NLT 90.0% and NMT 165.0% of the labeled amount of Vitamin D, as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$). They contain no other vitamins or minerals for which nutritional value is claimed. They may contain other labeled added substances or additional ingredients in amounts that are unobjectionable.

STRENGTH

- **CALCIUM**

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a *Calcium standard stock solution* is described in the following assay. Concentrations of the *Standard solutions* and the *Sample stock solution* may be modified to fit the linear or working range of the instrument.]

Solution A: Dissolve 26.7 g of lanthanum chloride in 0.125 N hydrochloric acid to make 100 mL.

Calcium standard stock solution: Weigh 1.001 g of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, and dissolve in 25 mL of 1 N hydrochloric acid. Boil to expel carbon dioxide, and dilute with water to 1000 mL to obtain a solution having a concentration of 400 µg/mL of calcium.

Standard stock solution: 100 µg/mL of calcium from a volume of *Calcium standard stock solution* in 0.125 N hydrochloric acid

Standard solutions: Into separate 100-mL volumetric flasks, separately pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the *Standard stock solution*. To each flask add 1.0 mL of *Solution A*, dilute with water to volume, and obtain *Standard solutions* having concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 µg/mL, of calcium.

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer the equivalent to 500 mg of calcium, in 25 mL of concentrated hydrochloric acid, and heat for 30 min on a steam bath. Cool, dilute with water to 1000 mL, and filter.

Sample solution: Quantitatively dilute a volume of *Sample stock solution* with 0.125 N hydrochloric acid to obtain a concentration of 100 µg/mL of calcium. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 1.0 mL of *Solution A*, and dilute with water to volume.

Spectrometric conditions

(See *Spectrophotometry and Light-scattering* ⟨851⟩.)

Mode: Atomic absorption spectrophotometer
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Analytical wavelength: Calcium emission line, 422.7 nm
Blank: 0.125 N hydrochloric acid containing 0.1% *Solution A*

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of both the solutions, using the blank. Plot the absorbances of the *Standard solution*s versus concentration, in µg/mL, of calcium, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg/mL, of calcium in the *Sample solution*.

Calcium in the portion of Tablets taken:

$$\text{Result} = C \times V \times D \times F_1 \times (100/L)$$

C = concentration of calcium in the *Sample solution* (µg/mL)
V = volume of the *Sample solution*, 1000 mL
D = dilution factor involved in the *Sample solution*
F_1 = conversion factor, 0.001 mg/µg
L = label claim (mg)

Acceptance criteria: 90.0%–125.0%

- **CHOLECALCIFEROL OR ERGOCALCIFEROL (VITAMIN D)**

[NOTE—Use low-actinic glassware throughout this procedure.]

Mobile phase: *n*-Hexane and isopropyl alcohol (99:1)

Standard solution: 2 µg/mL of USP Ergocalciferol RS or USP Cholecalciferol RS in *n*-hexane

System suitability solution: Heat a volume of *Standard solution* at 60° for 1 h to partially isomerize vitamin D (ergocalciferol or cholecalciferol) to its corresponding precursor.

Sample solution: Weigh, and grind NLT 20 Tablets. Transfer the equivalent to 20 µg of cholecalciferol or ergocalciferol to a container having a polytef-lined screw cap. Add 8 mL of dimethyl sulfoxide and 12 mL of *n*-hexane, and shake for 45 min on a wrist-action shaker with tubes in a water bath maintained at 60°. Centrifuge for 10 min, withdraw the hexane layer by means of a pipet, and transfer to an evaporation flask. Add 12 mL of *n*-hexane to the dimethyl sulfoxide layer, mix on a vortex mixer for 5 min, and again withdraw the hexane layer by means of a pipet, and add to the evaporation flask. Repeat this extraction with three additional 12-mL portions of *n*-hexane, adding the hexane extracts to the evaporation flask. Evaporate the combined hexane extracts in vacuum at room temperature to dryness. Dissolve in and dilute the residue in a volume of *n*-hexane to obtain a concentration of 2 µg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection size: 100 µL

System suitability

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

Suitability requirements

Resolution: NLT 10 between the vitamin D form present and its corresponding precursor, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Measure the responses for the vitamin D peaks. Calculate the percentage of $C_{27}H_{44}O$ or $C_{28}H_{44}O$ in the Tablets taken:

$$\text{Result} = 1.09 \times (r_U/r_S) \times (C_S/C_U) \times (100)$$

1.09 = correction factor to account for the average amount of previtamin D present in the formulation
r_U = peak heights for cholecalciferol or ergocalciferol from the *Sample solution*

In-Process Revision

r_S = peak heights for cholecalciferol or ergocalciferol from the *Standard solution*
C_S = concentration of USP Ergocalciferol RS or USP Cholecalciferol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ergocalciferol or cholecalciferol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–165.0%

PERFORMANCE TESTS

Change to read:

• **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS** ⟨**2040**⟩**:** Meet the requirements for *Dissolution* with respect to calcium

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Analysis: Determine the amount of calcium (Ca) dissolved, using the procedure in the assay for *Calcium*, making any necessary volumetric adjustments.
Tolerances: NLT 75% ~~(Q)~~▲*USP34* of the labeled amount of Ca is dissolved.

• **WEIGHT VARIATION OF DIETARY SUPPLEMENTS** ⟨**2091**⟩**:** Meet the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS—NUTRITIONAL DIETARY SUPPLEMENTS** ⟨**2021**⟩**:** The total aerobic microbial count does not exceed 3000 cfu/g, and the total combined molds and yeasts count does not exceed 300 cfu/g. Tablets also meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*, and for absence of *Staphylococcus aureus*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** The label states that the product is *Calcium with Vitamin D Tablets*. The label states also the quantities of calcium and Vitamin D in terms of metric units/Tablet, and the salt form of calcium and the chemical form of Vitamin D present in the Tablet.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Cholecalciferol RS
USP Ergocalciferol RS

BRIEFING

Excipients, USP and NF Excipients, Listed by Category, *NF 27* page 1143, page 4022 of the *First Supplement*, and page 1197 of *PF* 35(5) [Sept.–Oct. 2009]. It is proposed to add *Butyl Stearate* to the *Emollient* category to complement the proposed new monograph for *Butyl Stearate*, which appears elsewhere in this issue of *PF*.

(EM1; EM2) RTS—C70759

Change to read:

Antimicrobial Preservative

Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzethonium Chloride
Benzoic Acid
Benzyl Alcohol
Butylparaben
▲Calcium Propionate▲*NF28*
Cetrimonium Bromide
Cetylpyridinium Chloride
Chlorobutanol
Chlorocresol
Cresol
Dehydroacetic Acid
▲Erythorbic Acid▲*NF27*
Ethylparaben
Methylparaben
Methylparaben Sodium
Phenol
Phenoxyethanol
Phenylethyl Alcohol
Phenylmercuric Acetate
Phenylmercuric Nitrate
Potassium Benzoate
Potassium Sorbate
Propylparaben
Propylparaben Sodium
Sodium Benzoate
Sodium Dehydroacetate
Sodium Propionate
Sorbic Acid
Thimerosal
Thymol

Change to read:

Antioxidant

Ascorbic Acid
Ascorbyl Palmitate
Butylated Hydroxyanisole
Butylated Hydroxytoluene
Stannous Chloride
▲Erythorbic Acid▲*NF27*
Hypophosphorous Acid
■Lactobionic Acid■2S (*NF28*)
Monothioglycerol
Potassium Metabisulfite
Propyl Gallate
Sodium Bisulfite
Sodium Formaldehyde Sulfoxylate
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Sulfur Dioxide
Tocopherol
Tocopherols Excipient

Change to read:

Buffering Agent

Acetic Acid
Adipic Acid
Ammonium Carbonate
Ammonium Phosphate
Boric Acid
Citric Acid, Anhydrous
Citric Acid Monohydrate
■Alpha-Lactalbumin■1S (*NF28*)
Lactic Acid
Phosphoric Acid
Potassium Citrate
Potassium Metaphosphate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Citrate
Sodium Lactate Solution
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Succinic Acid

Change to read:

Bulking Agent for Freeze-Drying

Creatinine
■Alpha-Lactalbumin■1S (*NF28*)
Mannitol
Polydextrose
■Hydrogenated Polydextrose■2S (*NF28*)
Pullulan
■Trehalose■2S (*NF27*)

Change to read:

Coating Agent

Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carboxymethylcellulose, Sodium
▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*
Cellaburate
Cellacefate (formerly Cellulose Acetate Phthalate)
Cellulose Acetate

In-Process Revision

Cellulose Acetate Phthalate (see Cellacefate)

■Chitosan■1S *(NF28)*

Coconut Oil
■Hydrogenated Coconut Oil■1S *(NF27)*
Copovidone
Corn Syrup Solids
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose
Ethylcellulose Aqueous Dispersion

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S *(NF28)*

Gelatin
Glaze, Pharmaceutical
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hydroxypropyl Methylcellulose Phthalate (see Hypromellose Phthalate)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate
Hypromellose Phthalate (formerly Hydroxypropyl Methylcellulose Phthalate)

■Alpha-Lactalbumin■1S *(NF28)*

Maltodextrin

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S *(NF28)*

■Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer■2S *(NF28)*

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S *(NF28)*

Methacrylic Acid Copolymer
Methacrylic Acid Copolymer Dispersion
Methylcellulose
Palm Kernel Oil

■Palm Oil■2S *(NF27)*

■Hydrogenated Palm Oil■1S *(NF27)*

■Hydrogenated Polydextrose■2S *(NF28)*

Polyethylene Glycol

▲Polyvinyl Acetate▲*NF28*

▲Polyvinyl Acetate Dispersion▲*NF28*

Polyvinyl Acetate Phthalate
Pullulan
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Shellac
Starch, Pregelatinized Modified
Sucrose
Titanium Dioxide
Wax, Carnauba
Wax, Microcrystalline
Zein

Change to read:

Complexing Agent

Edetate Calcium Disodium
Edetate Disodium
Edetic Acid

■Alpha-Lactalbumin■1S *(NF28)*

Oxyquinoline Sulfate

Change to read:

Desiccant

Calcium Chloride
Calcium Sulfate

▲Polyvinyl Acetate▲*NF28*

Silicon Dioxide

Change to read:

Emollient

Alkyl (C12-15) Benzoate

▲Butyl Stearate▲*NF29*

Hydrogenated Soybean Oil
Hydrogenated Polydecene
Oleyl Oleate

Change to read:

Emulsifying and/or Solubilizing Agent

Acacia
Carbomer Copolymer
Carbomer Interpolymer
Cholesterol
Stannous Chloride
Coconut Oil

▲Desoxycholic Acid▲*NF28*

Diethanolamine (Adjunct)
Diethylene Glycol Stearates
Ethylene Glycol Stearates
Gamma Cyclodextrin
Glyceryl Distearate
Glyceryl Monolinoleate
Glyceryl Monooleate
Glyceryl Monostearate

■Alpha-Lactalbumin■1S *(NF28)*

Lanolin Alcohols
Lecithin
Mono- and Di-glycerides
Monoethanolamine (Adjunct)
Oleic Acid (Adjunct)
Oleyl Alcohol (Stabilizer)
Oleyl Oleate
Palm Kernel Oil

■Palm Oil■2S (*NF27*)

Poloxamer
Polyoxyethylene 50 Stearate
Polyoxyl 10 Oleyl Ether
Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polyoxyl Lauryl Ether
Polyoxyl Stearyl Ether
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Propylene Glycol Monostearate
Superglycerinated Fully Hydrogenated Rapeseed Oil
Sodium Cetostearyl Sulfate
Sodium Lauryl Sulfate
Sodium Stearate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Stearic Acid

■Sucrose Stearate■1S (*NF28*)

Trolamine
Wax, Emulsifying

Change to read:

Film-Forming Agent

■Chitosan■1S (*NF28*)

■Methacrylic Acid and Ethyl Acrylate Copolymer■2S (*NF28*)

■Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer■2S (*NF28*)

■Methacrylic Acid and Methyl Methacrylate Copolymer■2S (*NF28*)

Change to read:

Flavors and Perfumes

Almond Oil
Anethole
Benzaldehyde

■Diethyl Sebacate■2S (*NF28*)

Ethyl Acetate
Ethyl Vanillin

■L-Glutamic Acid, Hydrochloride■2S (*NF28*)

Lactitol
Maltol
Menthol
Methyl Salicylate
Monosodium Glutamate
Peppermint
Peppermint Oil
Peppermint Spirit
Rose Oil
Rose Water, Stronger
Thymol
Vanillin

Change to read:

Humectant

Corn Syrup Solids
Erythritol
Glycerin
Hexylene Glycol
Inositol
Maltitol
Polydextrose

■Hydrogenated Polydextrose■2S (*NF28*)

Propylene Glycol
Sorbitol
Sorbitol Sorbitan Solution

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

Tagatose

Change to read:

Solvent

Acetone
Alcohol
Alcohol, Diluted
Amylene Hydrate
Benzyl Benzoate
Butyl Alcohol
Canola Oil
Caprylocaproyl Polyoxylglycerides
Corn Oil
Cottonseed Oil
Diethylene Glycol Monoethyl Ether
Ethyl Acetate
Glycerin
Hexylene Glycol
Hydrogenated Polydecene
Isopropyl Alcohol

Lauroyl Polyoxylglycerides
Linoleoyl Polyoxylglycerides
Methyl Alcohol
Methylene Chloride
Methyl Isobutyl Ketone

■Methylpyrrolidone■2S (*NF28*)

Mineral Oil
Oleoyl Polyoxylglycerides
Peanut Oil
Polyethylene Glycol
Polyethylene Glycol Monomethyl Ether
Propylene Glycol
Sesame Oil
Stearoyl Polyoxylglycerides
Water for Injection
Water for Injection, Sterile
Water for Irrigation, Sterile
Water, Purified

Change to read:

Stiffening Agent

Castor Oil, Hydrogenated
Cetostearyl Alcohol
Cetyl Alcohol
Cetyl Esters Wax
Cetyl Palmitate
Hard Fat

■Alpha-Lactalbumin■1S (*NF28*)

Paraffin
Synthetic Paraffin
Fully Hydrogenated Rapeseed Oil
Superglycerinated Fully Hydrogenated Rapeseed Oil
Stearyl Alcohol
Wax, Emulsifying
Wax, White
Wax, Yellow

Change to read:

Suspending and/or Viscosity-Increasing Agent

Acacia
Agar
Alamic Acid
Alginic Acid
Aluminum Monostearate
Attapulgite, Activated
Attapulgite, Colloidal Activated
Bentonite
Bentonite, Purified
Bentonite Magma
Carbomer 910
Carbomer 934
Carbomer 934P
Carbomer 940
Carbomer 941
Carbomer 1342
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Calcium
Carboxymethylcellulose Sodium
Carboxymethylcellulose Sodium 12

▲Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium▲*NF28*

▲Carmellose▲*NF28*

Carrageenan
Cellulose, Microcrystalline, and Carboxymethylcellulose Sodium

■Chitosan■1S (*NF28*)

▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Gelatin
Gellan Gum
Guar Gum
Hydroxyethyl Cellulose
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)

■Alpha-Lactalbumin■1S (*NF28*)

Magnesium Aluminum Silicate
Maltodextrin
Methylcellulose
Pectin

■Hydrogenated Polydextrose■2S (*NF28*)

Polyethylene Oxide
Polyvinyl Alcohol
Povidone
Propylene Glycol Alginate
Pullulan
Hydrophobic Colloidal Silica
Silicon Dioxide
Silicon Dioxide, Colloidal
Sodium Alginate
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Tapioca
Starch, Wheat

■Sucrose Palmitate■1S (*NF28*)

Tragacanth
Xanthan Gum

Change to read:

Sweetening Agent

Acesulfame Potassium
Aspartame
Aspartame Acesulfame
▲Corn Syrup▲*NF27*
Corn Syrup Solids
High Fructose Corn Syrup
Dextrates
Dextrose
Dextrose Excipient
Erythritol

Fructose
Galactose
Maltitol
Maltose
Mannitol
Saccharin
Saccharin Calcium
Saccharin Sodium
Sorbitol
Sorbitol Solution

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

Sucralose
Sucrose
Sugar, Compressible
Sugar, Confectioner's
Syrup
Tagatose

■Trehalose■2S (*NF27*)

Change to read:

Tablet Binder

Acacia
Alginic Acid
Amino Methacrylate Copolymer
Ammonio Methacrylate Copolymer
Ammonio Methacrylate Copolymer Dispersion
Carbomer Copolymer
Carbomer Homopolymer
Carbomer Interpolymer
Carboxymethylcellulose Sodium
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

■Hydrogenated Coconut Oil■1S (*NF27*)
Copovidone
▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrin
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion
Ethylcellulose

■Ethylene Glycol and Vinyl Alcohol Graft Copolymer■1S (*NF28*)

Gelatin
Glucose, Liquid
Guar Gum
Low-Substituted Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose (see Hypromellose)
Hypromellose (formerly Hydroxypropyl Methylcellulose)
Hypromellose Acetate Succinate

■Alpha-Lactalbumin■1S (*NF28*)

Maltodextrin
Maltose
Methylcellulose
■Hydrogenated Palm Oil■1S (*NF27*)

■Hydrogenated Polydextrose■2S (*NF28*)

Polyethylene Oxide

▲Polyvinyl Acetate▲*NF28*

Povidone
Pullulan
Starch, Corn

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat
Syrup

■Trehalose■2S (*NF27*)

Change to read:

Tablet and/or Capsule Diluent

Calcium Carbonate
Calcium Phosphate, Dibasic
Calcium Phosphate, Tribasic
Calcium Sulfate
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

Cellulose, Powdered
▲Corn Syrup▲*NF27*
Corn Syrup Solids
Dextrates
Dextrin
Dextrose Excipient
Fructose
Kaolin

■Alpha-Lactalbumin■1S (*NF28*)

Lactitol
Lactose, Anhydrous
Lactose, Monohydrate
Maltitol
Maltodextrin
Maltose
Mannitol
Propylene Glycol Monocaprylate
Pullulan
Sorbitol
Starch
Starch, Corn

■Hydrogenated Starch Hydrolysate■1S (*NF28*)

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified

In-Process Revision

Starch, Tapioca
Starch, Wheat
Sucrose
Sugar, Compressible
Sugar, Confectioner's

■Trehalose■2S (*NF27*)

Change to read:

Tablet Disintegrant

Alginic Acid
Cellulose, Microcrystalline

■Silicified Microcrystalline Cellulose■2S (*NF27*)

Croscarmellose Sodium
Crospovidone
Low-Substituted Hydroxypropyl Cellulose
Maltose
Polacrilin Potassium
Pullulan
Sodium Starch Glycolate
Starch
Starch, Corn

▲Starch, Pea▲*NF28*

Starch, Potato
Starch, Pregelatinized
Starch, Pregelatinized Modified
Starch, Tapioca
Starch, Wheat

■Trehalose■2S (*NF27*)

Change to read:

Tablet and/or Capsule Lubricant

■Behenoyl Polyoxylglycerides■2S (*NF27*)

Calcium Stearate
■Hydrogenated Coconut Oil■1S (*NF27*)
Glyceryl Behenate
Magnesium Stearate
Mineral Oil, Light
■Hydrogenated Palm Oil■1S (*NF27*)
Polyethylene Glycol
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Sodium Lauryl Sulfate
Sodium Stearyl Fumarate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Starch
Stearic Acid
Stearic Acid, Purified

■Sucrose Stearate■1S (*NF28*)

Talc
Vegetable Oil, Hydrogenated, Type I
Zinc Stearate

Change to read:

Vehicle

FLAVORED AND/OR SWEETENED

Aromatic Elixir
Benzaldehyde Elixir, Compound
Corn Syrup Solids
Dextrose

■Ethyl Maltol■2S (*NF27*)

Peppermint Water
Sorbitol Solution
Syrup

■Trehalose■2S (*NF27*)

OLEAGINOUS

Alkyl (C12-15) Benzoate
Almond Oil
Canola Oil
Corn Oil
Cottonseed Oil
Ethyl Oleate
Hydrogenated Polydecene
Isopropyl Myristate
Isopropyl Palmitate
Mineral Oil
Mineral Oil, Light
Octyldodecanol
Olive Oil
Peanut Oil

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Safflower Oil
Sesame Oil
Soybean Oil
Squalane

SOLID CARRIER

■Chitosan■1S (*NF28*)

Corn Syrup Solids

■Alpha-Lactalbumin■1S (*NF28*)

Propylene Glycol Dicaprylate/Dicaprate
Propylene Glycol Monocaprylate
Sugar Spheres

STERILE

▲rAlbumin Human▲*NF27*
Sodium Chloride Injection, Bacteriostatic
Water for Injection, Bacteriostatic

Change to read:

Wetting and/or Solubilizing Agent

Benzalkonium Chloride
Benzethonium Chloride
Cetylpyridinium Chloride
Docusate Sodium
Nonoxynol 9
Octoxynol 9
Poloxamer
Polyoxyl 10 Oleyl Ether

▲Polyoxyl 15 Hydroxystearate▲*NF28*

Polyoxyl 20 Cetostearyl Ether
Polyoxyl 35 Castor Oil
Polyoxyl 40 Hydrogenated Castor Oil
Polyoxyl 40 Stearate
Polysorbate 20
Polysorbate 40
Polysorbate 60
Polysorbate 80
Pullulan
Sodium Lauryl Sulfate
Sorbitan Monolaurate
Sorbitan Monooleate
Sorbitan Monopalmitate
Sorbitan Monostearate
Sorbitan Sesquioleate
Sorbitan Trioleate
Tyloxapol

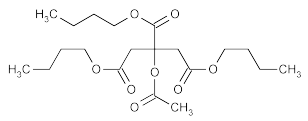
NF MONOGRAPHS

BRIEFING

Acetyltributyl Citrate, *NF 27* page 1153; **Triethyl Citrate,** *NF 27* page 1371. On the basis of comments and data received, it is proposed to revise the methods in the *Assay* sections for Acetyltributyl Citrate and Triethyl Citrate. Reports have indicated that the current method fails to separate the acetyltributyl citrate, tributyl citrate, triethyl citrate, and acetyltriethyl citrate peaks. Also, in the test for *Acidity*, it is proposed to add the preparation for neutralized isopropyl alcohol and to clarify the procedure.

(EM1: R. Lafaver.) RTS—C45943

Acetyltributyl Citrate



$C_{20}H_{34}O_8$ 402.48

DEFINITION
Acetyltributyl Citrate contains NLT 99.0% of $C_{20}H_{34}O_8$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197F**⟩
- **B.** The retention time of the *Sample solution* corresponds to that of a similar preparation of USP Acetyltriethyl Citrate RS, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

System suitability solution: 30 mg/mL each of USP Acetyltributyl Citrate RS and USP Tributyl Citrate RS in toluene

Sample solution: 30 mg/mL ▲of Acetyltributyl Citrate▲*NF29* in toluene

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC ~~, equipped with an on-column, temperature-programmable injector~~▲*NF29*

Detector: Flame ionization

Column: 0.32-mm × 30-m, bonded with a 0.5-µm layer of phase G42

Temperature

Injector: ~~See *Injector Temperature Program Table*.~~ ▲240°▲*NF29*

Detector: 280°

Column: See the temperature program table below.

~~Injector Temperature Program Table~~

~~Start Temperature (°)~~	~~Ramp (°)~~	~~End Temperature (°)~~	~~Hold Time (min)~~
~~85~~	~~=~~	~~85~~	~~0.5~~
~~85~~	~~20~~	~~225~~	~~10~~

▲▲*NF29*

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	0
80	20	230	~~10~~▲15▲*NF29*

Flow rate: 1.9 mL/min

Carrier gas: Helium

▲Injection type: Split, 30:1▲*NF29*

Injection size: 1 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for tributyl citrate and acetyl tributyl citrate are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between tributyl citrate and acetyl tributyl citrate

Relative standard deviation: NMT 2.0% determined from both the tributyl citrate and acetyl tributyl citrate peaks, based on area percent calculation

Analysis

Sample: *Sample solution*

[NOTE—Measure all of the peak areas, excluding the solvent peak.]

Calculate the percentage of $C_{20}H_{34}O_8$ in the portion of Acetyltributyl Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response of the *Sample solution*

r_T = sum of the area responses of all the peaks

Acceptance criteria: NLT 99.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨**841**⟩**:** 1.045–1.055
- **REFRACTIVE INDEX** ⟨**831**⟩**:** 1.4410–1.4425

Change to read:

- **ACIDITY**

▲Neutralized isopropyl alcohol: To a suitable quantity of isopropyl alcohol add 2–3 drops of bromothymol blue TS and just sufficient 0.10 N sodium hydroxide dropwise to produce a faint blue color. [NOTE—Prepare *Neutralized isopropyl alcohol* just prior to use.]▲*NF29*

Sample ▲solution:▲*NF29* 32.0 g ▲of Acetyltributyl Citrate in 30 mL of *Neutralized isopropyl alcohol*▲*NF29*

Analysis: ~~Dissolve the *Sample* in 30 mL of isopropyl alcohol, previously neutralized to bromothymol blue.~~▲▲*NF29*

Add bromothymol blue TS. Titrate with 0.10 N sodium hydroxide to a faint blue endpoint.

Acceptance criteria: NMT 1.0 mL ▲of 0.10 N▲*NF29* sodium hydroxide is required.

In-Process Revision

• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.25%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Acetyltributyl Citrate RS
USP Tributyl Citrate RS

BRIEFING

Benzalkonium Chloride, *NF 27* page 1173 and page 1012 of *PF* 34(4) [July–Aug. 2008]. On the basis of comments and data received and to align with the newly revised Benzalkonium Chloride monograph in the *European Pharmacopoeia, 6th Edition*, it is proposed to make the following revisions:

1. Add *Identification* test *D* based on peak identification and using USP Benzalkonium Chloride RS in the test for *Ratio of Alkyl Components*.
2. A requirement for column efficiency as part of the system suitability is deleted in the test for *Ratio of Alkyl Components*. An alternative analytical column, Alltech Nucleosil 100CN 4.6-mm × 25-cm, may also be used in this test. Calculation is revised for clarification.
3. The *Assay, Procedure 2: Total Alkylbenzyldimethylammonium Chlorides* is updated by introducing the addition of 0.1 N sodium hydroxide to help further titration, and by replacing chloroform with methylene chloride to improve occupational safety.
4. The test for *Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)benzene* in *Organic Impurities* is proposed based on analysis performed using a Macherey-Nagel Nucleosil 100-5 C18 column. Benzyl alcohol, benzaldehyde, and (chloromethyl)benzene elute at approximately 10, 13, and 24 min, respectively, on this system.
5. Add USP Benzyl Alcohol RS and USP Benzaldehyde RS in the *USP Reference Standards* section. These reference standards are used in the newly proposed test for *Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)benzene.*

(EM2: H. Wang.) RTS—C67223

Benzalkonium Chloride

Ammonium, alkyldimethyl(phenylmethyl)-, chloride; Alkylbenzyldimethylammonium chloride [8001-54-5].

DEFINITION
Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides of the general formula:

$$[C_6H_5CH_2N(CH_3)_2R]Cl$$

in which R represents a mixture of alkyls, including all or some of the group beginning with *n*-C_8H_{17} and extending through higher homologs, with *n*-$C_{12}H_{25}$, *n*-$C_{14}H_{29}$, and *n*-$C_{16}H_{33}$ composing the major portion. On the anhydrous basis, the content of the *n*-$C_{12}H_{25}$ homolog is NLT 40.0%, and the content of the *n*-$C_{14}H_{29}$ homolog is NLT 20.0% of the total alkylbenzyldimethylammonium chloride content. The amount of the *n*-$C_{12}H_{25}$ and *n*-$C_{14}H_{29}$ homolog components together is NLT 70.0% of the total alkylbenzyldimethylammonium chloride content. The total alkylbenzyldimethylammonium chloride content, calculated on the anhydrous basis, with allowance made for the amount of residue on ignition, is NLT 97.0% and NMT 103.0% of $[C_6H_5CH_2N(CH_3)_2R]Cl$.

IDENTIFICATION

Change to read:

• **A. ■PROCEDURE**
Analysis: To 2 mL of a solution (1 in 100) add 1 mL of 2 N nitric acid.
Acceptance criteria: A white precipitate is formed, and it is dissolved after adding 5 mL of alcohol.■2S (*NF27*)
• **B. PROCEDURE**
Analysis: Dissolve 200 mg in 1 mL of sulfuric acid, add 100 mg of sodium nitrate, and heat on a steam bath for 5 min. Cool, dilute with water to 10 mL, add 500 mg of zinc dust, and warm for 5 min on a steam bath. To 2 mL of the clear supernatant, add 1 mL of sodium nitrite solution (1 in 20), cool in ice water, and then add 3 mL of a solution of 500 mg of 2-naphthol in 10 mL of 6 N ammonium hydroxide.
Acceptance criteria: An orange-red color is produced.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** The solution in a mixture of equal volumes of water and alcohol meets the requirements of the tests.

Add the following:

▲• **D.** The retention times of the major peaks for benzalkonium chloride in the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Ratio of Alkyl Components.*▲*NF29*

ASSAY

Change to read:

• **PROCEDURE 1: RATIO OF ALKYL COMPONENTS**
Solution A: Adjust a 0.1 M solution of sodium acetate with glacial acetic acid to a pH of 5.0.
Mobile phase: Acetonitrile and *Solution A* (9:11). Acetonitrile and *Solution A* may be adjusted from (2:3) to (3:2) to meet system suitability requirements.
Standard solution: 4 mg/mL of USP Benzalkonium Chloride RS
Sample solution: 4 mg/mL of Benzalkonium Chloride
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L10, ▲or 4.6-mm × 25-cm; packing L10▲*NF29*
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
▲[NOTE—See the relative retention times in the table below.]

Component	Relative Retention Time (RRT)
C_{10} homolog	0.9
C_{12} homolog	1.0
C_{14} homolog	1.3
C_{16} homolog	1.7

▲*NF29*
Suitability requirements
Resolution: NLT 1.5 between the C_{12} and C_{14} peaks
~~**Column efficiency:** NLT 1000 theoretical plates from the C_{12} peak~~
▲▲*NF29*

In-Process Revision

Relative standard deviation: NMT 2.0% for the C_{12} peak

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the homolog peaks by comparison of the retention times with those from the *Standard solution*.

~~Calculate the percentage of each quaternary ammonium homolog taken:~~

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

~~A = product of the area obtained from the homolog multiplied by its molecular weight~~

~~B = sum of all of these products~~

~~The molecular weights of C_{10}, C_{12}, C_{14}, and C_{16} are 312, 340, 368, and 396, respectively.~~

▲Calculate the percentage of each quaternary ammonium homolog taken:

$$\text{Result} = \frac{r_U \times M_r}{\sum_i (r_U \times M_r)} \times 100$$

r_U = area of the peak due to a given homolog in the chromatogram from the *Sample solution*

M_r = molecular weight of a given homolog. The molecular weights of C_{10}, C_{12}, C_{14}, and C_{16} homologs are 312, 340, 368, and 396, respectively.

▲*NF29*

Acceptance criteria: On the anhydrous basis, the content of the *n*-$C_{12}H_{25}$ homolog is NLT 40.0%, and the content of the *n*-$C_{14}H_{29}$ homolog is NLT 20.0% of the total alkylbenzyldimethylammonium chloride content. The amount of the *n*-$C_{12}H_{25}$ and *n*-$C_{14}H_{29}$ homolog components together is NLT 70.0% of the total alkylbenzyldimethylammonium chloride content.

Change to read:

• **PROCEDURE 2: TOTAL ALKYLBENZYLDIMETHYLAMMONIUM CHLORIDES**

~~**Analysis:** Weigh a quantity of Benzalkonium Chloride equivalent to about 500 mg of anhydrous benzalkonium chloride, and transfer, with the aid of 35 mL of water, to a glass-stoppered, 250-mL conical separator containing 25 mL of chloroform. Add 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper in the separator, shake, allow the layers to separate, and discard the chloroform layer. Wash the aqueous layer with three 10-mL portions of chloroform, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold hydrochloric acid to the flask, mix, and titrate the with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of chloroform, insert the stopper into the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the chloroform layer becomes colorless and the aqueous layer is clear yellow. Perform a blank determination, using 20 mL of water as the sample. The difference between the two titrations represents the amount of potassium iodate equivalent to the weight of benzalkonium chloride in the sample. Each mL of 0.05 M potassium iodate is equivalent to X/10 mg of benzalkonium chloride, where X represents the average molecular weight of the sample, derived by summing, for all homologs, the products:~~

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

~~A = area of the peak produced by that homolog in the chromatogram from the *Ratio of alkyl components* test~~

~~B = total peak area for all homologs in that chromatogram from the *Ratio of alkyl components* test~~

~~M_r = molecular weight of a given homolog~~

▲**Sample:** Weigh a quantity of Benzalkonium Chloride equivalent to 500 mg of anhydrous benzalkonium chloride.

Analysis: Transfer the *Sample*, with the aid of 35 mL of water, to a glass-stoppered, 250-mL conical separator containing 25 mL of methylene chloride. Add 10 mL of 0.1 N sodium hydroxide, and 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper into the separator, shake, allow the layers to separate, and discard the methylene chloride layer. Wash the aqueous layer with three 10-mL portions of methylene chloride, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold hydrochloric acid to the flask, mix, and titrate with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of methylene chloride, insert the stopper into the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the methylene chloride layer becomes colorless and the aqueous layer is clear yellow. Record the titrant volume, V_t (mL). Perform a blank determination, using 20 mL of water as the sample, and record the titrant volume, V_b (mL). [NOTE—$V_b > V_t$.] The difference between the two titrations represents the amount of potassium iodate equivalent to the weight of benzalkonium chloride in the sample. Each mL of 0.05 M potassium iodate is equivalent to x/10 mg of benzalkonium chloride, where x represents the average molecular weight of the sample, derived by summing, for all homologs, the products:

$$\text{Result (x)} = \sum_i \left[(r_U / r_T) \times M_r \right]$$

r_U = area of the peak produced by a given homolog in the chromatogram from the *Ratio of Alkyl Components* test

r_T = sum of the peak areas for all homologs in the chromatogram from the *Ratio of Alkyl Components* test

M_r = molecular weight of a given homolog. The molecular weights of the C_{10}, C_{12}, C_{14}, and C_{16} homologs are 312, 340, 368, and 396, respectively.

▲*NF29*

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 2.0%

Change to read:

Organic Impurities

■• **PROCEDURE 1: LIMIT OF AMINES AND AMINE SALTS**

Sample: 5.0 g of Benzalkonium Chloride

Analysis and **Acceptance criteria:** Dissolve the *Sample* with heating ▲carefully on top of a steam bath with water as the steam source▲*NF29* in 20 mL of a mixture of methanol and 1 N hydrochloric acid VS (97:3). ▲[NOTE—However, the mixed solution must not reach the boiling point.]▲*NF29* Add 100 mL of isopropyl alcohol. Pass a stream of nitrogen slowly through the solution. Gradually add 12.0 mL of 0.1 N tetrabutylammonium hydroxide VS, while recording the potentiometric titration curve. If the curve shows two inflection points, the volume of titrant added between the two points is NMT 5.0 mL, corresponding to NMT 0.1 mmol/g of amines and amine salts. If the curve shows no point of inflection, the substance being examined does not comply with the test. If the curve shows one point of inflection, repeat the test, but add 3.0 mL of a 25.0 mg/mL solution of dimethyldecylamine in isopropyl alcohol before the titration. If after addition of 12.0 mL of the titrant, the titration curve shows only one point of inflection, the substance being examined does not comply with the test.■2S *(NF27)*

▲• **PROCEDURE 2: LIMIT OF BENZYL ALCOHOL, BENZALDEHYDE, AND (CHLOROMETHYL)BENZENE**

[NOTE—Prepare the solutions immediately before use.]

Solution A: Dissolve 1.09 g of sodium 1-hexanesulfonate and 6.9 g of monobasic sodium phosphate in water in a 1000-mL volumetric flask, adjust to pH 3.5 with phosphoric acid, and dilute with water to volume.

Solution B: Methanol

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	80	20
14	50	50
35	50	50
36	20	80
55	20	80
56	80	20
65	80	20

Standard solution A: 0.25 mg/mL of USP Benzyl Alcohol RS in methanol

Standard solution B: 0.075 mg/mL of USP Benzaldehyde RS in methanol

Standard solution C: 0.025 mg/mL of USP Benzyl Alcohol RS in methanol, prepared from *Standard solution A* and methanol

Sample solution: 50 mg/mL of Benzalkonium Chloride in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm for benzyl alcohol and (chloromethyl)benzene; UV 257 nm for benzaldehyde

Column: 4.6-mm × 15-cm analytical column; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample Solution*

[NOTE—See the relative retention times in the table below.]

Component	Relative Retention Time (RRT)
Benzyl alcohol	1.0
Benzaldehyde	1.3
(Chloromethyl)benzene	2.4

Suitability requirements

Relative standard deviation: NMT 5.0% for the benzyl alcohol peak obtained from *Standard solution A*

Signal-to-noise ratio: NLT 10 for the principal peak in the chromatogram from *Standard solution C*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

To calculate the content of (chloromethyl)benzene, multiply the peak area of (chloromethyl)benzene by 0.7.

Acceptance criteria

Benzyl alcohol: The response of the benzyl alcohol peak from the *Sample solution* is NMT that of the benzyl alcohol peak from the *Standard solution A*, corresponding to NMT 0.5%.

Benzaldehyde: The response of the benzaldehyde peak from the *Sample solution* is NMT that of the benzaldehyde peak from *Standard solution B*, corresponding to NMT 0.15%.

(Chloromethyl)benzene: The response of the (chloromethyl)benzene peak from the *Sample solution* is NMT 0.1 times that of the principal peak in the chromatogram from *Standard solution A*, corresponding to NMT 0.05%.▲*NF29*

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 15.0%

Add the following:

■• **ACIDITY OR ALKALINITY**

Sample: 0.5 g of Benzalkonium Chloride

Analysis: Dissolve the *Sample* in water, dilute with water to 50 mL, and mix. Add 0.1 mL of bromocresol purple TS.

Acceptance criteria: NMT 0.1 mL of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide is required to change the color of the indicator.■2S (*NF27*)

• **WATER-INSOLUBLE MATTER:** A solution (1 in 10) is free from turbidity and insoluble matter.

Delete the following:

~~■• **LIMIT OF FOREIGN AMINES:** To 5 mL of a solution (1 in 50) add 3 mL of 1 N sodium hydroxide: no precipitate is formed. Heat to boiling: the odor of amines is not perceptible.~~■2S (*NF27*)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ■No storage requirements specified.■2S (*NF27*)

Change to read:

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Benzalkonium Chloride RS

▲USP Benzyl Alcohol RS

USP Benzaldehyde RS▲*NF29*

In-Process Revision

BRIEFING

Benzalkonium Chloride Solution, *NF 27* page 1174. On the basis of comments and data received and to align with the newly revised Benzalkonium Chloride Solution monograph in the *European Pharmacopoeia, 6th Edition*, it is proposed to make the following revisions:

1. *Identification* test *A* is updated and modified to omit the use of mercuric chloride TS.
2. Add *Identification* test *D* based on peak identification and using USP Benzalkonium Chloride RS in the test for *Ratio of Alkyl Components*.
3. A requirement for column efficiency as part of the system suitability is deleted in the test for *Ratio of Alkyl Components*. An alternative analytical column, Alltech Nucleosil 100CN 4.6-mm × 25-cm, may also be used in this test. Calculation is revised for clarification.
4. The *Assay, Procedure 2: Total Alkylbenzyldimethylammonium Chlorides* is updated by introducing the addition of 0.1 N sodium hydroxide to help further titration, and by replacing chloroform with methylene chloride to improve occupational safety.
5. The test for *Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)benzene* in *Organic Impurities* is proposed based on the analysis performed using a Macherey-Nagel Nucleosil 100-5 C18 column. Benzyl alcohol, benzaldehyde, and (chloromethyl)benzene elute at approximately 10, 13, and 24 min, respectively, on this system.
6. Add a test for *Acidity or Alkalinity*.
7. Add a test for *Limit of Amines and Amine Salts* to replace the test for odor perception in *Limit of Foreign Amines*.
8. On the basis of the submitted method, the test for *Alcohol Determination* is replaced by a procedure using gas chromatography. This test is based on analyses performed with the J&W Scientific DB-624 brand of G43 column. The typical retention times for alcohol and tertiary butyl alcohol are 4.8 and 7.0 min, respectively.
9. Add a *Labeling* section to indicate the concentration of benzalkonium chloride, and the name and quantity of coloring agent added. The labeling should also indicate the concentration of alcohol.
10. Add USP Alcohol RS, USP Benzyl Alcohol RS, and USP Benzaldehyde RS in the *USP Reference Standards* section. These reference standards are used in the updated test for *Alcohol Content* and in the newly proposed test for *Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)benzene.*

Other changes are editorial in nature.

(EM2: H. Wang.) RTS—C68683

Benzalkonium Chloride Solution

DEFINITION

Change to read:

Benzalkonium Chloride Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of benzalkonium chloride in ~~concentrations of 1.0% or more~~▲solution that has a concentration of 1.0% or more;▲NF29 and NLT 93.0% and NMT 107.0% of the labeled amount ~~in concentrations of less than 1.0%~~▲in solution that has a concentration of less than 1.0%.▲NF29 It may contain a suitable coloring agent and may contain NMT 10% of alcohol.

[**CAUTION–**Mixing Benzalkonium Chloride Solution with ordinary soaps and with anionic detergents may decrease or destroy the bacteriostatic activity of the Solution.]

IDENTIFICATION

Change to read:

• **A. ▲PROCEDURE▲NF29** ~~Add 2 N nitric acid or mercuric chloride TS to a solution having an equivalent to 10 mg/mL of benzalkonium chloride. A white precipitate is formed, and it is soluble in alcohol.~~

▲Analysis: Add 1 mL of 2 N nitric acid to 2 mL of a solution having an equivalent to 10 mg/mL of benzalkonium chloride.

Acceptance criteria: A white precipitate is formed, and it is dissolved after adding 5 mL of alcohol.▲NF29

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** A solution of it in a mixture of equal volumes of water and alcohol meets the requirements.

• **C. PROCEDURE**

Analysis: Dissolve the residue obtained by evaporating, on a steam bath, a volume of Solution equivalent to 200 mg of benzalkonium chloride in 1 mL of sulfuric acid, add 100 mg of sodium nitrate, and heat on a steam bath for 5 min. Cool, dilute with water to 10 mL, add 500 mg of zinc dust, and warm for 5 min on a steam bath. To 2 mL of the clear supernatant add 1 mL of sodium nitrite solution (1 in 20), cool in ice water, then add 3 mL of a solution of 500 mg of 2-naphthol in 10 mL of 6 N ammonium hydroxide.

Acceptance criteria: An orange-red color is produced.

Add the following:

▲• **D.** The retention times of the major peaks for benzalkonium chloride in the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Ratio of Alkyl Components*.▲NF29

ASSAY

Change to read:

• **PROCEDURE 1: RATIO OF ALKYL COMPONENTS**

Solution A: Adjust a 0.1 M solution of sodium acetate with glacial acetic acid to a pH of 5.0.

Mobile phase: Acetonitrile and *Solution A* (9:11). Acetonitrile and *Solution A* may be adjusted from (2:3) to (3:2) to meet system suitability requirements.

Standard solution: 4 mg/mL of USP Benzalkonium Chloride RS

Sample solution: Transfer a volume of Solution, equivalent to 400 mg of benzalkonium chloride, to a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L10, ▲or 4.6-mm × 25-cm; packing L10▲NF29

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

▲[NOTE—See the relative retention time table below.]

Component	Relative Retention Time (RRT)
C_{10} homolog	0.9
C_{12} homolog	1.0
C_{14} homolog	1.3
C_{16} homolog	1.7

▲NF29

In-Process Revision

Suitability requirements

Resolution: NLT 1.5 between the C_{12} and C_{14} peaks

~~**Column efficiency:** NLT 1000 theoretical plates from the C_{12} peak~~

▲▲*NF29*

Relative standard deviation: NMT 2.0% for the C_{12} peak

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the homolog peaks by comparison of the retention times with those from the *Standard solution*.

~~Calculate the percentage of each quaternary ammonium homolog taken:~~

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

~~A = product of the area obtained from the homolog multiplied by its molecular weight~~

~~B = sum of all of these products~~

~~The molecular weights of C_{10}, C_{12}, C_{14}, and C_{16} are 312, 340, 368, and 396, respectively.~~

▲Calculate the percentage of each quaternary ammonium homolog taken:

$$\text{Result} = \frac{r_U \times M_r}{\sum_i (r_U \times M_r)} \times 100$$

r_U = area of the peak due to a given homolog in the chromatogram from the *Sample solution*

M_r = molecular weight of a given homolog. The molecular weights of the C_{10}, C_{12}, C_{14}, and C_{16} homologs are 312, 340, 368, and 396, respectively.

▲*NF29*

Acceptance criteria: On the anhydrous basis, the content of the *n*-$C_{12}H_{25}$ homolog is NLT 40.0%, and the content of the *n*-$C_{14}H_{29}$ homolog is NLT 20.0% of the total alkylbenzyldimethylammonium chloride content. The amount of the *n*-$C_{12}H_{25}$ and *n*-$C_{14}H_{29}$ homolog components together is NLT 70.0% of the total alkylbenzyldimethylammonium chloride content.

Change to read:

- **PROCEDURE 2: TOTAL ALKYLBENZYLDIMETHYLAMMONIUM CHLORIDES**

~~**Sample solution:** Evaporate or dilute with water to 30 mL a volume of Solution, equivalent to 275 mg of benzalkonium chloride. Transfer the solution with the aid of a minimum quantity of water to a glass-stoppered, 250-mL conical separator. Add 25 mL of chloroform. Add 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper in the separator, shake, allow the layers to separate, and discard the chloroform layer. Wash the aqueous layer with three 10-mL portions of chloroform, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold hydrochloric acid to the flask.~~

~~**Analysis:** Titrate with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of chloroform, insert the stopper into the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the chloroform layer becomes colorless and the aqueous layer is clear yellow. Perform a blank determination, using 20 mL of water as the sample. The difference between the two titrations represents the amount of potassium iodate equivalent to the weight of benzalkonium chloride in the sample. Each mL of 0.05 M potassium iodate is equivalent to x/10 mg of benzalkonium chloride, where x represents the average molecular weight of the sample, derived by summing, for all homologs, the products:~~

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

~~A = area of the peak produced by that homolog in the chromatogram from the *Ratio of Alkyl Components* test~~

~~B = total peak area for all homologs in that chromatogram~~

~~M_r = molecular weight of a given homolog~~

▲**Sample solution:** Evaporate or dilute with water to 30 mL a volume of Solution, equivalent to 275 mg of benzalkonium chloride.

Analysis: Transfer the *Sample solution* with the aid of a minimum quantity of water to a glass-stoppered, 250-mL conical separator. Transfer 25 mL of methylene chloride. Add 10 mL of 0.1 N sodium hydroxide, and 10.0 mL of freshly prepared potassium iodide solution (1 in 20), insert the stopper in the separator, shake, allow the layers to separate, and discard the methylene chloride layer. Wash the aqueous layer with three 10-mL portions of methylene chloride, and discard the washings. Transfer the aqueous layer to a glass-stoppered, 250-mL conical flask, and rinse the separator with three 5-mL portions of water, adding the washings to the flask. Add 40 mL of cold hydrochloric acid to the flask, mix, and titrate with 0.05 M potassium iodate VS until the solution becomes light brown in color. Add 5 mL of methylene chloride, insert the stopper into the flask, and shake vigorously. Continue the titration, dropwise, with shaking after each addition, until the methylene chloride layer becomes colorless and the aqueous layer is clear yellow. Record the titrant volume, V_t (mL). Perform a blank determination, using 20 mL of water as the sample, and record the titrant volume, V_b (mL). [NOTE—$V_b > V_t$.] The difference between the two titrations represents the amount of potassium iodate equivalent to the weight of benzalkonium chloride in the sample. Each mL of 0.05 M potassium iodate is equivalent to x/10 mg of benzalkonium chloride, where x represents the average molecular weight of the sample, derived by summing, for all homologs, the products:

$$\text{Result (x)} = \sum_i \left[(r_U / r_T) \times M_r \right]$$

r_U = area of the peak produced by a given homolog in the chromatogram from the *Ratio of Alkyl Components* test

r_T = sum of the peak areas for all homologs in the chromatogram from the *Ratio of Alkyl Components* test

M_r = molecular weight of a given homolog. The molecular weights of the C_{10}, C_{12}, C_{14}, and C_{16} homologs are 312, 340, 368, and 396, respectively.

▲*NF29*

Acceptance criteria

For labeled concentrations of NLT 1%: 95.0%–105.0%

For labeled concentrations less than 1%: 93.0%–107.0%

OTHER COMPONENTS

Add the following:

▲• **ALCOHOL CONTENT (IF ADDED)**

Internal standard solution: 0.06 mL/mL of tertiary butyl alcohol in water

Alcohol stock solution: 0.03 mL/mL of C_2H_5OH in water, using USP Alcohol RS

Standard solutions: Introduce 5 mL, 10 mL, and 20 mL, respectively, of *Alcohol stock solution* into three separate identical 50-mL volumetric flasks. To each flask, add a 5-mL portion of the *Internal standard solution*. Dilute with water to volume, and mix thoroughly. *Standard solutions* contain alcohol content of 0.003 mL/mL, 0.006 mL/mL, and 0.012 mL/mL, respectively.

Sample solution: Weigh an appropriate amount of Benzalkonium Chloride Solution into a 50-mL volumetric flask, and pipet 5 mL of *Internal standard solution*. Dilute with water

In-Process Revision

to volume, and mix thoroughly to obtain a solution containing an alcohol content of between 0.003 mL/mL and 0.012 mL/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m glass or quartz capillary; 1.4-µm layer of phase G43
Temperature
Detector: 250°
Injection port: 250°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	7
40	30	250	15

Run time: 29 min
Carrier gas: Helium or nitrogen
Flow rate: See the ramped flow program table below.

Initial Flow (mL/min)	Flow Ramp (mL/min²)	Final Flow (mL/min)	Hold Time at Final Flow (min)
1	—	1	8
1	10	3	21

Injection size: 1 µL
Injection mode: Split 75:1
System suitability
Sample: *Standard solution* containing alcohol content of 0.006 mL/mL
[NOTE—See the relative retention times in the table below.]

Component	Relative Retention Time (RRT)
Alcohol	0.7
Tertiary butyl alcohol	1.0

Suitability requirements
Resolution: NLT 2.0, between alcohol and tertiary butyl alcohol
Relative standard deviation: NMT 10%
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the peak response ratios of the alcohol to tertiary butyl alcohol in the *Standard solutions* versus the content, in mL/mL, of alcohol, and draw the straight line best fitting the plotted points. From the graph obtained, determine the content, C, in mL/mL, of alcohol in the *Sample solution*. Calculate the percentage of alcohol in the portion of Benzalkonium Chloride Solution (v/v) taken:

$$\text{Result} = V \times (C \times D/W) \times 100$$

V = volume of the *Sample solution*, 50 mL
D = density of Benzalkonium Chloride Solution (g/mL)
W = weight of Benzalkonium Chloride Solution taken to prepare the *Sample solution* (g)

Acceptance criteria: If present, between 95.0% and 105.0% of the labeled amount of C_2H_5OH ▲*NF29*

IMPURITIES

Add the following:

▲Organic Impurities

- **PROCEDURE 1: LIMIT OF AMINES AND AMINE SALTS**
Sample: A quantity of Benzalkonium Chloride Solution, equivalent to 5.0 g of benzalkonium chloride
Analysis and **Acceptance criteria:** Dissolve the *Sample* with heating carefully on top of a steam bath with water as the steam source in 20 mL of a mixture of methanol and 1 N hydrochloric acid VS (97:3). [NOTE—However, the mixed solution must not reach the boiling point.] Add 100 mL of isopropyl alcohol. Pass a stream of nitrogen slowly through the solution. Gradually add 12.0 mL of 0.1 N tetrabutylammonium hydroxide VS, while recording the potentiometric titration curve. If the curve shows two inflection points, the volume of titrant added between the two points is NMT 5.0 mL, corresponding to NMT 0.1 mmol/g of amines and amine salts. If the curve shows no point of inflection, the substance being examined does not comply with the test. If the curve shows one point of inflection, repeat the test, but add 3.0 mL of a 25.0 mg/mL solution of dimethyldecylamine in isopropyl alcohol before the titration. If after addition of 12.0 mL of the titrant, the titration curve shows only one point of inflection, the substance being examined does not comply with the test.
- **PROCEDURE 2: LIMIT OF BENZYL ALCOHOL, BENZALDEHYDE, AND (CHLOROMETHYL)BENZENE**
[NOTE—Prepare the solutions immediately before use.]
Solution A: Dissolve 1.09 g of sodium 1-hexanesulfonate and 6.9 g of monobasic sodium phosphate in water in a 1000-mL volumetric flask, adjust to pH 3.5 with phosphoric acid, and dilute with water to volume.
Solution B: Methanol
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	80	20
14	50	50
35	50	50
36	20	80
55	20	80
56	80	20
65	80	20

Standard solution A: 0.25 mg/mL of USP Benzyl Alcohol RS in methanol
Standard solution B: 0.075 mg/mL of USP Benzaldehyde RS in methanol
Standard solution C: 0.025 mg/mL of USP Benzyl Alcohol RS in methanol, prepared from *Standard solution A* and methanol
Sample solution: Determine the density of the Benzalkonium Chloride Solution. Dilute a quantity of the Solution equivalent to 2.5 g of benzalkonium chloride with methanol to 50.0 mL. This solution contains 50 mg/mL of benzalkonium chloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm for benzyl alcohol and (chloromethyl)benzene; UV 257 nm for benzaldehyde
Column: 4.6-mm × 15-cm analytical column; 5-µm packing L1

In-Process Revision

Column temperature: 30°
Flow rate: 1.0 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample Solution*
[NOTE—See the relative retention times in the table below.]

Component	Relative Retention Time (RRT)
Benzyl alcohol	1.0
Benzaldehyde	1.3
(Chloromethyl)benzene	2.4

Suitability requirements
Relative standard deviation: NMT 5.0% for the benzyl alcohol peak from *Standard solution A*
Signal-to-noise ratio: NLT 10 for the principal peak in the chromatogram from *Standard solution C*

Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
To calculate the content of (chloromethyl)benzene, multiply the peak area of (chloromethyl)benzene by 0.7.

Acceptance criteria
Benzyl alcohol: The response of the benzyl alcohol peak from the *Sample solution* is NMT that of the benzyl alcohol peak from the *Standard solution A*, corresponding to NMT 0.5%.
Benzaldehyde: The response of the benzaldehyde peak from the *Sample solution* is NMT that of the benzaldehyde peak from the *Standard solution B*, corresponding to NMT 0.15%.
(Chloromethyl)benzene: The response of the (chloromethyl)benzene peak from the *Sample solution* is NMT 0.1 times that of the principal peak in the chromatogram from the *Standard solution A*, corresponding to NMT 0.05%.▲*NF29*

SPECIFIC TESTS
- **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** Solution containing less than 5.0% of benzalkonium chloride meets the requirements of the test for absence of *Pseudomonas aeruginosa*

Add the following:

▲• **ACIDITY OR ALKALINITY**
Sample solution: 10 mg/mL of benzalkonium chloride, prepared from Benzalkonium Chloride Solution and carbon dioxide-free water
Analysis: To 50 mL of *Sample solution*, add 0.1 mL of bromocresol purple TS.
Acceptance criteria: NMT 0.1 mL of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide is required to change the color of the indicator.▲*NF29*

Delete the following:

~~▲• **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩ (if present)**:** 95.0%–105.0% of the labeled amount of C_2H_5OH▲*NF29*~~

Delete the following:

~~▲• **LIMIT OF FOREIGN AMINES:** A volume of Solution, equivalent to 100 mg of benzalkonium chloride, and adjusted to a concentration of 1 in 50. To 5 mL of this solution add 3 mL of 1 N sodium hydroxide: no precipitate is formed. Heat to boiling: the odor of amines is not perceptible.▲*NF29*~~

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and prevent contact with metals.

Add the following:

▲• **LABELING:** Label it to indicate the concentration of benzalkonium chloride, and to indicate the name and quantity of coloring agent added. The labeling also indicates the concentration of alcohol added.▲*NF29*

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
▲USP Alcohol RS
USP Benzyl Alcohol RS
USP Benzaldehyde RS▲*NF29*
USP Benzalkonium Chloride RS

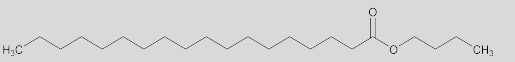
BRIEFING

Butyl Stearate. Because there is no existing *NF* monograph for this excipient, a new monograph, based on the monograph appearing in the *Food Chemical Codex, 6th Edition*, page 121 and supporting data is proposed. Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver. NOM: A. Wilk.) RTS—C70759

Add the following:

▲Butyl Stearate



Butyl Octadecanoate
$C_{22}H_{44}O_2$ 340.59
[123-95-5].

DEFINITION
Butyl Stearate consists chiefly of ester of butyl alcohol and stearic acid.

IDENTIFICATION
- **INFRARED ABSORPTION ⟨197F⟩**

SPECIFIC TESTS
- **SOLUBILITY IN ALCOHOL:** One mL dissolves in 6 mL of 95% alcohol.
- **SPECIFIC GRAVITY ⟨841⟩:** 0.850–0.870 at 20°
- **FATS AND FIXED OILS,** *Iodine Value* **⟨401⟩:** NMT 1
- **MELTING RANGE OR TEMPERATURE,** *Class III* **⟨741⟩:** 17°–25°
- **FATS AND FIXED OILS,** *Saponification Value* **⟨401⟩:** 165–180

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is intended for topical use only.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Butyl Stearate RS▲*NF29*

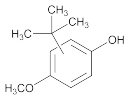
In-Process Revision

BRIEFING

Butylated Hydroxyanisole, *NF 27* page 1178. On the basis of comments received and supporting data, it is proposed in the *Assay* to replace the current GC procedure employing a packed column, which is no longer available, with a validated HPLC method. The HPLC chromatographic procedure in the *Assay* is based on analysis performed with a Waters Symemetry C18 brand of column with 3.5-µm L1 packing. The typical retention times for 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole are about 4.2 and 4.6 min, respectively. Interested parties are encouraged to comment on the proposal.

(EM1: R. Lafaver.) RTS—C59587

Butylated Hydroxyanisole



$C_{11}H_{16}O_2$ 180.24
Phenol, (1,1-dimethylethyl)-4-methoxy-;
tert-Butyl-4-methoxyphenol [25013-16-5].

DEFINITION
Butylated Hydroxyanisole contains NLT 98.5% of $C_{11}H_{16}O_2$.

IDENTIFICATION

Change to read:

• **▲A.▲*NF29* PROCEDURE**
Analysis: To 5 mL of a 0.1-mg/mL solution in 72% alcohol add 2 mL of a 20-mg/mL sodium borate solution and 1 mL of a 0.1-mg/mL solution of 2,6-dichloroquinone-chlorimide in dehydrated alcohol, and mix.
Acceptance criteria: A blue color is produced.

Add the following:

▲B. The retention times of 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.▲*NF29*

ASSAY

Change to read:

• **PROCEDURE**
~~**Internal standard solution:** 5 mg/mL of 4-*tert*-butylphenol in acetone~~
~~**Standard solution:** 9 mg/mL of USP 3-*tert*-Butyl-4-hydroxyanisole RS and 1 mg/mL of USP 2-*tert*-Butyl-4-hydroxyanisole RS in *Internal standard solution*~~
~~**Sample solution:** 10 mg/mL of Butylated Hydroxyanisole in *Internal standard solution*~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩*, System Suitability*.)~~
~~**Mode:** GC~~
~~**Detector:** Flame ionization detector~~
~~**Column:** 2-mm × 1.8-m stainless-steel column packed with 10% liquid phase G26 on support S1A~~
~~**Column temperature:** 175°–185°~~
~~**Injector temperature:** 200°~~
~~**Detector temperature:** 250°~~
~~**Carrier gas:** Helium~~
~~**Injection size:** 5 µL~~
~~**System suitability**~~
~~**Sample:** *Standard solution*~~
~~**Suitability requirements**~~
~~**Resolution:** NLT 1.3 between the 3-*tert*-butyl-4-hydroxyanisole isomer and the 2-*tert*-butyl-4-hydroxyanisole isomer~~
~~**Tailing factor:** NMT 2.0~~
~~**Relative standard deviation:** NMT 2.0% determined from the 3-*tert*-butyl-4-hydroxyanisole isomer and NMT 6.0% determined from the 2-*tert*-butyl-4-hydroxyanisole isomer~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Measure the peak areas for each isomer and the internal standard. Calculate the percentage of each isomer in the portion of Butylated Hydroxyanisole taken:~~

~~$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$~~

~~R_U = peak area ratio of the corresponding isomer to the internal standard of the *Sample solution*~~
~~R_S = peak area ratio of the corresponding isomer to the internal standard of the *Standard solution*~~
~~C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)~~
~~C_U = concentration of *Sample solution* (mg/mL)~~
~~[NOTE—Calculate the percentage of $C_{11}H_{16}O_2$ in the portion of Butylated Hydroxyanisole taken by adding the quantities of the two isomers.]~~
~~**Acceptance criteria:** NLT 98.5%~~

▲Solution A: 5% acetic acid
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard solution: 90 µg/mL of USP 3-*tert*-Butyl-4-hydroxyanisole RS and 10 µg/mL of USP 2-*tert*-Butyl-4-hydroxyanisole RS in *Mobile phase*
Sample solution: 100 µg/mL of Butylated Hydroxyanisole in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 75-mm column; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times of 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole are about 4.2 and 4.6 min, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the 3-*tert*-butyl-4-hydroxyanisole isomer and 2-*tert*-butyl-4-hydroxyanisole isomer peaks
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0% for the 3-*tert*-butyl-4-hydroxyanisole isomer and 2-*tert*-butyl-4-hydroxyanisole isomer peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the peak areas for each isomer. Calculate the percentage of each isomer in the portion of Butylated Hydroxyanisole taken:

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

r_U = peak area of the corresponding isomer in the *Sample solution*
r_S = peak area of the corresponding isomer in the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

In-Process Revision

[NOTE—Calculate the percentage of $C_{11}H_{16}O_2$ in the portion of Butylated Hydroxyanisole taken by adding the quantities of the two isomers.]
Acceptance criteria: NLT 98.5%▲*NF29*

IMPURITIES
Inorganic Impurities
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.01%, determined on a 10-g sample
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP 2-*tert*-Butyl-4-hydroxyanisole RS
  USP 3-*tert*-Butyl-4-hydroxyanisole RS

BRIEFING

Polyoxyl Stearyl Ether, *NF* 27 page 1318. On the basis of comments and data received, it is proposed to make the following revisions. Interested parties are encouraged to comment on the proposal.
1. The chemical formula is provided.
2. Data show that current USP Polyoxyl Stearyl Ether RS cannot be used to identify polyoxyl 2 stearyl ether and polyoxyl 20 stearyl ether in *Identification* test *A, Infrared Absorption*. Therefore, in the section *USP Reference Standards ⟨11⟩*, USP Polyoxyl Stearyl Ether RS is changed to USP Polyoxyl 10 Stearyl Ether RS, and two more Reference Standards are introduced: USP Polyoxyl 2 Stearyl Ether RS and USP Polyoxyl 20 Stearyl Ether RS. Articles with various oxyethylene units can be identified using their corresponding USP Reference Standards.
3. *Identification* test *C*, based on the article's hydroxyl value, is added.
4. A new test chapter, *Ethylene Oxide and Dioxane ⟨228⟩*, was proposed in *PF* 35(4) [July–Aug. 2009], page 917. The detailed procedure in this monograph in *Organic Impurities*, *Limit of Free Ethylene Oxide and Dioxane*, is deleted and now appears in the proposed chapter *⟨228⟩, Method I*. A new subsection is added to the monograph in *Organic Impurities*, in which the *Analysis* refers the reader to the chapter.

(EM2: H. Wang.) RTS—C72182

Polyoxyl Stearyl Ether

Change to read:

Polyethylene glycol monostearyl ether

▲$CH_3(CH_2)_{17}(OCH_2CH_2)_nOH$, n = 2–20▲*NF29* [9005-00-9].

DEFINITION
Polyoxyl Stearyl Ether is a mixture of the monostearyl ethers of mixed polyethylene glycols, the average polymer length being equivalent to NLT 2 and NMT 20 oxyethylene units (nominal value). It may contain various amounts of free stearyl alcohol and some free polyethylene glycol.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197F⟩:** Use a thin film of melted Polyoxyl Stearyl Ether.
- **B. PROCEDURE**
  Sample: 0.1 g
  Analysis: Dissolve or disperse the *Sample* in alcohol. Add 10 mL of diluted hydrochloric acid, 5 mL of barium chloride TS, and 10 mL of phosphomolybdic acid solution (1 in 10).
  Acceptance criteria: A precipitate is formed.

Add the following:

▲• **C.** It meets the requirements of the test for *Fats and Fixed Oils, Hydroxyl Value ⟨401⟩*.▲*NF29*

IMPURITIES

Change to read:

Organic Impurities
~~• **PROCEDURE: LIMIT OF FREE ETHYLENE OXIDE AND DIOXANE**~~

~~[**CAUTION**—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate at a temperature between 4° and 8°.]~~

~~[NOTE—Before using the Polyethylene Glycol 200 in this test, remove any volatile components from it by placing 500 mL of Polyethylene Glycol 200 in a 1000-mL round-bottom flask, attaching the flask to a rotary evaporator, and evaporating at a temperature of 60° and under a vacuum of 10–20 mm Hg for 6 h.]~~

~~**Solution A:** 10 µg/mL of acetaldehyde. [NOTE—Prepare *Solution A* fresh just before use.]~~

~~**Ethylene oxide stock solution:** Fill a chilled pressure bottle with liquid ethylene oxide, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Tare a glass-stoppered conical flask, add about 50 mL of Polyethylene Glycol 200, and reweigh the flask. Transfer about 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in a mixture of sodium chloride and wet ice (1:3). Using a gas-tight syringe that has been previously cooled to –10°, transfer 300 µL (corresponding to about 250 mg) of liquid ethylene oxide to the Polyethylene Glycol 200, and swirl gently to mix. Replace the stopper, reweigh the flask, and determine the amount of ethylene oxide absorbed by weight difference. Adjust the weight of the mixture with Polyethylene Glycol 200 to 100.0 g, replace the stopper, and swirl gently to mix. [NOTE—This stock solution contains 2.5 mg/g of ethylene oxide. Prepare this stock solution fresh just before use, and store in a refrigerator]~~

~~**Ethylene oxide solution:** Tare a glass-stoppered conical flask, and chill it in a refrigerator. Add 35 mL of Polyethylene Glycol 200, and reweigh the flask. Using a gas-tight gas chromatographic syringe that has been chilled in a refrigerator, transfer 1 g of the chilled *Ethylene oxide stock solution* to the tared, conical flask. Adjust the weight of the solution with Polyethylene Glycol 200 to 50.0 g, replace the stopper, and swirl gently to mix. Transfer about 10 g of this solution to a 50-mL volumetric flask. Add 30 mL of water. Dilute with water to volume to obtain a solution containing 10 µg/mL of ethylene oxide. [NOTE—Prepare this solution fresh just before use, and store in a refrigerator.]~~

~~**Dioxane solution:** 0.5 mg/mL of dioxane~~

~~**Standard solution A:** Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial, add 0.1 mL of *Solution A* and 0.1 mL of *Dioxane solution*, and seal the vial.~~

~~**Standard solution B:** Transfer 1.0 g of the substance under test to another 10-mL pressure headspace vial, add 0.1 mL of *Ethylene oxide solution*, 0.1 mL of *Dioxane solution*, and 1.0 mL of *N,N*-dimethylacetamide, and seal the vial.~~

In-Process Revision

~~**Sample solution:** Transfer 1.0 g to a 10-mL pressure headspace vial, add 1.0 mL of *N,N*-dimethylacetamide and 0.2 mL of water, and seal the vial.~~

~~**Chromatographic system**~~

~~(See *Chromatography* ⟨621⟩*, System Suitability*.)~~

~~**Mode:** GC~~

~~[NOTE—The use of a headspace apparatus that automatically transfers a measured amount of headspace is allowed.]~~

~~**Detector:** Flame ionization~~

~~**Column:** 0.32-mm × 30-m glass or quartz capillary column bonded with a 1.0-µm layer of phase G1~~

~~**Temperature**~~

~~**Injector:** 150°~~

~~**Detector:** 250°~~

~~**Column:** See the temperature program table below.~~

~~Initial Temperature (°)~~	~~Temperature Ramp (°/min)~~	~~Final Temperature (°)~~	~~Hold Time at Final Temperature (min)~~
~~50~~	~~—~~	~~50~~	~~5~~
~~50~~	~~5~~	~~180~~	~~—~~
~~180~~	~~30~~	~~230~~	~~5~~

~~**Carrier gas:** Helium flowing with a linear velocity of 20 cm/s~~

~~[NOTE—If the headspace apparatus is used, then an injection time of 12 s and a transfer line temperature of 150° are recommended.]~~

~~**Injection size:** 1 mL~~

~~**Injection type:** Split~~

~~**Split ratio:** 20:1~~

~~**System suitability**~~

~~**Sample:** Gaseous phase of *Standard solution A*~~

~~[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.94 and 1.0, respectively.]~~

~~**Suitability requirements**~~

~~**Resolution:** NLT 2.0 between acetaldehyde and ethylene oxide~~

~~**Signal-to-noise ratio:** NLT 5 for dioxane peak~~

~~**Relative standard deviation:** NMT 15%~~

~~**Analysis**~~

~~**Samples:** Gaseous headspace of *Standard solution B* and *Sample solution*~~

~~Using a heated, gas-tight gas chromatographic syringe, separately inject equal volumes of the *Samples*, record the chromatograms, and measure the areas for the major peaks: the mean areas of the ethylene oxide and dioxane peaks of the *Sample solution* are not greater than half the mean areas of the corresponding peaks of *Standard solution B*.~~

~~Calculate the concentration of ethylene oxide, in µg/g, in the portion of the substance under test taken:~~

$$\text{Result} = A r_U / [(r_S W_U) - (r_U W_S)]$$

~~A = quantity of ethylene oxide added to *Standard solution B* (µg)~~

~~r_U = peak response of ethylene oxide from the *Sample solution*~~

~~r_S = peak response of ethylene oxide from *Standard solution B*~~

~~W_U = weights of the substance under test taken to prepare the *Sample solution* (g)~~

~~W_S = weights of the substance to prepare *Standard solution B* (g)~~

~~**Acceptance criteria:** NMT 1 µg/g~~

~~Calculate the concentration of dioxane, in µg per g, in the portion of the substance under test taken:~~

$$\text{Result} = A_D r_U / 5[(r_S W_U) - (r_U W_S)]$$

~~A_D = amount of dioxane added to *Standard solution B* (µg)~~

~~r_U = dioxane peak response from the *Sample solution*~~

~~r_S = dioxane peak response from the *Standard solution B*~~

~~W_U and W_S are as defined above.~~

~~**Acceptance criteria:** NMT 10 µg/g~~

▲• **PROCEDURE: LIMIT OF FREE ETHYLENE OXIDE AND DIOXANE**

Analysis: Proceed as directed in *Ethylene Oxide and Dioxane* ⟨228⟩*, Method I*.

Acceptance criteria

Ethylene oxide: NMT 1 µg/g (ppm)

Dioxane: NMT 10 µg/g (ppm)▲*NF29*

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 1.0, determined on 5.0 g
- **FATS AND FIXED OILS,** *Hydroxyl Value* ⟨**401**⟩**:** Within the ranges specified in the table below

Oxyethylene Units/Molecule (Nominal Value)	Hydroxyl Value
2	150–180
10	75–90
20	40–60

- **FATS AND FIXED OILS,** *Iodine Value* ⟨**401**⟩**:** NMT 2.0
- **FATS AND FIXED OILS,** *Saponification Value* ⟨**401**⟩**:** NMT 3.0, determined on 10.0 g
- **ALKALINITY**

  Sample: 2.0 g of Polyoxyl Stearyl Ether

  Analysis: Transfer the *Sample* to a hot mixture of 10 mL of alcohol and 10 mL of water. Add 0.05 mL of bromothymol blue TS. Titrate with 0.1 N hydrochloric acid to a yellow endpoint.

  Acceptance criteria: NMT 0.5 mL of 0.1 N hydrochloric acid
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 3.0%
- **APPEARANCE OF SOLUTION:** 5.0 g of Polyoxyl Stearyl Ether in 50.0 mL of alcohol. The solution is not more intensely colored than a solution prepared immediately before use by mixing 12.0 mL of ferric chloride CS, 5.0 mL of cobaltous chloride CS, and 2.0 mL of cupric sulfate CS with dilute hydrochloric acid (10 g/L) to make 50.0 mL, and diluting 12.5 mL of this solution with dilute hydrochloric acid (10 g/L) to make 100.0 mL. Make the comparison by viewing the substance and the solution downward in matched color-comparison tubes against a white surface (see *Color and Achromicity* ⟨631⟩).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool, dry place.
- **LABELING:** Label it to indicate the average nominal number of oxyethylene units.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩

~~USP Polyoxyl Stearyl Ether RS~~

▲USP Polyoxyl 2 Stearyl Ether RS

USP Polyoxyl 10 Stearyl Ether RS

USP Polyoxyl 20 Stearyl Ether RS▲*NF29*

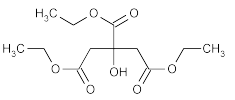
BRIEFING

Triethyl Citrate, *NF 27* page 1371. See briefing under *Acetyltributyl Citrate*.

(EM1: R. Lafaver.) RTS—C45943

In-Process Revision

Triethyl Citrate



$C_{12}H_{20}O_7$ 276.29

DEFINITION
Triethyl Citrate contains NLT 99.0% and NMT 100.5% of $C_{12}H_{20}O_7$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨**197F**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of a similar preparation of USP Triethyl Citrate RS, as obtained in the *Assay.*

ASSAY

Change to read:

- **PROCEDURE**

System suitability solution: 30 mg/mL each of USP Triethyl Citrate RS and USP Acetyltriethyl Citrate RS in toluene
Sample solution: 30 mg/mL of Triethyl Citrate in toluene
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; 0.5-µm layer of phase G42
Temperature
Injector: ~~See *Temperature program table 1.*~~ ▲225°▲*NF29*
Detector: 275°
Column: ~~See *Temperature program table 2.*~~ ▲See the temperature program table below.▲*NF29*

~~**Temperature program table 1**~~

~~Start Temperature (°)~~	~~Ramp (°)~~	~~End Temperature (°)~~	~~Hold Time (min)~~
~~85~~	~~—~~	~~85~~	~~0.5~~
~~85~~	~~20~~	~~225~~	~~10~~

~~**Temperature program table 2**~~▲*NF29*

Initial Temperature (°)	Temperature Ramp (°)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	0.5
80	20	220	~~10~~▲20▲*NF29*

Flow rate: 2.3 mL/min
Carrier gas: Helium
▲**Injection type:** Split, 30:1▲*NF29*
Injection size: 1 µL
~~**Injection type:** On-column, temperature-programmable injector~~
▲▲*NF29*
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for triethyl citrate and acetyltriethyl citrate are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between triethyl citrate and acetyltriethyl citrate
Relative standard deviation: NMT 2.0% (determined from both the triethyl citrate and acetyltriethyl citrate peaks, based on area percentage calculation)
Analysis
Sample: *Sample solution*
[NOTE—Measure all of the peak areas, excluding the solvent peak.]
Calculate the percentage of $C_{12}H_{20}O_7$ in the portion of Triethyl Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for triethyl citrate
r_T = sum of the area responses of all the peaks
Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES
Inorganic Impurities
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨**841**⟩**:** 1.135–1.139
- **REFRACTIVE INDEX** ⟨**831**⟩**:** 1.439–1.441

Change to read:

- **ACIDITY**

▲**Neutralized isopropyl alcohol:** To a suitable quantity of isopropyl alcohol add 2–3 drops of bromothymol blue TS and just sufficient 0.10 N sodium hydroxide dropwise to produce a faint blue color. [NOTE—Prepare *Neutralized isopropyl alcohol* just prior to use.]
Sample solution: 32.0 g of Triethyl Citrate in 30 mL of *Neutralized isopropyl alcohol*▲*NF29*
Analysis: ~~Dissolve 32.0 g in 30 mL of alcohol, previously neutralized to phenolphthalein, add bromothymol blue TS, and~~ ▲Add bromothymol blue TS.▲*NF29* Titrate with 0.10 N sodium hydroxide ▲to a faint blue endpoint.▲*NF29*
Acceptance criteria: NMT 1.0 mL ▲of 0.10 N sodium hydroxide▲*NF29* is required.

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.25%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Acetyltriethyl Citrate RS
USP Triethyl Citrate RS

In-Process Revision

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

BRIEFING

USP Reference Standards ⟨11⟩, *USP 32* page 35, page 3924 of the *First Supplement,* and page 507 of *PF* 31(2) [Mar.–Apr. 2005], page 1680 of *PF* 31(6) [Nov.–Dec. 2005], page 1161 of *PF* 32(4) [July–Aug. 2006], page 95 of *PF* 33(1) [Jan.–Feb. 2007], page 981 of *PF* 33(5) [Sept.–Oct. 2007], page 332 of *PF* 34(2) [Mar.–Apr. 2008], page 680 of *PF* 34(3) [May–June 2008], page 1021 of *PF* 34(4) [July–Aug. 2008], page 1230 of *PF* 34(5) [Sept.–Oct. 2008], page 1531 of *PF* 34(6) [Nov.–Dec. 2008], page 144 of *PF* 35(1) [Jan.–Feb. 2009], page 330 of *PF* 35(2) [Mar–Apr. 2009], page 612 of *PF* 35(3) [May–June 2009], page 913 of *PF* 35(4) [July–Aug. 2009], and page 1217 of *PF* 35(5) [Sept.–Oct. 2009]. With the endorsement of the Reference Standards Expert Committee (RSEC), a subcommittee has revised the general test chapter *USP Reference Standards* ⟨11⟩ to focus the chapter on information related to the use and handling of USP Reference Standards. The proposed chapter focuses on definition, required uses, and information the analyst needs to perform tests and assays in USP's compendia that require the use of USP Reference Standards. All process-oriented information has been removed from the chapter, including the role of the RSEC, internal USP processes for the development of USP Reference Standards, and historical information relating to the Reference Standards program. Current versions of chapter 11 include a listing of all USP Reference Standards and their associated chemical name and molecular weight. These will be moved to the associated monograph and removed from chapter 11 at the time the proposed revision becomes official. This is currently targeted to occur in *USP 34–NF 29*. The most current list of official USP Reference Standards is located on the USP Web site and in the USP Reference Standards Catalog. When final, the chapter will include only required information. This approach is being applied to many of USP's general test chapters [see *Stimuli* article on *General Chapter Management in the 2010–2015 Cycle* appearing in *PF* 35(5)]. As a result of this new alignment of the chapter, the RSEC has recommended the development of an above 1000 general informational chapter to include much of the removed information, with the exception of the list of Reference Standards. This new greater than 1000 chapter may be considered in the 2010–2015 cycle.

(RS: B. Jones) RTS—C74311

Change to read:

⟨11⟩ USP REFERENCE STANDARDS

~~USP Reference Standards are highly characterized specimens of drug substances, excipients, reportable impurities, degradation products, compendial reagents, and performance calibrators.~~

~~They are explicitly required in many Pharmacopeial assays and tests and are provided solely for such use. Assessment of the suitability for use in other application(s) rests with the purchaser.~~

~~AUTHORITY FOR ESTABLISHMENT AND RELEASE~~

~~USP Reference Standards are established and released under the authority of the USPC Board of Trustees upon recommendation of the USP Reference Standards Expert Committee, which approves each lot as being suitable for use in its compendial applications. For some Reference Standards a preliminary review and approval is sought from other Expert Committees of the Council of Experts.~~

~~The distribution of controlled substances is subject to the regulations and licensing provisions of the Drug Enforcement Administration of the Department of Justice.~~

~~Industry Advisory Panels and other expert groups (such as Project Teams) may be assembled to advise USP on various aspects of the Reference Standards Program.~~

~~HISTORY~~

~~Future availability of the first USP Reference Standards was announced in 1926 (*USP X*) "... in order to facilitate the adoption of the biological assay standards of the Pharmacopoeia, and to provide a greater degree of uniformity in their application." The list of USP Reference Standards that in 1936 comprised 6 items has grown to almost 1650 in 2004, and the collection has tracked the progress in pharmaceutical sciences: The first vitamins (Cod Liver Oil) and the first enzyme (Pepsin) in 1936; the first sulfonamide (Sulfanilamide) and the first hormones (Insulin; Posterior Pituitary) in 1942; the first performance standards (Melting Point Standards) in 1947; the first penicillin (Penicillin G Sodium) in 1950; the first recombinant DNA technology protein (Insulin Human) in 1985, etc.~~

~~The continuous increase in the number of USP Reference Standards (over 100 new standards are being developed yearly) reflects not only the increase in the number of monographs and General Chapters, but also the development and extensive use of modern analytical methodology (such as chromatography, spectrophotometry, biological and biochemical assays, etc.) which require measurements relative to a reference standard.~~

In-Process Revision

NOMENCLATURE

Standards designated as USP Reference Standards (USP RS) are, with a few exceptions, required for use in *USP–NF* monographs or General Chapters. The exceptions include current lots of USP and NF Reference Standards for which uses are no longer specified in the current *USP* or *NF* but for which sufficient demand remains (upon depletion of the current lots, future lots will be designated as Authentic Substances), Reference Standards specified in monographs developed by USP that are not intended for publication in the *USP–NF*, Reference Standards specified in the current edition of the *Food Chemicals Codex* (labeled with an additional designation ''FCC''), and Fluoride Dentifrices (evaluated and distributed by agreement with the FDA and the Cosmetics, Toiletries, and Fragrances Association). A USP Reference Standard required in a monograph or General Chapter proposed in *Pharmacopeial Forum* may be released in advance of the official date of the proposed *PF* revision.

Reference Standards currently labeled as ''NF Reference Standards'' will eventually all be designated and labeled as ''USP Reference Standards'' pursuant to the consolidation of *USP* and *NF* within the USPC as of January 2, 1975. Meanwhile, where a USP Reference Standard is called for, the corresponding substance labeled as an ''NF Reference Standard'' may be used.

As a service, the USPC tests and distributes additional Authentic Substances (designated by AS) not currently required for use in a USP monograph or General Chapter. These also are provided under the supervision of the USP Reference Standards Expert Committee. They are highly characterized samples of chemicals, including substances of abuse, which are collaboratively tested and made available as a service primarily to analytical, clinical, pharmaceutical, and research laboratories. Such materials may be used for identification, method development, evaluation of method performance, or other applications as found suitable and validated by the user.

Authentic Visual References

Unlike chemical reference standards, Authentic Visual References (AVRs) are not used in chemical analyses. Instead, the AVRs are visual images used by analysts to compare certain test articles to ensure that they meet compendial requirements and are incorporated by reference into the monograph. Approval of AVRs for use in a monograph is the decision of the Expert Committee that approves the specific monograph.

DIVERSITY AND IMPLICATIONS

The USP Reference Standards collection is very diverse in terms of appearance, chemical structure, composition, and uses. This diversity has significant implications for the way the materials are tested, packaged, stored, and utilized.

The USP Reference Standards may be crystalline or amorphous powders, volatile or viscous liquids, solutions or suspensions, gels or pastes, plastic sheets, etc. In chemical structure they vary from simple inorganic salts to proteins produced by recombinant DNA technology. Some are highly purified single components, while others are complex mixtures (in most cases extracted from plant or animal sources).

USES OF USP REFERENCE STANDARDS

The official and authorized uses of USP Reference Standards are specified in the USP monographs and General Chapters and they include the following:

- quantitative uses in assays for drug substances and formulations, limit tests, or blanks and controls
- qualitative uses, such as identification tests, system suitability tests, chromatographic peak markers, etc.
- performance standards and calibrators, such as dissolution calibrators, melting point standards, the particle count set, etc.

As discussed under *Nomenclature*, USP also establishes and distributes standards not specified for use in a USP monograph or General Chapter.

The most frequent applications of USP Reference Standards (USP RS) are in chromatographic and spectroscopic methodologies. However, they are also widely used in biological and biochemical applications, such as microbial assays for antibiotics, enzymatic reactions, cell culture tests, whole animal tests; in thermal analysis for polymers; and in titration, etc. Some of the most frequently used USP RS are those utilized in General Chapters tests such as *Dissolution* ⟨711⟩, *Bacterial Endotoxins Test* ⟨85⟩, *Total Organic Carbon* ⟨643⟩, and *Particulate Matter in Injections* ⟨788⟩.

STEPS IN ESTABLISHING A USP REFERENCE STANDARD

The establishment of a new USP Reference Standard is triggered by the proposal of a new monograph or of a revision of an existing monograph by the inclusion of a test requiring a new USP RS. The need for a new lot of an existing USP Reference Standard is identified when its inventory reaches a pre-established threshold. The new lot is designated as a ''replacement lot'' if a new bulk material is to be procured or as a ''continuation lot'' if the candidate material is another portion of the bulk used for the existing official lot.

USP scientists generate a set of documents including procurement specifications and a testing protocol. A bulk material is obtained, generally from a major manufacturer of the article. The material is tested and characterized in an inter-laboratory collaborative study organized according to the protocol designed at USP Headquarters. The results are evaluated by USP Staff, additional testing or investigations are performed when necessary, and a report is compiled and presented for review and approval to the USP Reference Standards Expert Committee. After approval the material is subdivided (if not packaged prior to the collaborative study), labeled, quality checks are performed, and the standard is made available for distribution. If a candidate material is found to be unsuitable by USP scientific staff or by the Reference Standards Committee, a new bulk is procured and tested.

COLLABORATIVE STUDY FOR THE EVALUATION OF A USP RS CANDIDATE MATERIAL

The goals of the evaluation study are to confirm the identity and assess the purity of the material, to determine its suitability for use in the official applications, to provide the user with all the necessary information and directions for use, and to acquire time-zero information for future continued suitability for use studies.

USP scientists design a detailed testing protocol that includes the following elements: types of tests, number of tests, number of collaborators, number of replicates, and references to the procedures to be used.

The following factors are considered when designing the study protocol: the compendial status of the standard, its official uses, the history of the standard, its composition and complexity, the characteristics of the methodology, and the availability of material and of competent laboratories.

The testing protocol may comprise visual and microscopic evaluation; identification tests (more elaborate for first-time standards); determination of physical–chemical constants (e.g., melting range, specific rotation, refractive index, specific gravity, etc); chromatographic and electrophoretic purity tests; inorganic contaminants determination; volatile tests (water, solvents); functional group analysis (such as titrations, UV absorptivity, elemental analysis); thermal analysis; and assays against another well-characterized standard (a previous lot,

In-Process Revision

an international standard, etc). Specialized testing is implemented where appropriate, such as for dissolution calibrators and the particle count set, for standards that define an attribute (negative and positive bioreaction, ion exchange capacity, permeability diameter), and for biological standards that define a Unit of activity (heparin, endotoxin, enzymes, complex antibiotics). Vapor sorption analysis may also be performed to assist in determining packaging and storage conditions, and directions for use. For lyophilized single use USP RS, acceptable vial content reproducibility and stability of the lyophilized form are demonstrated.

The number of collaborators is generally not less than three (two outside of USP); but it can increase significantly, especially when the methodology is complex or does not have a high level of precision or when potential users express an interest in participating in the evaluation of the candidate. (Participation in all evaluation studies is open to all competent, interested parties.) Where appropriate, statistical control is exercised in the design of the evaluation study and in the analysis of the results. The USP Reference Standards Laboratory and the FDA laboratories participate in almost all evaluation studies. Other collaborators include Health Canada, the USP Research and Development Laboratory, and industrial and academic laboratories from the United States and from abroad.

BIOLOGICAL REFERENCE STANDARDS

The World Health Organization, an agency of the United Nations, manages a program providing International Standards for biological materials.

USP collaborates closely with the WHO in the harmonization of analytical methodology, in the definition of the units of potency, and in some cases to share in the preparation of a reference standard. In many cases the USP Units and the International Units of potency are identical.

SUITABILITY FOR USE AND PURITY ASSIGNMENT

The data collected in the collaborative evaluation study are analyzed to determine whether the material is suitable for its monograph designated use. Characterization data and results must be considered as a whole when evaluating suitability for intended use, assignment strategy, and assigned value. For Reference Standards used in quantitative applications, this includes the determination of a calculation value to be used in the compendial utilization of the standard.

The method of choice in computing the assigned value is a mass balance analysis using independently determined components such as moisture, solvent residues, inorganic residues, chromatographic impurities, and ion content. The assay results against a previous lot or against another validated standard and the results of the functional group analysis are for confirmatory purposes only. Exceptions to the mass balance approach include many biological Reference Standards, especially those which define the Unit of activity.

The number of significant figures in the labeled calculation value is a function of the use of the standard and the number of significant figures in the acceptance range or limit. Generally, Reference Standards used in assays are labeled with three significant figures and standards used in limit tests with two significant figures. Reference Standards having multiple applications in different methodologies may require separate assay specific assignments.

The assigned value is labeled without any associated uncertainty. However, for calibration standards, the labeled value is a range, determined by a statistical analysis of the results.

Previous approaches used a purity threshold above which the content was no longer labeled, and the analyst was directed to use a default value of 100.0%. This approach is no longer in use, but older lots of standards have not been re-labeled, and users should continue to apply the default 100.0% value for compendial quantitative applications.

For antibiotics, the designation "µg/mg" is sometimes used as a unit of biological activity, and values greater than 1000 µg/mg may be assigned to some of these standards. This can happen when the first standard is assigned a value higher than its actual purity and subsequent standards of higher purity are defined relative to the previous lot. A relatively overstated assigned value can also result when less selective separation techniques are replaced with more selective modern methodologies. As a result, the original content might have been assumed to be higher than the actual level.

No value is assigned to standards having only qualitative applications.

A report compiling the results of the evaluation study and including the proposed label text is submitted for review and approval to the USP Reference Standards Expert Committee.

LABEL TEXT

The label text is designed to provide the user with all the information needed for the correct storage and usage of the Reference Standard in monograph application(s). The label includes directions for use, safety warnings, required information for controlled substances, and a calculation value for standards with quantitative applications. For calibrators, acceptance ranges are provided. Where necessary, USP Reference Standards are accompanied by additional documentation, such as Technical Sheets or Typical Chromatograms. USP generally does not provide Certificates of Analysis because all the information that the user needs for the official or authorized applications of the standard is provided in the label text and, where necessary, in the additional documentation provided.

Directions for use are lot specific, and they take precedence over any other indication in the compendium.

Material Safety Data Sheets are generated for every standard that USP distributes. They are available on the USP website.

USP REFERENCE STANDARDS EXPERT COMMITTEE

The USP Reference Standards Expert Committee comprises professionals from industry, government agencies and academia from the United States and abroad. It is organized in groups and may be assisted in the review of the evaluation studies by an Industry Advisory Panel. The approval of the evaluation report has to be unanimous.

PACKAGING

The USP Reference Standard production process operates under a registered ISO 9001:2000 Quality System and appropriate cGMP principles.

USP Reference Standards are packaged in individual units designed to maintain the integrity of the contained Reference Standard material. The packaging and storage conditions for USP Reference Standards provide protection for all materials even though the material may not need such exceptional protection due to its inherent stability. The most common packaging configurations are vials for solid materials and ampuls for liquids. The packaging environment is determined by the sensitivity of the material to light, oxidation, or atmospheric humidity, and by its toxicity. Where appropriate, containers are filled in a glove box under inert gas and in conditions of controlled low residual humidity. (The need to store such standards under inert gas protection is indicated on the label.) They may also be sealed in a foil bag as an added protective barrier. Ampuls are filled and sealed on an automated device and are typically purged with an inert gas. The most common ampul sizes are 2 mL and 5 mL. Vials may be filled by manual, semi-automated, or fully automated operations. Vials may be of different sizes depending upon the amount of material. The amount of material per individual container depends on the compendial

application of the standard and is generally sufficient for several replicates. Larger amounts are provided when additional experiments are required (such as a titrimetric determination of the water content at the time of use). In general, Reference Standard containers are slightly over filled so that the user can retrieve the labeled, nominal amount of material. Vials are closed with Teflon-lined stoppers and secured with aluminum crimps and a USP logo tamper-evident seal. Lots using prior vial closure configurations may still be in distribution.

Various considerations may determine the need to provide the standard in single-use containers, mainly for materials with significant handling issues or for those that are available only in small amounts. Such single-use containers are generally filled by lyophilization, and their content is labeled in mass or activity Units per container. If so labeled, the content of the container is to be reconstituted in its entirety without any additional weighing. Instructions for reconstitution are given either on the label or in the monographs where the standard is being used.

IMPURITY REFERENCE STANDARDS

The topic of impurities is addressed in several sections of USP, such as *General Notices,* General Chapters such as *Ordinary Impurities* ⟨466⟩, *Impurities in Official Articles* ⟨1086⟩, etc. In addition, most of the monographs for drug substances and many of those for formulations include specific tests for the identification or quantitation of impurities. Such tests generally require an official Reference Standard. The development of these impurity Reference Standards is one of the reasons for the continuous accelerated growth of the USP Reference Standards.

In many cases, the materials for impurity Reference Standards are expensive and difficult to procure. Only a limited amount of material may be available—procurement might require custom synthesis—and it may be of lesser quality than the Reference Standard for the official article, requiring purification. The limited amount of material available can affect the testing protocol and the packaging. Impurity Reference Standards might be available as purified single-component materials, solutions, or solid dispersions, or mixtures of more than one impurity. Other options include samples of the official article with a labeled content of impurity(ies), the in situ generation of the impurity from the official article by a validated specified procedure, the use of relative chromatographic mobilities and relative response factors, or of theoretical values such as UV absorptivities at selective wavelengths.

In earlier editions of the compendium, the impurities were designated by their chemical names. For ease of indexing and searching, these have been gradually replaced with the designation "X Related Compound Y RS," where X is the name of the official article and Y is a sequential alphabetical letter. Reference Standard impurity mixtures might be designated by their use, such as "X System Suitability RS". The conventional names and the chemical names are cross-referenced in the final section of this chapter and in a special section of the Official USP Reference Standards Catalog.

CONTINUED SUITABILITY FOR USE PROGRAM

To ensure that the Reference Standards maintain the properties determined at the initial evaluation, USP maintains a Continued Suitability for Use Program. The retesting intervals and protocols are a function of the uses and properties of the standard and of the information available about its stability. Abbreviated protocols use the stability-indicating methodology employed in the initial characterization of the material to confirm the consistency of attributes such as appearance, chromatographic purity, or volatiles content.

PROPER USE

Neither the Reference Standards nor the Authentic Substances are intended for use as drugs or as medical devices.

USP Reference Standards do not carry an expiration date as long as they are in distribution. A lot of USP RS may be used in its official applications as long as it is listed as "Current Lot" in the current (most recent) Official USP Reference Standards Catalog. Upon depletion, the lot is designated in the catalog as "Previous Lot" and a "Valid Use Date" is assigned. USP publishes the Official Catalog of Reference Standards (which also includes Authentic Substances) bimonthly as a separate brochure*. An updated version of the catalog can be found on the USP website at www.usp.org. It is the responsibility of the user to ascertain that a particular supply of USP Reference Standard has official status either as a "Current Lot" or as a "Previous Lot" within the valid use date.

Many Pharmacopeial tests and assays are based on comparison of a test specimen with a USP Reference Standard. In such cases, measurements are made on preparations of both the test specimen and the Reference Standard. Where it is directed that a Standard solution or a Standard preparation be prepared for a quantitative determination by stepwise dilution or otherwise, it is intended that the Reference Standard substance shall be accurately weighed (see *Weights and Balances* ⟨41⟩ and *Volumetric Apparatus* ⟨31⟩). Due account should also be taken of the relatively large errors associated with weighing small masses (see also *Dilution* under *Tests and Assays* in the *General Notices and Requirements*).

The label text provides the user with directions on the proper use of a Reference Standard. The directions include one of the following options. A Reference Standard may be used as follows:

- As is, i.e., without any prior treatment or correction for volatiles. This is the preferred option, and it is selected whenever validated data show that the volatiles content is constant over time.
- Immediately after a prior drying under stated conditions. Drying should not be performed in the original container. A portion of the material should be transferred to a separate drying vessel.
- With a correction for the water content or the loss on drying determined on a separate portion of material. Where the titrimetric determination of water is required at the time a Reference Standard is to be used, proceed as directed for *Method I* under *Water Determination* ⟨921⟩. Instrumental or microanalytical methods are acceptable for this purpose. When using typical amounts (about 50 mg of the Reference Standard), titrate with a two- to five-fold dilution of the reagent. Where the determination of the loss on drying on a separate portion of USP Reference Standard is required, proceed as directed on the label. Smaller sample sizes than those required in General Chapter *Loss on Drying* ⟨731⟩ may be used for a USP Reference Standard, provided that the user can obtain a sufficiently accurate result.

Whenever the labeled directions for use require a preliminary drying or a correction for volatiles, it should be performed "at the time" of use. Further experimental details should be controlled by the user's Standard Operating Procedures and good laboratory practices.

STORAGE

To serve its intended purpose, each USP Reference Standard must be properly stored, handled, and used. Generally, Reference Standards should be stored in their original stoppered containers away from heat and protected from light. Avoid humid storage areas in particular. Where special storage conditions are necessary, directions are given on the label.

* For nonsubscribers, the most recent Official Catalog is available from: U.S. Pharmacopeial Convention, Inc., Reference Standards Order Department, 12601 Twinbrook Parkway, Rockville, MD 20852. Telephone 1-301-881-0666. FAX 1-301-816-8148. Toll free telephone 1-800-227-USPC or access the Catalog on USP's website www.usp.org/dsd/refstd.

In-Process Revision

~~RELATIONSHIPS WITH OTHER STANDARDS-SETTING ORGANIZATIONS~~

~~USP maintains continuous contact with other organizations that establish Reference Materials for compendial and other purposes, such as the European and the Japanese Pharmacopoeias (through the Pharmacopeial Discussion Group), the World Health Organization, the National Institute for Science and Technology, the Reference Materials Committee of ISO (REMCO), etc.~~

~~The specific nature of pharmacopeial reference substances has been officially recognized by ISO REMCO in the introduction of the ISO Guide 34—General requirements for the competence of reference material producers (Second Edition 2000): "Pharmacopoeial standards and substances are established and distributed by pharmacopoeial authorities following the general principle of this guide. It should be noted, however, that a different approach is used by the pharmacopoeial authorities to give the user the information provided by certificate of analysis and the expiration dates. Also, the uncertainty of their assigned values is not stated since it is negligible in relation to the defined limits of the method-specific assays of the pharmacopoeias for which they are used."~~

~~The *USP Reference standards* section of an individual *USP* or *NF* monograph or general chapter names each USP Reference Standard required for assay and test procedures and refers to this chapter for additional information and instructions. It is especially important to refer to the current Supplement to *USP* and to *NF* for official revisions listed in the following section.~~

~~USP REFERENCE STANDARDS SPECIFIED IN USP AND NF MONOGRAPHS AND GENERAL CHAPTERS~~

~~NOTE—Consult the latest Supplement or *Interim Revision Announcement* pertaining to *USP* and to *NF* for revisions, additions, or deletions.~~

~~Revisions, additions, and deletions of individual USP Reference Standards are listed cumulatively in each Supplement to *USP–NF*. As a consequence, therefore, it is necessary to consult only the current edition of *USP–NF* and the latest Supplement for the complete list of USP Reference Standards currently specified in *USP–NF* monographs and general chapters. The list provides up-to-date and complete names and applicable chemical information for the USP Reference Standards that are in distribution as of the official date of that Supplement.~~

~~Revisions of this chapter are implemented continuously via the *Interim Revision Announcements* that are published in *Pharmacopeial Forum*. Those interim revisions of USP Reference Standards are cumulatively included in the next *USP–NF* Supplement.~~

~~The alphabetical list that follows constitutes an index of all revisions to this chapter. Thus, it is unnecessary to name repetitively the revised Reference Standards in the general index to the Supplement.~~

~~In the list that follows, chemical names are given for many substances (e.g., related compounds) that are not *USP* or *NF* monograph articles. Following the name of such a chemical substance RS, the empirical formula and molecular weight, separated by the ✧ symbol, may be given in parentheses if those data are available.~~

NOTE—For reader's ease of viewing, the pages of the list of crossed off USP Reference Standards are not printed; however, the complete document will be displayed for the online version of *PF*.

▲Reference Standards provided by the United States Pharmacopeial Convention (USP Reference Standards, or RS) are highly characterized specimens reflective of specified drugs and foods (drug substances, biologics, excipients, dietary supplements, food ingredients, impurities, degradation products, reagents, and performance verification standards). When approved as suitable for use as comparison standards for documentary tests or assays (i.e., as a monograph component) in the *United States Pharmacopeia (USP)* or *National Formulary (NF)*, USP RS also assume official status and legal recognition in the United States. Assessment of the suitability for use in other applications rests with the user. Official USP RS are primary standards in jurisdictions that so recognize them as such and, when appropriate, are calibrated relative to international reference materials such as those provided by the World Health Organization. USP RS are never intended for therapeutic use. USP's RS are provided for legal metrology purposes and can help ensure comparability of results and traceability to Systéme International d'Unités (SI) units whether certified or not. USP RS are Reference Materials as defined in the *International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM)*: 3rd Edition 2007.

TYPES OF REFERENCE STANDARDS

Reference Standards for *USP* or *NF* Articles

Reference Standards for official articles in *USP* or *NF* are provided as pure materials or as mixtures of chemicals reflective of the corresponding drug substances or excipients. The use of these materials is specified in the article's monograph, and these materials generally are necessary for use in the *Assay* and/or the *Identification* tests. The suitability of a USP RS for uses outside those specified in a monograph is the responsibility of the user. The property value or calculation value of the Reference Standard is stated on the label and should be included in calculations

used in the monograph and applicable general chapters. For Reference Standards that do not bear a property value or calculation value on the label or in accompanying documentation, assume the Reference Standard is 100.0% pure for compendial quantitative applications.

Impurity Reference Standards

Reference Standards for impurities may include the following:

- Organic impurities that may arise either during the manufacturing process or during the shelf-life storage of an article and may include starting materials, intermediates, by-products, reagents, catalysts, and/or degradation products.
- Inorganic impurities that normally result from a synthesis process and may include reagents, catalysts, heavy metals, or inorganic salts
- Residual solvents that may be either inorganic or organic liquids that are used to prepare solutions or suspensions during the synthesis of an article

Impurity Reference Standards may be presented as purified single-component materials or as mixtures of more than one impurity. Other options for controlling impurities may include presenting the official article with a labeled impurity content; using relative chromatographic retention times and response factors; or providing theoretical values such as UV absorptivities at selected wavelengths.

In earlier editions of the compendium, impurities were designated by their chemical names. For ease of indexing and searching, these have been gradually replaced with the designation ''X-related compound Y RS'' where X is the name of the official article and Y is a sequential alphabetical letter. The assignment of this letter does not necessarily match the naming schemes of other compendia. Reference Standard impurity mixtures may also be designated by their intended use, such as ''X System Suitability RS''. The conventional names and the chemical names are reproduced in the catalog and on the RS product label.

Certified Reference Materials

USP's Certified Reference Materials (CRMs) are Reference Standards that provide certified property values with associated uncertainties and metrological traceability, in accordance with International Organization for Standardization (ISO) Guides 30-35. Correct use of these CRMs support traceability of results to SI units and comparability of procedures.

USP Reference Standards for Biologicals

USP provides RS for biologic drugs and ancillary materials. For historical and other reasons, and as noted in Section 5.50.10 of the *General Notices and Requirements*, USP RS for biologicals may diverge in unitage, by definition, or otherwise from other internationally recognized standards. Unless so noted in the documentary standard, international reference standards generally are not interchangeable and the USP RS is required in the tests and assays of *USP–NF*.

NF Reference Standards

Reference Standards currently labeled as ''NF Reference Standards'' are intended to be designated and labeled as ''USP Reference Standards'' pursuant to the consolidation of *USP* and *NF* within the USP as of January 2, 1975. Where a USP Reference Standard is called for, the corresponding substance labeled as an ''NF Reference Standard'' may be used.

Transition of Authentic Substances to USP Reference Standards

Previously, highly characterized reference materials not required for use in a *USP–NF* monograph or general chapter were developed by USP as a service and were distributed as Authentic Substances (AS). AS typically are highly characterized chemicals that are collaboratively tested and made available as a service primarily to analytical, clinical, pharmaceutical, and research laboratories. Such materials may be used for identification, method development, evaluation of method performance, or other applications as found suitable and validated by the user. USP will no longer introduce materials labeled ''Authentic Substances.'' All reference materials released, whether or not required for use in a *USP–NF* monograph or general chapter, will be ''USP Reference Standards.''

Authentic Visual References

Authentic Visual References are USP Reference Standards, but unlike chemical reference materials, Authentic Visual References (AVR) are not used in chemical analyses. Instead, AVR are visual images used by analysts to compare certain test articles to ensure that they meet compendial requirements. AVR are incorporated by reference into the monograph.

USP Performance Verification Test Standards

These materials are provided to analyze and where appropriate to facilitate adjustment of the operation of an instrument to ensure that the results obtained are accurate and/or precise or otherwise give acceptable results. The use of these Reference Standards is generally described in associated general test chapters and allied information.

APPLICATIONS OF USP REFERENCE STANDARDS

Official applications of USP RS are specified in *USP–NF* monographs and general chapters. They include the following:

- quantitative uses in assays for drug substances and formulations, limit tests, or blanks and controls
- qualitative uses, (e.g., identification tests, system suitability tests, or chromatographic peak markers)
- method-specific uses, (e.g., performance verification standards, AVR, melting point standards, and the particle count set)

As described above, USP also provides Authentic Substances, not specified for use in a *USP* monograph or general chapter, which are used at the user's discretion.

PACKAGING

The amount of material per individual USP RS container depends on the compendial application of the standard and is generally sufficient for several replicates. Some standards (mainly materials with significant handling requirements or materials that are available only in small amounts) are provided in single-use containers. Such single-use products generally are lyophilized, and their content is labeled in mass or activity units per container. If so labeled, the content of the container should be reconstituted in its entirety without any additional weighing. Instructions for reconstitution are given either on the label or in the monographs where the standard is used.

LABELING

The label text provides all the information needed for the correct storage and use of the USP RS in monograph applications. The label includes directions for use, safety warnings, required information for controlled substances, and a property value or calculation value for standards with quantitative applications. For performance verifica-

tion standards, acceptance ranges are provided. Where necessary, USP RS are accompanied by additional documentation such as Technical Data Sheets or Typical Chromatograms.

Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP RS should be used in accordance with the instructions on the label of the Reference Standard. Material Safety Data Sheets for all USP reference materials are available on the USP Web site.

Although USP RS undergo retesting on a predefined schedule to determine continued suitability for use, USP RS do not carry an expiration date on the label. A lot of USP RS may be used in its official applications as long as it is listed as ''Current Lot'' in the current USP Reference Standards Catalog or has not reached its Valid Use Date. Upon depletion, the lot is designated in the catalog as ''Previous Lot'' and a ''Valid Use Date'' is assigned. USP publishes the Catalog of Reference Standards bimonthly. The most current version of the catalog can be found on the USP Web site at www.usp.org. The user is responsible for ascertaining before use that the USP RS lot of interest currently carries official status, either as a ''Current Lot'' or as a ''Previous Lot'' within the Valid Use Date.

PROPER USE

Many compendial tests and assays are based on comparison of a test specimen with a USP RS. In such cases, measurements are made on preparations of both the test specimen and the Reference Standard. Where it is directed that a Standard solution or a Standard preparation be prepared for a quantitative determination by stepwise dilution or otherwise, it is intended that the Reference Standard substance be accurately weighed (see *Weights and Balances* ⟨41⟩ and *Volumetric Apparatus* ⟨31⟩. Due account should also be taken of the potential errors associated with weighing small masses (see also *Dilution* under *Tests and Assays* in the *General Notices and Requirements*). Reference Standards that are defined on a content-per-container basis are an exception, as noted above.

USP RS instructions for use include the following:

- **As Is**: Use without any prior treatment or correction for volatiles. This is the preferred option, and is selected whenever valid data indicate that the volatiles content is constant over time.
- **Dry Before Use**: Use immediately after drying under stated conditions. Drying should not be performed in the original container. A portion of the material should be transferred to a separate drying vessel.
- **Determine Water Content Titrimetrically At Time of Use**: Use with a correction for the water content or the loss on drying, determined on a separate portion of material. Where the titrimetric determination of water is required at the time a Reference Standard is to be used, proceed as directed for *Method I* under *Water Determination* ⟨921⟩. Instrumental or microanalytical methods are acceptable for this purpose. When using typical amounts (about 50 mg of the Reference Standard), titrate with a 2- to 5-fold dilution of the reagent. Where the determination of the loss on drying on a separate portion of USP RS is required, proceed as directed on the label. Sample sizes smaller than those required in the general test chapter *Loss on Drying* ⟨731⟩ may be used for a USP RS provided that the user can obtain a sufficiently accurate result.

Whenever the labeled directions for use require drying or a correction for volatiles, it should be performed at the time of use. Further experimental details should be controlled by the user's Standard Operating Procedures and good laboratory practices.

STORAGE

USP RS should be stored in the packaging configuration provided by USP (e.g., vials that are packaged in hermetically sealed bags). When special storage conditions are specified, label directions should be followed. Unopened vials should be stored as indicated on the label. The user is responsible for ensuring that the contents of opened vials continue to be suitable for their intended use and that value assignment and uncertainty information are maintained.▲*USP34*

OTHER TESTS AND ASSAYS

BRIEFING

⟨**521**⟩ **Sulfonamides,** *USP 32* page 177. It is proposed to delete this general test chapter from *USP–NF* as it is not referenced in any *USP* monograph.

(MD-AA: B. Davani) RTS—C76341

Delete the following:

▲⟨521⟩ SULFONAMIDES

Identification of Individual Sulfonamides in Mixed Sulfonamides

NOTE—The following instructions for preparations and procedure are applicable to all sulfonamides except sulfadiazine. When testing for sulfadiazine proceed in the same manner, except to use sulfadiazine preparations having one-half the designated concentration, and apply twice the designated volumes of sulfadiazine preparations to the chromatographic plates.

Standard Preparation—Transfer a quantity of the pertinent USP Reference Standard to a suitable glass-stoppered, conical flask, dissolve in methanol to obtain a solution having a concentration of about 2 mg per mL, and mix. A separate *Standard Preparation* is required for each sulfonamide present in mixed sulfonamides.

Test Preparation—Transfer a portion of the thoroughly mixed suspension or finely powdered tablets, equivalent to about 100 mg of each sulfonamide, to a 50-mL volumetric flask containing 10 mL of ammonia TS, and swirl. Add methanol to volume, mix, filter, and use the filtrate in the *Procedure*.

Preparation of Chromatographic Plates—Prepare three identical chromatographic plates according to the following directions. Apply separately, and 2 cm apart along a spotting line 1.5 cm from the bottom of the plate and parallel to it, 2 µL of each *Standard Preparation* and 2 µL of the *Test Preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. On another spot, 2 cm along the spotting line from the application of the *Test Preparation*, apply, successively, 2 µL of each *Standard Preparation* to obtain a mixed standard. Dry the spots immediately with the aid of a stream of nitrogen.

Procedure—Prepare a chromatographic chamber lined with filter paper and containing a solvent system consisting of ethyl acetate, methanol, and a 1 in 4 aqueous solution of ammonium hydroxide (17 : 6 : 5), and allow to equilibrate for 1 hour. Similarly prepare a second chamber to contain a solvent system consisting of solvent hexane, chloroform, and butyl alcohol (1 : 1 : 1), and a third chamber to contain a solvent system consisting of chloroform and methanol (95 : 5). Place one prepared chromatographic plate in each equilibrated chamber, and develop the chromatograms until the solvent front has moved about three-fourths of the length of each plate. Remove each plate from its developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plates by viewing under short-wavelength UV light. Spray the plates with a 1 in 100 solution of *p*-dimethylaminobenzaldehyde in dilute hydrochloric acid (1 in 20), and heat at 110° for 5 minutes or until bright yellow spots become visible. The R_f values of the yellow spots obtained from each *Test Preparation* correspond to those obtained from the mixed *Standard Preparations* on the respective plates. The individual sulfonamides may be identified by comparison of the R_f values of the yellow spots obtained from the *Test Preparations* and individual *Standard Preparations* on the respective plates.

Determination of Individual Sulfonamides in Mixed Sulfonamides

Standard Preparation—A separate *Standard Preparation* is required for each sulfonamide being determined. Transfer about 50 mg, accurately weighed, of the pertinent USP Reference Standard to a 50-mL volumetric flask containing 1.5 mL of ammonium hydroxide, add methanol, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 100) to volume, and mix. [NOTE—Retain the methanol solutions for the *Mixed Standard Preparation*. The methanol solutions are stable for at least 1 week, and the acid solutions for at least 1 month.]

Mixed Standard Preparation—Transfer 1.0 mL of each methanol solution, prepared as required for each *Standard Preparation*, to a small glass-stoppered flask, and mix. [NOTE—This Standard is used to identify the components of the *Assay Preparation* on the chromatogram.]

Assay Preparation—Prepare as directed in the individual monograph.

Procedure—Prepare the necessary number of chromatographic sheets (Whatman No. 1 filter paper, or equivalent), about 20 × 20 cm in size, by drawing a pencil line parallel to and 2.5 cm from one edge of the paper. Mark the line at points 2.5 and 5 cm from each edge of the paper. Impregnate the paper by dipping it in the immobile solvent (prepared fresh by dissolving 30 mL of redistilled formamide in 70 mL of acetone) for 30 seconds. Remove the paper, drain for 10 seconds, and blot between filter paper. Place the impregnated paper on dry filter paper, and air-dry for 3 to 5 minutes. With a micropipet, and with repeated applications, streak 100 µL of the *Assay Preparation* along the starting line, applying the volume in five streaks of about 20 µL each and evaporating the solvent with a gentle stream of nitrogen between applications. [NOTE—Make the streak as narrow as possible along the starting line, and keep within the 5-cm border.] Rinse the tip of the pipet with a drop of methanol–ammonia TS mixture (9 : 1), and then streak the rinse along the starting line between the 5- and 2.5-cm points at the right edge. Repeat the rinsing with two additional drops, and then blow out the pipet.

Apply 10 µL of the *Mixed Standard Preparation* at the mark 2.5 cm from the left edge.

In-Process Revision

~~Place 50 mL of methylene chloride (mobile solvent) in a tray in a 23 × 23 × 7.5 cm chromatographic chamber arranged for ascending chromatography (see *Chromatography* ⟨621⟩), and allow the chamber to equilibrate for about 15 minutes. Remove the cover, place from 7 to 10 mL of water in a second tray, and without delay, suspend the prepared chromatographic paper sheet so that it dips into the mobile solvent. Cover and seal the chamber, and allow the chromatogram to develop for 1 hour. Remove the paper from the chamber, and allow to air-dry for 5 minutes. Place the chromatogram on a dry sheet of filter paper, and view it under short-wavelength UV light. [NOTE—Conduct the following identification and marking without delay to avoid excessive exposure of the sulfonamide spots to UV irradiation.] Identify and mark the respective spots by matching R_F values with those of the spots produced by the *Mixed-Standard Preparation*. [NOTE—Sulfadiazine and sulfamerazine are chromatographed with increasing R_F, respectively.]~~

~~Cut the marked zones from the paper, cut each zone into five or six pieces, and place the pieces from each spot in separate, glass-stoppered, 50-mL flasks. Add 20.0 mL of dilute hydrochloric acid (1 in 100) to each flask, and allow to stand for about 30 minutes, swirling each flask at least five times during this period. Filter the solutions through dry glass wool into separate test tubes, discarding the first 5 mL of the filtrate. Transfer 5.0 mL of the subsequent filtrate from each solution into separate 10-mL volumetric flasks. Transfer 3.0 mL of each required *Standard Preparation* into separate, 10-mL volumetric flasks. To each flask, and to a blank flask containing 5 mL of dilute hydrochloric acid (1 in 100), add 1.0 mL of sodium nitrite solution (1 in 1000) and 0.10 mL of hydrochloric acid, and allow to stand for 5 minutes with frequent swirling. To each flask add 1.0 mL of ammonium sulfamate solution (1 in 200), and allow to stand for 5 minutes, swirling frequently. Finally, to each flask add 1.0 mL of freshly prepared *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), mix, dilute with water to volume, and mix. Allow each solution to stand between 15 and 60 minutes, and then concomitantly determine the absorbances of the solutions, in 1-cm cells, recording the spectra from 440 to 700 nm, with a suitable spectrophotometer, using the blank to set the instrument. Draw a baseline, and determine the corrected absorbance for each solution at the wavelength of maximum absorbance at about 545 nm.~~

~~Calculate the concentration, in mg per mL, of each sulfonamide in the *Assay Preparation* by the formula:~~

$$0.12C(A_U/A_S)$$

~~in which C is the concentration, in µg per mL, of the pertinent USP Reference Standard in the *Standard Preparation*; A_U is the corrected absorbance of the *Assay Preparation*; and A_S is the corrected absorbance of the pertinent *Standard Preparation*. From the concentration of the *Assay Preparation* thus determined, and applying appropriate dilution factors, calculate the percentage of sulfonamide in the specimen taken.~~ ▲*USP34*

Physical Tests and Determinations

BRIEFING

⟨**621**⟩ **Chromatography,** *USP 32* page 227, page 3952 of the *First Supplement*, and page 1238 of *PF* 34(5) [Sept.–Oct. 2008]. The General Chapters Expert Committee proposes a revised version of *Chromatography* ⟨621⟩.

The revision retains in ⟨621⟩ all the required critical information needed in order to perform a monograph procedure (e.g., definitions, calculations, interpretation of chromatograms) and eliminates descriptive or noncritical information (e.g., theory of chromatography). The revision is also harmonized, to the extent possible, with the equivalent chapter in the *European Pharmacopoeia* (*Eur. Ph.*), 2.2.46, *Chromatographic Separation Techniques*.

The primary proposed changes are as follows:

1. A *General Procedures* section is added to describe basic procedures to be used when a monograph requires a chromatographic method.
2. A section titled *Definitions and Interpretation of Chromatograms* is added to define the main chromatographic terms and their application in *USP* monographs. To the extent possible, the symbols of the International Union of Pure and Applied Chemistry (IUPAC) are adopted. In this section, subsection changes are as follows:
   - In *Number of Theoretical Plates*, measuring column efficiency, and the section *Resolution*, calculations based on the tangent method are deleted, and calculations based on the half-height of the peak are provided.
   - In *Peak-to-Valley Ratio*, a definition and the calculation are aligned with *Eur. Ph.*
3. A section titled *Quantitation* is added to describe basic quantitation procedures.
4. In the section *System Suitability,* the revision adopts the procedure from *Eur. Ph.* to calculate repeatability requirements when they are not stated in monographs.

The General Chapters Expert Committee is considering the disposition of the information about different types of chromatography that has been removed from the chapter. This information is neither critical nor intended to be enforceable. The committee requests input from stakeholders regarding whether this noncritical information should be moved to chapters with numbers greater than ⟨1000⟩ to provide background for Chapter ⟨621⟩; or whether, considering the broad nature of the information and the availability of source information outside the compendia, the information should simply be removed from *USP–NF*.

Readers are encouraged to submit their comments and opinions.

NOTE—Because the large number of crossed-off pages would present the reader with the task of searching through them to review the revised text, the printed pages in this *PF* represent only the added proposed revision of ⟨621⟩. The complete text of this proposal is available using the online electronic version of *PF* 35(6).

(GC: H. Pappa) RTS—C77481

Change to read:

⟨621⟩ CHROMATOGRAPHY

▲INTRODUCTION

Chromatographic separation techniques are multistage separation methods in which the components of a sample are distributed between two phases, of which one is stationary and the other mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, distributed as a film, or applied

In-Process Revision

by other techniques. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), or ion exchange; or it may be based on differences among the physicochemical properties of the molecules, such as size, mass, and volume. This chapter contains general procedures, definitions, and calculations of common parameters and describes general requirements for system suitability. The types of chromatography useful in qualitative and quantitative analysis employed in *USP* procedures are column, gas, paper, thin-layer (including high-performance thin-layer chromatography), and pressurized liquid chromatography (commonly called high-pressure or high-performance liquid chromatography).

GENERAL PROCEDURES

This section describes the basic procedures used when a chromatographic method is described in a monograph. The following procedures are followed unless otherwise indicated in the individual monograph.

Paper Chromatography

Stationary Phase: The stationary phase is a sheet of paper of suitable texture and thickness. Development may be ascending, in which the solvent is carried up the paper by capillary forces, or descending, in which the solvent flow is also assisted by gravitational force. The orientation of paper grain with respect to solvent flow is to be kept constant in a series of chromatograms. (The machine direction is usually designated by the manufacturer.)

Apparatus: The essential equipment for paper chromatography consists of a vapor-tight chamber with inlets for addition of solvent and a rack of corrosion-resistant material about 5 cm shorter than the inside height of the chamber. The rack serves as a support for solvent troughs and for antisiphon rods that, in turn, hold up the chromatographic sheets. The bottom of the chamber is covered with the prescribed solvent system or mobile phase. Saturation of the chamber with solvent vapor is facilitated by lining the inside walls with paper wetted with the prescribed solvent system.

Spotting: The substance or substances analyzed are dissolved in a suitable solvent. Convenient volumes, delivered from suitable micropipets, of the resulting solution, normally containing 1–20 μg of the compound, are placed in 6- to 10-mm spots not less than 3 cm apart.

Descending Paper Chromatography Procedure

(1) A spotted chromatographic sheet is suspended in the apparatus, using the antisiphon rod to hold the upper end of the sheet in the solvent trough. [NOTE—Ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack, the chamber walls, or the fluid in the chamber.]
(2) The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
(3) After equilibration of the chamber, the prepared mobile phase is introduced into the trough through the inlet.
(4) The inlet is closed, and the mobile solvent phase is allowed to travel the desired distance down the paper.
(5) The sheet is removed from the chamber.
(6) The location of the solvent front is quickly marked, and the sheet is dried.
(7) The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated drug or drugs.

Ascending Paper Chromatography Procedure

(1) The mobile phase is added to the bottom of the chamber.

(2) The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
(3) The lower edge of the stationary phase is dipped into the mobile phase to permit the mobile phase to rise on the chromatographic sheet by capillary action.
(4) When the solvent front has reached the desired height, the chamber is opened, the sheet is removed, the location of the solvent front is quickly marked, and the sheet is dried.
(5) The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated drug or drugs.

Thin-Layer Chromatography

Stationary Phase: The stationary phase is a relatively thin, uniform layer of dry, finely powdered material applied to a glass, plastic, or metal sheet or plate (typically called the plate). The stationary phase of TLC plates has an average particle size of 10–15 μm, and that of high-performance TLC (HPTLC) plates has an average particle size of 5 μm. Commercial plates with a preadsorbent zone can be used if they are specified in a monograph. Sample applied to the preabsorbent region develops into sharp, narrow bands at the preabsorbent–sorbent interface. The separations achieved may be based on adsorption, partition, or a combination of both effects, depending on the particular type of stationary phase.

Apparatus: A chromatographic chamber made of inert, transparent material and having the following specifications is used: a flat-bottom or twin trough, a tightly fitted lid, and a size suitable for the plates. The chamber is lined on at least one wall with filter paper. Sufficient mobile phase or developing solvent is added to the chamber that, after impregnation of the filter paper, a depth appropriate to the dimensions of the plate used is available. The chromatographic chamber is closed and allowed to equilibrate. [NOTE—Unless otherwise indicated, the chromatographic separations are performed in a saturated chamber.]

Detection/Visualization: An ultraviolet (UV) light source suitable for observations under short- (254 nm) and long- (365 nm) wavelength UV light and a variety of other spray reagents used to make spots visible are often used.

Spotting: Solutions are spotted on the surface of the stationary phase (plate) at the prescribed volume in sufficiently small portions to obtain circular spots of 2–5 mm in diameter (1–2 mm on HPTLC plates) or bands of 10–20 mm × 1–2 mm (5–10 mm × 0.5–1 mm on HPTLC plates) at an appropriate distance from the lower edge of and sides of the plate. [NOTE—During development, the application position must be at least 5 mm (TLC) or 3 mm (HPTLC) above the level of the mobile phase.] The solutions are applied on a line parallel to the lower edge of the plate with an interval of at least 10 mm (5 mm on HPTLC plates) between the centers of spots, or 4 mm (2 mm on HPTLC plates) between the edges of bands, then allowed to dry.

Procedure

(1) Place the plate in the chamber, ensuring that the spots or bands are above the surface of the mobile phase.
(2) Close the chamber.
(3) Allow the mobile phase to ascend the plate until the solvent front has traveled three-quarters of the length of the plate, or the distance prescribed in the monograph.
(4) Remove the plate, mark the solvent front with a pencil, and allow to dry.
(5) Visualize the chromatograms as prescribed.
(6) Determine the chromatographic retardation factor (R_F) values for the principal spots or zones.

In-Process Revision

(7) Presumptive identification can be made by observation of spots or zones of identical R_F value and about equal magnitude obtained, respectively, with an unknown and a standard chromatographed on the same plate. A visual comparison of the size or intensity of the spots or zones may serve for semiquantitative estimation. Quantitative measurements are possible by means of densitometry (absorbence or fluorescence measurements).

Column Chromatography

Solid Support: Purified siliceous earth is used for normal-phase separation. Silanized chromatographic siliceous earth is used for reverse-phase partition chromatography.

Stationary Phase: The solid support is modified by the addition of a stationary phase specified in the individual monograph. If a mixture of liquids is used as the stationary phase, mix the liquids before the introduction of the solid support.

Mobile Phase: The mobile phase is specified in the individual monograph. If the stationary phase is an aqueous solution, equilibrate with water. If the stationary phase is a polar organic fluid, equilibrate with that fluid.

Apparatus: Unless otherwise specified in the individual monograph, the chromatographic tube is about 22 mm in inside diameter and 200–300 mm long. Attached to it is a delivery tube, without stopcock, about 4 mm in inside diameter and about 50 mm long.

APPARATUS PREPARATION: Pack a pledget of fine glass wool in the base of the tube. Combine the specified volume of stationary phase and the specified amount of solid support to produce a homogeneous, fluffy mixture. Transfer this mixture to the chromatographic tube, and tamp, using gentle pressure, to obtain a uniform mass. If the specified amount of solid support is more than 3 g, transfer the mixture to the column in portions of approximately 2 g, and tamp each portion. If the assay or test requires a multisegment column with a different stationary phase specified for each segment, tamp after the addition of each segment, and add each succeeding segment directly to the previous one. Pack a pledget of fine glass wool above the completed column packing. [NOTE—The mobile phase should flow through a properly packed column as a moderate stream or, if reverse-phase chromatography is applied, as a slow trickle.]

If a solution of the analyte is incorporated into the stationary phase, complete the quantitative transfer to the chromatographic tube by scrubbing the beaker used for the preparation of the test mixture with a mixture of about 1 g of *Solid Support* and several drops of the solvent used to prepare the sample solution before adding the final portion of glass wool.

Procedure

(1) Transfer the mobile phase to the column space above the column packing, and allow it to flow through the column under the influence of gravity.

(2) Rinse the tip of the chromatographic column with about 1 mL of mobile phase before each change in composition of mobile phase and after completion of the elution.

(3) If the analyte is introduced into the column as a solution in the mobile phase, allow it to pass completely into the column packing, then add mobile phase in several small portions, allowing each to drain completely, before adding the bulk of the mobile phase.

(4) Where the procedure indicates the use of multiple chromatographic columns mounted in series and the addition of mobile phase in divided portions is specified, allow each portion to drain completely through each column, and rinse the tip of each with mobile phase before the addition of each succeeding portion.

Gas Chromatography (GC)

Liquid Stationary Phase: This type of phase is available in packed or capillary columns.

Packed Column GC: The liquid stationary phase is deposited on a finely divided, inert solid support, such as diatomaceous earth, porous polymer, or graphitized carbon, which is packed into a column that is typically 2–4 mm in internal diameter and 1–3 m in length.

Capillary Column GC: In capillary columns, which contain no packed solid support, the liquid stationary phase is deposited on the inner surface of the column and may be chemically bonded to it.

Solid Stationary Phase: This type of phase is available only in packed columns. In these columns the solid phase is an active adsorbent, such as alumina, silica, or carbon, packed into a column. Polyaromatic porous resins, which are sometimes used in packed columns, are not coated with a liquid phase. [NOTE—Packed and capillary columns must be conditioned before use until the baseline and other characteristics are stable. The column or packing material supplier provides instructions for the recommended conditioning procedure.]

Apparatus: A gas chromatograph consists of a carrier gas source, injection port, column, detector, and recording device. The injection port, column, and detector are temperature controlled and may be varied as part of the analysis. The typical carrier gas is helium, nitrogen, or hydrogen, depending on the column and detector in use. The type of detector used depends on the nature of the compounds analyzed and is specified in the individual monograph. Detector output is recorded as a function of time, and the instrument response, measured as peak area or peak height, is a function of the amount present.

Temperature Program: The length and quality of a GC separation can be controlled by altering the temperature of the chromatographic column. When a temperature program is necessary, the individual monograph indicates the conditions in table format. The table indicates the initial temperature, rate of temperature change (ramp), final temperature, and hold time at the final temperature.

Procedure

(1) Equilibrate the column, injector, and detector with flowing carrier gas until a constant signal is received.
(2) Inject a sample through the injector septum, or use an autosampler.
(3) Begin the temperature program.
(4) Record the chromatogram.
(5) Analyze as indicated in the monograph.

Liquid Chromatography (LC)

The term *liquid chromatography*, as used in the compendia, is synonymous with high-pressure liquid chromatography and high-performance liquid chromatography. LC is a separation technique based on a solid stationary phase and a liquid mobile phase.

Stationary Phase: Separations are achieved by partition, adsorption, or ion-exchange processes, depending on the type of stationary phase used. The most commonly used stationary phases are modified silica or polymeric beads. The beads are modified by the addition of long-chain hydrocarbons. The specific type of packing needed to complete an analysis is indicated by the ''L'' designation in the individual monograph (see also the section *Chromatographic Columns*, below). The size of the beads is often described in the monograph as well. Changes in the packing type and size are covered in the *System Suitability* section of this chapter.

Chromatographic Column: The term *column* includes stainless steel, lined stainless steel, and polymeric columns, packed with a stationary phase. The length and inner diameter of the column affects the separation, and therefore typical column dimensions are included in the

individual monograph. Changes to column dimensions are discussed in the *System Suitability* section of this chapter. Compendial monographs do not include the name of appropriate columns; this omission avoids the appearance of endorsement of a vendor's product and natural changes in the marketplace. See the section *Chromatographic Columns* for more information.

Mobile Phase: The mobile phase is a solvent or a mixture of solvents, as defined in the individual monograph.

Apparatus: A liquid chromatograph consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device.

Gradient Elution: The technique of continuously changing the solvent composition during the chromatographic run is called gradient elution or solvent programming. The gradient elution profile is presented in the individual monograph as a gradient table, which lists the time and proportional composition of the mobile phase at the stated time.

Procedure

(1) Equilibrate the column and detector with mobile phase at the specified flow rate until a constant signal is received.
(2) Inject a sample through the injector, or use an autosampler.
(3) Begin the gradient program.
(4) Record the chromatogram.
(5) Analyze as directed in the monograph.

CHROMATOGRAPHIC COLUMNS

A complete list of packings (L), phases (G), and supports (S) used in *USP–NF* tests and assays is located in *USP–NF* and *PF*, *Reagents, Indicators, and Solutions—Chromatographic Columns*. This list is intended to be a convenient reference for the chromatographer in identifying the pertinent chromatographic column specified in the individual monograph.

DEFINITIONS AND INTERPRETATION OF CHROMATOGRAMS

Chromatogram: A chromatogram is a graphical representation of the detector response, concentration of analyte in the effluent, or other quantity used as a measure of effluent concentration versus effluent volume or time. In planar chromatography, *chromatogram* may refer to the paper or layer with the separated zones.

Figure 1 represents a typical chromatographic separation of two substances, 1 and 2. t_{R1} and t_{R2} are the respective retention times; and h is the height, h/2 the half-height, and $W_{h/2}$ the width at half-height, for peak 1. W_1 and W_2 are the respective widths of peaks 1 and 2 at the baseline. Air peaks are a feature of gas chromatograms and correspond to the solvent front in LC. The retention time of these air peaks, or unretained components, is designated as t_M.

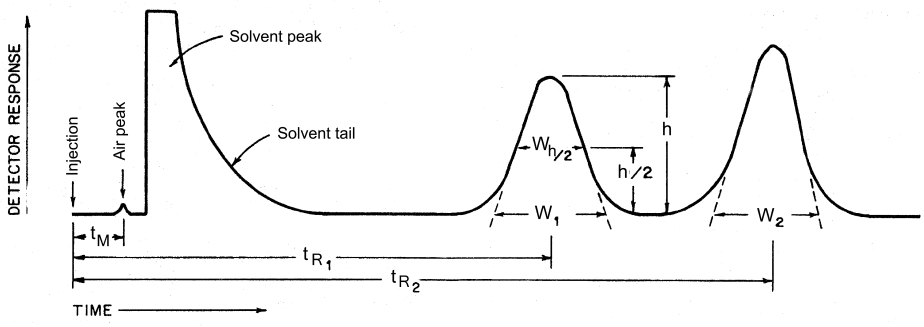


Figure 1. Chromatographic separation of two substances.

Dwell Volume (D): The dwell volume (also known as gradient delay volume) is the volume between the point at which the eluents meet and the top of the column.

Hold-Up Time (t_M): The hold-up time is the time required for elution of an unretained component (see *Figure 1,* shown as an air or unretained solvent peak, with the baseline scale in min).

Hold-Up Volume (V_M): The hold-up volume is the volume of mobile phase required for elution of an unretained component. It may be calculated from the hold-up time and the flow rate F, in mm/min:

$$V_M = t_M \times F$$

In size exclusion chromatography, the symbol V_O is used.

Number of Theoretical Plates (N)*: N is a measure of column efficiency, calculated by:

$$N = 5.54\left(\frac{t_R}{W_{h/2}}\right)^2$$

where t_R is the retention time of the substance, and $W_{h/2}$ is the peak width at half-height. The value of N depends upon the substance being chromatographed as well as the operating conditions, such as the flow rate and temperature of the mobile phase or carrier gas, the quality of the packing, the uniformity of the packing within the column, and, for capillary columns, the thickness of the stationary phase film and the internal diameter and length of the column.

Peak: The peak is the portion of the chromatographic recording of the detector response when a single component is eluted from the column. If separation is incomplete, two or more components may be eluted as one unresolved peak.

Peak-to-Valley Ratio (p/v): The p/v may be employed as a system suitability criterion in a test for related substances when baseline separation between two peaks is not achieved. *Figure 2* represents a partial separation of two substances, where H_p is the height above the extrapolated baseline of the minor peak and H_v is the height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks:

$$p/v = H_p / H_v$$

* The parameters k, N, r, and r_G were developed for isothermal GC separations and isocratic HPLC separations. Because these terms are thermodynamic parameters, they are valid only for separations made at constant temperature, mobile phase composition, and flow rate. However, for separations made with a temperature program or solvent gradient, these parameters may be used simply as comparative means to ensure that adequate chromatographic conditions exist to perform the methods as intended in the monographs.

In-Process Revision

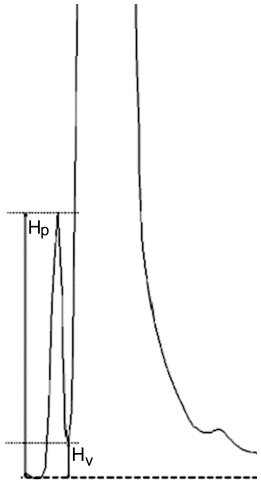


Figure 2. Peak-to-valley ratio determination.

Relative Retardation (R_{ret}) : The relative retardation is the ratio of the distance traveled by the analyte to the distance simultaneously traveled by a reference compound (see *Figure 3*) and is used in planar chromatography.

$$R_{rel} = b / c$$

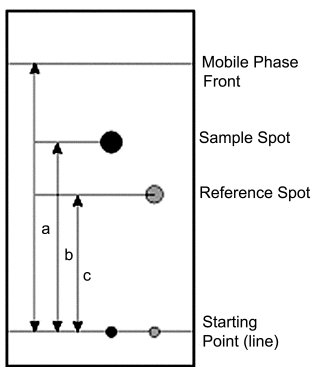


Figure 3. Typical planar chromatography.

Relative Retention (*r*)*: Retention time comparisons are normally made in terms of relative retention times to take into account any difference in experimental conditions within chromatograms:

$$r = t_{R2} - t_M / t_{R1} - t_M$$

where t_{R2} is the retention time measured from the point of injection of the compound of interest; t_{R1} is the retention time measured from the point of injection of the compound used as reference; and t_M is the retention time of a nonretained marker defined in the procedure, all determined under identical experimental conditions on the same column. Because in most procedures there is no need to identify an unretained peak, comparisons are normally made in terms of unadjusted relative retention, r_G:

$$r_G = t_{R2} / r_{R1}$$

Unless otherwise indicated, values for relative retention stated in monographs correspond to unadjusted relative retention.

Relative Standard Deviation in Percentage

$$\%RSD = \frac{100}{\bar{x}} \left(\frac{\sum_{i=1}^{N} \left(x_i - \bar{x} \right)^2}{N-1} \right)^{1/2}$$

Retardation Factor (R_F): The retardation factor is the ratio of the distance traveled by the center of the spot to the distance simultaneously traveled by the mobile phase and is used in planar chromatography. Using the symbols in *Figure 3*:

$$R_F = b / a$$

Retention Factor (k)*: The retention factor is also known as the capacity factor (k′). Defined as:

$$k = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in mobile phase}}$$

or

$$k = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}}$$

The retention factor of a component may be determined from the chromatogram:

$$k = t_R - t_M / t_M$$

Retention Time (t_R): In liquid chromatography and gas chromatography, the retention time, t_R, is defined as the time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone. t_R may be used as a parameter for identification. Chromatographic retention times are characteristic of the compounds they represent but are not unique. Coincidence of retention times of a sample and a reference substance can be used as a partial criterion in construction of an identity profile but may not be sufficient on its own to establish identity. Absolute retention times of a given compound may vary from one chromatogram to the next.

Retention Volume (V_R): The retention volume is the volume of mobile phase required for elution of a component. It may be calculated from the retention time and the flow rate in mL/min:

$$V_R = t_R \times F$$

Resolution (R_S): The resolution is the separation of two components in a mixture, calculated by:

$$R_S = 1.18(t_{R2} - t_{R1}) / (W_{1,h/2} + W_{2,h/2})$$

where t_{R2} and t_{R1} are the retention times of the two components; and $W_{1,h/2}$ and $W_{2,h/2}$ are the corresponding widths at half-height.

Separation Factor (α): The separation factor is the relative retention calculated for two adjacent peaks (by convention, the value of the separation factor is always >1):

$$\alpha = k_2/k_1$$

Symmetry Factor (A_S): The symmetry factor (also known as the tailing factor) of a peak (see *Figure 4*) is calculated by:

$$A_S = W_{0.05} / 2f$$

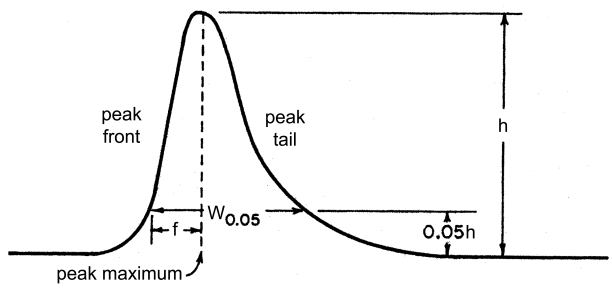


Figure 4. Asymmetrical chromatographic peak.

SYSTEM SUITABILITY

System suitability tests are an integral part of gas and liquid chromatographic methods. These tests are used to verify that the chromatographic system is adequate for the intended analysis.

In-Process Revision

The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such.

Factors that may affect chromatographic behavior include the following:

- Composition, ionic strength, temperature, and apparent pH of the mobile phase
- Flow rate, column dimensions, column temperature, and pressure
- Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area
- Reverse-phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, etc.)

The resolution, R_S, is a function of the number of theoretical plates, N (also referred to as efficiency), the separation factor, α, and the capacity factor, k. [NOTE—All terms and symbols are defined in the preceding section *Definitions and Interpretation of Chromatograms*.] For a given stationary phase and mobile phase, N may be specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug. This is a less reliable means to ensure resolution than is direct measurement. Column efficiency is, in part, a reflection of peak sharpness, which is important for the detection of trace components.

Replicate injections of a standard preparation or other standard solutions are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation, %RSD, if the requirement is 2.0% or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0%.

For the *Assay* in a drug substance monograph, where the value is 100% for the pure substance, and no maximum relative standard deviation is stated, the maximum permitted %RSD is calculated for a series of injections of the reference solution:

$$\%RSD = KB\sqrt{n} / t_{90\%,\, n-1}$$

where K is a constant (0.349), obtained from the expression $K = (0.6/\sqrt{2}) \times (t_{90\%}, 5/\sqrt{6})$, in which $0.6/\sqrt{2}$ represents the required percentage relative standard deviation after six injections for B = 1.0; B is the upper limit given in the definition of the individual monograph minus 100%; n is the number of replicate injections of the reference solution ($3 \leq n \leq 6$); and $t_{90\%,\, n-1}$ is the Student's t at the 90% probability level (double sided) with n – 1 degrees of freedom.

Unless otherwise prescribed, the maximum permitted relative standard deviation does not exceed the appropriate value given in the table of repeatability requirements. This requirement does not apply to tests for related substances.

Repeatability Requirements

	Number of Individual Injections			
	3	4	5	6
B (%)	**Maximum Permitted RSD**			
2.0	0.41	0.59	0.73	0.85

Repeatability Requirements *(Continued)*

	Number of Individual Injections			
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

The symmetry factor, A_S, a measure of peak symmetry, is unity for perfectly symmetrical peaks; and its value increases as tailing becomes more pronounced (see *Figure 4*). In some cases, values less than unity may be observed. As peak symmetry moves away from values of 1, integration, and hence precision, become less reliable.

The signal-to-noise ratio (S/N) is a useful system suitability parameter. The S/N is calculated as follows:

$$S/N = 2H/h$$

where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance $\geq$5 times the peak width at its half-height; and h is the difference between the largest and smallest noise values observed over a distance $\geq$5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest after the injection or application of a blank (see *Figure 5*).

These tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph.

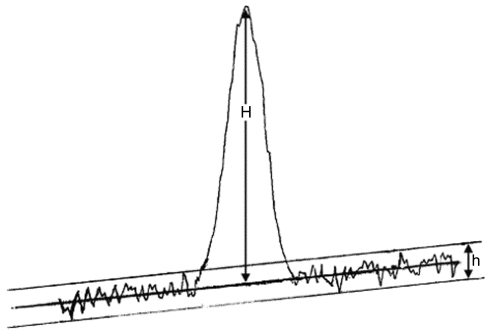


Figure 5. Noise and chromatographic peak, components of the S/N ratio.

The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions. Adjustments are permitted only when

- Suitable standards (including Reference Standards) are available for all compounds used in the suitability test; and
- Those standards show that the adjustments improved the quality of the chromatography with respect to the system suitability requirements.

Adjustments to chromatographic systems performed in order to comply with system suitability requirements are not to be made in order to compensate for column failure or system malfunction.

If adjustments of operating conditions are necessary in order to meet system suitability requirements, each of the items in the following list is the maximum variation that can be considered, unless otherwise directed in the monograph; these changes may require additional validation data. To verify the suitability of the method under the new conditions, assess the relevant analytical performance characteristics potentially affected by the change. Multiple adjustments can have a cumulative ef-

fect on the performance of the system and are to be considered carefully before implementation. Adjustments to the composition of the mobile phase in gradient elution are not recommended. If adjustments are necessary, only column changes (same packing material) or dwell volume adjustments are recommended.

pH of Mobile Phase (HPLC): The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 units of the value or range specified.

Concentration of Salts in Buffer (HPLC): The concentration of the salts used in the preparation of the aqueous buffer employed in the mobile phase can be adjusted to within ±10% if the permitted pH variation (see above) is met.

Ratio of Components in Mobile Phase (HPLC): The following adjustment limits apply to minor components of the mobile phase (specified at 50% or less). The amounts of these components can be adjusted by ±30% relative. However, the change in any component cannot exceed ±10% absolute (i.e., in relation to the total mobile phase). Adjustment can be made to one minor component in a ternary mixture. Examples of adjustments for binary and ternary mixtures are given below.

Binary Mixtures

SPECIFIED RATIO OF 50 : 50: 30% of 50 is 15% absolute, but this exceeds the maximum permitted change of ±10% absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40 : 60 to 60 : 40.

SPECIFIED RATIO OF 2 : 98: 30% of 2 is 0.6% absolute. Therefore the maximum allowed adjustment is within the range of 1.4 : 98.6 to 2.6 : 97.4.

Ternary Mixtures

SPECIFIED RATIO OF 60 : 35 : 5: For the second component, 30% of 35 is 10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore the second component may be adjusted only within the range of 25% to 45% absolute. For the third component, 30% of 5 is 1.5% absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100%. Therefore, mixture ranges of 50 : 45 : 5 to 70 : 25 : 5 or 58.5 : 35 : 6.5 to 61.5 : 35 : 3.5 would meet the requirement.

Wavelength of UV-Visible Detector (HPLC): Deviations from the wavelengths specified in the procedure are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is used to verify that error in the detector wavelength is, at most, ±3 nm.

Stationary Phase

COLUMN LENGTH (GC, HPLC): Can be adjusted by as much as ±70%.

COLUMN INNER DIAMETER (HPLC): Can be adjusted if the linear velocity is kept constant. See *Flow Rate (HPLC)* below.

COLUMN INNER DIAMETER (GC)—Can be adjusted by as much as ±50% for GC.

FILM THICKNESS (CAPILLARY CG)—Can be adjusted by as much as –50% to 100%.

Particle Size (HPLC): The particle size can be reduced by as much as 50%, but cannot be increased.

Particle Size (GC): Changing from a larger to a smaller or from a smaller to a larger particle size GC mesh support is acceptable if the chromatography meets the requirements of system suitability and the same particle size range ratio is maintained. The particle size range ratio is defined as the diameter of the largest particle divided by the diameter of the smallest particle.

Flow Rate (GC): The flow rate can be adjusted by as much as ±50%.

Flow Rate (HPLC): When column dimensions have been modified, the flow rate can be adjusted using:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

in which F_1 is the flow rate indicated in the monograph, in mL/min; F_2 is the adjusted flow rate, in mL/min; l_1 is the length of the column indicated in the monograph; l_2 is the length of the column used; d_1 is the column inner diameter indicated in the monograph; and d_2 is the internal diameter of the column used. Additionally, the flow rate can be adjusted by $\pm 50\%$.

Injection Volume (HPLC): The injection volume can be reduced as far as is consistent with accepted precision and detection limits; no increase is permitted.

Injection Volume and Split Volume (GC): The injection volume and split volume may be adjusted if detection and repeatability are satisfactory.

Column Temperature (HPLC): The column temperature can be adjusted by as much as $\pm 10^\circ$. Column thermostating is recommended to improve control and reproducibility of retention time.

Oven Temperature (GC): The oven temperature can be adjusted by as much as $\pm 10\%$.

Oven Temperature Program (GC): Adjustment of temperatures is permitted as stated above. When the specified temperature must be maintained or when the temperature must be changed from one value to another, an adjustment of up to $\pm 20\%$ is permitted.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

Measured values of R_r or R_F or t_R for the sample substance do not deviate from the values obtained for the reference compound and mixture by more than the statistically determined reliability estimates from replicate assays of the reference compound. Relative retention times may be provided in monographs for informational purposes only to aid in peak identification. There are no acceptance criteria applied to relative retention times.

Suitability testing is used to ascertain the effectiveness of the final operating system, which should be subjected to this testing. Make injections of the appropriate preparation(s) as required in order to demonstrate adequate system suitability (as described in the *Chromatographic system* section of the method in a monograph) throughout the run.

The preparation can be a standard preparation or a solution containing a known amount of analyte and any additional materials (e.g., excipients or impurities) useful in controlling the analytical system. Whenever there is a significant change in the chromatographic system (equipment, mobile phase component, or other components) or in a critical reagent, system suitability is to be reestablished. No sample analysis is acceptable unless the suitability of the system has been demonstrated.

QUANTITATION

During quantitation, disregard peaks caused by solvents and reagents or arising from the mobile phase or the sample matrix.

In the linear range, peak areas and peak heights are usually proportional to the quantity of compound eluting. The peak areas and peak heights are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs. The components measured are separated from any interfering components. Peak tailing and fronting is minimized, and the measurement of peaks on tails of other peaks are avoided when possible.

Although comparison of impurity peaks with those in the chromatogram of a standard at a similar concentration is preferred, impurity tests may be based on the measurement of the peak response due to impurities and expressed as a percentage of the area of the drug peak. The standard may be the drug itself at a level corresponding to, for example, 0.5% impurity, assuming similar peak responses. When impurities must be determined with greater certainty, use a standard of the impurity itself or apply a correction factor based on the response of the impurity relative to that of the main component.

External Standard Method: The concentration of the component(s) quantified is determined by comparing the response(s) obtained with the sample solution to the response(s) obtained with a standard solution.

Internal Standard Method: Equal amounts of the internal standard are introduced into the sample solution and a standard solution. The internal standard is chosen so that it does not react with the test material, is stable, is resolved from the component(s) quantified (analytes), and does not contain impurities with the same retention time as that of the analytes. The concentrations of the analytes are determined by comparing the ratios of their peak areas or peak heights and the internal standard in the sample solution with the ratios of their peak areas or peak heights and the internal standard in the standard solution.

Normalization Procedure: The percentage content of a component of the test material is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix and those at or below the limit at which they can be disregarded.

Calibration Procedure: The relationship between the measured or evaluated signal y and the quantity (e.g., concentration, mass) of substance x is determined, and the calibration function is calculated. The analytical results are calculated from the measured signal or evaluated signal of the analyte and its position on the calibration curve.

In tests for impurities for both the *External Standard Method,* when a dilution of the sample solution is used for comparison, and the *Normalization Procedure,* any correction factors indicated in the monograph are applied (e.g., when the response factor is outside the range 0.8–1.2).

When the impurity test prescribes the total of impurities or there is a quantitative determination of an impurity, choice of an appropriate threshold setting and appropriate conditions for the integration of the peak areas is important. In such tests the limit at or below which a peak is disregarded is generally 0.05%. Thus, the threshold setting of the data collection system corresponds to at least half of this limit. Integrate the peak area of any impurity that is not completely separated from the principal peak, preferably by valley-to-valley extrapolation (tangential skim).▲*USP34*

GENERAL CHAPTERS

General Information

BRIEFING

⟨**1121**⟩ **Nomenclature,** *USP 32* page 632. On the basis of comments received, it is proposed to revise the graphical examples illustrating the definition of the active moiety in the section *Monograph Naming Policy for Salt Drug Substances in Drug Products and Compounded Preparations*.

(NOM: A.Wilk) RTS—C77398

In-Process Revision

Change to read:

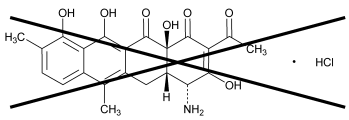
MONOGRAPH NAMING POLICY FOR SALT DRUG SUBSTANCES IN DRUG PRODUCTS AND COMPOUNDED PREPARATIONS

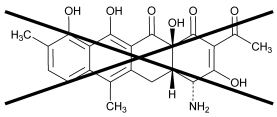
The titles of USP monographs for drug products and compounded preparations formulated with a salt of an acid or base use the name of the active moiety, as defined below. The strength of the product or preparation also is expressed in terms of the active moiety.

An active moiety is the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance, without regard to the actual charged state of the molecule in-vivo.

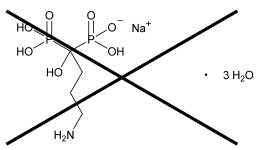
~~For example, the active moiety of a hydrochloride salt of a base will be the free base and not the protonated form of the base. The active moiety of a metal acid salt will be the free acid.~~

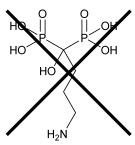
~~i. Example: Chelocardin Hydrochloride active moiety is Chelocardin~~





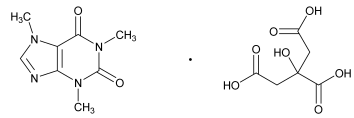
~~ii. Example: Alendronate Sodium active moiety is Alendronic Acid~~

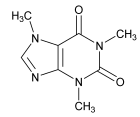




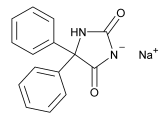
▲For example, the active moiety of a salt of a base will be the free base, and not the protonated form of the base. The active moiety of an acid salt will be the free acid.

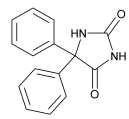
i. Example: Caffeine Citrate active moiety is Caffeine





ii. Example: Phenytoin Sodium active moiety is Phenytoin





▲*USP34*

This Policy is followed by USP in naming drug products and compounded preparations that are newly recognized in the USP. Revising existing monographs to conform to this Policy is not intended, except where the USP Council of Experts determines that, for reasons such as safety, a nomenclature change is warranted.

Related Issues

Labeling—The labeling clearly states the specific salt form of the active moiety that is present in the product/preparation, as this information may be useful to practitioners and patients. The names and strengths of both the active moiety and specific salt form (where applicable) are provided in the labeling.

Exceptions—In those rare cases in which the use of the specific salt form of the active moiety in the title provides vital information from a clinical perspective, an exception to this Policy may be considered. In such cases, where the monograph title contains the specific salt form of the active moiety, the strength of the product or preparation also is expressed in terms of the specific salt form.

In-Process Revision

BRIEFING

⟨1230⟩ Water for Health Applications, *USP 32* page 740. The monograph *Water for Hemodialysis* is being revised, and this companion informational chapter needs revisions to accommodate the monograph revisions. However, this informational chapter also needs other editorial contemporization and consistency changes to accommodate the terminology used in the new microbiological test chapters *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩ and *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* ⟨62⟩, as well as the revised AAMI standards.

(PW: A. Hernandez-Cardoso) RTS—C79382

Change to read:

⟨1230⟩ WATER FOR ~~HEALTH~~

▲HEMODIALYSIS▲USP34 APPLICATIONS

Change to read:

~~WATER FOR HEMODIALYSIS~~

▲GENERAL PURIFICATION CONSIDERATIONS▲USP34

Chemical and microbial components that can be found in drinking water meeting U.S. Environmental Protection Agency National Primary Drinking Water Regulations (or equivalent) may have the potential to produce significant negative effects in patients undergoing hemodialysis. It is, therefore, necessary to subject the water to further treatment to reduce these components to acceptable levels. The *Water for Hemodialysis* monograph provides bacterial and chemical tests that are required to ensure patient safety. Additional testing is recommended as follows:

(1) Excess levels of aluminum, fluorides, and ~~chlorides~~

▲chlorine▲USP34
may be found seasonally in drinking water as a result of chemicals used in water treatment. These components should be monitored in Water for Hemodialysis being produced in accordance with established standard operating procedures. The maximum acceptable levels of these

▲and other▲USP34
elements and compounds,

▲as proposed by AAMI (Association for the Advancement of Medical Instrumentation)▲USP34
are listed in *Table 1*.

▲These attributes should be periodically monitored to assure they are being controlled by the routine testing performed in accordance with the *Water for Hemodialysis* monograph.▲USP34

(2) A comprehensive validation testing of the system producing Water for Hemodialysis should be performed ~~, at least annually,~~

▲initially and periodically thereafter▲USP34
to ensure that the water treatment equipment ~~is functioning properly. The maximum acceptable levels of elements and compounds are listed in *Table 1*. Routine testing is performed in accordance with the monograph.~~

▲and system sanitization processes are functioning properly.▲USP34

Table 1. Maximum Allowable Chemical Levels in Water for Hemodialysis
(water used to prepare dialysate and concentrates from powder at a dialysis facility and to reprocess dialyzers for multiple use)*

Element or Compound	Maximum Concentration (mg/L)
▲*Contaminants with documented toxicity in hemodialysis*	
Aluminum	0.01
Chloramines	0.1
Free chlorine	0.5
Copper	0.1
Fluoride	0.2
Lead	0.005
Nitrate (as N)	2
Sulfate	100
Zinc	0.1
Contaminants normally included in dialysate▲USP34	
Calcium	2 (0.1 mEq/L)
Magnesium	4 (0.3 mEq/L)
Potassium	8 (0.2 mEq/L)
Sodium	70 (3.0 mEq/L)
▲*Other contaminants*▲USP34	
Antimony	0.006
Arsenic	0.005
Barium	0.1
Beryllium	0.0004
Cadmium	0.001
Chromium	0.014
~~Lead~~	~~0.005~~
▲▲USP34	
Mercury	0.0002
Selenium	0.09
Silver	0.005
~~Aluminum~~	~~0.01~~

In-Process Revision

Table 1. Maximum Allowable Chemical Levels in Water for Hemodialysis
(water used to prepare dialysate and concentrates from powder at a dialysis facility and to reprocess dialyzers for multiple use)*
(Continued)

Element or Compound	Maximum Concentration (mg/L)
~~Chloramines~~	~~0.1~~
~~Free chlorine~~	~~0.5~~
~~Copper~~	~~0.1~~
~~Fluoride~~	~~0.2~~
~~Nitrate (as N)~~	~~2~~
~~Sulfate~~	~~100~~
▲▲*USP34*	
Thallium	0.002
~~Zinc~~	~~0.1~~
▲▲*USP34*	

* Reprinted with permission from ANSI/AAMI RD62: ~~2001,~~ ▲2006,▲*USP34* ''Water treatment equipment for hemodialysis applications'', ©Association for the Advancement of Medical Instrumentation, Arlington, VA.

The chemical limits included in *Table 1* have been recognized by federal government agencies as standards for Water for Hemodialysis. Written standard operating procedures for water testing should be established by the physician in charge or the designated facility manager. The test frequency decision should be based upon historical data analysis, the quality of the source water as reported by the municipal water treatment facility or public health agency in the area, etc. Records should be maintained to document levels and any necessary ~~action taken.~~

▲remedial action taken promptly.▲*USP34*

Chemical analysis of water components listed should be performed using methods referenced in the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater,* ~~19th~~

▲21st▲*USP34* Edition,[1] those referenced in the U.S. Environmental Protection Agency's *Methods for the Determination of Metals in Environmental Samples,*[2] or equivalent methods as ~~described~~

▲referenced▲*USP34* in ANSI/AAMI RD 62:~~2001.~~

▲2006.▲*USP34*

MICROBIAL CONSIDERATIONS

The *Water for Hemodialysis* monograph includes ~~microbial limits~~

▲total aerobic microbial count (TAMC) limits▲*USP34* of 100 cfu/mL and endotoxin limits of ~~2~~

▲1▲*USP34* USP Endotoxin Unit/mL. ~~Culture media should be Soybean–Casein Digest Agar Medium or equivalent, and colonies should be counted after incubation at a temperature range between 30° and 35°, for no less than 48 hours. Sampling the water should be done at the end of the water purification cascade at the point where the water enters the dialysis equipment.~~

[1] American Public Health Association, Washington, DC 20005.

[2] U.S. Environmental Protection Agency Publication EPA-600-R-94-111, Cincinnati, OH.

▲In addition, the absence of *Pseudomonas aeruginosa* should be routinely determined because this is an opportunistic pathogen hazardous to acutely ill hemodialysis patients. Both the high microbial counts and the presence of *Pseudomonas aeruginosa* can be associated with inadequate water system maintenance and sanitization. Sampling the water should be done at all use points where the water enters the dialysis equipment.▲*USP34* Samples should be assayed within 30 minutes of collection or immediately refrigerated and then assayed within 24 hours of collection. ~~Quantification of bacterial endotoxins is performed using the Limulus Amebocyte Lysate (LAL) clotting method or any other LAL test found in the USP general test chapter *Bacterial Endotoxins Test* ⟨85⟩.~~

▲The microbial enumeration and absence tests are performed using procedures found in the USP general test chapters *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩. Quantification of bacterial endotoxins is performed using procedures found in the USP general test chapter *Bacterial Endotoxins Test* ⟨85⟩.▲*USP34*

Because of the incubation time required to obtain definitive microbiological results, water systems should be microbiologically monitored to confirm that they continue to produce water of acceptable quality. ''Alert'' and ''Action Levels'' are therefore necessary for the monitoring and control of the system. An Alert Level constitutes a warning and does not require a corrective action. An Action Level indicates a drift from normal operating conditions and requires that corrective action be taken to bring the process back into the normal operating range. Exceeding an Alert or Action Level does not imply that water quality has been compromised. ~~The recommended Action Level for a total viable microbial count in the product water is 50 cfu per mL, and the recommended Alert Level for bacterial endotoxins is 0.5 USP Endotoxin Unit per mL~~

▲The maximum recommended Action Level for a total viable microbial count in the product water should be no greater than 25 cfu/mL, and the maximum recommended Action Level for bacterial endotoxins should be no greater than 0.25 USP Endotoxin Unit/mL. As with all process control values, Action and Alert Levels should be established from normal system monitoring trends and process capabilities in a fashion that allow remedial actions to occur in response to process control level excursions well before specifications are exceeded▲*USP34* (also see *Microbial Considerations* under *Water for Pharmaceutical Purposes* ⟨1231⟩).

In-Process Revision

BRIEFING

⟨1788⟩ Methods for the Determination of Particulate Matter in Parenteral Injections and Ophthalmic Products. The Parenteral Products—Industrial Expert Committee proposed the general information chapter, ⟨1788⟩ *Particulate Matter Determination in Parenteral Ophthalmic Products* in *PF* 34(2) [Mar.–Apr. 2008], which reintroduced text that appeared in *Particulate Matter in Injections* ⟨788⟩, but was deleted when the chapter was harmonized. The Expert Committee thought that the deleted text was valuable information for end users in setting up equipment and measuring particulate matter. The Parenteral Products—Industrial Expert Committee is now resubmitting this new general information chapter in response to industry comments received from the *PF* 34(2) proposal and to include additions and edits that reflect current industry practice; therefore, the *PF* 32(4) text was cancelled.

(PPI: D. Hunt) RTS—C65454

Add the following:

▲⟨1788⟩ METHODS FOR THE DETERMINATION OF PARTICULATE MATTER IN PARENTERAL INJECTIONS AND OPHTHALMIC SOLUTIONS

INTRODUCTION

Methodology for the determination of particulate matter content in solution products is contained in this chapter. Also included are solutions constituted from sterile solids intended for parenteral use. Determination of the particulate matter content is an essential means to control the level contained in the final product and thus reduce risk to the recipient. *Injections* ⟨1⟩ specifies that these solutions are expected to be essentially free from particulate matter that can be observed on visual inspection. *Particulate Matter in Injections* ⟨788⟩ specifies limits for the particulate matter content in the product solutions in two subvisible size thresholds. Likewise, *Particulate Matter in Ophthalmic Solutions* ⟨789⟩ establishes these particle content expectations for ophthalmic solutions. The tests described herein are physical limits tests performed for the purpose of enumerating subvisible particles (particulate matter) within specific size ranges for both injections and ophthalmic solutions.

Particulate matter consists of mobile undissolved particles, other than gas bubbles, unintentionally present in the test solutions. Using the test methods, any semisolid to solid material (and even immiscible liquids) that trigger detector response above a selected size threshold will be tabulated.

Two general categories of particulate matter sources will be detected: extrinsic and intrinsic. Extrinsic material is additive and foreign, and not part of the formulation, package, or assembly process. Intrinsic material is that which is associated with the package, formulation ingredients and process, or assembly process.

Examples of extrinsic material are fibers, cellulosic matter, vegetative matter, corrosion products, paint/coatings, and building materials (gypsum, concrete, metal, plastic, etc.). Extrinsic particles are additive and generally nonchanging over the life of the product, unless by fragmentation, swelling (hydration), or degradation. Fragments of rubber, plastic, and metal are examples of extrinsic particulate matter deposited in the product during assembly or not removed in the container preparation process.

Intrinsic sources are inherent to the product and process—formulation, package, and commercial assembly steps. Intrinsic sources represent a variety of phenomena yielding unwanted substances, such as extraction, leaching, degradation of ingredient (active or excipient), change of ingredient by precipitation/salt form/crystalline form, change of package physical integrity, change of impurity level, change of micellar association, oligamerization, and package and process-related materials not removed during product assembly. Combinations of all of the above and physical phenomena such as aggregation, sedimentation, and coalescence by matrix (oils, semisolids) may bring smaller particles (<10 μm)

into the detection zone of the test method (≥10 μm). Intrinsic sources of detectable particulate matter are of great concern, because the substance may be present, but not evident until particles form over time, even long after lot release.

Regardless of material character or source, the simple intent of the determination is to quantitate the level of all detectable particulate matter. Two quite different methods are provided herein for the determination of particulate matter: light obscuration and membrane microscope, in a two-stage test approach. If possible, the product solution is first tested by the light obscuration (LO) procedure (stage 1). If it fails to meet the test limits, the product should then be tested by the membrane microscope (MM) procedure (stage 2) with its own set of test limits. These methods are for solution products; however, certain solution formulations may not be easily analyzed by LO. Any product that does not have a clarity and a viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Also, formulation characteristics such as color, viscosity, or inherent solution properties (shear-induced changes) may provide erroneous LO data. Similarly, products that produce air or gas bubbles when drawn into the LO sensor, such as bicarbonate-buffered formulations may provide erroneous data. For these product solutions, membrane microscope testing should be used exclusively. Documentation demonstrating that the light obscuration procedure is incapable of testing the test article or produces invalid results is required in each case. It is expected that most test articles will meet the requirements on the basis of the light obscuration test alone; however, it may be necessary to assay some test articles by the light obscuration method followed by the membrane microscope method in order to reach a conclusion. Refer to the specific monographs when a question of test applicability occurs. Higher limits may be appropriate for certain products and should be specified in the individual monograph.

Certain products may not be amenable to direct or simple analysis due to volumes of 1 mL or less or delivery characteristics. Examples include low-volume parenteral and ophthalmic products and those in novel packages intended for specific uses. The expectation is compliance for these products; however, the methods of the manufacturer may need to be validated to demonstrate conformance with the test limits. Special low-volume 'sippers' for LO sampling and the pooling of multiple containers may be necessary for these package presentations. Consider this example: a low-volume (100 μL) product is packaged in a prefilled sterile syringe. The nature of the package allows simple delivery of the solution product and may be used for direct sampling, but the 100-μL volume precludes the pooling of the larger volumes (~25 mL) for the LO method. Direct sampling to a small membrane for microscopical counting and evaluating single and pooled package particle content may be the optimal means to collect data. Also in this example, careful statistical evaluation of the batch population using small sample volumes (but not doses) will be necessary to validate product acceptability.

In some instances, the viscosity of a product may be sufficiently high so as to preclude its analysis by either test method. In this event, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

In the tests described below for all product forms, the results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans based on known operational factors should be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units. Sam-

pling plans should be based on consideration of product volume, dose evaluated, numbers of particles historically found to be present in comparison to limits, particle size distribution of particles present, and variability of particle counts between units.

LIGHT OBSCURATION PARTICLE COUNT TEST

USP Reference Standards ⟨11⟩—USP Particle Count RS for system suitability testing.

The test applies to large-volume injections (LVI) labeled as containing more than 100 mL, unless otherwise specified in the individual monograph. This test applies also to single-dose or multiple-dose, small-volume injections (SVI) labeled as containing 100 mL or less that are either in solution or in solution constituted from sterile solids, in which a test for particulate matter is specified in the individual monograph. The test applies to ophthalmic solutions.

Test Apparatus

The apparatus is a liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample-feeding device to deliver controlled aliquots of sample for analysis. Suspended particles in the sample fluid flowing between a light source and sensor produce changes in signal that are correlated to particle dimension. Due to the nature of the detection and counting system, air bubbles and immiscible liquids may block sufficient light from being recorded along with the target suspended particles. Air bubbles must be diminished through proper preparation techniques. Immiscible liquids may be inherent to the package, such as lubrication oils. Solutions with excessive immiscible liquids may not be amenable to light obscuration analysis. Improper handling of certain samples, such as protein formulations, may form shear-induced semisolids due to mixing effects and the resultant semisolids counted as ''particles''. Adequate training should be provided for those responsible for the technical performance of the test.

For the light obscuration method, the ideal sample consists of a clear, water-like fluid with individual, free particles of moderate buoyancy and high contrast (opacity, color, refractive index) such that each particle passing between the illuminator and sensor effectively blocks the illumination. It is the responsibility of those performing the test to ensure that the operating parameters of the instrumentation are appropriate to the required accuracy and precision of the test result and that the artifacts and interferences inherent in certain products and with certain methods of preparation are eliminated or accommodated. An example is a protein formulation that may form shear-induced semisolids due to mixing and counted as ''particles''. Adequate training should be provided for those responsible for the technical performance of the test.

It is important to note that for Pharmacopeial applications the ultimate goal is that the particle counter reproducibly size and count particles present in the material under investigation. The instruments available range from systems in which calibration and other components of standardization are carried out using manual procedures to sophisticated systems incorporating hardware- or software-based functions for the standardization procedures. Thus, it is not possible to specify exact methods to be followed for standardization of the instrument, and it is necessary to emphasize the required end result of a standardization procedure rather than a specific method for obtaining this result. This section is intended to emphasize the criteria that must be met by a system rather than specific methods to be used in their determination. It is the responsibility of the user to apply the various

methods of standardization applicable to a specific instrument. Critical operational criteria consist of the following.

SENSOR CONCENTRATION LIMITS

Use an instrument that has a concentration limit (the maximum number of particles per mL) identified by the manufacturer that is greater than the concentration of particles in the test specimen to be counted. The vendor-certified concentration limit for a sensor is specified as that count level at which coincidence counts due to simultaneous presence of two or more particles in the sensor view volume comprise less than 10% of the counts collected for 10-μm particles.

SENSOR DYNAMIC RANGE

The dynamic range of the instrument used (range of sizes of particles that can be accurately sized and counted) should include the smallest particle size to be enumerated in the products.

Instrument Standardization Tests

The following discussion of instrument standardization emphasizes performance criteria rather than specific methods for calibrating or standardizing a given instrument system. This approach is particularly evident in the description of calibration, in which allowance must be made for manual methods as well as those based on hardware, software, or the use of electronic testing instruments. Appropriate instrument qualification is essential to performance of the test according to requirements. Because different brands of instruments may be used in the test, the user is responsible for ensuring that the counter used is operated according to the manufacturer's specific instructions. The principles that should be followed to ensure that instruments operate within acceptable ranges are defined below. The following information for instrument standardization helps ensure that the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and count accuracy are appropriate to performance of the test. Procedures should be conducted at intervals of not more than 6 months.

SAMPLE VOLUME ACCURACY

Because the particle count from a sample aliquot varies directly with the volume of fluid sampled, it is important that the sampling accuracy is known to be within a certain range. For a sample volume determination, determine the dead (tare) volume in the sample feeder with particle-free water.[1] Transfer a volume of particle-free water that is greater than the sample volume to a container, and weigh. Using the sample feeding device, withdraw a volume that is appropriate for the specific sampler, and again weigh the container. Determine the sample volume by subtracting the tare volume from the combined sample plus tare volumes. Verify that the value obtained is within 5% of the appropriate sample volume for the test. Alternatively, the sample volume may be determined using a suitable Class A graduated cylinder (see *Volumetric Apparatus* ⟨31⟩). [NOTE—Instruments of this type require a variable tare volume. This is the amount of sample withdrawn prior to counting. This volume may be determined for syringe-operated samplers by setting the sample volume to zero and initiating sampling, so that the only volume of solution drawn is the tare. Subtract the tare volume from the total volume of solution drawn in the sampling cycle to determine the sample volume.]

[1] The particle-free water should be passed through a filter having a nominal pore size of 1.2 μm or finer.

SAMPLE FLOW RATE

Verify that the flow rate is within the manufacturer's specifications for the sensor used. This may be accomplished by using a calibrated stopwatch to measure the time required for the instrument to withdraw and count a specific sample volume (i.e., the time between beginning and ending of the count cycle as denoted by instrument indicator lights or other means). Sensors may be operated accurately over a range of flow rates. Perform the *Test Procedure* at the same flow rate as that selected for calibration of the instrument.

CALIBRATION

Use one of the following methods:

Manual Method—Calibrate the instrument with a minimum of three calibrators, each consisting of near-monosize polystyrene spheres having diameters of about 10, 15, and 25 μm, in an aqueous vehicle. The calibrator spheres should have a mean diameter of within 5% of the 10-, 15-, and 25-μm nominal diameters and be standardized against materials traceable to NIST standard reference materials.[2] The total number of spheres counted should be within the sensor's concentration limit. Prepare suspensions of the calibrator spheres in water at a concentration of 1000–5000 particles per mL, and determine the channel setting that corresponds to the highest count setting for the sphere distribution. This is determined by using the highest count threshold setting to split the distribution into two bins containing equal numbers of counts, with the instrument set in the differential count mode (moving window half-count method). Use only the central portion of the distribution in this calculation to avoid including asymmetrical portions of the peak. The portion of the distribution, which is divided equally, is the count window. The window is bounded by threshold settings that will define a threshold voltage window of $\pm$20% around the mean diameter of the test spheres. The window is intended to include all single spheres, taking into account the standard deviation of the spheres and the sensor resolution, while excluding noise and aggregates of spheres. The value of 20% was chosen based on the worst-case sensor resolution of 10% and the worst-case standard deviation of the spheres of 10%. Because the thresholds are proportional to the cross-sectional area of the spheres (and all particles) rather than the diameter, the lower and upper voltage settings are determined by the equations:

$$V_L = 0.64V_S$$

in which V_L is the lower voltage setting and V_S is the voltage at the peak center, and

$$V_U = 1.44V_S$$

in which V_U is the upper voltage setting. Once the center peak thresholds are determined, use these thresholds for the standards to create a regression of log voltage versus log particle size, from which the instrument settings for the 10- and 25-μm sizes can be determined.

Automated Method—The calibration (size response) curve may be determined for the instrument-sensor system by the use of validated software routines offered by instrument vendors; these may be included as part of the instrument software or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a response curve equivalent to that attained by the manual method and if the automated calibration is validated as necessary by the user.

Electronic Method—Using a multichannel peak height analyzer, determine the center channel of the particle counter pulse response for each standard suspen-

[2] ASTM standard F658-87 provides useful discussions pertaining to calibration procedures applying near-monosize latex spheres.

sion. This peak voltage setting becomes the threshold used for calculation of the voltage response curve for the instrument. The standard suspensions used for the calibration are run in order, and median pulse voltages for each are determined. These thresholds are then used to generate the size response curve manually or via software routines. The thresholds determined from the multichannel analyzer data are then transferred to the counter to complete the calibration.

SENSOR RESOLUTION

The particle size resolution of the instrumental particle counter is dependent upon the sensor used and may vary with individual sensors of the same model. Determine the resolution of the particle counter for 10-μm particles using the 10-μm calibrator spheres. The relative standard deviation of the size distribution of the standard particles used is not more than 5%. Acceptable methods of determining particle size resolution are manual determination of the amount of peak broadening due to instrument response; using an electronic method of measuring and sorting particle sensor voltage output with a multichannel analyzer; and automated methods.

Manual Method—Adjust the particle counter to operate in the cumulative mode or total count mode. Refer to the calibration curve obtained earlier, and determine the threshold voltage for the 10-μm spheres. Adjust 3 channels of the counter to be used in the calibration procedure as follows:

Channel 1 is set for 90% of the threshold voltage.

Channel 2 is set for the threshold voltage.

Channel 3 is set for 110% of the threshold voltage.

Draw a sample through the sensor, observing the count in *Channel 2*. When the particle count in that channel has reached approximately 1000, stop counting, and observe the counts in *Channels 1* and *3*. Check to see if the *Channel 1* and *3* counts are 1.68 ±10% and 0.32 ±10%, respectively, of the count in *Channel 2*. If not, adjust the *Channel 1* and *3* thresholds to meet these criteria. When these criteria have been satisfied, draw a sample of suspension through the counter until the counts in *Channel 2* have reached approximately 10,000, or until an appropriate volume (e.g., 10 mL) of the sphere suspension has been counted. Verify that the *Channel 1* and *3* counts are 1.68 ±3% and 0.32 ±3%, respectively, of the count in *Channel 2*. Record the particle size for the thresholds just determined for *Channels 1, 2,* and *3*. Subtract the particle size for *Channel 2* from the size for *Channel 3*. Subtract the particle size for *Channel 1* from the size for *Channel 2*. The values so determined are the observed standard deviations on the positive and negative side of the mean count for the 10-μm standard. Calculate the percentage of resolution of the sensor by the formula:

$$100\left(\sqrt{S_O^2 - S_S^2/D}\right)$$

in which S_O is the highest observed standard deviation determined for the sphere; S_S is the supplier's reported standard deviation for the spheres; and D is the diameter, in μm, of the spheres as specified by the supplier. The resolution is not more than 10%.

Automated Method—Software that allows for the automated determination of sensor resolution is available for some counters. This software may be included in the instrument or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a resolution determination equivalent to the manual method and if the automated resolution determination is validated as necessary by the user.

In-Process Revision

Electronic Method—Record the voltage output distribution of the particle sensor, using a multichannel analyzer while sampling a suspension of the 10-μm particle size standard. To determine resolution, move the cursor of the multichannel analyzer up and down the electric potential scale from the median pulse voltage to identify a channel on each side of the 10-μm peak that has approximately 61% of the counts observed in the center channel. Use of the counter size response curve to convert the mV values of these two channels to particle sizes provides the particle size at within one standard deviation of the 10-μm standard. Use these values to calculate the resolution as described under *Manual Method*.

PARTICLE COUNTING ACCURACY—SYSTEM SUITABILITY

Determine the particle counting accuracy of the instrument, using *Method 1* (for sensors requiring the moving window half-count (MWHC) method for calibration), *Method 2* (for multichannel sensors), or *Method 3* for any instrument (manual comparison to membrane microscope method).

Method 1 (MWHC Instruments)—

Procedure—Prepare the suspension and blank using the USP Particle Count RS. With the instrument set to count in the cumulative (total) mode, collect counts at settings of ≥10 μm and ≥15 μm. Prepare the blank and suspension sample in the same manner. Degas the mixture by one of three means: sonication (at 80–120 watts) for about 30 seconds, or by allowing to stand, or by vacuum. Gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis. Withdraw directly from the container three consecutive volumes of NLT 5 mL each, obtain the particle counts, and discard the data from the first portion. [NOTE—Complete the procedure within 5 minutes.] Repeat the procedure, using the suspension in place of the blank. From the averages of the counts resulting from the analysis of the two portions of the suspension at ≥10 μm and from the analysis of the two portions of the blank at ≥10 μm, calculate the number of particles in each mL by the formula:

$$(P_S - P_B)/V$$

in which P_S is the average particle count obtained from the suspension; P_B is the average particle count obtained from the blank; and V is the average volume, in mL, of the four portions tested. Repeat the calculations, using the results obtained at the setting of NLT 15 μm.

Interpretation—The MWHC instrument meets the requirements for *Particle Counting Accuracy* if the count obtained at ≥10 μm and the ratio of the counts obtained at ≥10 μm to those obtained at ≥15 μm conform to the values that accompany the USP Particle Count RS. If the instrument does not meet the requirements for *Particle Counting Accuracy*, and adequate test volumes remain, repeat the procedure with them. Prepare new suspension and blank if insufficient volumes remain and then repeat the procedure. If the results of the second test are within the limits given above, the instrument meets the requirements of the test for *Particle Counting Accuracy*. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures, and retest the instrument.

Method 2 (Multichannel Instruments)—

Procedure—Use either (a) a dilution of the USP Particle Count RS; or (b) a commercial preparation of standard calibrator spheres of nominal diameter 15–30 μm in a suspension containing between 50 and 200 particles per mL, certified by the manufacturer; or (c) a laboratory-prepared suspension of standard calibrator spheres having a nominal diameter of 15–30 μm, containing between 50 and 200 particles per mL. Use of non-USP stan-

dards ''b'' and ''c'' is acceptable when they are compliant with USP standardization criteria: five successive counts are NMT ±10% of stated size.

Degas the suspension by one of three means: sonication (at 80–120 watts) for about 30 seconds, or by allowing to stand, or by vacuum. Gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis and perform five counts on 5-mL volumes of the suspension, using the particle counter 10-μm size threshold. Obtain the mean cumulative particle count per mL.

Interpretation—The instrument meets the requirements for *Particle Counting Accuracy* if the count obtained at ≥10 μm conforms to the values that accompany the USP Particle Count RS. If the instrument does not meet the requirements for *Particle Counting Accuracy*, repeat the procedure. If the results of the second test are within the limits given above, the instrument meets the requirements of the test for *Particle Counting Accuracy*. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures, and retest the instrument.

Method 3 (Alternate Manual Method)—

Procedure—Prepare a suspension of standard calibrator spheres having a nominal diameter of 15–30 μm, containing between 50 and 200 particles per mL. Degas the suspension by one of three means: sonication (at 80–120 watts) for about 30 seconds, or by allowing to stand, or by vacuum. Gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis and perform five counts on 5-mL volumes of the suspension, using the particle counter 10-μm size threshold. Obtain the mean cumulative particle count per mL. Pipet a volume of this suspension containing 250–500 particles into a filter funnel prepared as described for *Filtration Apparatus* under *Membrane Microscope Particle Count Test*. After drying the membrane, count the total number of standard spheres collected on the membrane filter. This count should be within 20% of the mean instrumental count per mL for the suspension.

Test Environment

Perform the test in an environment that does not contribute any significant amount of particulate matter. Specimens should be cleaned to the extent that any level of extraneous particles added has a negligible effect on the outcome of the test. Preferably, the test specimen, glassware, closures, and other required equipment are prepared in an environment protected by high-efficiency particulate air (HEPA) filters, and nonshedding garments and powder-free gloves are worn throughout the preparation of samples.

Cleanse glassware, closures, and other required equipment, preferably by immersing and cleaning the items using warm, nonionic detergent solution. Rinse in flowing tap water, and then rinse again in flowing filtered water. Organic solvents may also be used to facilitate cleaning. [NOTE—These steps describe one way to clean equipment; alternatively, particulate-free equipment may be obtained from a suitable vendor.] Just before use, rinse the equipment in filtered water, using a hand-held pressure nozzle with final filter or other appropriate filtered water source. To collect blank counts, use a cleaned vessel of the type and volume representative of that to be used in the test. Place a 50-mL or more volume of filtered water in the vessel, and agitate the water sample in the cleaned glassware by inversion or swirling. [NOTE—A smaller volume, consistent with the article to be counted, can be used.] Degas by one of three means: sonication (at 80–120 watts) for about 30 seconds, or by vacuum, or by allowing to stand. Swirl the vessel containing the water sample by hand or agitate by mechanical

means to suspend particles. Determine the particulate matter in five samples of filtered water, each of 5 mL. If the number of particles of 10 μm or greater size exceeds 25 for the combined 25 mL (NMT 1/mL), the precautions taken for the test are not sufficient: the filtered water or glassware have not been properly prepared or the counter is generating spurious counts. In this case, repeat the preparatory steps until conditions of analysis are suitable for the test. It is recommended that when utilizing the test for the *Particulate Matter in Ophthalmic Solutions* ⟨789⟩ method, the blank test should be considered failed, if in addition, the number of particles of 25 μm or greater in size exceeds 3.

Test Procedure

TEST PREPARATION

Prepare the test specimens in the following sequence. Outside of the unidirectional airflow cabinet to be used for the test, remove outer closures, sealing bands, and remove or tape over labels. Rinse the exteriors of the containers with filtered water as directed under *Test Environment*. Protect the containers from environmental contamination until analyzed. Open and withdraw, pour, or otherwise sample the contents of the containers under test in a manner least likely to generate particles that could enter the test specimen. Contents of containers with removable stoppers may be withdrawn directly by removing the closures. Sampling devices having a needle to penetrate the unit closure may also be employed. Products packaged in flexible plastic containers may be sampled by cutting the medication or administration port tube or a corner from the unit with a suitably cleaned razor blade or scissors. Dry or lyophilized products may be constituted using their internal diluent, by removing the closure to add supplied product diluent or by injecting filtered water via hypodermic syringe. If test specimens are to be pooled, remove the closure and empty the contents into a clean container.

NUMBER OF TEST SPECIMENS

The number of test specimens should be adequate to provide a statistically sound assessment of whether a batch or other large group of units is adequately represented. At minimum, if the volume in the container is less than 25 mL, test a solution pool of 10 or more units to provide at least 25 mL. Small-volume parenteral injection product or ophthalmic product units may be tested individually if the unit volume is 25 mL or more. For large-volume injections, individual units are tested.

For all products, regardless of volume, comprehensive experience regarding the integrity and consistency of the batch is gained throughout development, allowing the proper sampling plans to be applied in commercial production that ensure sample selection is representative of batch quality. All batches should have sampling plans that accommodate desired statistical measures of batch quality and facilitate process control.

PRODUCT DETERMINATION

Depending upon the dosage form being tested, proceed as directed under the appropriate category below.

Liquid Preparations—

Volume in Container Less Than 25 mL—Prepare the containers as directed under *Test Preparation*. Mix and suspend the particulate matter in each unit by inverting the unit 20 times. [NOTE—Because of the small volume of some products, it may be necessary to agitate the solution more vigorously to suspend the particles properly.] Open and combine the contents of 10 or more units in a cleaned container, to obtain a volume of NLT 25 mL. De-

gas the pooled solution by one of three means: sonication for about 30 seconds, or by vacuum, or by allowing the solution to stand.

Gently stir the contents of the container by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Remove four portions, each of NLT 5 mL, and count the number of particles ≥10 μm and 25 μm. Disregard the result obtained for the first portion. [NOTE—For low-volume products, a pool of 15 or more units may be necessary to achieve a pool volume sufficient for four 5-mL sample aliquots. Smaller sample aliquots (i.e., less than 5 mL) can be used if the assay result obtained with the smaller aliquots is validated to give an assessment of batch suitability equivalent to that obtained with the 5-mL aliquots specified above.]

Volume in Container 25 mL or More—Prepare the containers as directed under *Test Preparation*. Mix and suspend the particulate matter in each unit by inverting the unit 20 times prior to opening the container for degassing. Degas the solution by one of three means: by sonication for about 30 seconds, or by vacuum, or by allowing the solution to stand. When sampling, ensure that the counter probe can be inserted into the middle of the solution. Gently stir the contents of the unit by hand-swirling or by mechanical means. Remove four portions, each of NLT 5 mL, and count the number of particles ≥10 μm and 25 μm. Disregard the result obtained for the first portion.

Dry or Lyophilized Preparations—Prepare the containers as directed under *Test Preparation*. Open each container, taking care not to contaminate the opening or cover. Constitute as directed under *Test Preparation*, using the specified volume of enclosed filtered water or an appropriate laboratory-filtered diluent if suitable. Replace the closure, and manually agitate the container sufficiently to dissolve the drug. [NOTE—For some dry or lyophilized products, it may be necessary to let the containers stand for a suitable interval, and then agitate again to dissolve.] After the drug in the constituted sample is completely dissolved, degas the solution by sonication for about 30 seconds, or by exposing to vacuum, or by allowing the solution to stand. When sampling, ensure that the counter probe can be inserted into the middle of the solution. Gently stir the contents of the unit by hand-swirling or by mechanical means to mix and suspend any particulate matter. Proceed as directed for the appropriate unit volume under *Liquid Preparations*, and analyze by withdrawing a minimum of four portions, each of NLT 5 mL, and count the number of particles ≥10 and 25 μm. Disregard the result obtained for the first portion.

Products Packaged with Dual Compartments Constructed to Hold the Drug Product and a Solvent in Separate Compartments—Prepare the units to be tested as directed under *Test Preparation* and according to product insert directions. Mix each unit as directed in the labeling, activating and agitating so as to ensure thorough mixing of the separate components and drug dissolution. Open and degas the units or pooled specimen to be tested by one of three means: sonication, or by vacuum, or by allowing the solution to stand. Proceed as directed for the appropriate unit volume under *Liquid Preparations*, mix and suspend the particulate matter present in each unit by inversion or swirling or by mechanical means and analyze by withdrawing a minimum of four portions, each of NLT 5 mL, and count the number of particles ≥10 and 25 μm. Disregard the result obtained for the first portion.

Products Labeled ''Pharmacy Bulk Package Not for Direct Infusion''—Proceed as directed for *Liquid Preparations* when the volume is 25 mL or more. Calculate the test result on a portion that is equivalent to the maximum dose given in the labeling. For example, if the total bulk package volume is 100 mL and the maximum dose volume is 10 mL, then the average light obscuration

particle count per mL would be multiplied by 10 to obtain the test result based on the 10-mL maximum dose. [NOTE—For the calculations of test results, consider this maximum dose portion to be the equivalent of the contents of one full container.]

LO Calculations

Note that the particle limits should be reported as all particles ≥10 μm and all particles ≥25 μm. If the instrument has been configured to count in differential bins, such as ≥10–25 μm, ≥25–50 μm, ≥50 μm, etc., all bins ≥10 μm are added to yield total ≥10 μm count; all bins ≥25 μm need to be added to yield total count ≥25 μm.

For example, the analyst has counted the test samples in eight bins: a) ≥10–15 μm, b) ≥15–25 μm, c) ≥25–40 μm, d) ≥40–75 μm, e) ≥75–100 μm, and f) ≥100 μm. $P_{\Sigma\geq 10}$ would then be calculated as:

$$P_{\Sigma\geq 10} = P_{\geq 10\text{–}15\ \mu m} + P_{\geq 15\text{–}25\ \mu m} + P_{\geq 25\text{–}40\ \mu m} + P_{\geq 40\text{–}75\ \mu m} + P_{\geq 75\text{–}100\ \mu m} + P_{\geq 100\ \mu m}$$

POOLED SAMPLES

Average the counts from the two or more aliquot portions analyzed. Calculate the number of particles in each container by the formulae:

$$P_{\Sigma 10} V_T / V_A n$$

$$P_{\Sigma 25} V_T / V_A n$$

in which $P_{\Sigma 10}$ is the average particle count per threshold obtained from all portions analyzed and $P_{\Sigma 25}$ is the average particle count per threshold obtained from all portions ≥25 μm analyzed. V_T is the volume, in mL, of pooled sample; V_A is the volume, in mL, of each portion analyzed; and n is the number of containers pooled.

INDIVIDUAL SAMPLES

Average the counts obtained for the 5-mL or greater aliquot portions from each separate unit analyzed, and calculate the number of particles in each container by the formulae:

$$P_{\Sigma 10} V / V_A$$

$$P_{\Sigma 25} V / V_A$$

in which $P_{\Sigma 10}$ is the average particle count obtained from all portions analyzed and $P_{\Sigma 25}$ is the average particle count obtained from all portions portions ≥25 μm analyzed. V is the volume, in mL, of the tested unit; and V_A is the volume, in mL, of each portion analyzed.

INDIVIDUAL UNIT SAMPLES

Average the counts obtained for the two or more 5-mL aliquot portions taken from the solution unit. Calculate the number of particles in each mL of product solution taken by the formulae:

$$P_{\Sigma 10} / V$$

$$P_{\Sigma 25} / V$$

in which $P_{\Sigma 10}$ is the average particle count per threshold obtained from all portions analyzed and $P_{\Sigma 25}$ is the average particle count per threshold obtained from all portions ≥25 μm analyzed. V is the volume, in mL, of the portion taken.

For all types of product, if the tested material has been diluted to decrease the viscosity, the dilution factor must be accounted for in the calculation of the final test result. For all test results, the particle count $\geq$10 μm represents all threshold bin counts.

MEMBRANE MICROSCOPE PARTICLE COUNT TEST

The microscope particulate matter test may be applied to all product types: both large-volume and small-volume parenteral injections and ophthalmic solution products. This test enumerates any material retained on the isolation membrane[3] $\geq$10 μm after rinsing and drying the membrane. Because a wide range of test aliquots may be utilized, particle counts may be determined on a per volume or per container basis without dilution or extrapolation. The MM method is often used in conjunction with LO methodology, providing isolated particles for further study. In some cases test articles cannot be tested meaningfully by stage 1 light obscuration, and so the MM assay is the sole method for particle enumeration.

In the performance of the MM assay, estimate the size of retained solids viewed at 100× magnification, tabulating them into specific size categories. In this process, materials may be encountered on the membrane surface that do not appear solid or substantial, showing little or no surface relief such as a ''stain'' or discontinuity on the membrane. For the purpose of this assay, do not attempt to size or enumerate such semisolid particles. However, from a formulation robustness aspect, consistent evidence of such materials may be indication that further development research is warranted to understand their content. The nature of these materials and subsequent decision to count or investigate must be based on product formulation experience. Interpretation of microscopical enumeration may be aided by testing a sample of the solution by the light obscuration particle count or a validated, alternate method.

Test Apparatus

MICROSCOPE

Use a compound binocular microscope that corrects for changes in interpupillary distance by maintaining a constant tube length. The objective and eyepiece combination of lenses should give a magnification of 100 ± 10×. The objective should be of 10× nominal magnification, a planar achromat or better in quality, with a minimum numerical aperture of 0.25. In addition, the objectives should be compatible with an episcopic illuminator attachment. The eyepieces should be matched. In addition, one eyepiece should be designed to accept and focus an eyepiece graticule. The microscope should have a mechanical stage capable of holding and traversing the entire filtration area of a 25- or 47-mm membrane filter.

ILLUMINATORS

Two illuminators are required. One is an external, focusable illuminator that can be adjusted to give incident oblique illumination at an angle of 10° to 20°. The other is an episcopic brightfield illuminator internal to the microscope. Both illuminators should be of sufficient output to provide a bright and even source of illumination and may be equipped with blue daylight filters to decrease operator fatigue during use.

[3] Soft particles and semisolid substances may also be retained.

CIRCULAR DIAMETER GRATICULE

Use a circular diameter graticule (see *Figure 1*) matched to the microscope model objective and eyepiece such that the sizing circles are within 2% of the stated size at the plane of the stage.

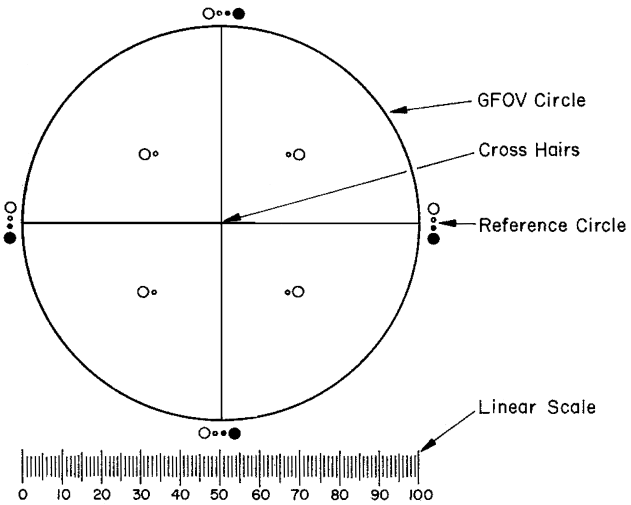


Figure 1. Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10- and 25-μm diameters at 100 × are provided as comparison scales for particle sizing.

STAGE MICROMETER

A commercial stage micrometer, such as 1 mm in 10-μm increments, is quite useful for day-of-use verification of 100 × set-up. The USP graticule will not change; however, optical elements of the microscope may have been altered, or the improper objective may been selected for the count—verification of the USP graticule linear scale with the stage micrometer linear scale is a simple and unequivocal verification of proper magnification.

FILTRATION APPARATUS

Use a filter funnel suitable for the volume to be tested, generally having an inner diameter of about 20 mm for 25 mm membranes or about 38 mm for 47 mm membranes. The funnel is made of plastic, glass, or stainless steel. Use a filter support made of stainless steel screen or sintered glass as the filtration diffuser. The filtration apparatus is equipped with a vacuum source, a solvent dispenser capable of delivering solvents filtered through a membrane filter at a range of pressures from 10–80 psi, and membrane filters (25- or 47-mm nongridded or gridded, black or dark gray (of a suitable material that is compatible with the product), with a nominal pore size of 1.0 μm or finer). The use of finer pore-size membranes for counting may be preferable because the membrane surface is much smoother and yields improved contrast for particle margin recognition. However, finer pore size selections may impede more viscous sample fluid during the assay. Finally, always use nonserrated forceps to handle membrane filters.

Test Environment

It is ideal to use two unidirectional airflow hoods (UAFH) or other unidirectional airflow enclosures, one for ''wet'' sample preparations, and the other an enclosure for the microscope counting phase. The UAFH has a capacity sufficient to envelop the area in which the analysis is prepared. The UAFH provides HEPA-filtered air, which typically contains NMT 100 particles (0.5 μm or larger) per cubic meter. A blank determination is necessary at the beginning of each test sequence to verify minimal contribution from the background, equipment, and personnel operations. To determine the blank count, assemble a clean filtration apparatus with a fresh membrane, rinse the interior with filtered water to drain, then deliver a 50-mL or more volume of filtered water to the filtration funnel while applying vacuum, and draw

the entire volume of water through the membrane filter. Remove the membrane from the filter funnel base, and place onto a holding device that will be used for test specimens, typically atop a strip of double-sided tape on a microscope slide or in a commercial membrane holder or Petri dish. After allowing the membrane to dry, examine the entire filtration area microscopically at a magnification of 100 ×. If NMT 20 particles ≥10 μm and NMT 5 particles ≥25 μm or larger are present within the filtration area, the background particle level is sufficiently low for performance of the microscope assay for *Particulate Matter in Injections* ⟨788⟩. If the particle load exceeds these limits, repeat the procedure.

There is value in further limiting the background for both injections and ophthalmic solutions testing in regard to good laboratory practice, and more specifically in regard to the *Particulate Matter in Ophthalmic Solutions* ⟨789⟩ ≥25 μm and ≥50 μm limits, which may be considered more restrictive than injectable limits in consideration of total particle content allowed for the (usually) small-unit volumes. Compare total particle load for a lower volume SVI and lower volume LVI with a 5-mL ophthalmic product in the following table.

Comparison of Total At-limit Load for Selected Products

Size Limit	Blank Count	SVI, 5 mL	LVI, 125 mL	Ophthalmic Product, 5 mL
≥10 μm	20	3000 particles	1500 particles	250 particles
≥25 μm	5	300 particles	250 particles	25 particles
≥50 μm	Not defined	N/A	N/A	10 particles

Therefore, for the smaller volume ophthalmic products and for a low-particle count injectable product, it may be beneficial for the laboratory to strive for consistent and low blank counts such as NMT 5 ≥10 μm, NMT 1 ≥25 μm and none ≥50 μm per blank.

Throughout this procedure, it is preferable to use powder-free gloves and thoroughly clean glassware and equipment. Prior to conducting the test, clean the work surfaces of the unidirectional flow enclosure with an appropriate filtered solvent. Glassware and equipment should be rinsed successively with a warm, residue-free solution of detergent, hot water, filtered distilled or deionized water, and isopropyl alcohol. [NOTE—Prior to use, pass the distilled or deionized water and the isopropyl alcohol through membrane filters having a nominal pore size of 0.2-μm or finer.] Perform the rinsing in the unidirectional airflow enclosure. Allow the glassware and filtration apparatus to dry in the unidirectional airflow enclosure, upstream of all other operations. Preferably, the enclosure is located in a separate room that is supplied with filtered air-conditioned air and maintained under positive pressure with respect to the surrounding areas.

MICROSCOPE PREPARATION

Two illuminators are necessary. First, select a filter membrane containing particles, and position it for counting on the microscope stage, for viewing at 100 ×. Next, using an auxiliary illuminator, bring the illumination close to the microscope stage to give a concentrated area of illumination on the filter membrane, and adjust the illuminator height so that the angle of incidence of the light is 10–20 with the horizontal, providing a full and even illumination when viewing the membrane at 100 ×. Next, using an internal episcopic brightfield illuminator, fully open the field and aperture diaphragms. Center the lamp filament, and focus the microscope on

the above membrane. Adjust the intensity of reflected illumination until particles are clearly visible and show pronounced shadows. This is often done by adjusting the intensity of episcopic illumination to the lowest setting, then increasing the intensity until shadows cast by particles show the least perceptible decrease in contrast.

USING THE CIRCULAR DIAMETER GRATICULE

The USP graticule is specifically fabricated for each microscope. The relative error of the graticule used is ±2% and is initially measured with a NIST-certified stage micrometer. To accomplish this, align the graticule micrometer scale with the stage micrometer so that they are parallel. (Compare the scales, using as large a number of graduations on each as possible.) Read the number of graticule scale divisions, GSD, compared to stage micrometer divisions, SMD. Calculate the relative error by the formula:

$$100[(GSD - SMD)/SMD]$$

A relative error of ±2% is acceptable and verifies good alignment, focus, and proper magnification. Thereafter, a day-of-use verification by the microscope operator with the NIST stage micrometer or commercial stage micrometer is sufficient to demonstrate proper setup.

The basic technique of measurement applied with the use of the circular diameter graticule is to count all particles 10 μm and larger, further categorizing into ≥10-μm and ≥25-μm particles. The circular zone or graticule field of view is a useful zone for active sizing and counting. Particles are compared to the linear scale and/or circles to determine their size in equivalent circular diameter. This is conducted by transforming mentally the image of each particle into a circle and then comparing to the 10- and 25-μm graticule reference circles. The sizing process is carried out without superimposing the particle on the reference circles; particles are not moved from their locations within the graticule field of view (the large circle) for comparison to the reference circles. Compare the area of the particle being sized to that of the black or transparent circles. Use the area of the clear graticule reference circles to size white or transparent particles. Use the area of the black reference circles to size dark particles. The intent of comparing particles to an equivalent circular diameter is correlation to the light obscuration particle sizing methodology, for which many manufacturers have extensive databases. In practice, particles with nearly circular areas will correlate well with the graticule circle diameters. For particles with one long axis, such as rods and needles, the conversion to circular area will produce more significant bias to smaller estimated sizes. It may be simpler, and most conservative, to count particles in the longest chord. To use an extreme example, the total count of mono-dispersions of fine needle crystals would vary greatly depending on the size determination utilized.

In order to properly focus the ocular lenses and attain balanced single-field view, each operator must bring the USP graticule lines into sharp focus by adjusting the eyepiece diopter ring (it helps to have an ''infinite'' view, or out-of-focus specimen). Next, focus the microscope on a specimen, through this same eyepiece, and then looking only through the other eyepiece, adjust its diopter ring to bring the specimen into sharp focus. The USP graticule and specimen particles are now in focus on a well-balanced illumination field.

PREPARATION OF FILTRATION APPARATUS

Preferably, wash the filtration funnel, base, and diffuser in a solution of liquid detergent and hot water. Rinse with hot water. Following the hot water rinse, apply a second rinse with filtered water, using a pressurized jet of water over the entire exterior and interior surfaces of the filtra-

tion apparatus. Repeat the pressurized rinse procedure using filtered isopropyl alcohol. Finally, using the pressurized system, rinse the apparatus with filtered water.

Remove a membrane filter from its container, using ultra-cleaned nonserrated forceps. Use a low pressurized stream of filtered purified water to wash both sides of the filter thoroughly by starting at the top and sweeping back and forth to the bottom. Assemble the cleaned filtration apparatus with the diffuser on top of the filtration base, placing the clean membrane filter on top of the diffuser. Place the funnel assembly on top of the filtration base, and lock it into place.

Test Procedure

TEST PREPARATIONS

Prepare the test specimens in the following sequence. Outside of the unidirectional airflow cabinet used for the test, remove outer closures, sealing bands, and remove or tape over labels. Rinse the exteriors of the containers with filtered water as directed under *Test Environment*. Protect the containers from environmental contamination until analyzed. Open and withdraw, pour, or otherwise sample the contents of the containers under test in a manner least likely to generate particles that could enter the test specimen. Contents of containers with removable stoppers may be withdrawn directly by removing the closures. Sampling devices having a needle to penetrate the unit closure may also be employed. Products packaged in flexible plastic containers may be sampled by cutting the medication or administration port tube or a corner from the unit with a suitably cleaned razor blade or scissors. Dry or lyophilized products may be constituted using their internal diluent, by removing the closure to add supplied product diluent or by injecting filtered water via hypodermic syringe. If test specimens are to be pooled, remove the closure and empty the contents into a clean container.

NUMBER OF TEST SPECIMENS

The number of test specimens should be adequate to provide a statistically sound assessment of whether a batch or other large group of units is adequately represented. At minimum, if the volume in the container is less than 25 mL, test a solution pool of 10 or more units to provide at least 25 mL. Small-volume parenteral injection product or ophthalmic product units may be tested individually if the unit volume is 25 mL or more. For LVI, individual units are tested.

For all products, regardless of volume, comprehensive experience regarding the integrity and consistency of the batch is gained throughout development, allowing the proper sampling plans to be applied in commercial production that ensure sample selection is representative of batch quality. All batches should have sampling plans that accommodate desired statistical measures of batch quality and facilitate process control.

PRODUCT PARTICLE COUNT DETERMINATION

Depending upon the dosage form being tested, proceed as directed under the appropriate category below.

Liquid Preparations—Thoroughly mix the units to be tested by inverting 20 times. Open the units in a manner consistent with the generation of the lowest possible numbers of background particles. For products <25 mL in volume, one may open them and drain to the filtration barrel individually, or combine the contents of 10 or more units in a cleaned container. [NOTE—When pooling containers, these are included in the blank determination step.] Filter LVI units individually. SVI units that have a volume of 25 mL or more may be filtered individually.

Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If the volume of solution to be filtered exceeds the volume of the filtration funnel, add, stepwise, a portion of the solution until the entire volume is filtered. It is prudent to maintain the liquid volume in the filtration funnel above one-half of the funnel volume between refills, especially if the partial count procedure is to be used (see *Partial Count Procedure* under *Enumeration of Particles*). [NOTE—This is necessary to ensure even distribution of particles on the analytical membrane.] After the last addition of solution, begin rinsing the walls of the funnel by directing a low-pressure stream of filtered water in a circular pattern along the walls of the funnel, and stop rinsing the funnel before the volume falls below about one-fourth of the fill level. Maintain the vacuum until all the liquid in the funnel is gone.

Remove the filtration funnel from the filtration base while maintaining vacuum, then turn the vacuum off, and remove the filter membrane with nonserrated forceps. Place the filter in the prepared holder and label with sample identification. Allow the filter to air-dry in the unidirectional airflow enclosure with the cover ajar.

Dry or Lyophilized Preparations—To test a dry powder vial or similar container of drug powder, constitute the material with an appropriate diluent, using the method least likely to introduce extraneous contamination, as directed for *Test Preparation* under *Light Obscuration Particle Count Test.* Using a solution pool of 10 or more units, or the desired number of individual units, proceed as directed for *Liquid Preparations.*

Products Packaged with Dual Compartments Constructed to Hold the Drug Product and a Solvent in Separate Compartments—Activate each unit as directed in the labeling, agitating the contents sufficiently to ensure thorough mixing of the separate components, and then proceed as directed for *Liquid Preparations.*

Pharmacy Bulk Packages or Multiple-Dose Containers—For *Products Labeled ''Pharmacy Bulk Package—Not for Direct Infusion''* or for multiple-dose containers, proceed as directed for *Liquid Preparations*, filtering the total unit volume.

Calculate the test result on a portion that is equal to the maximum dose given in the labeling. Consider this portion to be the equivalent of the contents of one full container. For example, if the total bulk package volume is 100 mL and the maximum dose listed is 10 mL, the microscopic total unit volume count test result would be multiplied by 0.1 to obtain the test result for the 10-mL dose volume. [NOTE—For calculation of the test result, consider this portion to be the equivalent of the contents of one full container.]

Enumeration of Particles

The microscope test described in this section is flexible in that typical artifacts such as air and immiscible liquids do not interfere with the final count. The method has a broad size detection and counting range, if applying the partial count procedure. This method may be used when all particles on an analysis membrane surface are counted or when only those particles on some fractional area of a membrane surface are counted.

TOTAL COUNT PROCEDURE

Note that the particle limits should be reported as all particles $\geq$10 μm and all particles $\geq$25 μm. If the lab method has been configured to tally the particle count in differential bins, such as $\geq$10–25 μm, $\geq$25–50 μm, $\geq$50 μm, etc., all tallies $\geq$10 μm are added to yield total $\geq$10 μm count; all bins $\geq$25 μm need to be added to yield total count $\geq$25 μm. Using a number of narrow size bins may be beneficial in product improvement efforts to separate particle groups.

In performance of a total count, the graticule field of view (GFOV) is defined by the large circle of the graticule and the vertical crosshair is used as a counting target. Scan the membrane in paths that cover the effective filtration area (EFA), adjoining but not overlapping previous scan paths. Repeat this procedure, tabulating particle counts minimally in the ≥10–25 μm and ≥25 μm thresholds, moving across the membrane until all particles on the membrane within the EFA are counted. More and narrower size range tallies may be deemed useful by the production team. Record the total number of particles that are ≥10–25 μm and the number that are ≥25 μm or larger.

For large-volume products, calculate the particle count, in particles per mL, for each unit tested by the formulae:

$$P_{\Sigma\geq 10}/V$$

$$P_{\Sigma\geq 25}/V$$

in which $P_{\Sigma\geq 10}$ is the total particle count obtained from all portions analyzed and $P_{\Sigma\geq 25}$ is the total particle count obtained from all portions ≥25 μm analyzed. V is the volume, in mL, of the solution tested.

For example, the analyst has counted the test samples in four tallies: a) ≥10–25 μm, b) ≥25 μm–50 μm, c) ≥50–100 μm, and d) ≥100 μm. $P_{\Sigma\geq 10}$ would then be calculated as:

$$P_{\Sigma\geq 10} = P_{\geq 10\text{–}25\ \mu m} + P_{\geq 25\text{–}50\ \mu m} + P_{\geq 50\text{–}100\ \mu m} + P_{\geq 100\ \mu m}$$

For small-volume injections, calculate the particle count, in particles per container, by the formulae:

$$P_{\Sigma\geq 10}/n$$

$$P_{\Sigma\geq 25}/n$$

in which $P_{\Sigma\geq 10}$ is the total particle count obtained from all portions analyzed and $P_{\Sigma\geq 25}$ is the total particle count obtained from all portions ≥25 μm analyzed. n is the number of units pooled (one in the case of an individual unit).

PARTIAL COUNT PROCEDURE

Note that the particle limits should be reported as all particles ≥10 μm and all particles ≥25 μm. When partial counting of particles on a membrane is performed, the analyst should first ensure that an even distribution of particles is present on the membrane. This is assessed by rapid scanning of the prepared membrane in order to detect clumps of particles. Significant clumping should not be present. Count the 10-μm or larger particles in one GFOV at the edge of the filtration area as well as those in the center of the membrane. The number of 10-μm or larger particles in the GFOV with the highest total particle count is NMT twice that of the GFOV with the lowest particle count. Reject a filter failing these criteria, and prepare another if a partial count procedure is used, or, alternatively, analyze this membrane by the total count method.

The normal number of GFOV counted for a partial count is 20. If a smaller confidence interval about the result is desired, a larger number of fields and particles may be counted. Count all particles that have a circular area diameter of 10 μm or larger and 25 μm or larger within the GFOV and those that are in contact with the right side of the GFOV circle. Do not count particles outside of the GFOV. Ignore those that touch the left side of the GFOV circle. The dividing line between right and left sides of the GFOV circle is the vertical cross hair. [NOTE—Make the best possible judgment on particle size without changing the microscope magnification or illumination.]

To perform a partial count of the particles on a membrane, start at the right center edge of the filtration area and begin counting adjacent GFOVs. When the left edge of the filtration area is reached, move one GFOV toward the top of the filter, and continue counting GFOVs by moving in the opposite direction. Moving from one GFOV to the next can be accomplished by one of two methods. One method is to define a landmark (particle or surface irregularity in the filter) and move over one GFOV in relation to the landmark. A second method is to use the vernier scale on the microscope stage to move 1 mm between GFOVs. To facilitate the latter, adjust the microscope x- and y-stage positioning controls to a whole number at the starting position at the center right edge of the filtration area, then each GFOV will be one whole division of movement of the x-stage positioning control. If the top of the filtration area is reached before the desired number of GFOVs is reached, begin again at the right center edge of the filtration area one GFOV lower than the first time. This time move downward on the membrane when the end of a row of GFOVs is reached. Continue as before until the number of GFOVs is complete.

For large-volume products, extrapolate the total count of particles per mL by the formulae:

$$P_{\Sigma 10}A_T/A_PV$$

$$P_{\Sigma 25}A_T/A_PV$$

in which $P_{\Sigma 10}$ is the total particle count obtained from all fields of view and all size thresholds and $P_{\Sigma 25}$ is the total particle count obtained from all fields of view and all size thresholds $\geq$25 μm; A_T is the filtration area, in mm², of the membrane (inner filtration barrel diameter); A_P is the partial area counted, in mm², based on the number of graticule fields counted (GFOV area × number GFOV counted); and V is the volume, in mL, of solution filtered.

For a solution pool (for small-volume product units containing less than 25 mL) or for a single unit of a small-volume product, extrapolate the total count of particles per unit by the formulae:

$$P_{\Sigma 10}A_T/A_Pn$$

$$P_{\Sigma 25}A_T/A_Pn$$

in which $P_{\Sigma 10}$ is the total particle count obtained from all fields of view and all size thresholds and $P_{\Sigma 25}$ is the total particle count obtained from all fields of view and all size thresholds $\geq$25 μm; n is the number of units counted (one in the case of an individual unit). For all types of product, if the tested material has been diluted to decrease viscosity, the dilution factor must be accounted for in the calculation of the final test result.▲*USP34*

DIETARY SUPPLEMENT CHAPTERS

BRIEFING

〈**2030**〉 **Supplemental Information for Articles of Botanical Origin,** *USP 32* page 777. The Dietary Supplements—General Chapters Expert Committee recommends to include an additional article, Elm, in this general chapter and to make editorial changes in the existing articles to maintain consistency throughout the chapter.

(DS-GC: Y. Tokiwa) RTS—C76366

Change to read:

PROTOCOL CONTENTS

Change to read:

SUPPLEMENTAL INFORMATION AND GENERAL GUIDANCE PROTOCOLS

Black Cohosh *Actaea racemosa* L. [*Cimicifuga racemosa* (L.) Nutt.] (Fam. Ranunculaceae)

Botanical Identification—*Actaea racemosa* L. Herbaceous perennial from rhizome.

Stem: Erect, solitary, to 2.5 m tall, glabrous.

Leaves: Basal and cauline, alternate, 2–4-ternately compound, petioles 15 to 60 cm long, bases clasping stem; leaflets 20 to 70; terminal leaflet of central division 3-lobed, 6 to 15 cm long, 6 to 16.5 cm wide, with 3 prominent veins arising from base; subterminal leaflets with blades ovate-lanceolate to obovate, 4 to 12 cm long and 3 to 8 cm wide; margins toothed to deeply incised; green above, paler below; glabrous or rarely pubescent along veins of undersurface.

Inflorescence: Terminal panicle of 4 to 9 slender branches, each 7 to 60 cm long, pubescent; 1 bract subtending each pedicel.

Flowers: Perfect, radially symmetric; sepals 4, greenish-white, caducous; petals 0; staminodes (1–) 4 (–8), petaloid, cream-colored, 2 to 3 mm long, clawed, apex bifid; stamens 55 to 110; pistils 1 (–3), glabrous to pubescent, ovary superior, style short, stigma 0.5 mm wide.

Fruit: Many-seeded follicle, 5 to 10 mm long, ovoid, laterally compressed with curved, stout beak (peristent style), pubescent; seeds hemispheric, brown, scales lacking. Chromosome number: n = 8.

There are currently two varieties of *A. racemosa* recognized based on differences in leaf morphology: var. *racemosa* and var. *dissecta*. The former variety has triternate-pinnate leaves with serrate margins, while the latter has quadriternate-pinnate leaves that are deeply incised with serrate lobes. Variety *dissecta* is only known from very few herbarium specimens, all of which were collected well over 100 years ago, making this taxon of uncertain taxonomic significance.

Compendial History—Black cohosh appeared on the secondary list of substances in the first *United States Pharmacopoeia (USP)* of 1820, where it was listed as an anti-inflammatory and antispasmodic. It soon rose to the primary list in 1830, a position it held until the 10th decennial revision of 1920. Black cohosh appeared in the first edition of the *United States Dispensatory (USD)* in 1833 and remained through 1955 for a total of 122 years. Carrying forward the traditional Native American use of black cohosh for women's ailments and Barton's use for throat complaints, current therapeutics finds the plant used in a number of preparations for coughs and for gynecological disorders. In 2001, both the rhizome and the dry rhizome extract of black cohosh were proposed once again for inclusion in the *United States Pharmacopeia–National Formulary (USP–NF).* (See revised proposal on page 1455 of *PF* 28(5) [Sept.–Oct. 2002].) The monograph became official in the *Second Supplement* to *USP 30–NF 25*.

Constituents—Major constituents of black cohosh are triterpene glycosides principally as beta-xylopyranosides and alpha-arabinopyranosides. The aglycones are mostly derived from acteol and cimigenol. The nomenclature of these compounds is quite confusing in the literature with different names often given to the same compounds. A cyclopropane ring is a common feature of these compounds, which are structurally related to cycloartenol. The isoflavone formononetin has been reported in some publications, however recent evidence indicates its absence in the roots and rhizomes of *Actaea racemosa.* Other constituents include tannins, resin, fatty acids, starch, sugars, and aromatic acids including ferulic acid, isoferulic acid, caffeic acid, and salicylic acid.

Sources

▲and Distribution—

Sources—▲*USP34*

Black cohosh can be found in moist deciduous forests, ravines, moist meadows, creek margins, and mountainous terrain. Black cohosh flowers from June to September and is native to eastern North America from Ontario south to Georgia and west to Missouri. The entire supply of Black Cohosh comes from the United States. The major producers of black cohosh are Kentucky and Tennessee, with additional supplies coming from Georgia, Ohio, North Carolina, Michigan, South Carolina, Virginia, West Virginia, and Wisconsin. Although there are reports of black cohosh being grown in China and India for export, the true identity of the cultivated material has not been verified and may well be an Asian species of *Actaea* such as *A. cimicifuga* (syn. *Cimicifuga foetida*). The vast majority of the commercial black cohosh is wild harvested. Concern over the conservation of black cohosh due to increasing demand makes this species a good candidate for cultivation.

▲*Distribution*—North America (Ontario, Kentucky, Tennessee, Georgia, Ohio, Missouri, North Carolina, Michigan, South Carolina, Virginia, West Virginia, and Wisconsin), China, and India.▲*USP34*

Collection and Cultivation—

Collection (Conservation and Ecology)—Traditionally, black cohosh has been harvested after plants become reproductive, which occurs anywhere from 2 to 8 years of age in cultivated plants depending on growing techniques (see *Cultivation*). A portion of the rhizome with a visible bud on it should be left in the ground to resprout the following year. There is no published information on the relationship between the constituent profile of the rhizome and its age, growing conditions, or place of origin, although such studies are underway. The impact of harvest on wild populations of black cohosh is currently unknown and sources differ in their opinion about it. While some maintain that current levels of harvest threaten the viability of wild populations, others feel that sustainable harvesting is possible at current levels of demand. A study of sustainable harvest limits is currently underway. The regulatory status regarding the trade of black cohosh is under review by the CITES. Refraining from harvesting plants until after they have set seed and leaving a portion of the rhizome in the ground to resprout are key components to sustainable harvesting.

Cultivation Practices—Black cohosh is grown from rhizome cuttings or seeds and requires some shading, depending on altitude and other environmental conditions. If grown from rhizome cuttings, a plant takes 2 to 3 years to become reproductive; grown from seed sown in the greenhouse and then planted, takes 4 to 6 years; direct-seeded may take from 6 to 8 years. Preliminary work indicates that black cohosh can be propagated successfully using in vitro techniques.

In-Process Revision

Optimal Times for Harvest—Rhizomes and roots should be harvested in autumn when the plant is dormant. At that time the underground portions of the plant have lower moisture content than in other seasons. Fall harvesting also allows plants to produce mature seeds before being uprooted.

~~**Drying, Storage, and Shipping**~~

▲Post-Harvest Handling—▲*USP34*

Optimal Handling and Processing Practices—Rhizomes with roots may be processed fresh or dried. They should be thoroughly washed directly after harvest and then laid out to dry. Freshly harvested roots should be solid but not woody.

Drying—Rhizomes with roots are cut and air-dried at 35° to 45°. They are fully dried when they are brittle and snap easily and when no moisture is evident in cross section, either visibly or to the touch.

Storage—Follow general guidelines for storage by packing in airtight containers protected from light, heat, moisture, and insect infestation.

Adulterants

▲and Contaminants—▲*USP34*

Other species of *Actaea,* especially yellow cohosh (*A. podocarpa* syn. *Cimicifuga americana*), have commonly been mixed with *A. racemosa* due to the similarity in above-ground appearance and common growing habitat between species. The two species can be distinguished by differences in their freshly harvested underground parts: the fresh rhizome of *A. podocarpa* has a distinct yellowish hue, whereas that of *A. racemosa* is black. The rhizomes of both species are far more difficult to tell apart when dry because *A. podocarpa* darkens upon drying. The underground portions of baneberry *(Actaea pachypoda* and *A. rubra)* occur as occasional adulterants of black cohosh supplies. Fruiting plants of baneberry may be distinguished from black cohosh by their fleshy white or red poisonous berries, which contrast with the dry follicles of black cohosh. No information was available on how to distinguish the underground portions of black cohosh and baneberry from each other. According to one herb dealer, the roots of baneberry are smaller than those of black cohosh, and therefore are not often harvested by wildcrafters. In the Pacific Northwest, *Actaea elata* (syn. *Cimicifuga elata*) is collected for medicinal use.

Ginger *Zingiber officinale* Roscoe (Fam. Zingiberaceae)

Botanical Identification—*Zingiber officinale* Roscoe. Herbaceous perennial from tuberous rhizome, aromatic due to the presence of volatile oils.

Stem: Erect, unbranched pseudostem formed by the tight overlap of sheathing leaf bases; 9 to 15 dm tall.

Leaf: Simple, alternate and two-ranked, sessile or petioles short with bases sheathing the stem and a ligule where the leaf base meets the stem; blade linear to narrowly lanceolate, 15 to 25 cm long, 1.5 to 3 cm wide; margin entire; glabrous to pubescent.

Inflorescence: Terminal spike, 3.5 to 8 cm long, 1.5 to 2 cm wide, with conspicuous spirally arranged primary bracts; usually borne on specialized leafless stems.

Flower: Perfect, bilaterally symmetric; calyx tubular with 3 lobes; corolla tube 2 to 2.5 cm long with lanceolate apical lobes, 1.5 to 2 cm long, 2 to 3.5 mm wide, greenish yellow; stamen 1, anther cream-colored with dark purple, elongated connective grasping upper part of style; staminodes 4, petaloid, 2 fused into an erect, ovate-oblong lip which is dull purple with cream mottling; ovary inferior; style 1, slender, exerted beyond connective.

Fruit: Loculicidal capsule; seeds shiny black with a white aril. Chromosome number: n = 11.

There are several different varieties and forms of *Zingiber.* The varying morphological characteristics of these are displayed in *Table 1*.

Table 1. Morphological and Key Characteristics of Ginger from Different Areas of Production

Source	Form	Aroma	Color (External)
Africa	Flat surfaces, mostly peeled, starchy and fibrous; 9 cm long, 1.5 cm wide	Poor quality is recognized by its camphoraceous aroma	Uncut surface dark grayish-brown; cut surface brownish-black
Australia		Citrus-like	Buff
Bengal	Flat surfaces, scraped		Gray-brown
China	Short stumpy lobes, unscraped, mostly sliced	Strong, floral to citrus	Pale brown
Cochin	Lateral surfaces lacking cork	Strong, floral to citrus	Cream color with numerous black resin dots
Jamaica (unbleached)	Up to 12 cm long, 1 cm wide; surfaces completely peeled; starchy and fibrous thin cortex	Delicate, citrus-like	All surfaces yellow-brown
Japan	Up to 7 cm long, 12 mm wide; flat surfaces usually completely peeled; starchy and fibrous thick cortex	Bergamot-like	Externally gray-white to light grayish-brown, often with white powder from being coated with lime
Malabar (Cochin and Calcutta)	Cork layer completely removed, mostly treated with chalk	Citrus-like	Almost white
Nigeria	Smaller in size than other varieties, rather less deeply scraped	Delicate	Somewhat darker than other varieties

Compendial History—Ginger was official in the *United States Pharmacopoeia* from the first edition of 1820 through the fourteenth revision of 1950, often appearing in multiple preparations. It also appeared in all editions of the *United States Dispensatory* from 1833 through the final edition of 1973 where it was described as ''a stimulant and carminative that has been used for treatment of dispepsia and flatulent colic''.

Constituents—The essential oils and the pungent principles make up some of the major components of the rhizome of ginger: 4.0% to 10.0% of the rhizome consists of an oleoresin composed of nonvolatile, pungent principles (phenols such as gingerols and their related dehydration products, shogaols); nonpungent fats and their waxes. The essential oil (1% to 3%) contains sesquiterpenes and monoterpenes, mainly geranial and nerals. Generally, but not always sesquiterpenes predominate (30% to 70%) such as zingiberene, sesquiphellandrene and beta-bisabolene, which decompose on drying and storage. The nonvolatile pungent principles include the phenylalkanones, gingerols, and the phenylalkanonols, shogaols with varying chain lengths.

Sources

▲and Distribution—

Sources—▲*USP34*

Zingiber is cultivated in most tropical and subtropical countries to greater or lesser degrees. The world production is estimated to be 100,000 tons. China and India are reported to be the primary areas of production. Approximately 5000 tons of *Zingiber* are imported into the United States. An estimated 80% of this comes from China. In China, Sichuan and Guizhou provinces reportedly produce the largest quantities and highest quality. It is also produced in Zhejian, Shandong, Hubei, Guangdong, and Shanxi provinces. Most of the dried ginger from China available in the United States has had the cortex scraped or rubbed off before it is dried. This gives it a whitish appearance. The freshly dug root is soaked overnight in water, scraped with a knife to remove the outer cortex, and then sun-dried. It has been reported that high arsenic levels in the soil of Changning County of Hunan Province, China has negatively effected *Zingiber* yields.

In India, *Zingiber* is grown on a large scale in the warm, moist regions of Madras and Cochin, and to a lesser extent in Bengal and the Punjab. Varieties grown in Bengal are reportedly the highest quality material in India. Other areas of production include Africa (Nigeria and Sierra Leone), Australia, Fiji, East Indies, Jamaica, and Hawaii. The morphological characteristics of *Zingiber* cultivated in these different areas are outlined in *Table 1*.

In older literature, Jamaican *Zingiber* is reported to be the highest quality and the most aromatic, though supplies are limited.

▲*Distribution*—Most tropical and subtropical countries, such as Australia, China (Sichuan, Guizhou, Zhejian, Shandong, Hubei, Guangdong, and Shanxi provinces), India (Cochin, Malabar, Bengal), Jamaica, Japan, Nigeria, Sierra Leon, Fiji, East Indies. Hawaii in the United States.▲*USP34*

Collection and Cultivation—

Collection (Conservation and Ecology)—When the stems wither and are white, the rhizomes are ready for collection. Usually ginger is harvested after 6 months of growth at the earliest, and sometimes not until as late as 20 months, or to obtain larger roots it is harvested in January or February of the second year of growth. In tropical and subtropical areas, roots are harvested as early as 4 months of growth as they tend to become fibrous and tough as they get older. As *Zingiber* matures it becomes more fibrous and stronger in flavor. Ginger harvest can be described in three stages:

1. Ginger that has been harvested early is known as green ginger and is traded as fresh ginger. It is succulent and tender, mellow, and mildly aromatic with a floral or lemony aroma and mild flavor.
2. Ginger harvested a few months later is more fibrous and drier and is collected for drying and may be sold as a full-flavored, pungent dried whole ginger.
3. The last harvest is usually around 9 months and yields the strongest ginger, which is quite dry and also richest in pungent components. This ginger is dried and then ground into powder.

Cultivation Practices—Ginger is a perennial herb that grows well at subtropical temperatures where the rainfall is at least 1.98 meters per year. The plant is sterile and is grown by vegetative means. Selected pieces of rhizome (''seed pieces'' or ''setts''), each bearing a bud, are planted in holes or trenches. Ideally the soil should be well-drained, rich clay loam. The growing conditions resemble those of potato cultivation. Mulching or manuring is necessary because the plant rapidly exhausts the soil of nutrients.

Ginger is susceptible to water logging and root rot. Preventive methods include using only the cleanest ginger for planting and washing it with fungicide before planting. A study growing *Zingiber* hydroponically yielded up to 125 tons per hectare in 6 to 7 months compared to 35 tons per hectare when grown in soil.

Optimal Times for Harvest—Typically in December or January.

~~*Optimal Handling and Processing Practices*—After harvesting, the rhizome is cleaned and stripped of its stems and roots. Each area processes its *Zingiber* differently after harvest. This results in the different quality and commercial grades available on the market. Green *Zingiber* consists of the rhizomes sent to market without drying. Unscraped or partially scraped varieties are traded as coated or black ginger. These roots have been scalded with boiling water and dried quickly. When dry, black *Zingiber* breaks with a horny, blackish, somewhat diaphanous fracture, due to the pasty condition of the starch. White *Zingiber* is bleached usually by rubbing with chalk or lime to lighten its color and to prevent insect infestation. Preserved ginger consists of soft, yellowish brown pieces obtained by steeping the fresh *Zingiber* in hot syrup and carefully bottling. It is soft, brown yellow and translucent. When baked, *Zingiber* loses its pungency and acquires a bitter taste.~~

▲▲*USP34*

~~**Drying, Storage, and Shipping**~~

▲Post-Harvest Handling—

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In-Process Revision

White *Zingiber* is bleached usually by rubbing with chalk or lime to lighten its color and to prevent insect infestation. Preserved ginger consists of soft, yellowish-brown pieces obtained by steeping the fresh *Zingiber* in hot syrup and carefully bottling. It is soft, brown-yellow and translucent. When baked, *Zingiber* loses its pungency and acquires a bitter taste.▲*USP34*

Drying—In general, after harvest, the fresh roots are washed and the whole dark outer skin, consisting of cork and a little underlying parenchyma, is scraped away. Scraping speeds up the drying time of the crude drug. However, excessive scraping can result in lower concentrations of essential oil that is lost with the discarded epidermal tissue. After scraping, the rhizomes are then laid out on clean floors and dried in the sun for 7 to 10 days. During this time they are occasionally turned and are piled up every night. If the fresh rhizomes are too fleshy or moist, drying will take longer and the product will end up looking shriveled. To obtain a whiter product, the ginger is moistened after 5 or 6 days and dried for another 2 days at which time it is ready for export. Dried ginger is more pungent and stronger in taste than fresh ginger.

Storage—Store in a tightly closed container, protected from light and moisture, in a cool area. A study was done on ginger harvested after 8, 9.5, 11, or 12 months. Samples were stored at 10° to 15° and 45% to 55% relative humidity or 25° to 30° and 75% relative humidity for 0, 4, or 8 weeks. Oil and oleoresin yields increased with the age of the ginger. Room temperature storage had adverse effects but refrigerated storage for up to 4 weeks had no affect on quality. When stored for extended periods of time, ground ginger loses its pungency.

Adulterants—Because *Zingiber* is so characteristic, unintentional adulterants are rare. However, in East Asia sometimes the much larger *Zedoary cassumer* and *Zedoary zerumbet* along with *Alpinia allughas* are used and found in European commerce. They are easy to distinguish due to their characteristic aromas. Occasionally, Chinese sugar-candied "ginger" is prepared from *Alpinia galangal*.

In older literature, other herbs have reportedly been used as adulterants. These include various species of *Curcuma, Capsicum,* and Grains of Paradise *(Amomum melegueta)* added to exhausted material in order to enhance the color and pungency.

Zingiber powder is sometimes adulterated with plant starches such as those from wheat middlings, potatoes, corn, barley, rice, legumes, acorns, flaxseed meal, mannihot, oil cakes from linseed, raps, mustard, almond meal, palm kernal or olives, hazelnut shells, and mineral additives. These may be easily identified microscopically. The extent of this type of adulteration in trade is unknown.

Exhausted material should be considered an adulterant.

Valerian *Valeriana officinalis* L. (Fam. Valerianaceae)

Botanical Identification—*Valeriana officinalis* L. Herbacious perennial, rhizomatous.

Stem: Solitary, hollow, 15 to 150 cm.

Leaf: Basal and cauline, opposite, oddly once pinnately lobed, lobes 11 to 21 lanceolate, entire or dentate, basal leaves petiolate, cauline leaves subsessile to clasping.

Inflorescence: Compound cyme, terminal or axillary, many pale pink to white, strongly scented flowers.

Flower: Calyx 5-lobed, lobes inconspicuous in flower, becoming elongate and pappus-like in fruit, corolla funnel-form, slightly saccate at the base, 5-lobed, tube 4 mm, lobes 1 mm, stamens 3, filaments attached to corolla tube alternate to corolla lobes, ovary inferior, tri-loculate, uni-ovulate, only 1 locule fertile, stigma tripartite.

Fruit: Achene crowned by persistent calyx, lanceolate-oblong, 4.5 to 5 mm, hairy or glabrous. Populations of *V. officinalis* range in ploidy level from diploid to tetraploid or octaploid. British *V. officinalis* is usually octaploid, and central European supplies are tetraploid.

There are three subspecies of *V. officinalis*: ssp. *officinalis,* ssp. *collina* (Wallr.) Nyman, and ssp. *sambucifolia* (Mikan fil.) Celak. All three of these subspecies, as well as the other European species of valerian, *V. repens* Host, have been considered acceptable source material for medicinal preparations.

Macroscopic Identification—Various chemotypes will have slightly different characteristics. When dried, the whole rhizome is up to 50 mm long and up to 30 mm in diameter, obconical to cylindrical, with an elongated or compressed base. It has a yellowish-brown to dark brown exterior with a circular stem and leaf scars. The rhizome contains numerous thick, light to dark brown rootlets that are located around a thin ligneous cord. The root is longitudinally wrinkled and approximately 100 mm long and 1 to 3 mm in diameter, almost cylindrical and almost the same color as the rhizome. In longitudinal section, the pith exhibits a central cavity transversed by septa. The stolons are 20 to 50 mm long, pale yellowish grey with prominent nodes separated by longitudinally striated internodes. It is commonly sliced in half for ease of cleaning. The rootlets, which contain the majority of the essential oil, are brittle and break in short, horny fractures and are whitish or yellowish internally. Aroma: when dried properly, *V. officinalis* L., s.l. has only a very faint characteristic, valeric acid-like aroma that becomes stronger as it ages. Improperly dried or old material possesses a strong and characteristic odor due to the enzymatic hydrolysis of esters of the valepotriates (isovaleric acid and hydroxyvaleric acid). Taste: mildly sweet and camphoraceous with a slightly bitter and spicy aftertaste.

Compendial History—Valerian was official in the *United States Pharmacopoeia* from the first edition of 1820 through the eleventh revision of 1930, often appearing in multiple preparations. At its peak from 1850 through 1880 it appeared six to seven times in different preparations. Valerian is among the top 30 most listed botanicals in the history of the *USP.* The root of valerian has been used as a sedative and spasmolytic in Europe since the 16th century.

Constituents—Major constituents of valerian have been identified as sesquiterpenes of volatile oils and iridoids (epoxy-triesters) known as valepotriates. The total content of volatile oil varies widely within a single species and between different species. European *Valeriana officinalis* L. usually contains 0.1% to 2.8% volatile oil. The oil consists of mixtures of monoterpene and sesquiterpene derivatives. The amount of valepotriates present also varies widely between species and genera and even within a species, generally ranging from 0.5% to 1.2%. Valepotriates are particularly unstable; they decompose easily under the effect of moisture, temperatures above 40° (104°F), or acidity (pH < 3).

Valerian also contains small amounts of aliphatic acids, alkaloids, amino acids, phenolic acids, flavonoids, free fatty acids, sugars, and salts. Valerian constituents that have possible sedative effects include acetoxyvalerenic acid, 1-acevaltrate, baldrinal, didrovaltrate, hydroxyvalerenic acid, kessane derivatives, valeranone, valerenal, valerenic acid, and valtrate.

Sources

▲and Distribution—

Sources—▲*USP34*

Valerian is found in damp or dry meadows, scrub, or woods in most of Europe, but rare in the south, and is cultivated and naturalized in North America. Valerian is cultivated in Britain, Belgium, Eastern Europe, France, Germany, ~~Holland,~~

▲▲*USP34*

Japan, the Netherlands, North America, and Russia. The majority of standardized extract products and crude cut and sifted material on the domestic market are prepared from European supplies. A large number of liquid extracts are prepared from domestically cultivated material. Many species other than *V. officinalis* are reported to be traded as medicinal valerian. These

include *V. edulis* Nutt. ex Torr. & A. Gray, *V. corneana* Briq. k, *V. stubendorfi* Kreyer ex Kom., *V. amurensis* P. Smirn. ex Kom., *V. hardwickii* Wall., *V. exaltata* Mikan, and *V. wallichi* DC. syn. *V. jatamansi* Jones.* The most frequently used North American species include *V. sitchensis* Bong and *V. edulis* Nutt.* = *V. edulis* Nutt. ex Torr. & Gray ssp. *procera.* Other species reported to be used locally include *V. arizonica* Gray, *V. capitata* Pall ex Link., *V. diocia* L., and *V. scouleri* Rydb. Detailed chemical analyses of most American species are lacking. A limited number of assays of material cultivated in the Pacific Northwest show varying levels of essential oil ranging from 0.4% to 1.3%. Valerenic acid and valepotriates have been found to be present in fresh and dry samples of *V. sitchensis* Bong. *V. sitchensis* Bong exhibits a strong pungency when fresh. High quality material is reported to contain from 1.0% to 1.5% essential oil, ≥30% extractable matter, and ≥0.5% valerenic acid.

▲*Distribution*—North America, Europe (Britain, Belgium, Eastern Europe, France, Germany, the Netherlands), Russia, and Japan.▲*USP34*

Collection and Cultivation—

Collection (Conservation and Ecology)—The majority of valerian in trade comes from cultivated material. Harvest times will vary geographically. The composition of the essential oil varies greatly among different populations of the same subspecies and even between the same population of plants from year to year. Essential oil content also varies with genotypes, harvest times, growing conditions, age of root, drying techniques, and method of analysis. It has been reported that valerian harvested in higher elevations, grown in dryer regions, or cultivated in phosphate-rich soil yields relatively high levels of essential oil.

Older literature reports that valerian should be harvested in the fall, between August and September, preferably in the second year of growth. Analyses of material cultivated in the Netherlands report that the majority of constituents, including the essential oil and valerenic acid, were highest in roots harvested in the first year of growth with essential oil being highest in September and November (1.2% to 2.1%). The next highest level of essential oil was reported for material harvested in March (0.9% to 1.6%). Valerenic acid and its derivatives were found to be highest in February and March (0.7% to 0.9%) followed by material harvested in September (0.5 to 0.7%) and then in January (0.3% to 0.4%). From a commercial standpoint, it is more cost effective to harvest the roots in the same year the plants are sown than in the second year.

Cultivation Practices—Sowing seeds has been reported to be preferred over planting of seedlings. Best results were achieved by flat field planting at row spacings of 50 cm and a seed rate of 3 kg per hectare. Cutting off the flowering tops before the plant has set seed causes the rhizome to develop more fully.

Optimal Times for Harvest—Wagner reports that harvest should take place in the morning during relatively cool weather, a general recommendation for roots rich in essential oils.

~~*Optimal Handling and Processing Practices*—The essential oil is located in the hypodermis of the rhizome in large thin walled cells. Therefore, care must be taken not to damage these cells during handling. Excess washing of the roots can result in a significant reduction of extractive matter. Because of the sensitivity of volatile oils to heat, it is necessary to minimize the amount of time generated in the grinding or powdering process by doing small lots at a time, with frequent interruptions in run times, or by utilizing a cryogenic grinder.~~

▲▲*USP34*

~~**Drying, Storage, and Shipping**~~

▲Post-Harvest Handling—

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Drying—For maximum preservation of the essential oils, valerian should be dried at 40° with a flow rate of 0.05 kg per sec per m^2. Alternatively, drying at 20° for approximately 10 days, shade drying at approximately 45°, low temperature vacuum-drying, and freeze-drying are also reported to be appropriate drying techniques.

Careless or prolonged drying produces a darker color in the roots and results in the hydrolysis of the isovalerianic esters and the liberation of isovaleric and hydroxyisovaleric acid. This produces the characteristic valerianic aroma. Properly dried valerian will produce this same aroma over time.

Storage—Store in closed containers protected from light, air, and moisture. Hydroxyvalerenic acid, a decomposition product of acetoxyvalerenic acid, is formed when the herb is stored at too high humidity.

Improper storage conditions can cause significant deterioration of the material. Although the essential oil is relatively stable, it can evaporate with excessive exposure to air. The essential oil can degrade quickly in powdered material. In powdered root, the essential oil content can decrease by 50% within 6 months.

Valepotriates are sensitive to humidity, temperatures above 40°, and acid media (pH <3) and are generally not detected in commercial products after 60 days.

Adulterants—Other species of valerian: An unidentified *Apiaceae* species may be found in valerian trade. Adulteration of valerian in the American market is not common. Many species other than *V. officinalis* are reported to be traded as medicinal valerian. These include *V. edulis* Nutt. ex Torr. & A. Gray, *V. coreana* Briq.k, *V. stubendorfi* Kreyer ex Kom., *V. amurensis* P. Smirn. ex Kom., *V. hardwickii* Wall, *V. exaltata* Mikan, and *V. wallichi* DC. syn. *V. jatamansi* Jones.

▲Elm *Ulmus rubra* Muhlenberg [*Ulmus fulva* Michaux] (Fam. Ulmaceae)

Botanical Identification—*Ulmus rubra* Muhlenberg; Tree to 35 m high, with spreading branches and open flat crown; preparations derived from inner bark. *U. rubra* appears to be more closely related to the introduced

* *V. wallichi* DC. and *V. edulis* Nutt. reportedly are lacking in valerenic acid and its derivatives.

In-Process Revision

Asian species *U. pumila* L. than to other native American species of *Ulmus*; where the two co-occur, interbreeding is common.

Trunk: 18 to 35 m high, to 1 m in diameter, the trunk rises free of branches until about 5 to 6 m.

Branches: Erect, spreading; young twigs are scabrous-pubescent.

Bark: Dark brown to reddish-brown, deeply furrowed. Inner bark is whitish (outer surface yellow-orange; inner surface pale yellow), fragrant (upon powdering a distinctive fenugreek-like odor) and very mucilaginous upon chewing or moistening.

Leaves: Alternate; simple; petiolate with petiole (3–)5–7(–9) mm long; 7–18(–23) cm long, 5–10(–15) cm broad; elliptical to ovate, oblong, or obovate with oblique base and acuminate apex; margins serrate towards base, elsewhere doubly serrate; upper surface scabrous, rough; lower surface tomentose; secondary veins parallel, slightly curved, running to tips of marginal teeth.

Inflorescence: Axillary fascicles, roughly hemispherical, to 1.5(–2.5) cm in diameter.

Flowers: Small, perfect; pedicels 1–2(–3) mm long; calyx campanulate, 5–9-lobed at apex, ca. 2.6–3.5 mm in diameter, reddish-pubescent; petals absent; stamens 5–9, exserted at flowering; styles 2. Flowers occur before the leaves from March through early May.

Fruit: Winged samara, yellowish, irregularly suborbicular or occasionally broadly elliptical or obovate, 10–20 mm in diameter, reddish-pubescent over seed; wing papery-textured.

Compendial History—Slippery elm (Ulmus) inner bark appeared in the list of materia medica in the first *United States Pharmacopoeia (USP)* of 1820 and remained official until it was removed from the *USP XI* (1936). The *USP 1820* included instructions for the preparation of *Infusion of Slippery Elm: ''Take of Slippery elm, sliced, one ounce. Boiling water, one pint. Infuse for twelve hours in a covered vessel, near the fire with frequent agitation, and strain.''* Immediately following its removal from the *USP XI* (official: June 1, 1936), slippery elm bark became an official monograph in the sixth edition of the *National Formulary* (*NF*; official: June 1, 1936) until its elimination from the 11th edition (official: October 1, 1961). It became official again, as *Elm,* on November 15, 1995 in the USP section of the *Third Supplement* of the *United States Pharmacopeia–National Formulary* (*USP 23–NF 18*). A revision was published in the *Seventh Supplement* on November 15, 1997.

In 1982, Elm bark appeared in the Food and Drug Administration (FDA) Advance Notice of Proposed Rulemaking (ANPR) for the establishment of a therapeutic monograph for oral health care drug products for over-the-counter (OTC) human use. In the ANPR (1982) as well as in the subsequent tentative final monograph of 1988 and in the amendment to the monograph of 1991, Elm bark was classified as a Category I (Generally Recognized as Safe and Effective (GRASE)) OTC oral demulcent active ingredient and appropriate standards were urged to be developed in the official compendia.

Aside from *USP–NF*, the monograph of Elm had already appeared in the second edition of *The Dispensatory of the United States of America* (1834) and its last appearance was in the 25th edition of 1960.

Constituents—Constituents of relevance for conformance to *Identification A* under *Elm* are mucilaginous substances. Elm inner bark mucilage is readily extractable by water and consists principally of a polysaccharide which on hydrolysis yields D-galactose, D-methyl galactose, L-rhamnose, and glucose. Borohydride reduction of the periodate-oxidized polysaccharide affords, on partial hydrolysis with hot acid, three oligosaccharides: *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose, *O*-(3-*O*-meth-

yl-β-D-galactopyranosyl)-(1→4)-L-rhamnose, and *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-3-*O*-methyl-D-galactose.

Other Elm constituents include traces of tannins including proanthocyanidins, some starch, traces of oxalate salts, beta-sitosterol, and minerals.

Sources and Distribution—

Sources—Slippery elm bark is harvested from wild populations in eastern Canada and the United States, from southern Quebec west to North Dakota, south to south-central Texas, and Florida. It is common throughout eastern, southern, and midwestern U.S., and grows in more than 25 states. An increasing amount of the commercial supply is being collected according to sustainable wild resource management plans as a condition of organic certification for wild crops. Conversely, Dutch elm disease has had a significant negative impact on elm populations, from 1930 when it was first found in the United States affecting over 50% of elm trees in the northern states.

Distribution—Canada (New Brunswick, Ontario, Quebec), the United States (Alabama, Arkansas, Connecticut, Delaware, the District of Columbia, Florida, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Louisiana, Massachusetts, Maryland, Maine, Michigan, Minnesota, Missouri, Mississippi, North Carolina, North Dakota, New Hampshire, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Virginia, Vermont, Wisconsin, West Virginia).

Collection and Cultivation—

Collection (Conservation and Ecology)—''Natural'' (both inner and outer bark) and ''rossed'' (inner bark only) are harvested but only the rossed bark will conform to the standards of the *USP* monograph. Harvesting should occur on dry, preferably warm days with no chance of precipitation and it usually commences late morning after the morning dew and humidity pass. This is because post-harvest processing (rossing) generally takes place at the collection site outdoors and humidity can damage the quality of the inner bark. Because the inner bark contains polysaccharide mucilage, when it comes in contact with moisture it begins to gel.

The minimum 10-year-old bark (some harvesters recommend selecting at least 12- to 15-year-old trees) is obtained mainly by pruning or trimming the lower limbs and branches, but can also be obtained from the bole (trunk) and, very rarely, even from the roots in cases where the entire tree is felled. While the inner bark of the root reportedly contains more mucilage than that of the trunk or branches, sustainable wild resource management dictates harvesting only the branches of mature trees. In practice, commercial bark collectors are more likely to select branches from trees that are at least 30 to 50 years old in order to obtain a high enough yield. After pruning, the tree will heal over where harvested and continue to grow, but the trunk and/or branches should never be girdled as this will kill the tree. Girdling is the stripping away of bark from the trunk or a branch all the way around. In practice, the most sustainable method of harvest from mature healthy trees is to prune off entire lower branches in a way that will not harm the tree. If sawn properly so that rain water will not drop directly into the exposed cut area, the tree will grow over the cut area within a couple of years. The proper cut is made just outside the ''branch collar'' and the ''branch bark ridge'' and does the least amount of damage to the trees.

While elm bark should not be gathered from already dead trees, selective collection from dying trees, for example those affected with Dutch elm disease, is feasible. The fungus that causes Dutch elm disease, *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* (syn: *Graphium ulmi* or *Ceratocystis ulmi*) is carried from tree to tree by the European elm bark beetle (*Scolytus multistriatus*), which arrived in North America on a boat of logs from Europe

around 1930, and to a much lesser extent by the native elm bark beetle (*Hylurgopinus rufipes*). Elms appear to succumb to the disease at about 10 years of age and then die off in a two-year period. Once a tree is diagnosed with Dutch elm disease, healthy bark can still be harvested for about two years, up until the tree is near death. To monitor how long harvesting can continue, one can use a drawknife to take samples. Once the inner bark is showing increasingly noticeable black streaking, and as more and more limbs die and debarking is evident, the tree is near death. The near dead tree can be dropped and the remaining healthy bark can be stripped from the entire tree. Discolored (black-streaked) inner bark should be separated out and discarded. Pruning lower limbs from healthy trees and/or selectively harvesting entire older trees that are near death can both be acceptable methods as part of a sustainable resource management plan for a specific area of forest under organic supervision.

Following organic production system rules (e.g., for harvest site selection, absence of prohibited substances, testing of soil and water, disallowed inputs, periodic residue testing, documentation control, independent inspection, etc.) will more likely result in botanical raw material that meets the general requirements for pesticide residue and heavy metals limits, among other potential contaminants. Certified organic production of wild elm bark requires producers to promote ecological balance and conserve biodiversity. Wild collected elm bark that is to be certified organic must be harvested from a designated area that has had no prohibited substance applied to it for a period of 3 years immediately preceding the harvest, and must be harvested in a manner that ensures that such harvesting or gathering will not be destructive to the environment and will sustain the growth and production of the wild crop. Wild crop producers must comply with the same organic system plan requirements and conditions, as applicable to their operation, as their counterparts who produce cultivated crops. The producer of organic wild harvested elm bark must initiate practices to support biodiversity and avoid, to the extent practicable, any activities that would diminish it. Production practices must maintain or improve the natural resources of the operation, including soil, water, wetlands, woodlands and wildlife. This is accomplished, in part, by developing and executing a resource management plan that requires wild harvest from stable populations, minimizing disruption of priority species/sensitive habitats, avoiding erosion, allowing re-establishment, and monitoring wild crop sustainability.

Cultivation Practices—Even though the commercial supply is harvested from wild populations, slippery elm trees can be propagated by cuttings or by seed. For propagation by seed, the ripe seeds are collected from April to June from healthy and successful (dominant) trees from an area similar to the proposed planting site. A ripeness indicator is when the samaras (fruits) are green. It is best to collect seed from trees within 160 km north or south of the planting site, as potential for success is optimal within this range from the parents. Twenty-five seeds per square foot can be scattered, 0.6 cm deep. Slippery elm may be sown as in its normal cycle in the spring in a raised peat moss soil and sand bed. The seedbeds may need a wire top to protect young seedlings. Germination rate is 10 to 25% with light germination in summer and increased germination the following spring. The young trees can be transplanted into tree tubes within the first month of germination and field planted after one or two years depending on the size of the tree tube. The tree saplings must be watered during times of drought as well as routine checking for insect predation and indications of fertilization needs.

Optimal Times for Harvest—Harvest should preferably occur in the spring (March to May), but can also take place in the autumn. In the spring, bark is harvested from mature trees (minimum 10 years) when the sap begins to rise.

Post-Harvest Handling—

Optimal Handling and Processing Practices—To produce pharmacopoeial quality elm inner bark, the outer corky layer of bark must be removed, exposing the inner bark. If post-harvest processing occurs at the wild collection site, the pruned limbs and branches should be placed onto clean tarps and not directly on the ground. The very small branches with leaves are stripped off of the pruned limbs by hand and discarded. To optimize conformance to standards for composition, identity, purity, and quality (e.g., NMT 2% of adhering outer bark, NMT 2% foreign organic matter, NMT 10% total ash, and NMT 0.65% acid-insoluble ash), a clean bark rosser (hand tool with handle and knife blade) should be used to shave off the outer bark. The rough, scaly matter on the surface of the bark is called ''ross'' and to ross bark is to scrape or shave the outer bark from the limb. An experienced rosser can visually discern that at least 98% of the outer bark has been shaved off. The inner bark is white in color (in the spring; reddish later in season) in obvious visible contrast to the brown outer bark layer. After rossing most of the outer bark off, greater care must be exercised to very carefully slice off the remaining thin layer of outer bark so as not to waste any of the inner bark in the process. After removal of the outer bark, the inner bark can then be removed in strips, squares, or chips. An incision is made with a clean knife down the center of the limb. Then a clean crow bar is slipped underneath the incision in order to lift and peel the inner bark off from the cambium. The strips of inner bark are stacked on a clean tarp and later bundled for transport to the drying facility.

Drying—Elm USP requires a loss on drying limit of NMT 12%. So long as rain is not expected, fresh elm inner bark can be sun-cured within a temperature range of 32° to 60°. Drying can also be carried out in a warm room with airflow or in a greenhouse. Greenhouse drying takes about 3 to 4 days. Drying indoors can take 5 to 7 days depending on the heat source. Drying at commercial scale, however, is done typically in enclosed drying chambers wherein time and temperature can be better controlled. The strips of elm bark are placed onto a clean screen floor and dried over about 2 days time at about 50° with fan forced heat through the floor. Due to additional phytosanitary requirements for export of tree barks to Europe, higher heat exposure is necessary, usually at least 65° but up to 93° for up to two days. Post-drying, the strips of inner bark can be cut or sawn into pieces of equal length and bound into bundles with wire. The bundles usually consist of flat, oblong pieces, about 30 cm in length and from 10 to 15 cm in width. The bark strips can be stored this way until further processing (e.g., cutting or powdering) is scheduled.

Storage—To maintain pharmacopoeial purity and quality (e.g., to prevent accumulation of excess moisture), dried elm inner bark should be preserved in well-closed containers, and stored in a cool, dry place.

Adulterants and Contaminants—Common contaminants that could cause a material not to conform with the *Elm USP* identification tests would include other plant parts, for example greater than 2% outer bark which lacks mucilage. Insufficient shaving or rossing of outer bark could cause the material to exceed the monograph limit of NMT 2% of adhering outer bark. Other possible contaminants would include visible discolored inner bark, although no maximum limit has been established (for example, inner bark with visible black streaking obtained from a diseased tree). Powdered bark can also be adulterated with corn meal, rice flour, starch, or other starchy substances. Consequences of contamination with outer bark or adulteration with flour or starch are lower mucilage content, lower swelling index value, and correspondingly less of a therapeutic demulcent effect that is mucilage-dependent. Excess outer bark could also cause the material to fail the quantitative standard of NMT 10% total ash. Methods to determine presence of adulterants include microscopic examination in order to

determine the presence of excess outer bark or any other adulterant, and the concentration of mucilage cells. The Elm mucilage test (*Identification* test *A*) as well as a modified swelling volume test (based on the Psyllium Husk USP monograph) may also be useful to investigate if adulteration is suspected.▲*USP34*

BRIEFING

⟨2040⟩ Disintegration and Dissolution of Dietary Supplements, *USP 32* page 782, page 4007 of the *First Supplement,* a *Revision Bulletin* posted on USP's website on May 1, 2009, and page 984 of *PF* 35(4) [July–Aug. 2009]. In order to clarify acceptance criteria for *Dissolution* testing of *Botanical Dosage Forms*, the following revision is proposed: the existing *Interpretation* section is being deleted and replaced with *Tolerances* where dissolution requirements for botanical dosage forms are defined.

(DS-PS: Natalia Davydova) RTS—C78869

Change to read:

DISINTEGRATION

This test is provided to determine whether dietary supplement tablets or capsules disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below. Compliance with the limits on *Disintegration* stated in the individual monographs for dietary supplements is required except where the label states that the products are intended for use as troches, are to be chewed, or are designed as extended-release dosage forms. Dietary supplements claiming to be extended-release dosage forms must comply with standards other than disintegration to verify that the release of the dietary ingredients from the dosage form is for a defined period of time. Dietary supplements claiming to be extended-release dosage forms shall not be labeled as in compliance with USP unless a USP monograph exists for such product. Determine the type of units under test from the labeling and from observation, and apply the appropriate procedure to 6 or more units.

For purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disk, if used, is a soft mass having no palpably firm core.

Apparatus

Apparatus A—Use the *Apparatus* described under *Disintegration* ⟨701⟩ for tablets or capsules that are not greater than 18 mm long. For larger tablets or capsules, use *Apparatus B.*

Apparatus B—The apparatus[1] consists of a basket-rack assembly, a 1000-mL low-form beaker for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-Rack Assembly—The basket-rack assembly

■(*Figure 1*)■2S *(USP33)* consists of three open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 32.0 to 34.6 mm and a wall 2.0 to 3.0 mm thick; the tubes are held in a vertical position by two plastic plates, each ~~about~~

■■2S *(USP33)* 97

■ ± 2■2S *(USP33)* mm in diameter and 7.5 to 10.5 mm in thickness, with three holes, ~~each about 33 to 34~~

■36.0 to 40.6■2S *(USP33)* mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the undersurface of the lower plate is 10-mesh No. 23 (0.025-inch) W. and M. gauge woven stainless-steel wire cloth having a plain square weave. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device, using a point on its axis.

The design of the basket-rack assembly may be varied somewhat, provided that the specifications for the glass tubes and the screen mesh size are maintained.

[1] An apparatus and disks meeting these specifications are available from Varian Inc., 13000 Weston Parkway, Cary, NC 27513, or from laboratory supply houses.

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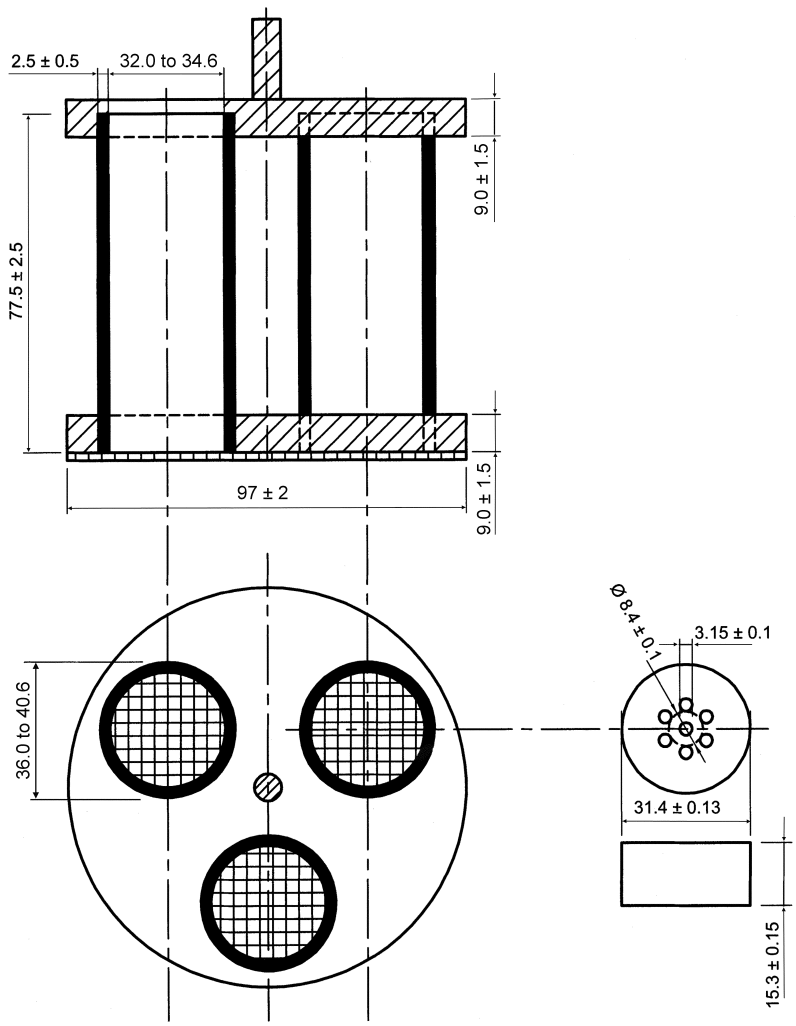

Figure 1. Basket-rack assembly, *Disintegration, Apparatus B* (dimensions in mm).

Beaker—Low form, 1000 mL; the difference between the diameter of the plastic plates, which hold the tubes in a vertical position, and the inside diameter of the beaker should not be more than 6 mm.[2] ■2S (*USP33*)

Disks—Each tube is provided with a perforated cylindrical disk 15.3 ± 0.15 mm thick and 31.4 ± 0.13 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Seven 3.15 ± 0.1-mm holes extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it and equally spaced on a 4.2 ± 0.1-mm radius from it. All surfaces of the disk are smooth.[3]

Procedure

Uncoated Tablets—Place 1 tablet in each of the tubes of the basket and, if prescribed, add a disk to each tube. Operate the apparatus, using water or the specified medium as the immersion fluid, maintained at 37 ± 2°. At the end of 30 minutes, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The requirement is met if not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Plain Coated Tablets—Place 1 tablet in each of the tubes of the basket and, if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 minutes. Then, if prescribed, add a disk to each tube, and operate the apparatus, using water or the specified medium as the immersion fluid, maintained at 37 ± 2°. At the end of 30 minutes, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The requirement is met if not fewer than 16 of the total of 18 tablets tested disintegrate completely.

■[2] 1000-mL low-form beakers, designed in compliance with the current ASTM E 960 Type I or Type II or ISO 3819 specifications, are suitable.■2S (USP33)

[3] The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimensions given in this chapter.

In-Process Revision

Delayed-Release (Enteric-Coated) Tablets—Place 1 tablet in each of the six tubes of the basket, and if the tablet has a soluble external sugar coating, immerse the basket in water at room temperature for 5 minutes. Then operate the apparatus using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 1 hour of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the tablets: the tablets show no evidence of disintegration, cracking, or softening. Operate the apparatus, using simulated intestinal fluid TS, maintained at 37 ± 2°, as the immersion fluid for the time specified in the monograph. Lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

▪Delayed-Release (Enteric-Coated) Soft Shell Capsules—Place 1 softgel capsule in each of the six tubes of the basket. Use two baskets for a total of six tubes for *Apparatus B*. Omit the use of a disk. Operate the apparatus using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 1 hour of operation in simulated gastric fluid TS, lift the basket from the fluid and observe the softgels: the softgels show no evidence of disintegration or rupture permitting the escape of the contents. Operate the apparatus with disks, using simulated intestinal fluid TS, maintained at 37 ± 2°, as the immersion fluid. Lift the basket from the fluid, and observe the capsules. All the capsules disintegrate completely within 60 minutes. If 1 or 2 capsules fail to disintegrate completely, repeat the test on 12 additional capsules: not fewer than 16 of a total of 18 capsules tested disintegrate completely.▪2S (*USP33*)

Buccal Tablets—Apply the test for *Uncoated Tablets*. After 4 hours, lift the basket from the fluid, and observe the tablets: all of the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Sublingual Tablets—Apply the test for *Uncoated Tablets*. At the end of the time limit specified in the individual monograph, all the tablets disintegrate completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not fewer than 16 of the total of 18 tablets tested disintegrate completely.

Hard Shell Capsules—Apply the test for *Uncoated Tablets,* using as the immersion fluid, maintained at 37 ± 2°, a 0.05 M acetate buffer prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of 4.50 ± 0.05. Attach a removable wire cloth, as described under *Basket-Rack Assembly,* to the surface of the upper plate of the basket-rack assembly. At the end of 30 minutes, lift the basket from the fluid, and observe the capsules: all of the capsules disintegrate except for fragments from the capsule shell. If 1 or 2 capsules fail to disintegrate completely, repeat the test on 12 additional capsules: not fewer than 16 of the total of 18 capsules tested disintegrate completely.

Soft Shell Capsules—Proceed as directed under *Rupture Test for Soft Shell Capsules.*

Use of Disks—

VITAMIN–MINERAL DOSAGE FORMS—Add a disk to each tube unless otherwise specified in the individual monograph.

BOTANICAL DOSAGE FORMS—Omit the use of disks unless otherwise specified in the individual monograph.

DIETARY SUPPLEMENTS OTHER THAN VITAMIN–MINERAL AND BOTANICAL DOSAGE FORMS—Omit the use of disks unless otherwise specified in the individual monograph.

NOTE—The use of disks for enteric-coated tablets is not permitted.

Change to read:

DISSOLUTION

This test is provided to determine compliance with the *Dissolution* requirements where stated in the individual monograph for dietary supplements, except where the label states that tablets are to be chewed.

See *Dissolution* ⟨711⟩ for a description of the apparatus used, the *Apparatus Suitability Test,* and other related information.

•Soft gelatin capsule preparations of dietary supplements meet the requirements for *Disintegration.*

Official until May 1, 2010• (RB 1-May-2009)

▪*Figure 2* shows the schematic view of a flow-through cell specifically intended for lipid-filled soft gelatin capsules. It consists of three transparent parts that fit into each other (USP *Apparatus 4*). The lower part (1) is made up of two adjacent chambers connected to an overflow device. The dissolution medium passes through chamber A and is subjected to an upward flow. The flow in chamber B is directed downward to a small-size bore exit that leads upward to a filter assembly. The middle part (2) of the cell has a cavity designed to collect lipophilic excipients that float on the dissolution medium. A metal grid serves as a rough filter. The upper part (3) holds a filter unit for paper, glass fiber, or cellulose filters.▪2S (*USP33*)

Of the types of apparatus described in ⟨711⟩, use the one specified in the individual monograph.

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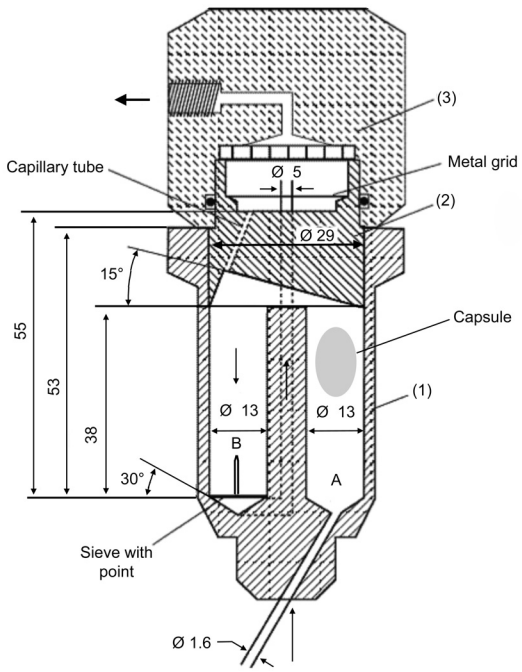

Figure 2. Flow-through cell designed for lipid-filled soft gelatin capsules (dimensions in mm).■2S (*USP33*)

For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the dissolution specification, repeat the test as follows. Where water or a medium with a pH of less than 6.8 is specified as the *Medium* in the individual monograph, the same *Medium* specified may be used with the addition of purified pepsin that results in an activity of 750,000 Units or less per 1000 mL. For media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1750 USP Units of protease activity per 1000 mL.

This nonspecific dissolution is intended to be diagnostic of known technological problems that may arise as a result of coatings, lubricants, disintegrants, and other substances inherent in the manufacturing process. For dosage forms containing botanical extracts, this dissolution measurement allows an assessment of the extent of decomposition of the extract to polymeric or other nondissoluble compounds that may have been produced by excessive drying or other manipulations involved in the manufacture of botanical extracts. The operative assumption inherent in this procedure is that if the index or marker compound(s) or the extract is demonstrated to have dissolved within the time frame and under the conditions specified, the dosage form does not suffer from any of the above formulation or manufacturing related problems.

Vitamin–Mineral Dosage Forms

All dietary supplements belonging to USP *Classes II* to *VI,* prepared as tablets or capsules, are subject to the dissolution test and criteria described in this chapter for folic acid (if present) and for index vitamins and index minerals. This test is required because of the importance of the relationship between folate deficiency and the risk of neural tube defects. The accompanying table lists the dissolution requirements for the individual USP classes of dietary supplements. *Class I* dietary supplements are combinations of oil-soluble vitamins for which dissolution standards are not established; hence, dissolution requirements do not apply to the oil-soluble vitamins contained in formulations belonging to *Class IV* or *Class V.* Vitamin–mineral combinations that may not be strictly covered by USP *Class I* to *Class VI* are subject to the dissolution test and criteria specified in the individual monographs.

Dietary Supplements—Vitamin–Mineral Dosage Forms

USP Class	Combination of Vitamins or Minerals Present	Dissolution Requirement
I	Oil-Soluble Vitamins	not applicable
II	Water-Soluble Vitamins	one index vitamin; folic acid (if present)
III	Water-Soluble Vitamins with Minerals	one index vitamin and one index element; folic acid (if present)
IV	Oil- and Water-Soluble Vitamins	one index water-soluble vitamin; folic acid (if present)
V	Oil- and Water-Soluble Vitamins with Minerals	one index water-soluble vitamin and one index element; folic acid (if present)
VI	Minerals	one index element

Unless otherwise stated in the individual monograph, test six dosage units for dissolution as directed under *Dissolution* ⟨711⟩.

DISSOLUTION CONDITIONS FOR FOLIC ACID

NOTE—Perform this test under light conditions that minimize photodegradation.

Medium: water; 900 mL. If the units tested do not meet the requirements for dissolution in water, test six additional dosage units for dissolution in a medium of 900 mL of 0.05 M pH 6.0 citrate buffer solution, prepared by mixing 9.5 mL of 0.1 M citric acid monohydrate and 40.5 mL of 0.1 M sodium citrate dihydrate in a 100-mL volumetric flask, diluting with water to volume, mixing, and adjusting to a pH of 6.0 by using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution.

Apparatus 1: 100 rpm, for capsules.

Apparatus 2: 75 rpm, for tablets.

Time: 1 hour.

NOTE—Compliance with the dissolution requirements for folic acid does not exempt the product from dissolution testing of the pertinent index vitamin or the corresponding index mineral.

In-Process Revision

DISSOLUTION CONDITIONS FOR INDEX VITAMINS AND INDEX MINERALS

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm, for capsules.

Apparatus 2: 75 rpm, for tablets.

Time: 1 hour.

For formulations containing 25 mg or more of the index vitamin, riboflavin, use the following conditions:

Medium: 0.1 N hydrochloric acid; 1800 mL.

Apparatus 1: 100 rpm, for capsules.

Apparatus 2: 75 rpm, for tablets.

Time: 1 hour.

NOTE—Compliance with dissolution requirements for the pertinent index vitamin or index mineral does not exempt the product from dissolution testing of folic acid, if present.

SELECTION OF INDEX VITAMINS AND INDEX ELEMENTS

Compliance with the dissolution requirements for dietary supplements representing combinations of water-soluble vitamins (*Water-Soluble Vitamins Capsules* and *Water-Soluble Vitamins Tablets*) and combinations of oil- and water-soluble vitamins (*Oil- and Water-Soluble Vitamins Capsules* and *Oil- and Water-Soluble Vitamins Tablets*) is determined by measuring the dissolution of a single index vitamin from the water-soluble vitamins present. Riboflavin is the index vitamin when present in the formulation. For formulations that do not contain riboflavin, pyridoxine is the index vitamin. If neither riboflavin nor pyridoxine is present in the formulation, the index vitamin is niacinamide (or niacin), and in the absence of niacinamide (or niacin), the index vitamin is thiamine. If none of the above four water-soluble vitamins is present in the formulation, the index vitamin is ascorbic acid.

Compliance with the dissolution requirements for dietary supplements representing combinations of minerals (*Minerals Capsules* and *Minerals Tablets*) is determined by measuring the dissolution of only one index element. Iron is the index element when present in the formulation. For formulations that do not contain iron, the index element is calcium. If neither iron nor calcium is present, the index element is zinc, and in the absence of all three of these elements, magnesium is the index element.

Compliance with dissolution requirements for dietary supplements representing combinations of water-soluble vitamins and minerals (*Water-Soluble Vitamins with Minerals Capsules* and *Water-Soluble Vitamins with Minerals Tablets*) and combinations of oil- and water-soluble vitamins and minerals (*Oil- and Water-Soluble Vitamins with Minerals Capsules* and *Oil- and Water-Soluble Vitamins with Minerals Tablets*) is determined by measuring the dissolution of one index water-soluble vitamin and one index element, designated according to the respective hierarchies described above.

PROCEDURES

In the following procedures, combine equal volumes of the filtered solutions of the six individual specimens withdrawn, and determine the amount of folic acid or the index vitamin or element dissolved, based on the average of six units tested. Make any necessary modifications, including concentration of the analyte in the volume of test solution taken. Use the *Medium* for preparation of the Standard solution and dilution, if necessary, of the test solution.

Folic Acid—Determine the amount of $C_{19}H_{19}N_7O_6$ dissolved by employing the procedure set forth in the *Assay for folic acid* under *Oil- and Water-Soluble Vitamins with Minerals Tablets*, in comparison with a Standard solution having a known concentration of USP Folic Acid RS in the same *Medium*.

Niacin or Niacinamide, Pyridoxine, Riboflavin, and Thiamine—Determine the amount of the designated index vitamin dissolved by employing the procedure set forth in the *Assay for niacin or niacinamide, pyridoxine, riboflavin, and thiamine* under *Water-Soluble Vitamins Tablets.*

Ascorbic Acid—Determine the amount of $C_6H_8O_6$ dissolved by adding 10 mL of 1.0 N sulfuric acid and 3 mL of starch TS to 100.0 mL of test solution, and titrating immediately with 0.01 N iodine VS. Perform a blank determination, and make any necessary correction.

Iron, Calcium, Magnesium, and Zinc—Determine the amount of the designated index element dissolved by employing the procedure set forth in the appropriate *Assay* under *Minerals Capsules*.

TOLERANCES

The requirements are met if not less than 75% of the labeled content of folic acid and not less than 75% of the labeled content of the index vitamin or the index element from the units tested is dissolved in 1 hour.

Botanical Dosage Forms

Compliance with dissolution requirements necessitates the testing of six dosage units individually, or testing two or more dosage units in each of the six vessels of the dissolution apparatus, and measuring the dissolution of one or more index/marker compound(s) or the extract specified in the individual monograph.

PROCEDURES

Combine equal volumes of the filtered solutions of the six or more individual specimens withdrawn, and use the pooled sample as the test solution. Determine the average amount of index or marker compound(s) or the extract dissolved in the pooled sample by the *Procedure* specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the test solution taken. Use the *Medium* for preparation of the Standard solution and dilution, if necessary, of the test solution.

~~INTERPRETATION~~

~~*Pooled Sample*—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of the index or marker compound(s) or the extract dissolved from the pooled sample conform to the accompanying acceptance table. The quantity, *Q*, is the amount of dissolved index or marker compound(s) or the extract specified in the individual monograph, expressed as a percentage of the labeled content. The 5%, 15%, and 25% values in the acceptance table are percentages of the labeled content so that these values and *Q* are in the same terms.~~

~~**Acceptance Table for a Pooled Sample**~~

~~Stage~~	~~Number Tested~~	~~Acceptance Criteria~~
~~S_1~~	~~6~~	~~Average amount dissolved is not less than Q + 10%~~
~~S_2~~	~~6~~	~~Average amount dissolved (S_1 + S_2) is equal to or greater than Q + 5%~~
~~S_3~~	~~12~~	~~Average amount dissolved (S_1 + S_2 + S_3) is equal to or greater than Q~~

▲TOLERANCES

Unless otherwise specified in the individual monograph, the requirements are met if not less than 75% of the labeled content of the index or marker compound(s) or the extract from the units tested is dissolved in 1 hour.▲*USP34*

Dietary Supplements Other Than Vitamin–Mineral and Botanical Dosage Forms

Unless otherwise stated in the individual monographs for dietary supplement dosage forms in this category, compliance requires the testing of six individual units, measuring the dissolution of the dietary ingredient as the average of the six units tested.

PROCEDURES

Combine equal volumes of the filtered solutions of the six specimens withdrawn, and use the pooled sample as the test solution. Determine the average amount of dietary ingredient dissolved in the pooled sample by the *Procedure* specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the test solution taken. Use the *Medium* for preparation of the Standard solution and for dilution, if necessary, of the test solution.

TOLERANCES

Because of the diversity of chemical characteristics and solubilities of dietary ingredients pertaining to this category, general tolerances cannot be established. See individual monographs for *Tolerances*.

In-Process Revision

REAGENTS, INDICATORS, AND SOLUTIONS

Reagent Specifications

BRIEFING

Ammonium Bicarbonate. It is proposed to add this new reagent used in *Identification* test *B* in the monograph for *Glucagon*.

(HDQ: M. Marques.) RTS—C54723

Add the following:

▲**Ammonium Bicarbonate** *(Ammonium Hydrogen Carbonate)*, NH_4HCO_3—**79.06** [*1066-33-7*]—Use a suitable grade with a content of NLT 99.0%.▲*USP34*

BRIEFING

2-Chloroethanol. It is proposed to add this new reagent used in the *Limit of 2-Chloroethanol* test in the monograph for *Carmustine*.

(HDQ: M. Marques) RTS—C52248

Add the following:

▲**2-Chloroethanol** *(Ethylene Chlorohydrin)*, C_2H_5ClO—**80.51** [*107-07-3*]—Use a suitable grade with a content of NLT 99%.▲*USP34*

BRIEFING

Alpha-Chymotrypsin. It is proposed to add this new reagent used in *Identification* test *B* in the monograph for *Glucagon*.

(HDQ: M. Marques) RTS—C54723

Add the following:

▲**Alpha-Chymotrypsin—25 kDa** [*9004-07-3*]—Use a suitable salt-free grade for protein sequencing.

NOTE— A suitable grade is available as catalog number 4423 from www.sigma-aldrich.com.▲*USP34*

BRIEFING

Glycolic Acid, *USP 32* page 826. It is proposed to include the sample silanization procedure.

(HDQ: M. Marques) RTS—C78511

Change to read:

Glycolic Acid, $C_2H_4O_3$—**76.05** [*79-14-1*]—White crystalline powder or chunks.

~~*Assay*—Inject an appropriate volume (silanized) into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2. The injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; the column temperature is maintained at 100° and programmed to rise 10° per minute to 250°. The area of the $C_2H_4O_3$ peak is not less than 98.5% of the total peak area.~~

▲*Assay*—

SILYLATING REAGENT: Pyridine, hexamethyldisilazane, chlorotrimethylsilane (9 : 3 : 1)

SAMPLE PREPARATION: Weigh approximately 25 mg (about 3 drops) of the sample into a test tube. Add 2 mL of the *Silylating reagent* and cap the test tube. Mix well and allow to incubate at room temperature for a minimum of 2 h. A white precipitate of ammonium chloride will form. Centrifuge and use the clear supernatant for injection.

PROCEDURE: Inject an appropriate volume of the *Sample preparation* into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2. The injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; the column temperature is maintained at 100° and programmed to rise 10° per min to 250°. The area of the peak corresponding to $C_2H_4O_3$ is NLT 98.5% of the total peak area.▲*USP34*

BRIEFING

Lead Acetate Cotton. It is proposed to add this new reagent used in the *Limit of Arsenic* test in the monograph for *Aluminum Oxide*, which appears elsewhere in this issue of *PF*.

(HDQ: M. Marques.) RTS—C77141

Add the following:

▲**Lead Acetate Cotton**—Immerse absorbent cotton in a mixture of 1 volume of dilute acetic acid and 10 volumes of lead acetate TS. Drain off the excess of liquid, without squeezing the cotton, by placing it on several layers of filter paper. Allow to dry in air. Store in airtight containers.▲*USP34*

BRIEFING

Mercuric Bromide Test Paper, *USP 32* page 861. It is proposed to add more information on how to prepare this test paper.

(HDQ: M. Marques) RTS—C78623

Mercuric Bromide Test Paper ~~Use alcoholic mercuric bromide TS. Store protected from light.~~

▲Place a 50 mg/mL solution of mercuric bromide in dehydrated alcohol in a dish, and immerse in it pieces of white filter paper weighing 80 g/m^2 (speed of filtration = filtration time expressed in s for 100 mL of water at 20° with a filter surface of 10 cm^2 and a constant pressure of 6.7 kPa; 40–60 s), each measuring 1.5 cm by 20 cm and folded in the middle. Allow the excess of liquid to drain, and allow the paper to dry, protected from light, suspended over a nonmetallic thread. Discard 1 cm from each end of each strip, and cut the remainder into 1.5-cm squares or discs of 1.5-cm diameter. Store in a glass-stoppered container wrapped with black paper.▲*USP34*

BRIEFING

Potassium Arsenate Monobasic. It is proposed to add this new reagent used to evaluate the pullulanase enzyme used in the monograph for *Pullulan*.

(HDQ: M. Marques) RTS—C54621

Add the following:

▲**Potassium Arsenate Monobasic,** KH_2AsO_4—**180.03** [*7784-41-0*]—Use a suitable grade with a content of NLT 98%.▲*USP34*

BRIEFING

Zinc, Activated. It is proposed to add this new reagent used in *Limit of Arsenic* test in the monograph for *Aluminum Oxide*.

(HDQ: M. Marques.) RTS—C77141

Add the following:

▲**Zinc, Activated**—Place zinc pellets in a conical flask and add a sufficient quantity of a 50-ppm solution of chloroplatinic acid to cover all pellets. Allow the metal to remain in contact with the solution for 10 min, drain, wash, and dry immediately.

Assay—To 5 g of activated zinc add 15 mL of hydrochloric acid, 25 mL of water, 0.1 mL of stannous chloride solution (235 mg/mL in hydrochloric acid) and 5 mL of potassium iodide solution (166 mg/mL in water). No stain is produced when in contact with mercurc bromide paper. Repeat the test for arsenic using the same reagents and adding a solution containing 1 μg of arsenic. An appreciable stain appears when in contact with mercuric bromide paper.▲*USP34*

REFERENCE TABLES

BRIEFING

Container Specifications for Capsules and Tablets, *USP 32* page 881, page 4014 of the *First Supplement*, and page 1341 of *PF* 35(5) [Sept.–Oct. 2009].

(HDQ) RTS—C55635; C67684; C77735

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	*Container Specification*
Add the following:	
▲Acetaminophen and Tramadol Hydrochloride Tablets	$T_{▲USP33}$
Add the following:	
■Amlodipine Besylate Tablets	T, $LR_{■2S\ (USP32)}$
Add the following:	
■Azithromycin Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Balsalazide Disodium Capsules	$T_{■2S\ (USP33)}$
Add the following:	
■Carbidopa and Levodopa Tablets, Extended-Release	W, $LR_{■1S\ (USP33)}$
Add the following:	
■*Crypthecodinium cohnii* Oil Capsules	T, $LR_{■2S\ (USP33)}$

Container Specifications for Capsules and Tablets *(Continued)*

Monograph Title	*Container Specification*
Change to read:	
Dantrolene Sodium Capsules	T, ~~LR~~ $■_{■2S\ (USP32)}$
Add the following:	
■Fluconazole Tablets	$W_{■2S\ (USP32)}$
Add the following:	
■Granisetron Hydrochloride Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Guggul Tablets	W, $LR_{■2S\ (USP32)}$
Add the following:	
■Ketoprofen Capsules, Extended-Release	$T_{■2S\ (USP32)}$
Add the following:	
■Lamivudine and Zidovudine Tablets	W, $LR_{■1S\ (USP33)}$
Add the following:	
■Lisinopril Tablets	$T_{■2S\ (USP32)}$
Add the following:	
■Loratadine and Pseudoephedrine Sulfate Tablets, Extended-Release	$LR_{■1S\ (USP33)}$
Add the following:	
■Loratadine Orally Disintegrating Tablets	$T_{■1S\ (USP33)}$
Add the following:	
■Losartan Potassium Tablets	$T_{■2S\ (USP32)}$

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Morphine Sulfate Tablets, Extended-Release	T, LR■2S (*USP33*)
Add the following:	
■Mycophenolate Mofetil Capsules	W, LR■2S (*USP33*)
Add the following:	
■Mycophenolate Mofetil Tablets	W, LR■2S (*USP33*)
Add the following:	
■Nateglinide Tablets	T■1S (*USP33*)
Add the following:	
■Olanzapine Tablets	T, LR■1S (*USP33*)
Add the following:	
■Ondansetron Tablets	T, LR■2S (*USP32*)
Add the following:	
■Orlistat Capsules	T■2S (*USP33*)
Add the following:	
▲Oseltamivir Phosphate Capsules	W▲*USP33*
Add the following:	
■Oxcarbazepine Tablets	W■1S (*USP33*)
Delete the following:	
▲Paramethasone Acetate Tablets	W▲*USP34*
Add the following:	
■Ramipril Capsules	W■2S (*USP33*)

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Ribavirin Capsules	W■1S (*USP33*)
Add the following:	
■Riluzole Tablets	W, LR■2S (*USP33*)
Add the following:	
■Risedronate Sodium Tablets	W■2S (*USP32*)
Add the following:	
■Schizochytrium Oil Capsules	T, LR■2S (*USP33*)
Add the following:	
■Sumatriptan Tablets	W■2S (*USP33*)
Add the following:	
■Tacrolimus Capsules	T■1S (*USP33*)
Add the following:	
■Tamsulosin Hydrochloride Capsules	T■2S (*USP32*)
Add the following:	
■Telmisartan Tablets	W■1S (*USP33*)
Add the following:	
■Telmisartan and Hydrochlorothiazide Tablets	W■*USP34*
Add the following:	
■Terazosin Capsules	W, LR■2S (*USP33*)
Add the following:	
■Terazosin Tablets	W, LR■2S (*USP32*)

In-Process Revision

Container Specifications for Capsules and Tablets
(Continued)

Monograph Title	*Container Specification*
Add the following:	
■Ticlopidine Hydrochloride Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Tablets	W■1S (*USP33*)
Add the following:	
■Tranylcypromine Sulfate Tablets	W■1S (*USP33*)
Add the following:	
■Valacyclovir Tablets	T■2S (*USP33*)
Add the following:	
■Valganciclovir Tablets	T■2S (*USP32*)
Add the following:	
▲Venlafaxine Tablets	W▲*USP34*
Add the following:	
■Zinc Gluconate Tablets	T, LR■1S (*USP33*)
Add the following:	
■Zolpidem Tartrate Tablets	W■2S (*USP33*)
Add the following:	
■Zolpidem Tartrate Extended-Release Tablets	W■1S (*USP33*)

BRIEFING

Description and Relative Solubility of USP and NF Articles, *USP 32* page 890, page 4014 of the *First Supplement*, page 817 of *PF* 34(3) [May–June 2008], page 1322 of *PF* 34(5) [Sept.–Oct. 2008], page 1565 of *PF* 34(6) [Nov.–Dec. 2008], page 188 of *PF* 35(1) [Jan.–Feb. 2009], page 464 of *PF* 35(2) [Mar.–Apr. 2009], page 651 of *PF* 35(3) [May–June 2009], page 993 of *PF* 35(4) [July–Aug. 2009], and page 1343 of *PF* 35(5) [Sept.–Oct. 2009].

(HDQ) RTS—C49074; C54956; C63974; C67356; C70759; C74528; C77133; C77734; C77792; C78462

Add the following:

▲**Butyl Stearate:** Butyl Stearate occurs as a colorless, waxy solid at temperatures below approximately 25° or as a clear liquid at temperatures above approximately 25°. Soluble in alcohol, most fixed oils; insoluble or practically insoluble in propylene glycol and water. *NF category:* Emollient.▲*NF29*

Change to read:

Cefoperazone Sodium: White to pale buff crystalline powder. Freely soluble in water; ~~and in methanol;~~

▲soluble in methanol;▲*USP34*
slightly soluble in dehydrated alcohol; insoluble in acetone, in ethyl acetate, and in ether.

Change to read:

Doxycycline: Yellow, crystalline powder. Very slightly soluble in water; freely soluble in dilute acid and in alkali hydroxide solutions; ~~sparingly soluble in alcohol;~~

▲very slightly soluble in alcohol;▲*USP34*
practically insoluble in chloroform and in ether.

Change to read:

Ferric Sulfate: Grayish-white or yellowish powder or fawn-colored pearls. Hygroscopic. ~~Rapidly soluble in the presence of a trace of ferrous sulfate; slowly soluble in water; sparingly soluble in alcohol; practically insoluble in acetone and in ethyl acetate.~~

▲Slightly soluble in water and in ethanol (96%). Practically insoluble in acetone and in ethyl acetate.▲*USP34*
Hydrolyzes slowly in aqueous solution.

In-Process Revision

Add the following:

▲**Halobetasol Propionate:** White to off-white powder. Freely soluble in dichloromethane and acetone; practically insoluble in water.▲*USP34*

Add the following:

▲**Levofloxacin:** Light yellowish-white to yellow-white crystals or crystalline powder. Soluble in dimethylsulfoxide and in acetic acid; sparingly soluble in water, in acetone, and in methanol; practically insoluble in glyerin and in *n*-octanol.▲*USP34*

Delete the following:

~~▲Paramethasone Acetate: Fluffy, white to creamy white, odorless, crystalline powder. Melts at about 240°, with decomposition. Insoluble in water; soluble in chloroform, in ether, and in methanol.~~▲*USP34*

Change to read:

Pravastatin Sodium: White to yellowish white, hygroscopic powder. Freely soluble in water and in methanol; soluble in

▲dehydrated▲*USP34*
alcohol; ~~very slightly soluble in acetonitrile;~~

▲▲*USP34*
practically insoluble ~~in ether, in ethyl acetate,~~

▲in acetonitrile▲*USP34*
and in chloroform.

Add the following:

▲**Rivastigmine Tartrate:** White to off-white powder. Very soluble in water and in methanol; very slightly soluble in ethyl acetate.▲*USP34*

Add the following:

▲**Venlafaxine Hydrochloride:** Off-white to white crystalline powder. Soluble in methanol and in water.▲*USP34*

In-Process Revision

Pending Proposals

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

In order for an item to be adopted into the *USP–NF* and become officially binding, it must first be proposed and published in the *Pharmacopeial Forum (PF)* to allow the public an opportunity to review and comment upon it. When an item is adopted, it is published in the *USP–NF,* its *Supplements,* an *IRA* , or a *Revision Bulletin.* Those items that have not yet been adopted are marked as *Pending Proposals.*

The *Pending Proposals* list contains these items separated into the following categories: General Notices and Requirements; *USP* monographs; Dietary Supplements Monographs; General Chapters; Reagents; Indicators; and Solutions; Reference Tables; Excipients; and *NF* Monographs. Each entry in the *Pending Proposals* list contains the monograph title and the citation of the most recent publication of the monograph. Reprints of *PF* proposals may be purchased from USP by sending a written request for information to custsvc@usp.org.

To check the status of a *Pending Proposal,* please contact USP as directed below.

- The briefing accompanying the monograph or general chapter lists the names of the Scientific Liaisons responsible for the proposed revisions. The contact information (phone number and email) for the Scientific Liaison is available in the *Staff Directory* section of *How to Use PF.* For *USP–NF Online* subscribers, the name and contact information for the assigned Scientific Liaison is available in the *Auxiliary Information* portion of each monograph.
- Call USP at 301-816-8344 and ask to speak with the Scientific Liaison assigned to the monograph or general chapter of interest.
- Submit questions by email to stdsmonographs@usp.org. Please indicate the name of the monograph or general chapter in the subject line of the email.

Following these lists the reader will find the *Canceled Proposals* list. These are items that were published in *PF* and were pending, but have since been canceled. This list contains cumulative entries for the six issues per volume of *PF* [i.e., 35(1) through 35(6)]. Note that canceled proposals may be republished in *PF* to be considered for future adoption into the *USP–NF.*

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
General Notices (entire *General Notices and Requirements* revised)	34	1	40
USP Monographs			
Acetaminophen—*Readily carbonizable substances*	34	5	1136
Acetaminophen and Tramadol Hydrochloride Tablets (new)	35	1	56
Acetylcysteine—*USP Reference standards, Assay*	31	3	726
Acitretin—*Assay, Heavy Metals*	35	5	1102
Acitretin Capsules—*Assay*	35	5	1095
Medical Air—*Identification* (add); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide, Sulfur Dioxide, Limit of Nitric Acid and Nitrogen Dioxide; Packaging and Storage; Labeling*	35	4	828
Albumin Human—*Definition, Packaging and storage, Expiration date, Labeling, USP Reference standards* (add), *Identification A, B* (add), *Bacterial endotoxins* (add), *Safety* (add), *Sterility* (add), *pH* (add), *Molecular size distribution* (add), *Heat stability* (add), *Incubation* (add) *Prekallikrein activator* (add), *Protein content* (add), *Heme content* (add), *Potassium content* (add), *Sodium content* (add)	31	5	1338
Albuterol Tablets—*Assay*	31	3	726
Alendronate Sodium Tablets—*Dissolution*	35	1	59
Alprazolam Tablets—*Assay*	33	1	41
Alumina, Magnesia, and Calcium Carbonate Chewable Tablets (new)	29	6	1836
Amifostine—*X-ray diffraction* (delete)	34	5	1136
Amiodarone Hydrochloride (new)	34	6	1429
Amlodipine Besylate—*Chemical information, Definition, Labeling* (add), *Water*	34	5	1136
Amlodipine Besylate Tablets (new)	35	1	62
Amphetamine Sulfate—*USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	902
Amphetamine Sulfate Tablets—*Identification, Assay*	34	4	904

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ampicillin—*Definition, USP Reference standards, Related compounds* (add), *Assay*	34	5	1140
Ampicillin Sodium—*Dimethylaniline*	35	1	65
Aprotinin (new)	31	3	732
Aprotinin Injection (new)	31	3	736
Articaine Hydrochloride (new)	35	3	544
Aspirin—*Readily carbonizable substances*	34	5	1143
Atenolol—*Identification B*	35	3	545
Atenolol Tablets—*Dissolution*	35	1	66
Atorvastatin Calcium (new)	35	1	66
Atovaquone Oral Suspension—*Assay*	34	2	247
Atracurium Besylate Injection—*Related compounds*	34	4	905
Atropine Sulfate—*Identification C* (add); *Organic Impurities—Other Alkaloids* (delete), *Procedure* (add); *Melting Range or Temperature* (delete); *Optical Rotation* (delete); *Optical Rotation* (add); *Acidity* (delete); *Packaging and Storage; USP Reference Standards*	35	4	829
Aurothioglucose Injectable Suspension—*Bacterial endotoxins* (add), *Sterility* (add)	34	4	906
Azithromycin—*USP Reference standards, Limit of related substances* (delete), *Related compounds* (add)	34	3	559
Azithromycin for Injection (new)	34	3	562
Azithromycin Tablets (new)	34	5	1143
Aztreonam—*Definition, Assay, Organic Impurities* (add), *Limit of Alcohol* (add), *USP Reference Standards*	35	5	1103
Aztreonam for Injection—*Assay*	34	4	906
Soluble Bacitracin Methylene Disalicylate—*Title change, Definition*	35	5	1105
Bacitracin Methylene Disalicylate Soluble Powder—*Title change, Definition*	35	5	1106
Balsalazide Disodium (new)	35	4	830
Balsalazide Disodium Capsules (new)	35	4	832
Bendroflumethiazide Tablets—*Dissolution*	35	5	1106
Benzethonium Chloride—*Identification B, C* (delete), *Identification B* (add), *USP Reference Standards* (add)	35	4	833
Benzocaine—*Readily carbonizable substances*	34	5	1147
Benzoic Acid—*Readily carbonizable substances*	34	5	1147
Benzoin—*Botanic characteristics, Identification*	35	1	70
Bicalutamide—*Organic Impurities*	35	5	1107
Bicalutamide Tablets—*Labeling, Dissolution*	34	5	1147
Biperiden Hydrochloride Tablets—*Dissolution*	35	5	1108
Bisoctrizole (new)	32	2	309
Bleomycin for Injection—*Identification A, B* (add), *Other requirements*	34	5	1150
Budesonide—*Related compounds*	35	3	539
Bupropion Hydrochloride Extended-Release Tablets—*Dissolution*	35	5	1109
Buspirone Hydrochloride—*Content of chloride*	31	3	742
Caffeine—*Identification B, Melting range* (delete), *Readily carbonizable substances* (delete), *Other alkaloids* (delete)	34	5	1150
Camphor—*Water*	31	3	742
Capecitabine—*Assay, Organic Impurities*	35	4	834
Capecitabine Tablets—*Assay, Dissolution, Organic Impurities*	35	5	1113
Carbidopa—*Specific rotation*	35	1	73
Carbidopa and Levodopa Extended-Release Tablets (new)	34	6	1433
Carmustine (new)	35	3	546
Carmustine for Injection (new)	35	3	548
Cefazolin Sodium—*Chemical information, Related compounds* (add)	34	6	1438
Cefdinir Capsules—*Organic Impurities*	35	5	1115
Cefdinir for Oral Suspension—*Organic Impurities*	35	5	1118
Cefotetan for Injection—*Identification A* (add), *B* (add), *Other Requirements*	35	5	1122
Cefixime for Oral Suspension—*Water* (delete)	34	6	1441
Ceftazidime Injection—*USP Reference standards, Pyrogen* (delete), *Bacterial endotoxins* (add)	34	4	907
Ceftiofur Hydrochloride (new)	34	4	908
Ceftiofur Sodium (new)	34	4	912
Cefuroxime Axetil for Oral Suspension—*pH*	35	5	1123
Chloral Hydrate—*Readily carbonizable substances*	34	5	1150
Ciprofloxacin—*Identification B*	35	4	837
Ciprofloxacin Hydrochloride—*Identification B*	35	4	839

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ciprofloxacin Injection—*Identification A*	35	4	840
Ciprofloxacin Ophthalmic Solution—*Identification A*	35	4	842
Ciprofloxacin Tablets—*Identification A, B* (delete)	35	4	843
Citalopram Hydrobromide—*Identification, Related compounds*	34	4	917
Citalopram Tablets—*Identification A, Uniformity of Dosage Units, Organic Impurities*	35	4	844
Anhydrous Citric Acid *(Harmonization)—Sulfate*	31	3	749
Anhydrous Citric Acid—*Readily carbonizable substances*	34	5	1150
Citric Acid Monohydrate *(Harmonization)—Sulfate*	31	3	750
Citric Acid Monohydrate—*Readily carbonizable substances*	34	5	1151
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation—*USP Reference standards, Assay for citric acid (delayed implementation to January 1, 2009)*	31	2	394
Cladribine—*Specific rotation, Related compounds*	33	1	49
Clarithromycin Tablets—*Dissolution*	35	5	1124
Clavulanate Potassium—*Limit of aliphatic amines, Limit of 2-ethylhexanoic acid*	34	6	1441
Clenbuterol Hydrochloride (new)	35	5	1125
Clindamycin Palmitate Hydrochloride—*Chemical information, Assay*	34	6	1442
Clobetasol Propionate—*Residue on Ignition, Heavy Metals*	35	5	1125
Clopidogrel Tablets—*Related compounds*	33	1	50
Cloprostenol Injection (new)	34	4	918
Cloprostenol Sodium (new)	34	4	920
Cocaine—*Readily carbonizable substances*	34	5	1151
Cocaine Hydrochloride—*Readily carbonizable substances*	34	5	1151
Codeine—*Readily carbonizable substances*	34	5	1151
Codeine Sulfate—*Readily carbonizable substances*	34	5	1151
Cyclophosphamide—*Identification B, Assay, Organic Impurities—Procedure 1 and Procedure 2* (add), *Limit of Chloride* (add), *Limit of Phosphate* (add), *Bacterial Endotoxins Test* (add), *Sterility Tests* (add), *Labeling* (add), *USP Reference Standards*	35	5	1126
Dalteparin Sodium (new)	30	5	1598
Dantrolene Sodium Capsules—*Packaging and storage*	34	5	1151
Dapsone—*Assay*	31	3	750
Deferoxamine Mesylate—*Definition, Identification* (delete), *A* (add), *B* (add), *Assay, Organic Impurities* (add), *Packaging and Storage*	35	4	847
Human Acellular Dermal Matrix (new)	35	3	558
Microsized Human Acellular Dermal Matrix (new)	35	3	561
Desmopressin Nasal Spray Solution (new)	31	4	1059
Dextroamphetamine Sulfate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Related compounds* (add), *Organic volatile impurities* (delete), *Assay*	34	4	921
Dextrose—*Harmonization*	34	6	1585
Diazepam Extended-Release Capsules—*USP Reference standards, Assay*	32	2	330
Diclazuril (new)	35	1	73
Diclofenac Sodium Delayed-Release Tablets—*Dissolution*	35	5	1129
Diclofenac Sodium Extended-Release Tablets—*Assay, Dissolution, Organic Impurities*	35	5	1130
Dicyclomine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Didanosine for Oral Solution—*Assay*	34	6	1443
Dihydroxyaluminum Sodium Carbonate Chewable Tablets (new)	29	6	1873
Dinoprostone—*Organic Impurities*	35	5	1132
Disulfiram—*Assay, Inorganic Impurities—Selenium*	35	4	848
Docetaxel (new)	35	5	1133
Docusate Sodium—*Residue on Ignition*	35	5	1095
Dofetilide (new)	35	5	1135
Dolasetron Mesylate—*Impurities*	35	2	272
Dopamine Hydrochloride—*Readily carbonizable substances*	34	5	1152
Doxazosin Mesylate—*Assay*	34	5	1152
Dronabinol Capsules—*Definition, Assay, USP Reference Standards*	35	3	549
Ecamsule Solution (new)	34	5	1153
Egg Phospholipids (new)	31	3	757
Endotoxin Indicator for Depyrogenation (new)	34	6	1444
Enrofloxacin (new)	34	4	924
Ensulizole—*USP Reference Standards*	35	4	849
Epirubicin Hydrochloride (new)	35	2	273

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Erythromycin Pledgets—*Identification* (add), *Other requirements*	34	5	1157
Sterile Erythromycin Ethylsuccinate—*Identification* (add), *Other requirements*	34	5	1158
Esomeprazole Magnesium—*Identification B, Other Components—Content of Magnesium, Optical Rotation* (delete)	35	3	550
Esterified Estrogens—*Identification, Free steroids, Assay*	32	6	1678
Esterified Estrogens Tablets—*USP Reference standards, Assay*	32	6	1680
Estradiol Transdermal System—*Drug Release*	35	5	1136
Estradiol and Norethindrone Acetate Tablets—*Assay, Dissolution, Uniformity of Dosage Units, Organic Impurities*	35	5	1139
Conjugated Estrogens—*Assay*	35	5	1142
Ethotoin Tablets—*USP Reference standards, Assay*	32	2	332
Famotidine Injection (new)	32	2	333
Famotidine for Oral Suspension—*Uniformity of dosage units*	34	5	1158
Fenofibrate—*Definition, Assay*	35	2	275
Fentanyl (new)	34	3	602
Fexofenadine Hydrochloride Tablets—*Dissolution*	34	4	931
Fluconazole Injection (new)	35	3	552
Fluconazole Tablets (new)	34	4	932
Fludarabine Phosphate for Injection—*Related compounds*	34	4	933
Flurazepam Hydrochloride—*Identification*	31	3	766
Fluvestrant (new)	33	5	99
Fosinopril Sodium—*Organic Impurities—Procedure 1*	35	5	1144
Gabapentin Tablets—*Labeling* (add), *Dissolution*	34	4	934
Galantamine Tablets—*Organic Impurities*	35	5	1145
Glimepiride Tablets—*Dissolution*	33	3	411
Glucagon—*Chemical information, Definition, Identification* (delete), *A* (add), *B* (add), *Assay, Nitrogen Determination* (delete), *Residue on Ignition* (delete), *Zinc Determination* (delete), *Organic Impurities, Water Determination, Bacterial Endotoxins Test* (add), *Packaging and Storage, USP Reference Standards*	35	5	1148
Glucagon for Injection—*Definition, Identification* (add), *Assay, Organic Impurities* (add), *Water Determination* (add), *pH and Clarity of Solution* (delete), *Bacterial Endotoxins Test, Sterility Tests, Other Requirements* (delete), *Labeling* (add), *USP Reference Standards*	35	5	1152
Glutaral Concentrate—*Specific gravity*	31	3	766
Glyburide and Metformin Hydrochloride Tablets—*Identification A*	34	5	1163
Goserelin Acetate (new)	32	3	792
Granisetron Hydrochloride Injection (new)	34	4	935
Granisetron Hydrochloride Oral Suspension (new)	34	6	1454
Granisetron Hydrochloride Tablets (new)	34	4	937
Halazone—*Readily carbonizable substances*	34	5	1163
Helium—*Identification A* (delete), *B* (delete), *Identification* (add), *Assay, Inorganic Impurities—Carbon Monoxide, Odor, Packaging and Storage, Labeling* (add)	35	4	850
Hydromorphone Hydrochloride—*Organic Impurities, USP Reference Standards*	35	5	1156
Hydromorphone Hydrochloride Oral Solution (new)	35	4	851
Hydroxychloroquine Sulfate Tablets—*Identification*	34	4	940
Hydroxypropyl Cellulose Ocular System—*Assay*	35	4	852
Ibuprofen—*Chromatographic purity*	34	4	941
Ibuprofen Tablets—*Limit of ibuprofen related compound C*	34	4	941
Imipramine Hydrochloride—*Melting range* (delete)	34	5	1164
Biphasic Isophane Insulin Human Suspension (new)	31	4	1033
Human Insulin Isophane Suspension and Human Insulin Injection—*Soluble insulin human content*	34	4	941
Irbesartan—*Limit of azide*	34	5	1164
Itraconazole (new)	34	4	947
Ketoprofen Extended-Release Capsules (new)	34	4	951
Lactic Acid—*Readily carbonizable substances*	34	5	1164
Lamivudine and Zidovudine Tablets (new)	35	2	277
Leflunomide—*Organic Impurities—Procedure 1: Limit of Leflunomide Related Compound A, Loss on Drying*	35	5	1158
Leflunomide Tablets—*Water Determination* (delete)	35	5	1159
Levothyroxine Sodium—*Organic Impurities—Procedure 1, Procedure 2* (add), *Packaging and Storage, Labeling* (add), *USP Reference Standards*	35	3	555
Levothyroxine Sodium Oral Powder—*Identification* (add)	34	4	954
Levothyroxine Sodium Tablets—*Definition, Identification*	34	4	954
Lindane—*Assay*	34	2	280

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Liothyronine Sodium Tablets—*Identification*	34	4	955
Liotrix Tablets—*Identification*	34	4	955
Lisinopril Tablets—*Dissolution*	34	4	956
Lisinopril and Hydrochlorothiazide Tablets (new)	34	4	956
Loratadine Orally Disintegrating Tablets (new)	34	3	624
Loratadine and Pseudoephedrine Sulfate Extended-Release Tablets (new)	32	6	1715
Losartan Potassium Tablets (new)	34	5	1164
Losartan Potassium and Hydrochlorothiazide Tablets (new)	34	6	1455
Magnesium Carbonate and Citric Acid for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	419
Magnesium Citrate Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	420
Magnesium Citrate for Oral Solution—*USP Reference standards* (add), *Content of anhydrous citric acid, Other requirements (delayed implementation to January 1, 2009)*	31	2	421
Mannitol—*Harmonization*	34	6	1588
Mannitol Injection—*Labeling*	32	2	263
Mefloquine Hydrochloride—*Assay*	35	5	1160
Megestrol Acetate Oral Suspension—*Dissolution*	35	1	75
Meloxicam—*Impurities, Procedure 1*	35	2	278
Mesna (new)	34	5	1168
Metformin Hydrochloride Extended-Release Tablets—*Dissolution*	35	1	76
Methylbenzethonium Chloride—*Identification B* (delete), *C* (delete), *D* (delete), *B* (add); *USP Reference Standards* (add)	35	4	853
Methylcellulose (new)—*Stage 6 Harmonization*	35	3	683
Methylcellulose Ophthalmic Solution—*Identification*	31	3	780
Methylcellulose Oral Solution—*Identification*	31	3	780
Methylcellulose Tablets—*Identification*	31	3	780
Methylene Blue Injection, Veterinary (new)	34	6	1461
Methylphenidate Hydrochloride Extended-Release Tablets—*Identification B* (add), *Organic Impurities* (add), *USP Reference Standards*	35	5	1162
Metronidazole Benzoate—*USP Reference standards, Related compounds*	31	3	781
Midazolam (new)	34	4	961
Midazolam Injection (new)	34	3	635
Minocycline Periodontal System (new)	34	4	963
Mirtazapine—*USP Reference standards, Water, Chromatographic purity, Assay*	34	4	964
Misoprostol (new)	35	3	564
Mometasone Furoate Cream—*Packaging and storage, Related compounds* (add), *Assay*	35	1	82
Mometasone Furoate Ointment—*Packaging and storage, Related compounds* (add), *Assay*	35	1	84
Mometasone Furoate Topical Solution—*Packaging and storage, Related compounds* (add), *Assay*	35	1	87
Morantel Tartrate—*pH*	32	6	1735
Morphine Sulfate Extended-Release Capsules—*Assay, Organic Impurities, USP Reference Standards*	35	3	565
Morphine Sulfate Extended-Release Tablets (new)	35	5	1164
Moxifloxacin Hydrochloride (new)	34	5	1170
Moxifloxacin Ophthalmic Solution (new)	34	5	1173
Mupirocin Nasal Ointment (new)	34	4	966
Mycophenolate Mofetil—*Identification, Melting range* (delete), *Related compounds, Assay*	35	1	89
Mycophenolate Mofetil Capsules (new)	35	4	854
Mycophenolate Mofetil Tablets (new)	35	4	856
Naratriptan Hydrochloride Oral Suspension (new)	35	1	90
Nevirapine Oral Suspension—*Organic Impurities*	35	4	857
Nateglinide (new)	34	6	1463
Nateglinide Tablets (new)	35	2	281
Niacinamide—*Readily carbonizable substances*	34	5	1176
Nitrofurantoin Capsules—*Packaging and storage*	35	1	92

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Nitrous Oxide—*Definition; Identification A, B* (delete), *C* (delete); *Assay; Inorganic Impurities—Ammonia, Nitric Oxide, Nitrogen Dioxide, Halogens, Carbon Monoxide, Carbon Dioxide; Water; Packaging and Storage; Labeling* (add)	35	4	859
Norepinephrine Bitartrate—*Residue on Ignition*	35	5	1165
Norethindrone Acetate—*Chromatographic purity*	34	4	969
Norethynodrel (delete entire monograph)	35	1	92
Octisalate—*Assay*	34	4	970
Ofloxacin—*Chromatographic purity* (delete), *Related compounds* (add)	30	4	1274
Ofloxacin Tablets—*Uniformity of dosage units*	34	6	1467
Olanzapine Tablets (new)	35	2	282
Olopatadine Hydrochloride (new)	35	3	567
Olopatadine Hydrochloride Ophthalmic Solution (new)	35	3	568
Ondansetron Hydrochloride—*Limit of ondansetron related compound D, Assay*	32	1	126
Ondansetron Tablets (new)	34	4	971
Ondansetron Orally Disintegrating Tablets—*Labeling* (add), *Disintegration, Dissolution, Water* (delete)	34	6	1467
Orlistat (new)	35	5	1166
Orlistat Capsules (new)	35	5	1169
Oseltamivir Phosphate (new)	34	6	1468
Oseltamivir Phosphate Capsules (new)	34	6	1471
Oxaliplatin (new)	34	4	973
Oxaliplatin Injection (new)	35	2	284
Oxaliplatin for Injection (new)	34	6	1473
Oxazepam Capsules—*Assay, Dissolution, Organic Impurities* (add)	35	5	1170
Oxcarbazepine (new)	34	5	1177
Oxcarbazepine Tablets (new)	34	6	1478
Oxybutynin Chloride Tablets—*Dissolution*	35	1	93
Oxycodone Hydrochloride—*USP Reference standards, Limit of oxycodone related compound A (14-hydroxycodeinone) and oxycodone related compound C (codeinone)* (add), *Chromatographic purity*	34	6	1480
Oxygen—*Identification—Procedure, B* (delete); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide; Packaging and Storage; Labeling*	35	4	861
Oxygen 93 Percent—*Identification A, B* (delete); *Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide; Packaging and Storage; Labeling*	35	4	862
Pamidronate Disodium—*Alcohol content* (delete)	34	5	1179
Pamidronate Disodium for Injection—*Definition*	33	1	81
Pancuronium Bromide Injection (new)	32	4	1097
Pantoprazole Oral Suspension (new)	35	4	863
Paricalcitol—*Identification, Assay*	33	2	252
Pectin—*Chemical information; Definition; Identification—A, B, C, D* (delete), *Procedure* (add); *Assay—Methoxy Groups* (name change), *Galacturonic Acid, Methoxy Groups* (add); *Impurities—Lead, Procedure 1, Procedure 2* (add), *Procedure 3* (add); *Microbial Enumeration Tests; Packaging and Storage; Labeling; USP Reference Standards* (add)	35	2	287
Penicillamine Capsules—*Dissolution*	31	2	436
Pentamidine Isethionate (new)	35	3	570
Pentobarbital—*Identification B* (delete), *C, Assay, Organic Impurities, Melting Range or Temperature* (delete)	35	4	864
Pentobarbital Sodium—*Labeling* (add), *USP Reference standards, Other requirements* (add)	31	1	73
Petrolatum (new)—*Stage 4 Harmonization*	35	5	1363
White Petrolatum (new)—*Stage 4 Harmonization*	35	5	1364
Liquefied Phenol—*Identification* (add), *Other requirements*	35	1	93
Phenytoin Chewable Tablets (new)	29	6	1965
Physostigmine—*Readily carbonizable substances*	34	5	1179
Physostigmine Salicylate—*Readily carbonizable substances*	34	5	1179
Physostigmine Sulfate—*Readily carbonizable substances*	34	5	1179
Pilocarpine Hydrochloride—*Readily carbonizable substances*	34	5	1179
Pilocarpine Nitrate—*Readily carbonizable substances*	34	5	1179
Piperacillin and Tazobactam for Injection (new)	34	4	980

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution—*USP Reference standards* (add), *Assay for anhydrous citric acid (delayed implementation to January 1, 2009)*	31	2	440
Potassium Bitartrate—*Heavy metals*	34	5	1180
Potassium Citrate Extended-Release Tablets—*USP Reference standards* (add), *Assay (delayed implementation to January 1, 2009)*	31	2	443
Potassium Citrate and Citric Acid Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	444
Potassium Iodide Delayed-Release Tablets—*Identification* (add), *Other requirements*	34	6	1481
Potassium Iodide Oral Solution—*Definition*	31	3	786
Potassium Sodium Tartrate—*Limit of ammonia*	31	3	787
Pralidoxime Chloride for Injection—*Identification A, B, C* (add), *Other requirements*	34	5	1180
Pravastatin Sodium Tablets—*USP Reference standards, Related compounds*	34	5	1180
Praziquantel Tablets—*Dissolution*	35	2	291
Primidone—*Identification B, C* (delete), *Assay, Organic Impurities, Melting Range or Temperature* (delete), *USP Reference Standards*	35	3	571
Primidone Tablets—*Assay, Organic Impurities* (add), *USP Reference Standards*	35	3	573
Promethazine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	292
Promethazine Hydrochloride and Dextromethorphan Hydrobromide Oral Solution (new)	35	2	295
Promethazine and Phenylephrine Hydrochloride Oral Solution (new)	35	2	298
Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution (new)	35	2	301
Propafenone Hydrochloride—*USP Reference standards, Chromatographic purity* (delete), *Related compounds* (add)	35	1	94
Propranolol Hydrochloride Extended-Release Capsules—*Dissolution*	35	5	1096
Propoxyphene Hydrochloride—*Definition, Assay, Organic Impurities, Melting Range or Temperature* (delete)	35	4	865
Propoxyphene Hydrochloride Capsules—*Identification B* (delete), *Identification C*	35	3	574
Psyllium Husk—*Impurities—Heavy Metals* (add), *Procedure 3* (add)	35	2	304
Pyrantel Pamoate—*USP Reference standards, Related compounds*	34	6	1482
Quinapril Tablets—*Related compounds*	34	5	1182
Ractopamine Hydrochloride Suspension (new)	35	5	1171
Ramipril—*Definition, Assay*	31	3	787
Ramipril Capsules (new)	35	4	867
Oral Rehydration Salts—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	5	1399
Repaglinide Tablets—*Loss on Drying* (delete)	35	2	306
Ribavirin Capsules (new)	35	3	576
Riluzole (new)	35	5	1173
Riluzole Tablets (new)	35	5	1174
Risedronate Sodium (new)	34	5	1183
Risedronate Sodium Tablets (new)	34	5	1186
Risperidone Oral Solution (new)	35	4	870
Ritonavir—*Identification*	35	1	95
Salmeterol Xinafoate (new)	35	2	307
Salsalate Tablets—*Assay*	33	6	1211
Secobarbital Sodium—*Chemical structure, Definition, Identification, Related compounds* (add), *Isomer content* (delete), *Assay*	34	4	984
Sennosides—*Content of Sennosides A and B* (add), *USP Reference Standards* (add)	35	2	308
Sertraline Hydrochloride (new)	34	5	1189
Sibutramine Hydrochloride (new)	34	4	986
Sodium Chloride—*Identification, Loss on drying, Limit of potassium (postponed indefinitely)*	32	2	264
Sodium Fluoride Gel (new)	35	5	1175
Sodium Sulfate—*Assay*	34	5	1192

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Soybean Oil—*CAS number* (add), *Labeling, Identification* (add), *Specific gravity* (delete), *Refractive index* (delete), *Heavy metals, Free fatty acids* (delete), *Acid value* (add), *Fatty acid composition, Iodine value* (delete), *Saponification value* (delete), *Cottonseed oil* (delete), *Peroxide value, Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	4	989
Spectinomycin for Injectable Suspension—*Identification* (add), *Other requirements*	34	5	1193
Streptomycin Injection—*Identification* (add), *Other requirements*	34	5	1193
Sucralfate—*Identification*	33	2	254
Sulfadiazine Tablets—*Dissolution*	35	3	577
Sulfadoxine—*Identification, Assay*	34	2	300
Sulfamethazine Granulated—*Assay*	31	3	797
Sulfinpyrazone—*Identification A, B* (add), *Melting Range or Temperature* (delete), *Solubility in acetone* (delete), *Solubility in 0.50 N sodium hydroxide* (delete)	35	3	577
Sumatriptan Tablets (new)	35	4	871
Tacrolimus (new)	35	2	310
Tacrolimus Capsules (new)	35	2	312
Tamsulosin Hydrochloride (new)	35	3	578
Tamsulosin Hydrochloride Capsules (new)	34	5	1193
Telmisartan (new)	35	3	580
Telmisartan Tablets (new)	35	3	581
Terazosin Capsules (new)	35	4	872
Terazosin Tablets (new)	35	4	874
Terbinafine Oral Suspension (new)	35	1	96
Terbutaline Oral Suspension (new)	35	1	97
Terbutaline Sulfate Inhalation Aerosol—*USP Reference standards, Assay*	31	2	450
Terconazole (new)	34	4	991
Thiabendazole Chewable Tablets (new)	29	6	1991
Thimerosal—*Readily carbonizable substances*	34	5	1197
Thioguanine—*USP Reference standards, Identification, Limit of guanine*	34	2	305
Thioridazine Hydrochloride—*Identification*	31	3	798
Tiagabine Hydrochloride Oral Suspension (new)	35	1	98
Ticlopidine Hydrochloride (new)	35	3	582
Ticlopidine Hydrochloride Tablets (new)	35	3	584
Tilmicosin—*Definition, Related compounds, Assay*	31	3	798
Tioconazole—*Assay*	35	4	875
Tizanidine Tablets—*Dissolution*	35	3	585
Topiramate Tablets (new)	34	5	1197
Tramadol Hydrochloride (new)	34	5	1200
Tramadol Hydrochloride Tablets (new)	31	2	462
Tranexamic Acid (new)	34	6	1484
Tranylcypromine Sulfate (new)	35	2	314
Tranylcypromine Tablets (new)	35	3	587
Trenbolone Acetate—*Definition, USP Reference standards, Identification, Chromatographic purity* (delete), *Limit of trenbolone acetate 17α-isomer* (delete), *Related compounds* (add), *Assay*	35	1	100
Tretinoin Gel—*Identification, Assay*	34	6	1485
Triamcinolone Acetonide—*USP Reference standards, Assay*	31	3	800
Tricitrates Oral Solution—*USP Reference standards* (add), *Assay for citrate (delayed implementation to January 1, 2009)*	31	2	465
Tromethamine—*Melting Range or Temperature*	35	2	316
Tryptophan—*Chromatographic purity* (add), *Limit of tryptophan related compound A* (add)	33	6	1214
Tylosin Injection (new)	34	5	1205
Ursodiol Capsules—*Dissolution*	31	3	800
Ursodiol Tablets—*Identification, Assay*	35	4	876
Valacyclovir Hydrochloride (new)	35	3	589
Valacyclovir Tablets (new)	35	4	878
Valganciclovir Tablets (new)	33	1	89
Valproic Acid Capsules—*Disintegration* (delete)	35	3	591
Valrubicin—*Definition, USP Reference standards, Identification, Loss on drying* (delete), *Water* (add), *Limit of residual solvents* (delete), *Related compounds, Assay*	35	1	103
Valrubicin Intravesical Solution—*USP Reference standards, Related compounds*	34	6	1486

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Valsartan and Hydrochlorothiazide Tablets—*Organic Impurities*	35	5	1176
Vancomycin Hydrochloride—*Identification, Inorganic Impurities—Heavy Metals* (add), *Organic Impurities—Procedure: Limit of Monodechlorovancomycin, Sterility Tests* (add), *Bacterial Endotoxins Test* (add), *Composition of Vancomycin, Labeling* (add)	35	4	879
Vancomycin Hydrochloride Capsules—*Identification*	34	6	1487
Sterile Vancomycin Hydrochloride—(delete the entire monograph)	34	1	112
Vancomycin Hydrochloride for Injection—*Definition, Identification* (add), *Assay, Uniformity of Dosage Units* (add), *Inorganic Impurities—Heavy Metals* (delete), *pH* (add), *Water Determination* (add), *Composition of Vancomycin, Other Requirements, Labeling* (add)	35	4	881
Vasopressin—*Chemical information, Definition, USP Reference standards, Oxytocic activity* (delete), *Water* (add), *Acetic acid content* (add), *Assay*	34	4	994
Vasopressin Injection—*Assay*	34	4	995
Vinblastine Sulfate for Injection—*Identification* (add), *Other requirements*	34	4	995
Vincristine Sulfate Injection—*Identification*	35	1	106
Vincristine Sulfate for Injection—*Identification*	35	1	106
Pure Steam (new)	31	2	467
Water for Hemodialysis—*Bacterial endotoxins*	31	2	468
Water for Injection—*Definition, Bacterial Endotoxins Test, Water Conductivity, Sterility Tests* (add), *Packaging and Storage* (add), *Labeling* (add)	35	2	316
Purified Water—*Definition, Packaging and Storage* (add), *Labeling* (add)	35	2	317
Sterile Water for Inhalation—*Definition, Water Conductivity*	35	5	1178
Sterile Water for Injection—*Calcium* (delete), *Carbon Dioxide* (delete), *Sulfate* (delete), *Chloride* (delete), *Ammonia* (delete), *pH* (delete), *Water Conductivity*	35	5	1178
Sterile Water for Irrigation—*Water Conductivity*	35	5	1179
Sterile Purified Water—*Water Conductivity*	35	5	1180
Xylose—*USP Reference standards, Identification, Chromatographic purity, Assay*	34	4	995
Zidovudine Oral Solution—*Identification A* (delete), *B* (delete), *Identification* (add), *Organic Impurities, Labeling* (add), *USP Reference Standards*	35	5	1180
Ziprasidone Hydrochloride (new)	35	3	592
Zolpidem Tartrate (new)	34	6	1487
Zolpidem Tartrate Extended-Release Tablets (new)	35	3	595
Zolpidem Tartrate Tablets (new)	35	4	883
Zonisamide (new)	34	6	1489
Dietary Supplements Monographs			
Acesulfame Potassium—*Packaging and storage* (add), *Limit of fluoride*	31	3	811
N-Acetyltyrosine (new)	35	1	107
Andrographis (new)	35	5	1183
Powdered Andrographis (new)	35	5	1184
Powdered Andrographis Extract (new)	35	5	1186
Ashwagandha (new)	35	4	885
Powdered Ashwagandha (new)	35	4	886
Powdered Ashwagandha Extract (new)	35	4	888
Boswellia Serrata (new)	35	4	890
Boswellia Serrata Extract (new)	35	4	891
Calcium and Vitamin D with Minerals Tablets—*Assay for calcium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for zinc; Assay for calcium, copper, magnesium, manganese, and zinc, Method 2* (add)	34	6	1491
Crypthecodinium cohnii Oil (new)	35	4	892
Crypthecodinium cohnii Oil Capsules (new)	35	5	1187
Fish Oil Containing Omega-3 Acids—*Content of EPA and DHA*	34	5	1207
Glutamic Acid (new)	34	4	997
Grape Seeds Oligomeric Proanthocyanidins (new)	34	3	659
Guggul (new)	34	4	1000
Native Guggul Extract (new)	34	4	1002
Purified Guggul Extract (new)	34	4	1003
Guggul Tablets (new)	34	4	1004

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Ground Limestone (new)	34	4	998
Alpha Lipoic Acid—*Limit of 6,8-epitrithiooctanoic acid* (delete), *Limit of polymer content* (delete), *Chromatographic purity* (add), *Assay*	34	5	1209
Maleic Acid—*Identification*	31	3	815
Maltose—*Water*	31	3	815
Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1493
Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1495
Olive Oil—*Definition, Labeling* (add), *Teaseed oil*	31	3	815
Omega-3 Acid Ethyl Esters (new)	35	5	1190
Phenoxyethanol—*Chromatographic purity, Assay*	31	3	816
Polyethylene Glycol (new)—*Harmonization*	31	3	897
Polyoxyl 10 Oleyl Ether—*Free ethylene oxide*	31	3	816
Polyoxyl 20 Oleyl Cetostearyl Ether—*Free ethylene oxide*	31	3	817
Schizochytrium Oil (new)	35	4	894
Schizochytrium Oil Capsules (new)	35	5	1192
Sodium Benzoate—*USP Reference standards* (add), *Identification*	31	3	818
Sucrose (new)—*Harmonization*	31	3	902
Sugar Spheres—*Identification, Specific rotation*	31	3	819
Tagatose (new)	31	3	819
Thymol—*USP Reference standards* (add), *Identification*	31	3	821
Tumeric (new)	33	6	1229
Powdered Tumeric (new)	33	6	1232
Powdered Tumeric Extract (new)	33	6	1232
Ubidecarenone—*USP Reference standards, Assay*	31	1	86
Valerian Capsules (new)	27	1	1825
Vinpocetine (new)	35	5	1195
Vitamin A Oral Liquid Preparation (new)	35	3	596
Oil- and Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1499
Oil- and Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1500
Water-Soluble Vitamins with Minerals Capsules—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1505

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Water-Soluble Vitamins with Minerals Tablets—*Definition, Assay for calcium; Assay for chromium; Assay for copper; Assay for iron; Assay for magnesium; Assay for manganese; Assay for phosphorus; Assay for zinc; Assay for boron, nickel, tin, and vanadium, Method I; calcium, chromium, copper, iron, magnesium, manganese, phosphorus, and zinc, Method 2; molybdenum and selenium, Method 3* (add)	34	6	1507
Xanthan Gum—*Assay*	31	3	821
Zinc Gluconate Tablets (new)	35	3	597
USP General Test Chapters			
⟨1⟩ Injections—*Ingredients, Packaging—Labeling on Ferrules and Cap Overseals* (delayed date to May 1, 2010); *Foreign and Particulate Matter*	35	5	1214
⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests (new)	35	3	602
⟨11⟩ USP Reference Standards	31	2	507
	31	6	1680
	32	4	1161
	33	1	95
	34	2	332
	34	3	680
	34	4	1021
	34	5	1230
	34	6	1531
	35	1	144
	35	2	330
	35	3	612
	35	4	913
	35	5	1217
⟨11⟩ USP Reference Standards—*Stage 6 Harmonization*	35	4	1022
⟨41⟩ Weights and Balances—*Introduction, Repeatability, Verification of Accuracy, Calibration Check*	35	2	331
⟨63⟩ Mycoplasma Tests (new)	35	1	146
⟨85⟩ Bacterial Endotoxins Test—*Stage 6 Harmonization*	35	3	695
⟨90⟩ Fetal Bovine Serum—Quality Attributes and Functionality Tests (new)	35	5	1219
⟨92⟩ Growth Factors and Cytokines Used in Cell Therapy Manufacturing (new)	35	4	915
⟨111⟩ Design and Analysis of Biological Assays (entire chapter revised)	34	3	685
⟨121⟩ Insulin Assays—*Appendix* (add)	30	5	1675
⟨197⟩ Spectrophotometric Identification Tests (entire chapter revised)	35	1	153
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium (new)	34	1	143
⟨223⟩ Dimethylaniline—*Chromatographic System, Procedure*	35	1	156
⟨228⟩ Ethylene Oxide and Dioxane (new)	35	4	917
⟨231⟩ Heavy Metals—*Method II*	32	1	182
⟨331⟩ Amphetamine Assay (delete entire chapter)	35	4	920
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate (new)	31	2	514
⟨381⟩ Elastomeric Closures for Injections—*Introduction*	35	5	1225
⟨413⟩ Impurities Testing in Medical Gases (new)	35	4	920
⟨415⟩ Medical Gases Assay (new)	35	4	921
⟨429⟩ Light Diffraction Measurement of Particle Size (new)—*Stage 6 Harmonization*	35	3	707
⟨467⟩ Residual Solvents—*Identification, Control, and Quantification of Residual Solvents; Other Analytical Procedures* (delete)	35	2	334
⟨525⟩ Sulfur Dioxide—*Method IV* (add), *Method V* (add)	35	2	341
⟨601⟩ Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers—*Harmonization*	33	3	550
⟨616⟩ Bulk Density and Tapped Density (new)—*Stage 6 Harmonization*	35	3	715
⟨643⟩ Total Organic Carbon—*Introduction, Apparatus Requirements, Glassware Preparation, Standard Solution, Test Solution* (delete), *Water Sample* (add), *Other Control Solutions, System Suitability, Procedure*	34	5	1241
⟨645⟩ Water Conductivity—*Introduction, Instrument Specifications and Operating Parameters, Bulk Water, Packaged Water*	35	5	1226
⟨670⟩ Containers—Auxiliary Packaging Components (new)	34	6	1533
⟨696⟩ Characterization of Crystallinity Determination by Solution Calorimetry—*Stage 4 Harmonization*	35	3	675
⟨699⟩ Density of Solids (new)—*Harmonization*	31	3	912

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
⟨711⟩ Dissolution—*Introduction, USP Reference Standards, Apparatus, Procedure, Interpretation*	34	5	1243
⟨711⟩ Dissolution—*Stage 6 Harmonization*	35	3	719
⟨725⟩ Topical and Transdermal Drug Products—Product Performance Tests (new)	35	3	615
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions—*Method II—Measurement of Large Globule Content by Light Obscuration or Extinction Method*	35	3	626
⟨741⟩ Melting Range or Temperature—*Introduction; Procedure for Class I, Apparatus II; Procedure for Class Ia, Apparatus II* (add); *Procedure for Class Ib, Apparatus II* (add)	35	4	925
⟨785⟩ Osmolality and Osmolarity—*Measurement of Osmolality*	34	5	1251
⟨788⟩ Particulate Matter in Injections—*Introduction*	35	3	628
⟨795⟩ Pharmaceutical Compounding—Nonsterile Preparations (entire chapter revised)	35	4	926
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations—*Environmental Monitoring* (add)	32	3	852
⟨811⟩ Powder Fineness—*Harmonization*	31	1	228
⟨851⟩ Spectrophotometry and Light-Scattering (entire chapter revised)	35	1	157
⟨853⟩ Fluorescence Spectroscopy (new)	34	5	1252
⟨854⟩ Mid-Infrared Spectroscopy (new)	34	5	1266
⟨857⟩ Ultraviolet-Visible Spectroscopy (new)	34	5	1282
⟨891⟩ Thermal Analysis—*Introduction, Transition Temperature, Determination of Transition Temperature (Melt Onset Temperature) and Melting Point Temperature* (add), *Thermogravimetric Analysis, Hot-Stage Microscopy* (add), *Eutetic Impurity Analysis*	34	4	1023
⟨905⟩ Uniformity of Dosage Units—*Introduction, Content Uniformity, Weight Variation, Criteria*	34	5	1290
⟨905⟩ Uniformity of Dosage Units—*Stage 6 Harmonization*	35	3	724
⟨911⟩ Viscosity (entire chapter revised)	34	6	1536
⟨912⟩ Non-Newtonian Rheology (new)	34	6	1541
⟨921⟩ Water Determination—*Method I (Titrimetric)*	35	2	346
⟨941⟩ X-Ray Diffraction (new)—*Stage 6 Harmonization*	35	3	731
General Information Chapters			
⟨1024⟩ Bovine Serum (new)	35	3	628
⟨1033⟩ Biological Assay Validation (new)	35	2	349
⟨1059⟩ Excipient Performance (new)	35	5	1228
⟨1066⟩ Physical Environments that Promote Safe Medication Use (new)	34	6	1549
⟨1072⟩ Disinfectants and Antiseptics—*Classification of Disinfectants, Selection of a Disinfectant for Use in a Pharmaceutical Manufacturing Environment, Theoretical Discussion of Disinfectant Activity, Mechanism of Disinfectant Activity, Microbial Resistance to Disinfectants, Disinfectant Challenge Testing, Disinfectants in a Cleaning and Sanitization Program*	35	5	1250
⟨1075⟩ Good Compounding Practices (delete entire chapter)	35	4	942
⟨1082⟩ Genotoxicity Testing (new)	30	1	264
⟨1086⟩ Impurities in Official Articles—*Introduction, Initial IND Filing* (delete), *Drug Substance* (add), *NDA Filing* (delete), *Drug Product* (add), *Post-NDA Approval* (delete), *ANDA Filing* (delete), *Definitions*	35	5	1254
⟨1090⟩ In Vivo Bioequivalence Guidances (entire chapter revised)	34	4	1028
⟨1097⟩ Bulk Powder Sampling Procedures (new)	35	2	367
⟨1113⟩ Microbial Identification (new)	35	1	167
⟨1117⟩ Microbiological Best Laboratory Practices—*Introduction, Media Preparation and Quality Control, Maintenance of Microbiological Cultures, Maintenance of Laboratory Equipment, Laboratory Layout and Operations, Sample Handling* (add), *Microbiological Media Incubation Times* (add), *Training of Personnel, Laboratory Resources* (add), *Documentation, Maintenance of Laboratory Records, Interpretation of Assay Results*	35	4	945
⟨1151⟩ Pharmaceutical Dosage Forms (entire chapter revised)	35	5	1260
⟨1160⟩ Pharmaceutical Calculations in Prescription Compounding—*Basic Pharmaceutical Calculations*	31	3	847
⟨1180⟩ Human Plasma (new)	35	2	388

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles—*Introduction; Methods of Sterilization; Sterility Testing of Lots; Performance, Observation, and Interpretation* (delete)	35	4	952
⟨1225⟩ Validation of Compendial Procedures—*Validation*	35	2	444
⟨1231⟩ Water for Pharmaceutical Purposes—*Introduction, Types of Water, Chemical Considerations*	35	5	1310
⟨1232⟩ Instrumentation for Analysis of High Purity Pharmaceutical Waters (new)	30	5	1806
⟨1235⟩ Vaccines for Human Use—General Considerations (new)	35	4	960
⟨1251⟩ Weighing on an Analytical Balance (entire chapter revised)	35	2	448
Dietary Supplements Chapters			
⟨2040⟩ Disintegration and Dissolution of Dietary Supplements—*Disintegration, Dissolution*	35	4	984
⟨2750⟩ Manufacturing Practices for Dietary Supplements—*Introduction* (delete); *General Provisions* (add); *Organization and Personnel; Grounds, Buildings, and Facilities; Equipment; Raw Materials, Product Containers, and Closures; Production and Process Controls; Labeling and Packaging; Quality Control Operations; Records and Reports; Returned and Salvaged Products; Glossary*	35	5	1319
Reagents, Indicators, and Solutions			
Reagents, Indicators, and Solutions—*Introduction*	35	1	176
Alcohol	35	1	177
Ammonium Molybdate	35	1	177
t-Butylthiol (new)	35	3	648
Calcium Acetate	35	4	990
Chromotropic Acid	35	1	177
Chromotropic Acid Disodium Salt	35	1	177
Cobalt Chloride	35	5	1339
Cobalt Nitrate	35	3	648
Diaveridine	35	3	648
1,3-Dicaffeoylquinic Acid (new)	35	4	990
N,*N*-Dimethyldecylamine (new)	34	4	1041
4′4-Dipyridyl Dihydrochloride	33	5	1047
Ethylene Oxide in Methylene Chloride (50 mg/mL) (new)	31	3	859
Heptyl *p*-Hydroxybenzoate (new)	35	2	460
Methylbenzothiazolone Hydrazone Hydrochloride	34	5	1319
Methyl Red	35	4	990
p-Naphtholbenzein	35	3	648
Nitrogen Certified Standard (new)	35	4	990
Oxygen Certified Standard (new)	35	5	1339
93.0% Oxygen Certified Standard (new)	35	4	991
Oxygen in Nitrogen Certified Standard (new)	35	4	991
3.0% Oxygen in Nitrogen Certified Standard (new)	35	4	991
21.0% Oxygen in Nitrogen Certified Standard (new)	35	4	991
Oxygen–Helium Certified Standard (delete)	35	4	991
Pectate Lysate (new)	35	2	460
Phosphorous Acid (new)	35	1	178
Potassium Metabisulfite (new)	35	1	178
Potassium Sodium Tartrate	35	1	178
Sodium Acetate	35	2	461
Sodium Biphenyl	35	3	648
Sodium 1-Decanesulfonate	34	5	1319
Stannous Chloride	35	3	649
Sulfuric Acid, Nitrogen Free (new)	35	3	649
Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent (new)	34	4	1041
Tetramethylbenzidine (new)	35	5	1339
Delta-8-tetrahydrocannabinol (new)	35	4	991
Test Solutions			
Acetic Acid, Glacial, TS	35	1	179
Denatured Alcoholic TS (new)	35	1	179
Cupric Citrate TS 2, Alkaline	35	1	179
Lead Subacetate TS	35	5	1339
Dibasic Sodium Phosphate TS	35	3	649
Starch TS	35	5	1340
Volumetric Solutions			
Hydrochloric Acid, Normal (1 N)	35	1	180
Hydrochloric Acid, Half-Normal (0.5 N)	35	1	180

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Hydrochloric Acid, Half-Normal (0.5 N) in Methanol	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Dioxane	35	1	180
Perchloric Acid, Tenth-Normal (0.1 N) in Glacial Acetic Acid	35	1	181
Potassium Thiocyanate, Tenth-Normal (0.1 N) (new)	34	4	1043
Chromatographc Columns			
Chromatographic Columns—*Packings*	35	1	182
L## (Emtricitabine, Chirobiotic V) (new)	35	5	1340
Reference Tables			
Container Specifications for Capsules and Tablets	35	5	1341
Description and Solubility	29	1	266
	34	3	817
	34	4	1046
	34	5	1322
	34	6	1565
	35	1	188
	35	2	464
	35	3	651
	35	4	993
	35	5	1343
Description and Solubility—*Stage 6 Harmonization*	35	4	1022
Atomic Weights—*Standard Atomic Weights of the Elements*	35	1	189
Excipients			
USP and *NF* Excipients, Listed by Category	35	5	1197
USP and *NF* Excipients, Listed by Category—*Stage 6 Harmonization*	35	4	1017
NF General Notices and Requirements—*Title* (delete), ''*Official*'' *and* ''*Official Articles*'' (delete), *Storage under Nonspecific Conditions* (delete), *Other General Notices* (delete)	34	1	119
NF Monographs			
Agar—*CAS number* (add), *Definition, Botanic characteristics, Packaging and storage* (add), *USP Reference standards* (add), *Identification, Microbial limits, Limit of foreign insoluble matter*	33	4	702
Alpha-Lactalbumin (new)	34	3	670
Amylene Hydrate—*Identification A, B, C* (delete)	35	4	903
Behenoyl Polyoxylglycerides (new)	34	5	1217
Benzalkonium Chloride—*Packaging and storage, Identification, Acidity or alkalinity* (add), *Limit of foreign amines* (delete), *Limit of amines and amine salts* (add)	34	4	1012
Benzyl Alcohol—*Stage 6 Harmonization*	35	3	685
Butylparaben—*Harmonization*	34	6	1592
Calcium Propionate (new)	34	6	1517
Caprylocaproyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add), *Limit of free glycerol*	34	4	1012
Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium (new)	34	6	1519
Carmellose (new)—*Stage 6 Harmonization*	35	4	1018
Silicified Microcrystalline Cellulose (new)	34	5	1218
Chitosan (new)	35	1	115
Copovidone—*Harmonization*	32	6	1843
Corn Oil—*CAS number* (add), *Labeling* (add), *Identification* (add), *Specific gravity* (delete), *Heavy metals, Cottonseed oil* (delete), *Fatty acid composition, Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add), *Other requirements* (add)	34	5	1220
Corn Syrup (new)	33	6	1240
Cottonseed Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Iodine value* (delete), *Water* (add), *Heavy metals, Alkaline impurities* (add), *Other requirements* (add)	34	5	1222
Crospovidone (new)—*Stage 4 Harmonization*	35	3	671
Cystine (new)	35	1	122
Desoxycholic Acid (new)	34	6	1523
Diethyl Sebacate (new)	35	5	1203

In-Process Revision

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	PF Volume, Issue, and Page Numbers of Pending Proposals Vol.	No.	Page(s)
Egg Phospholipids (new)	33	4	703
Ethyl Acetate—*Readily carbonizable substances*	34	5	1223
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity, Coagulum content*	35	1	123
Ethyl Maltol (new)	34	5	1224
Ethylene Glycol and Vinyl Alcohol Graft Copolymer (new)	35	2	324
Ethylparaben—*Harmonization*	34	6	1594
Fumaric Acid—*Identification*	35	3	598
L-Glutamic Acid, Hydrochloride (new)	35	5	1203
Hydrogenated Polydecene (new)	33	3	485
Hydroxyethyl Cellulose (new)—*Harmonization*	34	6	1595
Hydroxypropyl Cellulose—*Identification*	35	1	124
Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	672
Low-Substituted Hydroxypropyl Cellulose (new)—*Stage 4 Harmonization*	35	3	673
Lactobionic Acid (new)	35	4	904
Anhydrous Lactose—*Stage 4 Harmonization*	35	4	1013
Lanolin Alcohols—*CAS number* (add), *Packaging and storage, Labeling* (add), *Acid value, Hydroxyl value* (add), *Peroxide value* (add)	34	4	1014
Lauroyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1224
Linoleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Refractive index* (delete), *Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1015
Magnesium Stearate—*Harmonization*	30	1	340
Methacrylic Acid Copolymer—*Title change, Chemical information* (add), *Definition, Identification B, Assay, Organic Impurities—Procedure: Limit of Monomers, Viscosity, Packaging and Storage, Labeling*	35	4	905
Methacrylic Acid and Ethyl Acrylate Copolymer (new)	35	4	907
Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer (new)	35	5	1204
Methacrylic Acid and Methyl Methacrylate Copolymer (new)	35	4	909
Methylacrylic Acid Copolymer Dispersion—*Packaging and storage, Viscosity, Limit of monomers, Coagulum content*	35	1	124
Methyl Alcohol—*Readily carbonizable substances*	34	5	1226
Methylparaben—*Harmonization*	34	6	1601
Methylpyrrolidone (new)	35	5	1205
Light Mineral Oil—*Neutrality*	33	5	972
Nitrogen—*Identification; Assay; Inorganic Impurities—Carbon Monoxide, Limit of Oxygen; Odor; Packaging and Storage; Labeling*	35	4	910
Nitrogen 97 Percent—*Definition; Identification; Assay; Inorganic Impurities—Carbon Dioxide, Carbon Monoxide, Sulfur Dioxide, Limit of Nitric Oxide and Nitrogen Dioxide; Packaging and Storage*	35	4	911
Oleoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	4	1016
Olive Oil—*CAS number* (add), *Definition, Packaging and storage, Identification* (add), *Fatty acid composition* (add), *Specific gravity* (delete), *Cottonseed oil* (delete), *Peanut oil* (delete), *Sesame oil* (delete), *Teaseed oil* (delete), *Absence of sesame oil* (add), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Unsaponifiable matter* (add), *Specific absorbance* (add), *Iodine value* (delete), *Saponification value* (delete), *Water* (add), *Alkaline impurities* (add), *Sterol composition* (add)	35	1	126
Palm Oil (new)	34	4	1018
Peanut Oil—*CAS number* (add), *Definition, Labeling* (add), *Identification, Specific gravity* (delete), *Cottonseed oil* (delete), *Solidification range of fatty acids* (delete), *Free fatty acids* (delete), *Acid value* (add), *Peroxide value* (add), *Iodine value* (delete), *Saponification value* (delete), *Refractive index* (delete), *Heavy metals, Water* (add), *Alkaline impurities* (add), *Other requirements* (add)	34	6	1525
Poloxamer—*Packaging and storage, USP Reference standards* (add), *Identification* (add), *Limit of free ethylene oxide, propylene oxide, and 1,4-dioxane*	33	4	714
Hydrogenated Polydecene—*Readily carbonizable substances*	34	5	1227

Pending Proposals (*continued*)

(Items from earlier numbers of *PF* that have not yet been adopted and become official)

Title and Proposal	***PF Volume, Issue, and Page Numbers of Pending Proposals***		
	Vol.	***No.***	***Page(s)***
Polydextrose—*Assay, Organic Impurities—Procedure 2: Limit of Monomers, Molecular Weight Limit, USP Reference Standards*	35	5	1206
Hydrogenated Polydextrose (new)	35	5	1210
Polyethylene Glycol—*Harmonization*	31	3	897
Polyoxyl 15 Hydroxystearate (new)	35	1	128
Polypropylene Glycol Monolaurate—*USP Reference standards, Identification*	34	1	140
Polysorbate 80—*Stage 6 Harmonization*	35	4	1019
Polyvinyl Acetate (new)	34	6	1526
Polyvinyl Acetate Dispersion (new)	35	1	134
Propylene Glycol (new)—*Harmonization*	33	2	317
Propylene Glycol Dilaurate—*Chemical information, Identification A, Assay*	35	3	599
Propylparaben—*Harmonization*	34	6	1603
Silicon Dioxide (new)—*Harmonization*	31	4	1229
Colloidal Silicon Dioxide (new)—*Harmonization*	31	4	1233
Corn Starch—*Stage 6 Harmonization*	35	3	687
Hydrogenated Starch Hydrolysate (new)	35	1	136
Pea Starch (new)	35	1	140
Potato Starch (new)—*Stage 6 Harmonization*	35	3	689
Rice Starch (new)—*Stage 6 Harmonization*	35	3	690
Wheat Starch (new)—*Stage 6 Harmonization*	35	3	692
Stearoyl Polyoxylglycerides—*Title, Definition, Labeling, Identification, Hydroxyl value, Saponification value, Fatty acid composition, Water, Total ash, Heavy metals, Alkaline impurities* (add)	34	5	1228
Sucrose—*Harmonization*	31	3	902
Sucrose Palmitate (new)	35	2	326
Sucrose Stearate (new)	35	2	328
Tagatose (new)	30	5	1672
Tartaric Acid—*Identification B, USP Reference Standards* (add)	35	5	1212
Tetrafluoroethane (new)	31	6	1672
Trehalose (new)	34	3	677
Zein—*CAS number* (add), *Packaging and storage, Residue on ignition, Nitrogen content* (delete), *Protein content* (add)	34	4	1019

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)]

Title and Proposal	*PF Volume, Issue, and Page Numbers of Canceled Proposals* Vol.	No.	Page(s)
USP Monographs			
† Anecortave Acetate Injectable Suspension (entire submission)	30	2	447
Carvedilol Tablets—*Title* (add), *Definition* (add), *Packaging and storage* (add), *USP Reference standards* (add), *Identification* (add), *Uniformity of dosage units* (add), *Related compounds* (add), *Assay* (add)	33	5	888
† Climbazole (entire submission)	33	5	891
Conjugated Estrogens—*Definition*	30	3	840
Desogestrel and Ethinyl Estradiol Tablets—*Related compounds*	30	5	1604
Estradiol Vaginal Inserts—*Dissolution*	31	6	1617
† Ethinyl Estradiol Tablets—*Dissolution* (add)	31	4	1067
† Ethyl Acrylate and Methyl Methacrylate Coploymer Dispersion—*Viscosity*	33	6	1247
Flavoxate Hydrochloride Tablets—*Dissolution* (add)	33	6	1174
Hydrocodone Bitartrate and Homatropine Methylbromide Tablets—*Dissolution*	30	3	853
Isotretinoin Capsules—*Labeling (add), Dissolution*	34	4	942
Ketoprofen Extended-Release Capsules—*Drug release*	31	5	1378
Leflunomide Tablets—*Dissolution*	31	5	1383
† Methacrylic Acid Copolymer—*Definition, Packaging and storage, Labeling, Viscosity, Heavy Metals, Limit of monomers*	33	6	1251
† Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
†Mirtazapine Orally Disintegrating Tablets—*Water, Method Ia* (add); *Related compounds* (add)	33	6	1189
† Nitrofurantoin—*Packaging and storage*	35	1	92
† Nitrofurantoin Oral Suspension—*Packaging and storage*	35	1	92
† Nitrofurantoin Tablets—*Packaging and storage*	35	1	92
Norethindrone Tablets—*Dissolution* (add)	32	6	1736
Norethindrone Tablets—*Dissolution* (add)	33	6	1193
Orlistat Capsules (entire submission)	32	6	1739
† Oxycodone Hydrochloride Extended-Release Tablets—*Related compounds*	31	4	1104
† Oxymetazoline Hydrochloride Nasal Solution—*pH*	33	5	932
† Permethrin (entire submission)	32	4	1100
† Permethrin Cream (entire submission)	34	1	103
Piperacillin and Tazobactam for Injection—*Definition* (add), *USP Reference standards* (add), *pH* (add), *Particulate matter* (add), *Assay* (add)	31	2	439
Promethazine Hydrochloride—*USP Reference standards, Related substances*	32	2	365
Promethazine Hydrochloride—*USP Reference standards, Related compounds*	32	4	1105
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add), *Assay*	32	2	367
Promethazine Hydrochloride Tablets—*USP Reference standards, Related compounds* (add)	32	4	1107
Terbinafine Hydrochloride—*Melting range*	34	5	1197
Dietary Supplements			
Asian Ginseng Capsules (entire submission)	30	2	571
USP General Test Chapters			
⟨11⟩ USP Reference Standards			
† *USP Climbazole RS*	33	5	981
† *USP Permethrin RS*	32	4	1161
† *USP Permethrin Related Compound A RS*	32	4	1161
† *USP Permethrin Related Compound B RS*	32	4	1161
⟨191⟩ Identification Tests—General—*Acetate, Ammonium*	33	4	719
USP General Information Chapters			
⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments (entire submission)	31	2	524
⟨1024⟩ Bovine Serum (entire submission)	34	3	776
⟨1235⟩ Vaccines for Human Use—General Considerations (entire submission)	34	5	1297

Proposed Revisions and New Text Previously Presented in *PF* but Now Canceled
(Canceled proposals may be republished at any time in a future number of *Pharmacopeial Forum*.)
[*PF* 35(1)–*PF* 35(6)] *(Continued)*

Title and Proposal	***PF Volume, Issue, and Page Numbers of Canceled Proposals***		
	Vol.	***No.***	***Page(s)***
† ⟨1788⟩ Partculate Matter Determination in Parenteral and Ophthalmic Products (entire submission)	34	2	421
NF Monographs			
Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion—*Viscosity*	33	6	1247
Methacrylic Acid Copolymer (entire submission)	33	6	1251
Methacrylic Acid Copolymer Dispersion—*Viscosity*	33	6	1254
Sucralose—*Related compounds*	33	6	1255
Reagents, Indicators, and Solutions			
† L## (Ethylhexyl Triazone, Fluofix)	34	6	1561
Description and Solubility			
† Climbazole	33	5	1053

† New cancellation in *PF* 35(6).

STAGE 4 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). Stage 4 drafts are available for comments. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF,* this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STAGE 6 HARMONIZATION

This section contains monographs or chapters undergoing harmonization by the Pharmacopeial Discussion Group (PDG). The PDG consists of the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP). The process of harmonization consists of several steps (Stages 1 through 7, as defined below). This section includes Stage 6 adopted text which is provided for information. USP cannot incorporate public comments at Stage 6 without consulting PDG partners. Below are descriptions of the developmental Stages as defined by the United States Pharmacopeia, the European Pharmacopoeia, and the Japanese Pharmacopoeia.

Stage 1: Identification The PDG identifies items to be harmonized and designates a coordinating pharmacopeia for each item. The PDG distributes the work by consensus among the three participating pharmacopeias. Harmonization may be carried out retrospectively for existing monographs or chapters, or prospectively for new monographs or chapters.

Stage 2: Investigation The investigation process conducted by the coordinating pharmacopeia results in the preparation of a Stage 3 draft monograph or chapter accompanied by a report giving the rationale for the proposal and including validation data where appropriate. This report is based on input that comes from users, authorities, producers, associations, literature, experts, and staff.

Stage 3: Proposal The Stage 3 draft is reviewed and commented on by the other two pharmacopeias. The coordinating pharmacopeia reviews those comments, prepares a harmonized Stage 4 draft, and sends it to the other two participating pharmacopeias.

Stage 4: Official Inquiry The Stage 4 draft is published in the Forum of each pharmacopeia. In *PF*, this stage appears as OFFICIAL INQUIRY STAGE 4 in the *Harmonization* section. Each pharmacopeia analyzes the comments it receives and submits the consolidated comments to the coordinating pharmacopeia, which then reviews those comments, prepares a harmonized Stage 5A draft, and sends it to the other two participating pharmacopeias.

Stage 5: Consensus

A. Provisional

The Stage 5A draft is reviewed and commented on by the other two pharmacopeias. When consensus is reached, a CONSENSUS STAGE 5B document is prepared by the coordinating pharmacopeia.

B. Final

The Stage 5B draft (consensus document) is sent by the coordinating pharmacopeia to the other two participating pharmacopeias for final approval.

Stage 6: Adoption Each pharmacopeia incorporates the harmonized Stage 5B draft according to its own procedure. Adopted items are published by the three pharmacopeias in their Supplements or, where applicable, in a new edition of their Pharmacopeias.

Stage 7: Date of Implementation The pharmacopeias inform each other of the date of implementation in the particular region.

STIMULI TO THE REVISION PROCESS

This section may contain the following:

- reports or statements of Expert Committees
- original research reports
- evaluations of new and existing pharmacopeial methods
- commentaries
- articles relevant to compendial issues.

These items are published to stimulate discussion and continual review of Pharmacopeial standards. Generally, if an Expert Committee publishes an article on which they are specifically seeking comment, this will be clearly stated in the article. Readers may submit comments on issues raised in this section, but comment is not as critical as that for the *In-Process Revision* section. Readers interested in submitting comments should see *Instructions to Authors.*

INSTRUCTIONS TO AUTHORS

Contributions in the form of original research reports, evaluations of new and existing compendial methods, and other commentaries and articles relevant to drug standards or to *USP–NF* revision will be considered for publication in *Pharmacopeial Forum* under the section *Stimuli to the Revision Process*. Manuscripts are received with the explicit understanding that they have not been published previously in any language or medium and that they are not simultaneously under consideration by any other publication.

All manuscripts are subject to review by USP headquarters staff, Committee members, or qualified outside referees, and if accepted for publication they will be subject to editing by USP staff. Accepted manuscripts become the property of the USP Convention (USPC) and may not be subsequently published elsewhere without written permission from the USPC. Authors are also responsible for obtaining permission for reprinting any illustrations that have been published elsewhere.

Abstract—Include an abstract of not more than 250 words stating the purpose and the results or conclusions of the article.

Style and Usage—*Stimuli* articles generally follow the current *Chicago Manual of Style* except in scientific usage (numbers, abbreviations, etc.). For the latter, authors should use the current *AMA Manual of Style* or the current *ACS Style Guide*. Authors may usefully consult a current copy of *Pharmacopeial Forum*.

References—Consult the current *AMA Manual of Style*, which is generally consistent with the National Library of Medicine's *Recommended Formats for Bibliographic Citation*. A current copy of *Pharmacopeial Forum* will offer examples of reference formats.

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Submission Instructions—Manuscripts must be submitted both as an electronic file and as a printed copy of the electronic file. Submit the text in Microsoft® Word or another current word-processing application. The preferred format for graphics submitted electronically is tagged image file format (TIFF). Photocopies are not acceptable. Manuscripts submitted for publication should be addressed to:

Pharmacopeial Forum
Executive Secretariat, USP
12601 Twinbrook Pkwy.
Rockville, MD 20852

Pharmacopeial Standards for the Subdivision Characteristics of Scored Tablets

Geoff Green, Carolyn Berg, Accu-Break Pharmaceuticals Inc.; James E. Polli, University of Maryland School of Pharmacy; Dirk M. Barends, RIVM—National Institute for Public Health and the Environment, Bilthoven, The Netherlands[a]

ABSTRACT The practice of tablet splitting as a way to reduce prescription medication costs has become increasingly prevalent. The United States Pharmacopeial Convention (USPC) has no standards for the subdivision characteristics of scored tablets. Literature results show that many tablets on the US market exhibit unacceptable subdivision characteristics. The *European Pharmacopoeia* (*EP*) provides requirements for subdivision accuracy of scored tablets, if subdivision is indicated in order to comply with the product label. This *Stimuli* article provides a rationale for why standards should be included in *USP* to address the accuracy of subdivision, as well as to account for loss of mass upon subdivision. We propose that for accuracy of subdivision current *EP* standards be adopted, applicable only to any tablet that bears a score mark. For loss of mass, we propose an average of $\leq$ 3% of the intact tablet mass. From data reported in the literature we estimate that as many as half of the scored tablets on the US market would be in compliance with these standards. Generally, we do not advocate such standards be tested on a batch-to-batch basis but rather that the testing should be conducted as part of the development process before marketing approval. We also discuss a third, related, quality attribute: ease of subdivision. Although future research and discussion in this area are warranted, we believe that not only should scored tablets break into accurate partial doses with minimal loss of mass, but also that the tablets should be breakable by a representative sample of the population, including the elderly.

INTRODUCTION

Tablets intended for oral administration are the most common pharmaceutical dosage form in the US, and many tablets bear score mark(s) (*1*). The presence of a score mark implies that the tablet can be subdivided into smaller doses. Patients split tablets for a variety of reasons, including to adjust the dose, to ease swallowing, and to save money. As healthcare costs rise, tablet splitting to save money has become more prevalent in the US. Because some manufacturers have established the same or similar prices for different strengths of tablets of the same medication, consumers can purchase double the strength needed and divide the tablets in half for twice the number of doses (*2*). This has led many healthcare plans to establish mandatory tablet splitting policies as a means to reduce costs, a practice that has drawn opposition from several organizations, including the American Society of Consultant Pharmacists, the American Medical Association, and the American Pharmacists Association. The opposition stems from concerns about the potential for unpredictable dosing, particularly for the elderly (*3–5*).

The most important advantage of score lines, however, is dose flexibility—the ability to adjust a dose up or down in response to medication effects or to comply with the labeled dosage and administration instructions (posology). Dose flexibility can be especially important for medications that typically are titrated to achieve a therapeutic goal or for those that have a narrow therapeutic index, such as warfarin or levothyroxine.

Therefore, scored tablets play an important role in providing dose flexibility, among other benefits. Many studies have shown that scored tablets can be difficult—or even impossible—to break and often display large variations in the mass of the subdivided parts (*6–8*). A research group of the Dutch National Institute for Public Health and the Environment (RIVM) conducted a comprehensive literature review of articles that investigated the subdivision performance of scored tablets and identified several studies that reported unacceptable subdivision characteristics (*9*). Problems included large variations in the mass of the subdivided parts when split by hand, tablet splitter, or other means (*9*). A later study published by the same research group investigated patients' experiences and perceptions with breaking scored tablets. This study reported that 39% of patients were in some way dissatisfied with the subdivision characteristics of their scored tablets and that poorly functioning score lines were perceived as a quality defect. The authors concluded that the subdivision performance of scored tablets cannot be interpreted as a purely technical quality attribute and that badly performing score lines may lead to reduced patient compliance with medication.

Based on available literature, this *Stimuli* article examines: a) the ease or lack thereof in splitting scored tablets, b) the accuracy of the splitting process, and c) the loss of mass of split scored tablets sold in the US. The *Stimuli* article then proposes *USP* standards for the subdivision of scored tablets.

[a] Correspondence should be addressed to: William E. Brown, Senior Scientist, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8380; e-mail web@usp.org.

SUBDIVISION PERFORMANCE OF SCORED TABLETS ON THE US MARKET

The RIVM research group concluded that the performance of score lines should be defined by the following three quality attributes: accuracy of subdivision (i.e., uniformity of the mass of the subdivided tablets), ease of breaking, and loss of mass resulting from subdivision (*9*). We follow the same classification to explore the subdivision performance of scored tablets on the US market.

We reviewed published studies that were selected according to the following criteria: (a) the research was conducted in US laboratories or institutions, which presumably supported the assumption of probability that the tablets studied were approved by FDA and marketed in the US, and (b) the research included reports about the measuring subdivision accuracy for scored tablets. Eight studies (''US studies'') satisfied both requirements (*10–17*). In six of the eight US studies, the tablets were obtained from commercial suppliers. One of the studies used tablets donated by the manufacturer (*15*), and one study used professional samples (*16*).

Accuracy of Subdivision

Each of the US studies focused on assessing subdivision accuracy by measuring the whole tablet and the subsequent subdivided tablets' mass as a surrogate for estimating the content uniformity of a split tablet (see General Chapter *Uniformity of Dosage Units* ⟨905⟩ for information about relying on mass as a surrogate for content). Four of the US studies tested the accuracy of subdivision of scored tablets from manual splitting (*Table 1*), and six tested tablet splitting accuracy using a tablet-splitting device (*Table 2*). Of the six studies involving a tablet splitter, four cited cost containment as the rationale for testing the accuracy of tablet splitters; one study reported concern about obtaining accurate pediatric doses; and one study involved obtaining nonmarketed doses of a popular anti-hypertensive drug. In the absence of FDA and/or *USP* standards for uniformity of mass of the subdivided parts, seven of eight (88%) US studies adapted and applied the *USP 24* criteria for the uniformity of dosage units (*11*). The term *uniformity of dosage unit* is defined as the degree of uniformity in the amount of the drug substance among dosage units. This *USP* criterion states all units tested must be within 85.0% to 115.0% of label claim and no units outside the range of 75.0% to 125.0% of label claim with a maximum batch standard deviation (SD) no greater than 6%.

Results of the US studies are comparable to those previously summarized and reported in the RIVM review (*9*). A comparison of *Tables 1* and *2* shows that a tablet-splitting device may improve the accuracy of subdivision—but not in all cases—and accuracy varies with different devices, users, and tablet shapes. In general, oblong-shaped tablets performed slightly better, but this also depended on the user and device.

With respect to the subdivision accuracy of scored tablets putatively on the US market, we conclude: (a) the situation is comparable to that reported in other parts of the world; (b) for many tablets on the US market, significant variation can occur in the mass of subdivided tablet parts, regardless of the splitting method or person; (c) although tablet splitters somewhat improve subdivision accuracy, this accuracy is still unacceptable for scored tablets, and the results can vary widely depending on the device and the user; and (d) the presence of a score mark on a tablet does not necessarily imply that the tablet can be split into accurate partial doses.

Loss of Mass

Four of the eight US studies reported data on the loss of tablet mass upon subdivision (*Table 3*). Of the scored tablets tested, the largest mean percent loss of mass occurred when users split glyburide tablets with a razor blade. In this case the loss was 2.6%, which was approximately 6.5 times higher than the loss of glyburide tablet mass after manual splitting (*14*). McDevitt et al. reported a maximum loss of mass from manually splitting hydrochlorothiazide tablets (nearly 20%, with an average of 1.06%) (*13*). Sertraline tablets split manually and with a tablet splitter showed comparable loss of mass (0.08% using a tablet splitter compared to 0.06% when split manually) (*16*). Although there are limited data available for US-marketed products, the results of these US studies are consistent with those reported by the RIVM review, in which the authors concluded that most tablets, on average, lost less than 1% of the intact tablet mass upon subdivision (*9*).

Ease of Subdivision

Another quality attribute that researchers should consider when assessing the efficacy of a score mark is ease of subdivision (*9*), that is, individuals' ability to subdivide tablets regardless of accuracy or loss of mass. Although this attribute has been studied in some detail by the RIVM research group, including the development of an in vivo test, only two of the eight US studies included this attribute in their splitting accuracy studies (*10*, *14*). Wilson et al. asked elderly diabetic patients to manually subdivide micronized glyburide tablets and to rate the degree of splitting difficulty using a visual analog scale. On average, the rating was 7.7 on a scale of 1 to 10 where 10 was considered the most difficult (*10*). Teng et al. reported that because of their hardness 50-mg hydrochlorothiazide tablets were''difficult to split by hand'' (*14*).

PHARMACOPEIAL STANDARDS FOR THE SUBDIVISON PERFORMANCE OF SCORED TABLETS

In 2002, *EP* presented pharmacopeial standards for the subdivision performance of scored tablets (*12*). This marked the first time a pharmacopeial requirement for the functioning of a score mark was established. Since that date, several proposals for revision to that standard have been published (*6*, *13–18*). Some of these proposals were adopted in subsequent revisions, e.g., in *EP 5.0* (*19*) and in *Supplement EP 5.5* (*20*). The text of *EP 5.5* remained unchanged until *EP 6.4*, which is now cur-

rent (*21*). A historical overview of proposed standards and enforced *EP* standards relative to scored dosage forms is depicted in *Table 4*. With the exception of the proposed revisions in December 2006, which called for the adoption of additional tests and standards for ease of breaking and loss of mass, accuracy of subdivision was the only quality attribute addressed. In other words, *EP*currently presents standards for accuracy of subdivision of scored tablets but has not yet adopted standards for ease of subdivision nor for loss of mass upon subdivision.

PROPOSED STANDARD FOR ACCURACY OF SUBDIVISION FOR SCORED TABLETS

Among the two quality attributes for which standards are being proposed, we view splitting accuracy as primary in importance. If sufficient accuracy is not achievable even under ideal conditions, other aspects become less important because splitting is no longer a viable option for dose adjustment. With respect to accuracy of subdivision, several questions arise:

(a) Which standards should be set?
(b) Which test methods should be used?
(c) Should all scored tablets be forced to comply with these standards?

Answering question (a) suggests two very different approaches that can be taken. The first is that the subdivided tablet parts should conform to uniformity of mass or weight variation standards. In this respect, the ⟨905⟩ standards currently in place for whole dosage forms could be adapted and applied to subdivided tablet parts. Many of the researchers of the US studies adapted a variation of these standards to the studies in *Tables 1* and *2*. Or one could adopt the current enforced *EP* standard for uniformity of mass of subdivided parts of scored tablets. The advantage of adopting the *EP* standard is the ability to leverage the considerable work, research, debate, and discussion that have already taken place.

A second approach to answering question (a) would be to apply the uniformity of content standards where the amount of drug substance within the subdivided tablet parts is assayed. Interestingly, both approaches were adopted in the first *EP* standard, which stated that subdivided tablet parts should comply with ''... either Uniformity of Mass (2.9.5) or Uniformity of Content (2.9.6), as appropriate,'' leaving it up to regulatory authorities to decide which test to apply (*12*). This first *EP* standard for scored tablets proved to be insufficient because it raised a number of questions such as whether or not chemical analyses were necessary for uniformity of mass studies, whether the uniformity of content standards could be used with the uniformity of mass test, and how the tablets should be subdivided (i.e., manually or by a tablet cutter).

Gradually, the standard for uniformity of content variability was dropped by *EP*, perhaps because very few scored tablets could comply with that standard, and the requirement of mass determinations replaced the requirement of chemical analyses. Later, *EP* introduced a provision stating that only one part from every subdivided tablet should be allowed in the final test.

The current *EP* requirement for subdivision accuracy (6.4) has been unchanged for a number of years and appears to work well in practice. This requirement specifies subdivision by hand, using only one part of each subdivided tablet, and limits deviations to ± 15% of the average mass of the subdivided parts. Thus, questions (a) and (b) are answered by the current *EP* standard.

We recognize that the present *EP* standards for accuracy of subdivision evolved from a long process and seem to reflect both the interest of the patient in receiving the correct dose while taking into account current technological/manufacturing capabilities. Also, from the point of view of pharmacopeial harmonization, it is sensible to adopt the present *EP* standards for accuracy of subdivision.

With respect to question (c), Should all scored tablets be forced to comply with these standards? We do not support the restricted applicability of the current *EP* standards that require that only tablets with break marks need to comply with labeling (posology) and should be tested for uniformity of mass. To ''comply with posology'' means that the tablet must be subdivided to arrive at a dose stated in the Product Information Leaflet (PIL), or Package Insert (PI) as it is referred to in the US, for some indications, conditions, and/or patients. We do not support this restricted applicability of the *EP* standard in scored tablets for the reasons outlined below.

First, the presence of a score line in a tablet implies the tablet is meant to be subdivided. Thus, no matter what the reason for subdividing, the score line should perform well. Patients perceive badly functioning score lines as a quality defect, regardless of the reason for the subdivision (*7*). Second, from a practical point of view it is unusual and cumbersome to make the applicability of pharmacopeial standards depend on dosage schemes in the PI. In such a situation, any decision to declare an article either in compliance or out of compliance can be made only in combination with an interpretation of the current PI of that particular drug. Third, the criterion of tablets that ''may be subdivided in parts to comply with posology'' appears not to be entirely objective (*6*). Fourth, off-label use of drug products is common, particularly for pediatric and geriatric patients. Hence, the PI of those medications may not reflect dosing instructions for those patient groups and, therefore, those tablets will not be subject to the compendial subdivision requirements and thus will not be tested for compliance. In practice, standards are needed most for these particular medications because doses for pediatric and geriatric patients are frequently adjusted, and a lack of standards could potentially compromise patients who receive an incorrect dose based on the characteristics of tablet subdivision.

In summary, we recommend that USP adopt the present *EP* standards for accuracy of subdivision but enforce these standards for all scored tablets. *Table 5* depicts our proposals for *USP* standards.

PROPOSED STANDARD FOR LOSS OF MASS FOR SCORED TABLETS

No pharmacopeial standard exists today for loss of mass upon tablet subdivision. A standard of $\leq$ 1% average loss of mass compared to the mass of the intact tablet was proposed in December of 2006, although this standard has not been adopted at the time of this writing (*6*). This proposed standard was tested by the RIVM research group for 29 scored tablets on the European market, and 25 products (86%) were in compliance. Products that exceeded the 1% threshold had a loss of mass of 1.10%, 1.14%, 1.21%, and 2.68%, and all were round, single scored, and between 5.9 and 9.0 mm in diameter. Based on the data from the US studies in *Table 3* and the European data, we propose a limit of $\leq$ 3%. The proposed standard and test method can be found in *Table 5*. The test method mandates subdivision by hand and calculating the loss of mass for each of the subdivided tablets. As in the case for the accuracy of subdivision standard, the loss of mass standard should apply to all scored tablets.

ATTAINABILITY OF THESE STANDARDS

Because the eight US studies were conducted using a variety of methodologies and criteria, one finds it difficult to make any conclusions how many products on the US market would be in compliance if the proposed standards for accuracy of subdivision were adopted. However, in 2006, the RIVM research group conducted a market surveillance study applying the enforced *EP* standards at the time to tablets available in the European market. They concluded that 7 of the 29 (24%) products tested complied with the proposed standards of accuracy of subdivision. Four of the seven products were oblong, and three were round (*6*). For the proposed *USP* loss of mass standard ($\leq$ 3%), all 29 products tested in the RIVM research study complied.

It seems reasonable to assume that the attainability rate for scored tablets on the US market would be comparable to that observed in RIVM research study.

FREQUENCY OF TESTING OF PROPOSED PHARMACOPEIAL STANDARDS

A related question is ''Under what situations should the two standards be tested?'' We advocate that all scored tablets be characterized in terms of accuracy and loss of mass during registrations in a New Drug Application (NDA) or Abbreviated New Drug Application (ANDA). Further, both accuracy and loss of mass should be pharmacopeial standards that are tested during registration and under scale-up and post-approval change (SUPAC) situations when such changes may influence splitting performance. We recognize that not all SUPAC situations would trigger the need for accuracy and loss of mass testing, but it currently may not be easy to discern when testing is needed and when testing is not needed. We suspect that tests for accuracy and loss of mass need not be performed for each batch, although we are not aware of batch-to-batch data that suggests otherwise.

Pharmacopeial standards are not necessarily batch-to-batch tests. Until May 2009, *USP*'s General Notices stated that

> every compendial article in commerce shall be so constituted that when examined ... it meets all the requirements in the monograph defining it. However, it is not to be inferred that the application of every analytical procedure in the monograph to samples from every production batch is necessarily a prerequisite for ensuring compliance with pharmacopeial standards before the batch is released for distribution. Data derived from manufacturing *process validation* studies and from *in-process controls* may provide greater assurance that a batch meets a particular monograph requirement than analytical data derived from an examination of finished units drawn from that batch. On the basis of such assurances, the analytical procedures in the monograph may be omitted by the manufacturer in judging compliance of the batch with the Pharmacopeial standards (*22*).

Thus, the standards proposed in *Table 5* can be enforced as pharmacopeial without necessarily implying the need for batch-to-batch testing. However, even in the absence of batch-to-batch testing, each batch should be compliant.

IN VIVO TEST PROCEDURES FOR EASE OF SUBDIVISION

We do not propose a standard at this time for the ease of subdivision but do advocate further research on this topic. To date, pharmacopeial standards for ease of subdivision have not been adopted in any country/region. However, in view of the unsatisfactory performance of scored tablets previously presented in The Netherlands by the RIVM research group with respect to ease of subdivision (*6*, *8*, *9*) and the data available in the US studies (*10*, *14*), we believe USP should consider adopting a standard for this attribute. As stated previously, patients perceive poorly breaking tablets to have a quality defect, which may lead to reduced patient compliance (*7*). Thus, we hold the view that USP could and should take the lead in enforcing standards for this attribute.

Until recently, very few tests to evaluate the ease of breaking of scored tablets have been proposed. The RIVM published an in vivo test using a panel of elderly volunteers who manually split scored tablets (*8*). This ease of breaking test subsequently was applied in the 2006 market surveillance study in The Netherlands, and it concluded that the proposed test procedure for ease of subdivision was workable (*6*).

Depending on the statistical power required, between 39% and 52% of the tested tablets were in compliance. Of note, 100% of all oblong tablets were considered to be in compliance based on the proposed criteria (*6*). The published test procedure proposed that no less than 80% of the elderly panelists be able to break the tablet, with a minimum confidence level of 90%, one sided (*8*). The authors also concluded that large panels are sometimes needed to meet the minimum statistical power.

It is uncommon to define the statistical power of a pharmacopeial test, but it is not uncommon to define the sample size. Consequently, we reworked this test into a more common two-tier test (*Table 6*). In the first tier, 10 elderly panelists break one tablet each. In order to comply, all 10 tablets must be deemed breakable by hand. At a 90% confidence level, this requirement corresponds to at least 79% of the elderly population being able to break that tablet. If the tablets do not comply with the first-tier test, the elderly panel is expanded from 10 to 30 for the second tier. When a total of 30 tablets have been tested, at least 27 tablets (78%) must be deemed breakable by hand. With a 90% confidence level this corresponds to a minimum of 81% of the elderly population being able to break the tablet.

Although we believe that for the time being, a testing method for the ease of breaking of scored tablets requires additional study, the RIVM research group in The Netherlands has proposed this quality attribute be considered for inclusion within the *EP* standards. In the US, 51.6% of the population > 65 years takes three or more prescription drugs, and by the year 2020 the age group will consume 40% of all prescription drugs. The number of persons 75 years and older will increase from 18.1 million in 2005 to 33.5 million in the year 2030. Thus, a criterion of a mean age $\geq$ 75 years and none < 65 years is representative of the US population that consumes the largest portion of pharmaceutical tablets (*23*). Other procedures, such as panel participants breaking the tablets by hand without having a tryout of the tablet to be tested, stem from real-world experiences. Patients would rarely be able to practice breaking tablets and discarding those that do not break well without jeopardizing the duration of their prescription. Also, breaking by hand is the most common situation. The test anticipates that 20% of the elderly panel will be incapable of subdividing the tablet by hand, so for this subset, use of a tablet splitter may be indicated.

In summary, the standard and criteria proposed by the RIVM research group for ease of subdivision appears to be workable but will need to be validated in future US studies. Although we do not study it in this *Stimuli* article, the authors suggest that any future ease of subdivision testing by the panel of elderly would be unworkable for batch-to-batch testing. The RIVM research group already concluded that this test typically should be performed only once, i.e., during the development phase of a tablet (*8*).

CONCLUSIONS AND RECOMMENDATIONS

In ⟨905⟩, *USP* provides content uniformity requirements for drug products where applicable to ensure that patients receive an accurate dose with minimal variability. However, no such standards or tests are mandated for the subdivided parts of tablets that bear score marks. Upon subdivision, such tablets have shown significant variability in the mass of the subdivided parts. By inference, this should be interpreted as variability in dose, a result contrary to the intent of the whole dosage form standards developed by USP. In an increasingly cost-conscious environment when patients are frequently subdividing dosage forms, we see considerable risk related to unpredictable dosing resulting from inaccurate splitting and the loss of mass. Therefore, we propose that USP should adopt the current EP standards, but USP should make the standards applicable to all scored tablets. The purpose of this proposal would be to ensure adequate content uniformity for divided tablets with a score. For loss of mass, we propose a limit of $\leq$ 3% of the intact tablet mass. We do not believe such standards generally should be tested on a batch-to-batch basis but rather should be determined as part of the development and registration processes. These proposed standards for accuracy of subdivision and loss of mass are attainable both from a pharmaceutical and a technological point of view. We advocate future investigation of the ability of representative populations to demonstrate ease of splitting scored tablets.

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APPENDIX

Table 1. Accuracy of Subdivision of Scored Tablets on US Market Split Manually

Reference	Panel	Products	Tablet Shape[a]	Result (% of parts >115% of ideal mass)
Matuschka and Graves 2001	Volunteers	Sertraline 100 mg	Capsule	0
Wilson et al. 2001	Elderly diabetics	Micronized glyburide 3 mg	Oval	12
McDevitt et al. 2002	Volunteers	HydroDIURIL 25 mg	Round	24
Teng et al. 2002	Trained pharmacy student	HydroDIURIL 50 mg	Round	40
		Glyburide 5 mg	Rectangle	15
		Oretic 50 mg	Round	55

[a] All tablets single-scored on one side only.

Table 2. Accuracy of Subdivision of Scored Tablets on US Market Split by Splitter

Author	Panel and Splitting device	Products	Tablet Shape[a]	Result (% of parts > 115% of ideal mass)
Horn et al. 1999	Pharmacists EZ Dose tablet cutter	Catapres 0.1 mg	Round	12
		Clonidine 0.1 mg	Round	43
		Capoten 12.5 mg	Capsule	2
		Sertraline 50 mg	Capsule	3
		Tegretol 100 mg	Round	32
	Pharmacists Health Care Logistics tablet cutter	Catapres 0.1 mg	Round	22
		Clonidine 0.1 mg	Round	42
		Capoten 12.5 mg	Capsule	26
		Amlodipine 5 mg	Octagon (modified)	17
		Tenormin 25 mg	Round	18
		Sertraline 50 mg	Capsule	0
		Tegretol 100 mg	Round	9

[a] All tablets single scored on one side only
[b] Tablet mass reported for Rosenberg et al. in the ''Products'' column are the ideal half tablet mass.

Table 2. Accuracy of Subdivision of Scored Tablets on US Market Split by Splitter *(continued)*

Author	Panel and Splitting device	Products	Tablet Shape[a]	Result (% of parts > 115% of ideal mass)
Matuschka and Graves 2001	Volunteers. LGS Health Products pill cutter	Sertraline 100 mg	Capsule	0
Rosenberg et al. 2002[b]	Pharmacists Splitter not specified	Buspar 5 mg	Modified Rectangle	3
		Captopril 6.25 mg	Capsule	13
		Doxazosin (Apotex) 0.5 mg	Capsule	10
		Cardura 2 mg	Round	0
		Luvox 50 mg	Oval	0
		Glipizide 2.5 mg	Round	13
		Hydrocholorothiazide 12.5 mg	Round	0
		Metoprolol (Caraco) 25 mg	Capsule	7
		Metoprolol (Mylan)	Round	0
		Toprol XL 25 mg	Oval	0
		Oxybutynin 2.5 mg	Round	13
		Zoloft 25 mg	Capsule	3
		Zoloft Sample A 50 mg	Capsule	0
		Zoloft Sample B 50 mg	Capsule	0
		Trazodone (Geneva) 25 mg	Round	14
		Trazodone (Mutual) 25 mg	Round	0
		Effexor 25 mg	Pentagon	45
		Coumadin 0.5 mg	Round	0

[a] All tablets single scored on one side only
[b] Tablet mass reported for Rosenberg et al. in the ''Products'' column are the ideal half tablet mass.

Table 2. Accuracy of Subdivision of Scored Tablets on US Market Split by Splitter *(continued)*

Author	Panel and Splitting device	Products	Tablet Shape[a]	Result (% of parts > 115% of ideal mass)
Teng et al. 2002	Trained pharmacy student Razor blade	Hydrodiuril 50 mg	Round	15
		Glyburide 5 mg	Rectangle	15
		Oretic 25 mg	Round	45
		Oretic 50 mg	Round	20
		Zoloft 100 mg	Capsule	0
Polli et al. 2003	Trained pharmacy student	Coumadin 5 mg—orientation 1	Round	0
		Coumadin 5 mg—orientation 2	Round	0
	ACE-LIFE Pill Cutter	Furosemide 40 mg—orientation 1	Round	0
		Furosemide 40 mg—orientation 2	Round	0
		Glipizide 10 mg	Round	0
		Metoprolol 50 mg	Capsule	0
		Zoloft 100 mg	Capsule	0
Peek et al. 2002	Elderly patients using cutter A; brand not specified	Metoprolol 50 mg	Capsule	Tablet portions deviated 9% from their intended ideal mass
	Elderly patients using cutter A; brand not specified	Warfarin 5 mg	Round	Tablet portions deviated 9% from their intended ideal mass
	Elderly patients using cutter B; brand not specified	Metoprolol 50 mg	Capsule	Tablet portions deviated 20% from their intended ideal mass
	Elderly patients using cutter B; brand not specified	Warfarin 5 mg	Round	Tablet portions deviated 26% from their intended ideal mass

[a] All tablets single scored on one side only
[b] Tablet mass reported for Rosenberg et al. in the ''Products'' column are the ideal half tablet mass.

Table 3: Loss of Mass on Subdivision of Scored Tablets on US Market

Author	Panel	Splitting method[a]	Product	Percent Loss of Mass[b] (Range)
McDevitt et al. 1998	Volunteers	Manual	Hydrochlorothiazide 25 mg	1.06 (0 to 19.4)
Matuschka and Graves 2001	Volunteers	LGS Health Products Cutter	Sertraline 100 mg	0.08 (NR)
	Volunteers	Manual	Sertraline 100 mg	0.06 (NR)
Polli et al. 2003	Trained pharmacy student	ACE-LIFE tablet cutter	Coumadin 5 mg—orientation 1 Coumadin 5 mg—orientation 2 Furosemide 40 mg—orientation 1 Furosemide 40 mg—orientation 2 Glipizide10 mg Metoprolol 50 mg Zoloft 100 mg	0.0 (NR to 0.18) 0.5 (NR to 1.4) 0.8 (NR to 1.7) 1.3 (NR to 7.3) 0.08 (NR to 0.95) 0.1 (NR to 0.4) 0.1 (NR to 0.3)
Teng et al. 2002	Trained individual in laboratory conditions	Razor blade	Zoloft (sertraline) 100 mg Glyburide 5 mg Hydrodiuril (hydrochlorothiazide) 50 mg Oretic (hydrocholorthiazide) 50 mg	0.4 (NR to 1.2) 2.6 (NR to 6.7) 0.8 (NR to 3.0) 0.8 (NR to 2.0)
	Trained individual in laboratory conditions	Manual	Glyburide 5 mg Hydrodiuril (hydrochlorothiazide) 50 mg Oretic (hydrocholorthiazide) 50 mg	0.4 (NR to 1.2) 0.3 (NR to 0.7) 0.4 (NR to 0.5)

[a] All tablets split into halves.
[b] Mean loss of mass calculated by dividing the total unaccounted mass for all tablets split by the sum of theoretical weight of all whole tablets. NR = Not reported.

Table 4. History of EP Standards Proposed and Enforced for the Subdivision Characteristics of Scored Tablets

Date Published	Period Enforced	Action	Test and Standard(s)	Reference
October 2001	April 1, 2002 – June 30, 2002	First implementation of tablet subdivision accuracy standards.	For tablets for which subdivision is authorized, it is demonstrated to the satisfaction of the competent authority that the subdivided parts comply with either Test A for *Uniformity of content of single-dose preparations (2.9.6)* or with the test for *Uniformity of mass (2.9.5)*, as appropriate. **Uniformity of Content:** Subdivide 10 tablets and randomly select 10 parts from 10 subdivided tablets and, using a suitable analytical method, determine the content of active substance(s) in each individual part. The preparation complies with the test if each individual content is between 85% and 115% of the average content. The preparation fails to comply with the test if more than one individual content is outside these limits or if one individual content is outside the limits of 75% to 125% of the average content. If one individual content is outside the limits of 85% to 115% but within the limits of 75% to 125%, determine the individual contents of another 20 units (subdivided tablet parts) taken at random. The preparation complies with the test if not more than one of the individual contents of the 30 units is outside 85% to 115% of the average content and none is outside the limits of 75% to 125% of the average content. **Uniformity of Mass:** Subdivide 20 tablets and weigh individually 20 parts selected randomly and determine the average mass. The preparation complies with the test if not more than 2 of the individual masses deviate from the average mass by more than the following percentage deviations *and* no individual mass deviates by more than twice that percentage: 10% for tablets < 80 mg; 7.5% for tablets between 81 and 249 mg; 5% for tablets > 250 mg.	Monograph 0478. *Ph. Eur. Suppl. 4.1*; 2002:2433–2436.

[a] No change in *Ph. Eur. Supplements 5.6, 5.7, 5.8, 6.0, 6.1,* and *6.2 5* to current *Ph. Eur. 6.4*.

Table 4. History of EP Standards Proposed and Enforced for the Subdivision Characteristics of Scored Tablets *(continued)*

Date Published	Period Enforced	Action	Test and Standard(s)	Reference
April 2002	NA	Proposed Revision to Monograph 0478 (*Ph. Eur. 4.1*): Clarify breaking method.	Tablet should be ''broken by hand.''	*Pharmeuropa* 2002 Apr; 14(2):302–304.
October 2002	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 4.1*): Clarify prevailing test method.	**Content of Uniformity** test can be replaced by Uniformity of Mass test for tablets containing 25 mg or greater of an active substance that comprises 25% or more (by weight) of one tablet.	*Pharmeuropa* 2002 Oct; 14(4):725–728.
April 2003	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 4.1*): Clarify prevailing test method and sampling procedure.	If the subdivided parts of the tablet contain < 2 mg of active substance or the content of the active ingredient in the subdivided parts is < 2 % of the total mass of the subdivided tablet, the test of **Uniformity of Content** prevails. Two halves of the same tablet should be included in the test.	*Pharmeuropa* 2003 Apr; 15(2):322–324.
January 2004	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 4.1*): Clarify prevailing test method and sampling procedure.	Two halves of the same tablet should be included in the test. Subdivided tablets should comply with test 2.9.6A (**Uniformity of Content**) irrespective of their content of active ingredient.	*Pharmeuropa* 2004 Jan; 16(1):51–55.
April 2004	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 4.1*): Elimination of **Uniformity of Content** requirement and propose new **Uniformity of Mass** test.	Take 30 tablets at random. Take 10 tablets from among these and break them in parts by hand, mix the parts. Take 10 parts at random and determine the average mass. Weigh each part individually. The tablets comply with the test if the mass of each part is between 85% and 115% of the average mass. The tablets fail to comply with the test if more than one individual mass is outside these limits, or if one individual mass is outside the limits of 75% to 125% of the average mass. If one individual mass is outside the limits of 85% to 115% of the averagemass but within the limits of 75% to 125%, determine the individual massof another 20 parts taken at random from the remaining 20 tablets. The tablets comply with the test if not more than one of the individual masses of the 30 parts is outside 85% to 115% of the average mass and none is outside the limits of 75% to 125%.	*Pharmeuropa* 2004 Apr; 16(2):250–252.

[a] No change in *Ph. Eur. Supplements 5.6, 5.7, 5.8, 6.0, 6.1,* and *6.2 5* to current *Ph. Eur. 6.4*.

Table 4. History of EP Standards Proposed and Enforced for the Subdivision Characteristics of Scored Tablets *(continued)*

Date Published	Period Enforced	Action	Test and Standard(s)	Reference
June 2004	January 1, 2005 to March 31, 2005	Revised Monograph 0478: Revised guidance on prevailing test method and revision to the test and standards for **Uniformity of Content**.	**Uniformity of Content** test prevails if tablets with a content of active substance of tablet less than 2 mg or less than 2% of the total mass. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the conditions above. *Pharmeuropa* 16(2) proposed testing method and standard adopted for assessing **Uniformity of Content** for subdivided tablets. **Uniformity of Mass** test prevails in all other instances. Original *Ph. Eur.* 4.1 test method and standard remains in place.	Monograph 0478. *Ph. Eur. 5.0.*; 2005: 626–628.
October 2005	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 5.0*): Clarification on timing of testing efficacy of break mark(s) and proposal to eliminate **Uniformity of Content** standards.	In order to ensure that the patient will receive the intended dose, the efficacy of break-mark(s) must be assessed during the development of the product, in respect to uniformity of mass of the subdivided parts. Each authorized dose must be tested using the following test: **Uniformity of Mass**: Take 30 tablets at random, break them by hand and, from all the parts obtained from one tablet, take one part for the test and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. The tablets comply with the test if not more than one individual mass is between 85% and 115% of the average mass. The tablets fail to comply with the test if more than one individual mass is outside these limits, or if one individual mass is outside the limits of 75% to 125% of the average mass.	*Pharmeuropa* 2005 Oct; 17(4):512–514.
December 2005	July 1, 2006 to December 31, 2006	Revised Monograph 0478 (*Ph. Eur. 5.0*): Clarification of which tablets are subject to the subdivision standards' elimination of **Uniformity of Content** tests and standards, and revision to **Uniformity of Mass** test.	Only tablets for which the break mark is needed to comply with the posology should be tested. **Uniformity of Mass**: Testing and standards proposed in *Pharmeuropa* 17(4) are adopted and enforced. **Uniformity of Content** requirement is eliminated.	Monograph 0478. *Ph. Eur. Suppl.* 5.5; 2006: 4166–4168.

[a] No change in *Ph. Eur. Supplements 5.6, 5.7, 5.8, 6.0, 6.1,* and *6.2 5* to current *Ph. Eur. 6.4*.

Table 4. History of EP Standards Proposed and Enforced for the Subdivision Characteristics of Scored Tablets *(continued)*

Date Published	Period Enforced	Action	Test and Standard(s)	Reference
December 2006	NA	Proposed revision to Monograph 0478 (*Ph. Eur. 5.5*): Clarification of which tablets are subject to subdivision standards and tests, instructions for handling broken/crumbled tablets, and proposal of additional standards.	Remove restriction of *Ph. Eur. 5.5*. which states that only tablets for which break marks needed to comply with posology should be tested for **Uniformity of Mass**. **Uniformity of Mass**: instructions should be given about how to handle tablets that cannot be broken or that crumble upon subdivision for their inclusion in the calculation of Uniformity of Mass. Proposed additional testing and standards for efficacy of score marks including **Ease of Subdivision and Loss of Mass upon subdivision.**	*Pharmeuropa Scientific Notes*. 2006(2): 1–7.
June 2008[a]	January 1, 2009 to March 31, 2009[a]	Monograph 0478 unchanged from previous published standards (*Ph. Eur. 5.5*).	Tablets may bear a break-mark or break-marks and may be subdivided in parts, either to ease the intake of the medicinal product or to comply with the posology. In the latter case, subdivision must be assessed and authorized by the competent authority. In order to ensure that the patient will receive the intended dose, the efficacy of the break-mark(s) must be assessed during the development of the product, in respect of uniformity of mass of the subdivided parts. Each authorized dose must be tested using the following test. Take 30 tablets at random and, from all the parts obtained from 1 tablet, take 1 part for the test and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. The tablets comply with the test if not more than 1 individual mass is outside the limits of 85% to 115% of the average mass. The tablets fail to comply with the test if more than 1 individual mass is outside these limits, or if 1 individual mass is outside the limits of 75% to 125% of the average mass.	Monograph 0478. *Ph. Eur. Suppl. 6.4*; 2008.

[a] No change in *Ph. Eur. Supplements 5.6, 5.7, 5.8, 6.0, 6.1,* and *6.2 5* to current *Ph. Eur. 6.4*.

Table 5. Standards Proposed to *USP* for the Subdivision Characteristics of Scored Tablets

Loss of Mass	Take 30 tablets at random. Weigh each tablet. Break each tablet by hand, and weigh each of the subdivided parts. Calculate the loss of mass for that tablet. Repeat the procedure for the other 29 tablets, and calculate the mean loss of mass. **Criterion for Loss of Mass**: The tablets comply with the test if the mean loss of mass is not more than 3.0%.
Accuracy of Subdivision	Take 30 tablets at random and break them by hand. From all the parts obtained from one tablet, select one part and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. **Criterion for Accuracy of Subdivision**: The tablets comply with the test if not more than 1 individual mass is outside the limits of 85% to 115% of the average mass. The tablets fail to comply with the test if more than 1 individual mass is outside these limits or if 1 individual mass is outside the limits of 75% to 125% of the average mass.

Table 6. Test and Criteria for Ease of Subdivision of Scored Tablets

Ease of subdivision
Panel: Select a panel of 10 healthy elderly volunteers with mean age $\geq$ 75 y and none < 65 y. There are no restrictions on male/female ratio, and previous membership on a breaking panel is not an exclusion criterion, but impaired use of hand and/or fingers is an exclusion criterion.
Procedure: Each panelist is instructed to break by hand one tablet in a way he/she would do if he/she were a patient, without having a ''tryout'' of the tablet to be tested.
The investigator scores every tablet given to the panelist either ''breakable'' or ''not breakable.''
Criterion for Ease of Subdivision: Each of the 10 panelists should be able to subdivide the tablet. If the tablets do not comply with this test, the elderly panel is expanded from 10 to 30 using the same inclusion criteria and procedure. When a total of 30 tablets have been tested, at least 27 tablets (78%) must be deemed ''breakable'' by hand.
Notes: • Each panelist should not break more than 10 tablets during one session. • During the test, panelists must not know the results from other panelists. • Care should be taken that the panelist does not swallow the tablet before or after breaking. • Care should be taken that the panelist washes his/her hands after the session is completed.

A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin

Bruce Loverock, Barry Simon, Allen Burgenson, and Alan Baines, *Lonza Walkersville, Inc.*[a]

ABSTRACT This *Stimuli* article addresses a modified photometric procedure for the detection of gram-negative bacterial endotoxin (lipopolysaccharide). This procedure is based on the activation of recombinant Factor C (rFC), the endotoxin-sensitive protease that initiates the traditional *Limulus* Amebocyte Lysate (LAL) cascade. The rFC procedure described in this *Stimuli* article is a single-step, quantitative endpoint procedure that measures the enzyme-mediated cleavage of a fluorogenic substrate. This study shows that the rFC procedure is equivalent to LAL in its ability to measure endotoxin and has a quantitation range comparable to that of quantitative photometric LAL procedures. However, unlike LAL, which uses an extract from the blood cells of the horseshoe crab *Limulus polyphemus* that contains Factor C, the rFC procedure uses an rFC cloned from the horseshoe crab, *Carcinoscorpius rotundicauda*, thereby reducing the number of false-positive results involving glucans.

RATIONALE

The majority of parenteral drugs and implantable medical devices are tested for gram-negative bacterial endotoxin using reagents prepared from circulating amebocytes found in the blood of the American horseshoe crab, *Limulus polyphemus*. Variations of this procedure are described in *US Pharmacopeia* (*USP*) General Chapter *Bacterial Endotoxins Test* ⟨85⟩ (*1*). This chapter describes the gel-clot *Limulus* Amebocyte Lysate (LAL) procedure and the various kinetic and endpoint photometric LAL procedures. A comparison of a kinetic turbidimetric LAL, kinetic chromogenic LAL, and the proposed recombinant quantitative photometric procedure that is not subject to false-positive reactions involving glucans, is the subject of this *Stimuli* article. This alternative procedure has been successfully validated according to the requirements described in *USP* General Information Chapter *Validation of Compendial Procedures* ⟨1225⟩ (*2*).

Mechanism of Reaction

The protease cascade and rationale of traditional LAL procedures have been extensively investigated (*3–7*). Factor C (FC), the first component in the LAL cascade, is a protease zymogen that is activated by endotoxin binding (*5, 6, 8*). Following a series of cascading events, a dose-dependent response is seen in the presence of endotoxin (*Figure 1a*, LAL–Endotoxin). In the kinetic turbidimetric variation of the LAL procedure, in the presence of endotoxin the protein coagulogen is converted to coagulin, resulting in a dose-dependent increase in turbidity. In the kinetic chromogenic version of the LAL procedure, a synthetic chromogenic substrate is cleaved to produce a yellow color. An alternative LAL-based pathway triggered by glucans can also invoke a similar response, thus causing a false-positive result (*Figure 1*, LAL–Glucan).

[a] Correspondence should be addressed to: Radhakrishna Tirumalai, PhD, Senior Scientist, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8339; e-mail rst@usp.org.

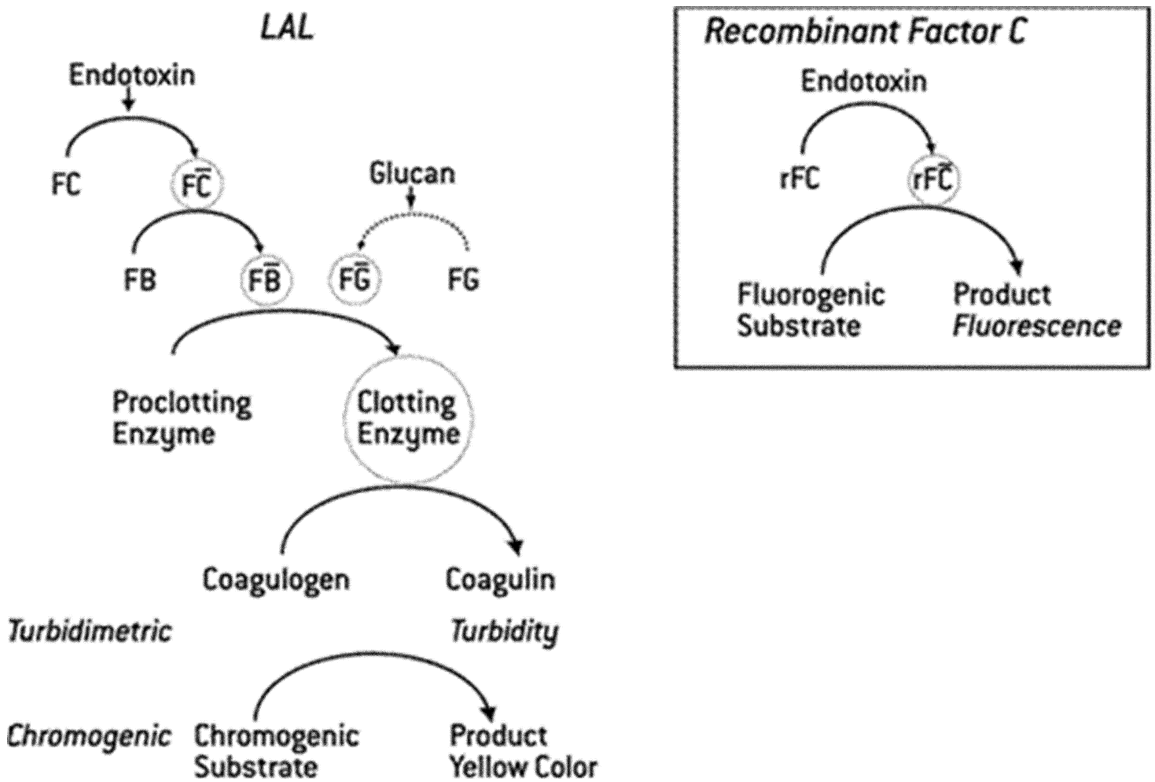

Figure 1a. LAL procedure. Figure 1b. (insert) rFC procedure.

The rFC procedure (*Figure 1b*) utilizes a single protein as its active ingredient, rFC, cloned from the horseshoe crab *Carcinoscorpius rotundicauda* (*8–9*). In the rFC procedure endotoxin binding activates rFC. This activated molecule then acts to cleave a fluorogenic substrate, resulting in the generation of a fluorogenic compound. The rFC endotoxin detection reaction is not subject to false-positive reactions involving glucans (*Figure 1b*, Recombinant Factor C). The rFC protein derived from *C. rotundicauda* shares a 90.5% identical amino acid sequence with the native Factor C protein derived from *L. polyphemus* (unpublished data).

ANALYTICAL PROCEDURE

Precaution: All materials used in this procedure must be endotoxin free.

Recombinant FC is used in a single-step, fluorogenic endpoint procedure to measure endotoxin. One hundred microliters of water blank, standard, and sample are added to a microplate. The microplate is then preincubated in a fluorescent microplate reader at 37° ± 1 °C for 10 min, followed by addition of 100 µL rFC/substrate working reagent to each well. A time zero reading is taken (excitation/emission wavelengths 380/440 nm). The microplate is further incubated at 37° ± 1 °C for 60 min in the fluorescent microplate reader. At elapsed incubation time of 60 min, the microplate is read a second time (excitation/emission wavelengths 380/440 nm). Corrected fluorescence (fluorescence reading at 60 min minus fluorescence reading at time zero) of endotoxin standards and samples are then normalized by the corrected fluorescence of the water blank. The log of the normalized fluorescence of the endotoxin standards is plotted vs the log of endotoxin concentration. Generation of the standard curve is performed using linear regression (least squares). The log increase in fluorescence (net change in relative fluorescence units) vs the log endotoxin concentration is linear over the 0.01 to 10 EU/mL range (EU = endotoxin unit). Endotoxin in samples is predicted using the endotoxin standard curve.

DATA ELEMENTS

Specificity

As defined in ⟨1225⟩, specificity is the ''ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.'' In the case of analytical procedures for impurities, specificity may be established ''by spiking the drug substance or product with appropriate levels of impurities.''

A multicenter study (6 centers, 3 analysts/center) was initiated to test 10 pharmaceutical or related products using both the rFC and the kinetic chromogenic LAL procedures. Products were tested at maximum valid dilution (MVD), MVD/2, and MVD/10 (see ⟨85⟩ for further details), except for Water for Injection, which was tested undiluted. Parallel samples were tested with endotoxin spiked to a final concentration of 0.1 EU/mL. One hun-

dred sixty-eight measurements were made with the unspiked samples across the 6 study sites for each procedure. For both the rFC and kinetic chromogenic procedures, no detectable endotoxin was observed in any of the unspiked samples (data not shown). This was expected because all test samples were pharmaceuticals released to the marketplace (except for hemodialysate). For measurements of samples spiked to a nominal value of 0.1 EU/mL, spike recoveries in the range of 0.05 to 0.20 EU/mL (50%–200%) are considered acceptable, or "passing" according to requirements in ⟨85⟩. Except for the Human Albumin (HA) samples, all of the measurements made with each procedure were within the acceptable range of 0.05 to 0.20 EU/mL (*Figure 2*).

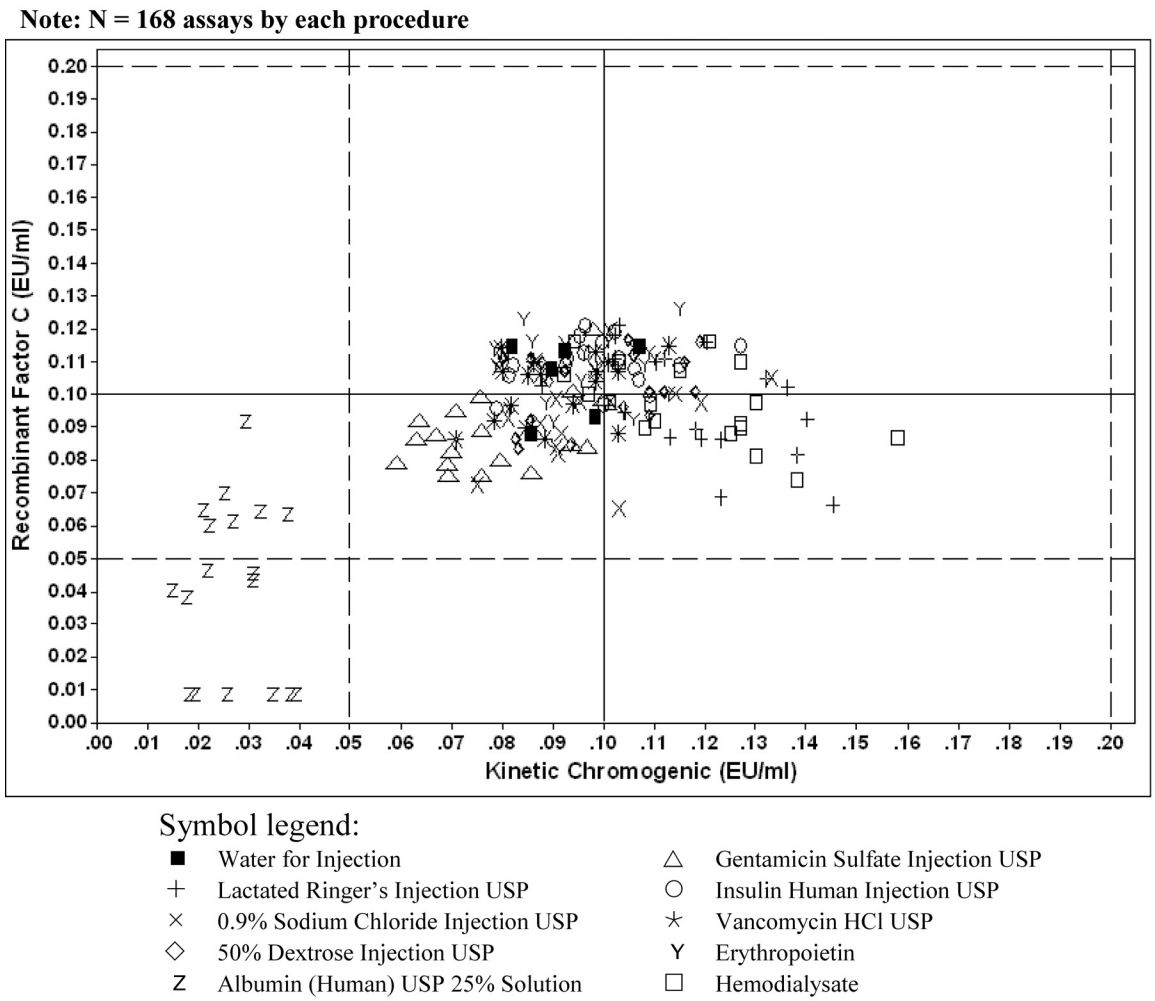

Figure 2. rFC vs kinetic chromogenic LAL: comparison of results for 10 products spiked to 0.1 EU/mL.

With the kinetic chromogenic procedure, all 18 measurements on the HA sample were outside the acceptable range and below 0.05 EU/mL. With the rFC procedure, 7 of the HA measurements were within the acceptable range, and 11 were outside and below 0.05 EU/mL. HA is known to be inhibitory in the LAL-endotoxin reaction due to proteinacious components in the preparation. Other means of circumventing this inhibitory property of HA were not included as part of this study, offering a more direct examination of any inhibitory/enhancement effects across the two analytical procedures.

The two procedures were further compared by determining for each procedure the percent of assay results that were within ± 25% of the nominal 0.1 EU/mL spike. *Table 1* shows that a significantly ($P < 0.01$) greater proportion of the rFC results (85.7%) were within ± 25% of the target concentration than were the proportion of chromogenic results (75.0%).

Table 1. rFC and Chromogenic Assay Results within ± 25% of Spike Value

Note: N = 168 assay results by each procedure		
	Number and % of Results within ± 25% of 0.1 EU/mL Spike	**Number and % of Results beyond ± 25% of 0.1 EU/mL Spike**
rFC	144 85.7%	24 14.3%
Chromogenic	126 75.0%	42 25.0%

The proportion of results within ± 25% of 0.1 EU/mL is greater for the rFC procedure than for chromogenic results; $P < 0.01$, 1-tailed Fisher's Exact Test.

LAL-reactive Material

LAL-reactive Material (LRM) consists of cellulosic material derived from sources such as Cuprophan hemodialysis filters (CF Capillary Flow Dialyzer, Model CF23, Baxter, Deerfield, IL). LRM contains glucans of various linkages, and the β-1,3 linkage is a significant contributor to the LRM false-positive responses in LAL-based procedures (*10*). With an LRM preparation prepared according to the procedure described by Pearson et al. (*11*), the rFC assay has been previously shown to have no significant response to LRM when compared to an LAL-based kinetic chromogenic procedure (*12*).

Response to Different Endotoxin Sources

A comparison was made regarding the response of rFC, kinetic chromogenic LAL, and turbidimetric LAL photometric procedures to different sources of purified endotoxin. Sources tested were: EC-6 (the internationally recognized Reference Standard Endotoxin which is distributed by USP and is derived from *Escherichia coli* stain O113:H10:K(-), *E. coli* O55:B5, *Pseudomonas aeruginosa* F-D Type 1, and *Salmonella minnesota* R595 (Re). Three assays were performed for each endotoxin–procedure combination. The results are summarized in *Figure 3* and *Table 2*.

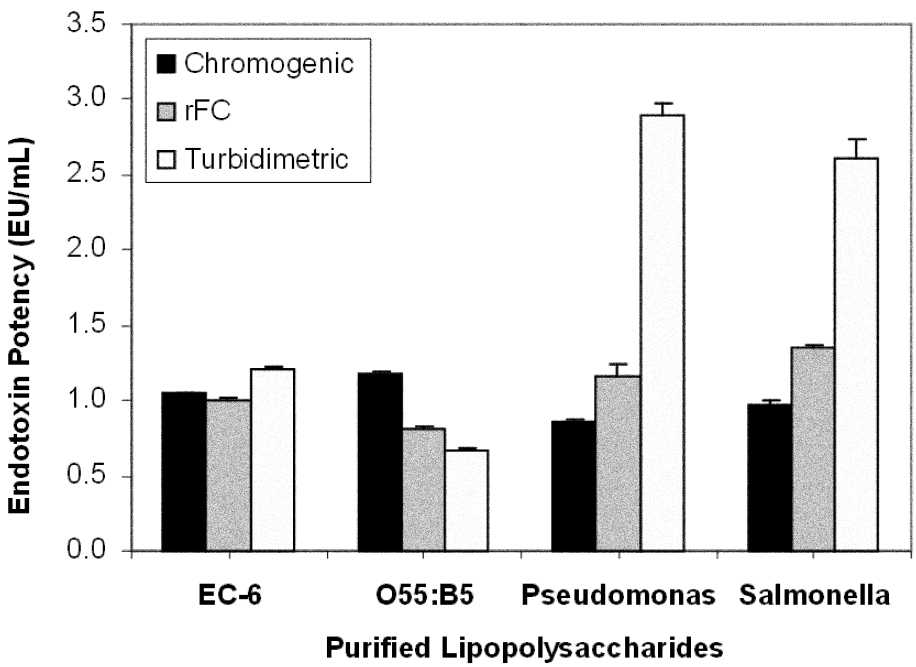

Figure 3. Endotoxin response to different source materials.

Table 2. Results for Different Endotoxin Source Materials

Endotoxin Source	Procedure	Mean[a] (EU/mL)	Standard Error (EU/mL)	Mean Comparisons[b]
EC-6	Chromogenic	1.04	0.012	Turbidimetric Mean > Chromogenic and rFCMean ($P < 0.05$)
	rFC	1.00	0.017	
	Turbidimetric	1.20	0.015	
O55:B5	Chromogenic	1.18	0.023	Chromogenic Mean > rFC Mean > Turbidimetric Mean ($P < 0.05$)
	rFC	0.81	0.021	
	Turbidimetric	0.67	0.007	
Pseudomonas	Chromogenic	0.86	0.014	Turbidimetric Mean > rFC Mean > Chromogenic Mean ($P < 0.05$)
	rFC	1.16	0.071	
	Turbidimetric	2.90	0.081	
Salmonella	Chromogenic	0.98	0.029	Turbidimetric Mean > rFC Mean > Chromogenic Mean ($P < 0.05$)
	rFC	1.36	0.015	
	Turbidimetric	2.61	0.131	

[a] N = 3 assays for each endotoxin–procedure combination.
[b] Multiple comparisons among each pair using Student's *t*-statistic.

The two LAL-based procedures gave statistically significant different mean responses for all four endotoxin sources. The turbidimetric response was greater than the chromogenic response for all sources except O55:B5, where the chromogenic response was greater.

For the O55:B5, *Pseudomonas*, and *Salmonella* sources the rFC mean response was significantly different from the two LAL-based procedures but fell between the two. With EC-6 there was not a significant difference between the rFC and chromogenic procedures, but both of these were significantly less than the turbidimetric results although the magnitude of the difference was small.

Recombinant FC recognizes endotoxin from different source materials as do other photometric procedures, demonstrating equivalency to the LAL-based procedures.

Precision

As defined in ⟨1225⟩, the precision of an analytical procedure ''is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.''

Comparative precision testing was performed with the rFC and kinetic chromogenic procedures at three test centers, by three analysts at each test center. Each analyst measured endotoxin samples from the three regions of the 0.01 to 10 EU standard curve (low = 0.0316 EU/mL, medium = 0.316 EU/mL, and high = 3.16 EU/mL) over the course of three different days.

Table 3 summarizes the precision study results. Note that total variation [measured standard deviation or percent coefficient of variation (%CV)] from this study is the sum of the variation among replicates within assay, variation among days, variation among analysts, and variation among locations. For the rFC procedure, observed total CV was 20% for the low concentration, 18% for the medium concentration, and 11% for the high concentration. Each of these is less than the corresponding CVs obtained with the kinetic chromogenic procedure. A significant difference between procedures was not detected for the low and medium concentrations, but at the high concentration rFC total variation was significantly less than chromogenic total variation ($P < 0.02$).

Table 3. Precision Study Analysis Summary

rFC		Low Sample 0.0316 EU/mL			Medium Sample 0.316 EU/mL			High Sample 3.16 EU/mL		
Location	N	Mean (EU/mL)	Standard (EU/mL)	%CV[a]	Mean (EU/mL)	Standard (EU/mL)	%CV	Mean (EU/mL)	Standard (EU/mL)	%CV
1	18	0.0291	0.0045	14	0.403	0.049	16	3.52	0.32	10
2	18	0.0276	0.0050	16	0.365	0.052	16	3.45	0.34	11
3	18	0.0365	0.0056	18	0.410	0.058	18	3.65	0.40	13
Over Locations	54	0.0311	0.0051[b]	16	0.392	0.053	17	3.54	0.35	11
			0.0063[c]	20		0.056	18		0.36[d]	11
Chromogenic		**Low Sample 0.0316 EU/mL**			**Medium Sample 0.316 EU/mL**			**High Sample 3.16 EU/mL**		
Location	N	Mean (EU/mL)	Standard (EU/mL)	%CV[a]	Mean (EU/mL)	Standard (EU/mL)	%CV	Mean (EU/mL)	Standard (EU/mL)	%CV
1	18	0.0350	0.0033	10	0.446	0.060	19	3.22	0.54	17
2	18	0.0327	0.0069	22	0.403	0.066	21	3.02	0.42	13
3	18	0.0420	0.0067	21	0.416	0.064	20	3.41	0.49	16
Over Locations	54	0.0366	0.0059[b]	19	0.421	0.063	20	3.22	0.49	16
			0.0070[c]	22		0.065	21		0.50[d]	16

[a] All %CVs were calculated using the known concentrations of the samples rather than the measured mean responses.
[b] Pooled within location standard deviation.
[c] Total standard deviation over locations.
[d] For the High sample, rFC total variation is significantly less than chromogenic total variation ($P < 0.02$). There is not a significant difference in total variation for the Low and Medium samples.

Accuracy of Individual Results

As defined in ⟨1225⟩, the accuracy of an analytical procedure ''is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range.''

Accuracy of individual assay results was assessed using the data from the precision study, which used three known concentrations of EC-6 (the current Reference Standard Endotoxin) as an endotoxin source. The three samples tested had concentrations of 0.0316 EU/mL, 0.316 EU/mL, and 3.16 EU/mL and were tested at three locations, by a total of nine different analysts, and over three different days by each analyst.

As a measure of ''closeness'' of individual assay results to the known concentration, each assay result was classified as either within ± 25% of the known sample concentration (i.e., ''close'') or beyond ± 25%. *Table 4* shows that at each of the three sample concentrations, the rFC procedure had a greater proportion of assay results within ± 25% of the known concentration than did the chromogenic procedure. The difference, however, was not statistically significant.

Table 4. Accuracy Relative to Known Concentration[a]

		rFC	Chromogenic
0.0316 EU/mL Sample	**Mean standard deviation**	0.0311 EU/mL 0.0063 EU/mL	0.0366 EU/mL 0.0070 EU/mL
	% of results within ± 25% of known concentration[b]	81.5%	74.1%
0.316 EU/mL Sample	**Mean standard deviation**	0.392 EU/mL 0.056 EU/mL	0.421 EU/mL 0.065 EU/mL
	% of results within ± 25% of known concentration[b]	44.4%	29.6%
3.16 EU/mL Sample	**Mean standard deviation**	3.54 EU/mL 0.36 EU/mL	3.22 EU/mL 0.50 EU/mL
	% of results within ± 25% of known concentration[b]	92.6%	87.0%

[a] Results were obtained from the precision study (see *Table 3*) and are based on N = 54 assays of each sample, by each procedure.
[b] The differences between procedures are not significant based on Fisher's Exact Test.

Linearity

As defined in ⟨1225⟩, the linearity of an analytical procedure "is its ability to elicit results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range."

A series of 18 rFC standard curves were generated as part of the 6-site multicenter study. Each curve included four standards (0.01, 0.1, 1.0, and 10 EU/mL) in replicates of three. The log net change in RFU (difference of the 60-min time point minus the zero time point corrected for the blank) was plotted against log endotoxin concentration to generate a standard curve. *Table 5* summarizes the linear regression statistics from the 18 curves.

Table 5. rFC Linear Regression Summary Statistics[a]

Standard Curve Characteristic	Mean	CV%	Range	
			Minimum	Maximum
Correlation	0.996	0.1%	0.995	0.998
Slope	0.865	1.9%	0.831	0.901
Y-intercept	3.398	1.1%	3.307	3.450

[a] Based on 18 standard curves, by six analysts at six sites.

The correlation coefficient was greater than 0.995 for all 18 standard curves, meeting FDA requirement ($\geq$ |0.980|) for LAL-based procedures (*13*). Standard curve slopes and *y*-intercepts were also highly consistent among the 18 standard curves, with CVs of 1.9% and 1.1% respectively.

Range

As defined in ⟨1225⟩, the range of a procedure ''is validated by verifying that the analytical procedure provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.'' Data obtained from the precision study (*Table 3*) show similar variation between the rFC and kinetic chromogenic procedures. The total variation of the rFC procedure was either the same as or lower than the kinetic chromogenic procedure for the three regions (low, middle, and high) of the standard curve. For the three regions the accuracy of the rFC procedure was equivalent to that of the kinetic chromogenic procedure (*Table 4*).

Quantitation Limit

According to ⟨1225⟩, the quantitation limit is the ''lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is expressed as the concentration of analyte in the sample.''

We evaluated three distinct lots of rFC enzyme to determine the quantitation limit of the rFC procedure. Reference Standard Endotoxin EC-6 was used to prepare the endotoxin standard curve (0.01 to 10 EU/mL). Three separate assays were performed, each with one of the three rFC enzyme lots. Five separate samples of both LAL Reagent Water (control blank) and 0.01 EU/mL EC-6 endotoxin were tested as unknowns in replicates of three with each of the three enzyme lots.

Table 6 contains a summary of the results. The assay results on the 0.01 EU/mL sample show acceptable precision and accuracy, demonstrating that the quantitation limit is at least 0.01 EU/mL. Furthermore, the 99% tolerance intervals for assays on the water blank and 0.01 EU/mL-samples do not overlap, indicating that assays on a sample with 0.01 EU/mL are clearly distinct from assays on a blank sample.

Table 6. rFC Quantitation Limit Summary Statistics

	Number of Assay Results	Mean (EU/mL)	Standard Deviation (EU/mL)	%CV	99% Tolerance Interval (95% Confidence)
0.01 EU/mL sample	15	0.009	0.0007	7.8%	0.006–0.012 EU/mL
Blank water sample	15	0.002	0.0008	n/a[a]	0–0.005 EU/mL

[a] n/a = not applicable

CONCLUSION

The rFC procedure for the detection of gram-negative bacterial endotoxin has been shown to be equivalent to LAL in its ability to quantifiably measure endotoxin and has a quantitative range comparable to that of quantitative photometric LAL procedures. However, unlike the LAL endotoxin procedure that uses Factor C derived from horseshoe crabs, the rFC procedure uses a recombinant form of Factor C. It provides another means of detecting endotoxin by other than traditional LAL procedures. The rFC does not contain the glucan-sensitive Factor G, and therefore the rFC-based procedure is not subject to false positives that can be caused by β-1,3-glucans (*12*). It utilizes material of nonanimal origin as its active ingredient. The requirements for validation of Compendial Methods as specified in ⟨1225⟩ for specificity, precision, accuracy, linearity, range, and quantitation limit have been met for the rFC procedure and are equivalent to current *USP* procedures for endotoxin measurement.

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Transfer of HPLC Procedures to Suitable Columns of Reduced Dimensions and Particle Sizes

Uwe D. Neue,[a] Doug McCabe,[a] Vijaya Ramesh,[b] Horacio Pappa,[d] Jim DeMuth[c]

ABSTRACT This *Stimuli* article contains proposals to help the analyst adjust HPLC column length and particle size to achieve separation power at least equivalent to that used in the original procedure, markedly increasing the range of options currently allowed in *Chromatography* ⟨621⟩. The article presents the scientific rationale for application of these proposals to isocratic procedures and follows with gradient procedures.

INTRODUCTION

Users of compendial chromatographic procedures increasingly need to develop analytically equivalent procedures that decrease analysis time and solvent consumption. In this process they face limitations because USP does not provide the necessary flexibility to change the chromatographic column without revalidation of the method. The *United States Pharmacopeia* (*USP*) General Chapter *Chromatography* ⟨621⟩ describes in detail the range of adjustments allowed in the system when the suitability test failed. These adjustments in the operating conditions, when needed, are the maximum variations that can be made without the need for validation rather than verification of method performance under the new conditions. Included, among others, are changes in column length (± 70%), changes in column diameter (± 25%), particle size (can be reduced by as much as 50%), and flow rate (± 50%). Additional changes are being implemented (*1*): The column diameter can be changed freely provided that the linear velocity is kept constant, following the formula: where *F*, *l*, and *d* are the flow rates, the column lengths, and the column diameters, respectively, before the change (subscript 1) and after the change (subscript 2). An adjustment of the flow rate by ± 50% is also allowed.

$$F_2 = F_1 \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (1)$$

Except for this flexibility, ⟨621⟩ is silent on changes allowed to the column specified in the monograph. In some cases *USP* chromatographic procedures prescribe the use of a column that is no longer available and needs to be replaced with another of the same stationary phase but different dimensions. In others cases, switching to a column with different particle size and dimensions may provide a more rapid separation with equivalent chromatographic performance. Both these situations currently require revalidation. This article proposes allowing the flexibility to change column dimensions or particle size as long as equivalent or better column performance is maintained, and it provides guidance to ensure that this is achieved in a scientifically rigorous manner.

PROPOSED CHANGES TO THE *SYSTEM SUITABILITY* SECTION OF ⟨621⟩ WITH RESPECT TO PARTICLE SIZE AND COLUMN LENGTH

This *Stimuli* article proposes a new approach that will both preserve the quality of the separation as well as expand the changes in particle size beyond the current twofold decrease. The intent of this proposal is to allow the chromatographer a reduction in analysis time without sacrificing column performance or impairing the separation capability for a procedure.

Chromatography defines the relationships by which particle size, column length, and flow rate can be changed without affecting the quality of the separation (*2–8*). The column plate count *N* is determined as follows:

$$N = (l / H) = l / (d_p \cdot h) \qquad (2)$$

where *l* is the column length, *H* is the theoretical plate height, d_p is the particle diameter, and *h* is the reduced plate height. The quality of the separation is determined primarily by the plate count, which is why most *USP* chromatographic procedures require a minimum plate count. The plate count remains constant if the ratio of column length to particle diameter remains constant, provided that the reduced plate height remains the same (see equation 2).

The reduced plate height *h* depends exclusively on the reduced velocity υ, which in turn is a function of the particle diameter and the flow rate.

$$h = H / d_p \qquad (3)$$

$$\upsilon = u d_p / D_M \qquad (4)$$

[a] Waters Corporation

[b] USP General Chapter Expert Committee Member

[c] USP General Chapter Expert Committee Chair

[d] To whom all correspondence should be addressed: Horacio Pappa, PhD, Senior Scientist, US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; 301.816.8319; e-mail hp@usp.org.

where u is the average linear velocity and D_M is the solute diffusion coefficient. To the practicing chromatographer, this means that the flow rate needs to increase in inverse proportion to the change in particle size in order to maintain the same reduced plate height h:

$$h_2 = h_1 \text{ if } \upsilon_2 = \upsilon_1 \qquad (5a)$$

and therefore

$$F_2 \cdot d_{p,2} = F_1 \cdot d_{p,1} \qquad (5b)$$

and

$$F_2 = F_1 \, (d_{p,1} / d_{p,2}) \qquad (5c)$$

As an example, if the particle size is reduced from 5 μm to 3.5 μm, the flow rate will need to be increased by slightly more than 40%. The combination of the shorter column (equation 2) and the increased flow rate (equation 5c) results in a reduction in analysis time while preserving the performance of the separation.

Analysis time decreases with both the shorter column length l and the higher flow rate F, as the square of the reduction in particle diameter: In addition, because the column length is reduced at the same time as the particle size is decreased, the quantity of solvent per analysis is reduced with the shorter column.

$$t_{a,2} = t_{a,1} \cdot \frac{l_2 \cdot F_1}{l_1 \cdot F_2} = \frac{d_{p,2}^2}{d_{p,1}^2} \qquad (6)$$

Table 1 contains sets of conditions required to maintain the quality of the separation (the same plate count), when users change the particle size.

Table 1. Change in Conditions for Achieving the Same Plate Count

4-mm columns:				
Particle Size (μm)	Column Length (cm)	Flow Rate (mL/min)	Reduction in Analysis Time	Reduction in Solvent Use
5	15	1	1×	1×
3.5	10	1.5	2×	1.5×
2.5	7.5	2	4×	2×
1.7	5	3	9×	3×
2.1-mm columns:				
Particle Size (μm)	Column Length (cm)	Flow Rate (mL/min)	Reduction in Analysis Time	Reduction in Solvent Use
5	15	0.2	1×	1×
3.5	10	0.3	2×	1.5×
2.5	7.5	0.5	4×	2×
1.7	5	0.6	9×	3×

As noted, an increase in flow rate is associated with a reduction in analysis time, and a reduction in the column length is associated with a reduction in solvent consumption. *Figure 1* provides examples from the implementation of this procedure.

From *Figure 1* it can be seen that the separation is quantitatively maintained with each change, in excellent agreement with the theoretical predictions. The good agreement between theory and practice supports allowing this degree of flexibility in the pharmacopeia.

The following rule for conversion between different particle sizes may be used to ensure the plate count remains approximately the same:

> The column length and the particle diameter should be changed approximately in proportion to each other. The flow rate should be increased or decreased in inverse proportion to the change in particle size.

This last change will be beneficial to analysts because current rules for changing the particle diameter do not provide any guidance about how to maintain the quality of the separation.

In some cases, a perfect match of column length and particle size may not be possible. For example, the change in particle size from a 10-μm packing in a 25-cm column to a 5-μm packing would require a 12.5-cm column, which is not commonly available. For such a case, some flexibility can be provided by the following rule:

> If an exact match is not readily available, the ratio of column length to particle size can be changed by ± 25%.

Additional changes in flow rate of ± 50% are already permitted by ⟨621⟩.

As the column volume is reduced, the injection volume should be reduced proportionally. This is consistent with the existing ⟨621⟩ statement that ''the injection volume can be reduced as far as is consistent with accepted precision and detection limits.'' Because scaling to a large diameter and a larger column volume is also possible, we propose changing the statement in ⟨621⟩ as follows:

> *Injection Volume (GC, HPLC)*: Can be adjusted as far as is consistent with accepted precision and detection limits.

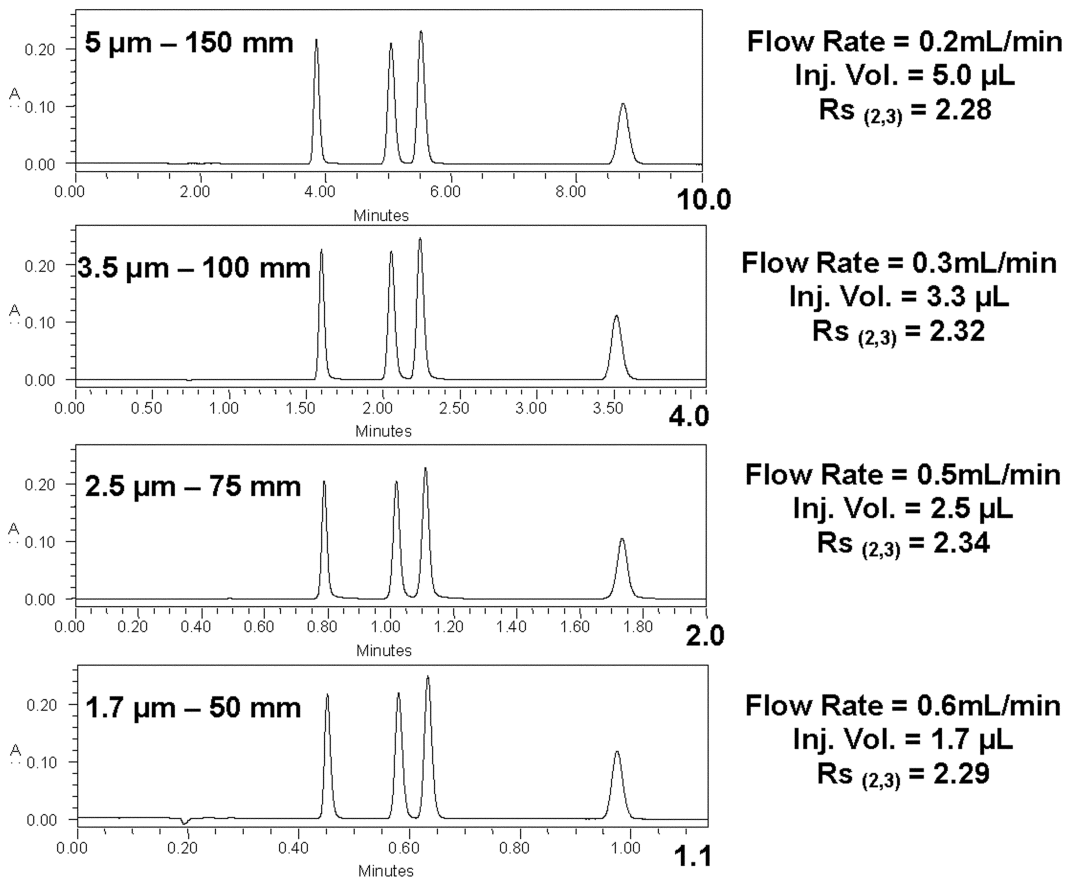

Figure 1. Scaling of a separation at a fixed ratio of column length and particle size using columns with a 2.1-mm internal diameter (reproduced with permission from Reference 7).

As a guide for accomplishing this change, chromatographers may scale the injection volume V_i relative to the column volume V_c, as shown below: The requirements to this point cover the scaling of isocratic methods, which encompass most *USP* methods. However, modern methods are increasingly gradient methods, so we propose the extension of these concepts to this area. In general, all rules written to this point for isocratic methods also apply to gradient methods. In addition, a gradient method is scaled properly if all segments of the gradient $V_{g,s}$ are scaled in proportion to the column volume V_c: Flow rates and injection volumes are scaled as in the isocratic method (see equation 5c—a faster flow rate is used and the particle diameter is reduced). The gradient segment volume $V_{g,s}$ is defined as the time programmed for the gradient segment $t_{g,s}$ multiplied by the flow rate F. This leads to the following formula for the scaling of the time for each gradient segment:

$$t_{g,s,2} = t_{g,s,1}\,[(V_{g,s,2} / F_2) / (V_{g,s,1} / F_1)] \qquad (9)$$

As was true for the isocratic method, this results in a 4-fold reduction in every gradient segment, and thus in the analysis time, for every 2-fold change in particle diameter (and column length).

$$V_{i,2} = V_{i,1} \cdot \frac{V_{c,2}}{V_{c,1}} = V_{i,1} \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (7)$$

$$V_{g,s,2} = V_{g,s,1} \cdot \frac{V_{c,2}}{V_{c,1}} = V_{g,s,1} \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (8)$$

Thus, we propose the following requirement for changing a gradient:

> Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies also to every gradient segment volume.

This simple procedure may be complicated by instrument-dependent gradient delay volumes. The gradient delay volume results in an isocratic section before the beginning of the gradient proper. For a well-functioning instrument and a well-designed gradient, the gradient delay volume is small relative to the column volume, and the analysis is not affected. Alternatively, modern instruments allow for delayed injection to adjust the gradient delay volume to the column volume. An example of a well-scaled gradient method is shown in *Figure 2*. In this case, the method was scaled from a 4.6-mm × 150-mm column packed with 5-μm particles to a 2.1-mm × 50-mm column packed with 1.7-μm particles. Of course, the separation chemistry of both packings was held constant. Following the rules outlined above, the user adjusted the flow rate for both the column diameter and the particle diameter. The gradient times were adjusted to maintain a constant ratio of the gradient segment volumes to the column volumes. As a consequence, the elution pattern and the resolution remain constant, and the analysis time decreased by a factor of 8 with the shorter column.

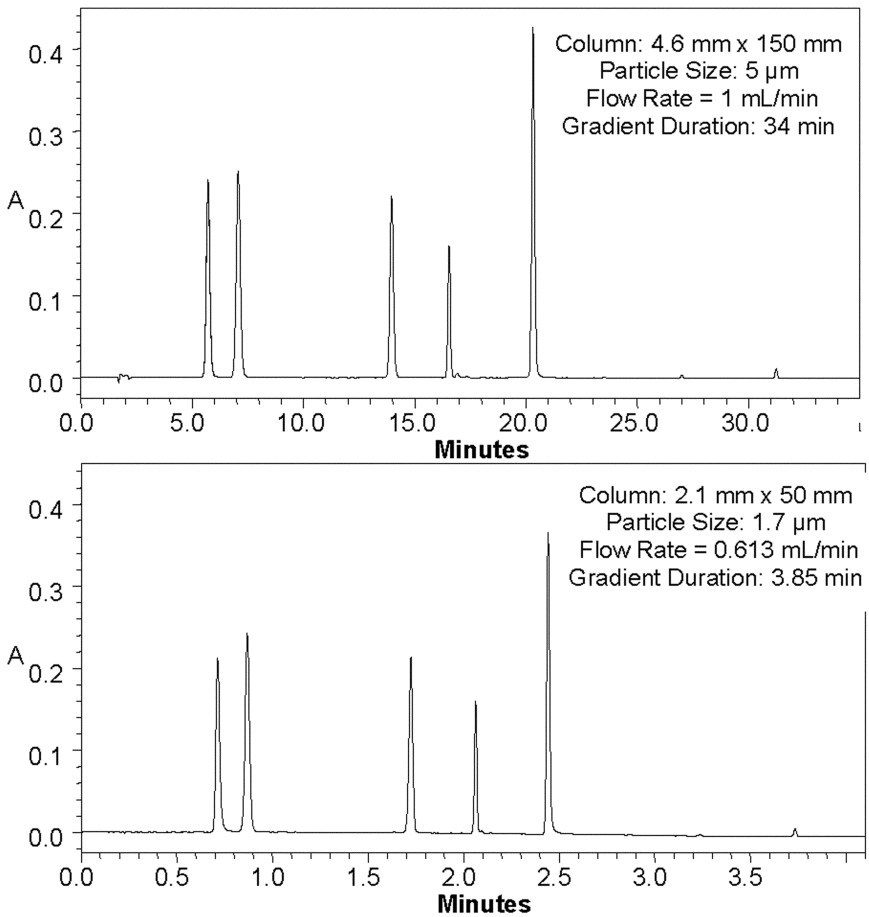

Figure 2. Comparison of two gradient chromatograms scaled by column dimension and particle diameter according to the rules described herein (reproduced with permission from Reference 9).

SUMMARY

The proposed changes to ⟨621⟩ will expand the analyst's ability to reduce solvent consumption and decrease analysis time while maintaining the quality of a chromatographic separation. By adhering to the proposed requirements, analysts can maintain the quality of the separation for the entire range of possible particle diameters, extending and improving on the rule that limits the particle diameter change to – 50% and the change in column length to ± 70%.

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NOMENCLATURE

This section includes supplements to the latest edition of the *USP Dictionary of USAN and International Drug Names* that incorporate new United States Adopted Names (USAN) and revisions to existing Dictionary names. Also listed are Proposed and Recommended International Nonproprietary Names (INN) when they have been announced by the World Health Organization.

Possible names suggested for use as USAN and INN are listed for public review and comment along with information on how nonproprietary names are devised. In addition, readers may find articles relevant to current compendial nomenclature issues that also occasionally report on related matters pertaining to USAN and INN.

Nomenclature

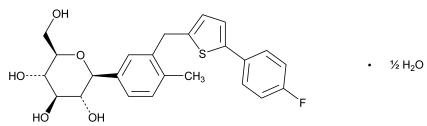
USP Dictionary of USAN and International Drug Names
2009 USP DICTIONARY SUPPLEMENT 4

IMPORTANT—Save this Supplement. This and all supplements appearing in *PF* are needed to keep the 2009 edition of the USP Dictionary (USPD) up-to-date. The cumulative contents of the supplements to the current (2009) edition will be included in the next complete edition of the Dictionary.

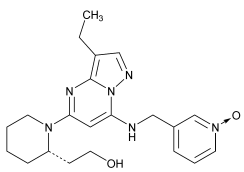
Newly Approved United States Adopted Names (USAN), Released for Publication

The following are newly established United States Adopted Names (USAN). These names will not be listed cumulatively; see preceding and succeeding numbers of *PF* for other new USAN to supplement the Dictionary main volume.

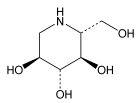
Canagliflozin [*2009*] (kan′′ a gli floe′ zin). $C_{24}H_{25}FO_5S.H_2O$. 453.53. (1) D-Glucitol, 1,5-anhydro-1-C-[3-[[5-(4-fluorophenyl)-2-thienyl]methyl]-4-methylphenyl]-, hydrate (2:1), (1*S*)-; (2) (1*S*)-1,5-Anhydro-1-C-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-D-glucitol hemihydrate; (3) (1*S*)-1,5-Anhydro-1-[3-[[5-(4-fluorophenyl)-2-thienyl]methyl]-4-methylphenyl]-D-glucitol hemihydrate. *CAS-928672-86-0*. *Antidiabetic.* ✧*JNJ-28431754; JNJ-28431754-AAA; JNJ-24831754-ZAE; TA-7284*



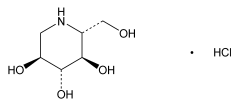
Dinaciclib [*2009*] (din′′ a sye′ klib). $C_{21}H_{28}N_6O_2$. 396.50. (1) 2-Piperidineethanol, 1-[3-ethyl-7-[[(1-oxido-3-pyridinyl)methyl]amino]pyrazolo[1,5-*a*]pyrimidin-5-yl]-, (2*S*)-; (2) 3-[[[3-Ethyl-5-[(2*S*)-2-(2-hydroxyethyl)piperidin-1-yl]pyrazolo[1,5-*a*]pyrimidin-7-yl]amino]methyl]pyridine 1-oxide. *CAS-779353-01-4*. *Treatment of cancer.* ✧*SCH 727965*



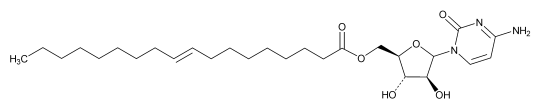
Duvoglustat [*2009*] (doo′′ voe gloo′ stat). $C_6H_{13}NO_4$. 163.20. (1) 3,4,5-Piperidinetriol, 2-(hydroxymethyl)-, (2*R*,3*R*,4*R*,5*S*)-; (2) (2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)piperidine-3,4,5-triol. *CAS-19130-96-2*. *Treatment of Pompe disease.*



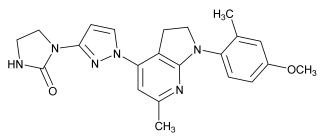
Duvoglustat Hydrochloride[*2009*] (doo′′ voe gloo′ stat hye′′ droe klor′ ide). $C_6H_{13}NO_4.HCl$. 199.60. (1) 3,4,5-Piperidinetriol, 2-(hydroxymethyl)-, hydrochloride (1:1), (2*R*,3*R*,4*R*,5*S*)-; (2) (2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)piperidine-3,4,5-triol hydrochloride. *CAS-73285-50-4*. *Treatment of Pompe disease.* ✧*AT2220*



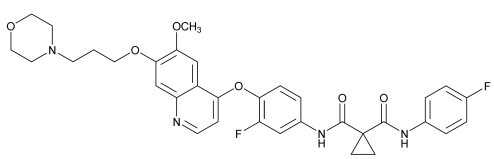
Elacytarabine [*2009*] (el′′ a sye tar′ a been). $C_{27}H_{45}N_3O_6$. 507.66. (1) 2(1*H*)-Pyrimidinone, 4-amino-1-[5-*O*-[(9*E*)-1-oxo-9-octadecenyl]-β-D-arabinofuranosyl]-; (2) 4-Amino-1-[5-*O*-[(9*E*)-octadec-9-enoyl]-β-D-arabinofuranosyl]pyrimidin-2(1*H*)-one. *CAS-188181-42-2*. INN. *Treatment of cancer.* ✧*CP-4055*



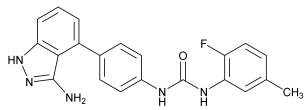
Emicerfont [*2009*] (em′′ i ser′ font). $C_{22}H_{24}N_6O_2$. 404.50. (1) 2-Imidazolidinone, 1-[1-[2,3-dihydro-1-(4-methoxy-2-methylphenyl)-6-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl]-1*H*-pyrazol-3-yl]-; (2) 1-{1-[1-(4-Methoxy-2-methylphenyl)-6-methyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl]-1*H*-pyrazol-3-yl}imidazolidin-2-one. *CAS-786701-13-1*. *Treatment of anxiety disorders, depression, and IBS.* ✧*GW876008X*



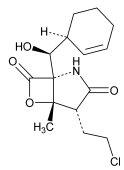
Foretinib [*2009*] (for e′ ti nib). $C_{34}H_{34}F_2N_4O_6$. 632.70. (1) 1,1-Cyclopropanedicarboxamide, *N*-[3-fluoro-4-[[6-methoxy-7-[3-(4-morpholinyl)propoxy]-4-quinolinyl]oxy]phenyl]-*N*′-(4-fluorophenyl)-; (2) *N*-[3-Fluoro-4-({6-methoxy-7-[3-(morpholin-4-yl)propoxy]quinolin-4-yl}oxy)phenyl]-*N*′-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide. *CAS-849217-64-7*. *Antineoplastic.* ✧*GSK1363089G*



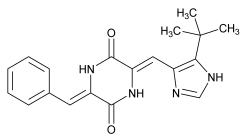
Linifanib [*2009*] (lin if′ a nib). $C_{21}H_{18}FN_5O$. 375.40. (1) Urea, *N*-[4-(3-amino-1*H*-indazol-4-yl)phenyl]-*N*′-(2-fluoro-5-methylphenyl)-; (2)1-[4-(3-Amino-1*H*-indazol-4-yl)phenyl]-3-(2-fluoro-5-methylphenyl)urea. *CAS-796967-16-3*. *Antineoplastic.* ✧*ABT-869*



Marizomib [*2009*] (mar iz′ oh mib). $C_{15}H_{20}ClNO_4$. 313.80. (1) 6-Oxa-2-azabicyclo[3.2.0]heptane-3,7-dione, 4-(2-chloroethyl)-1-[(*S*)-(1*S*)-2-cyclohexen-1-ylhydroxymethyl]-5-methyl-, (1*R*,4*R*,5*S*)-; (2) (1*R*,4*R*,5*S*)-4-(2-Chloroethyl)-1-{(*S*)-[(1*S*)-cyclohex-2-en-1-yl]hydroxymethyl}-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione.*CAS-437742-34-2*. *Antineoplastic.* ✧*NPI-0052*



Plinabulin [*2009*] (plin′′ a bue′ lin). $C_{19}H_{20}N_4O_2$. 336.40. (1) 2,5-Piperazinedione, 3-[[5-(1,1-dimethylethyl)-1*H*-imidazol-4-yl]methylene]-6-(phenylmethylene)-, (3*Z*,6*Z*)-; (2) (3*Z*,6*Z*)-6-Benzylidene-3-{[5-(1,1-dimethylethyl)-1*H*-imidazol-4-yl]methylidene}piperazine-2,5-dione. *CAS-714272-27-2*. *Antineoplastic.* ✧*NPI-2358*

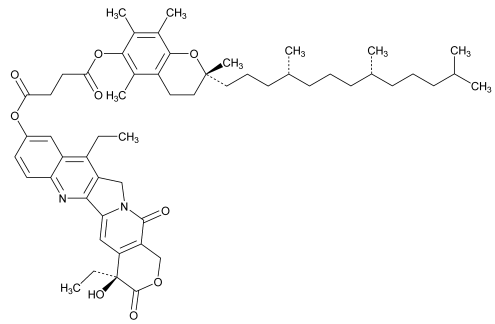


Potassium Sulfate [*2009*] (poe tas′ ee um sul′ fate). K_2SO_4. 174.26. (1) Sulfuric acid potassium salt (1 : 2); (2) Potassium sulfate. *CAS-7778-80-5*. JAN. *Oral bowel cleansing solution.*

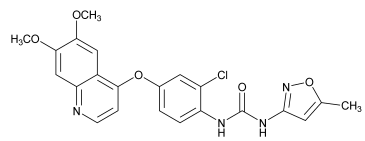
Rintatolimod [*2009*] (rin′′ ta tol′ i mod). $[[C_{10}H_{11}N_4O_7P]_{13}]_n$. $[[C_9H_{12}N_3O_7P]_{12}[C_9H_{11}N_2O_8P]]_n$. (1) 5′-Inosinic acid, homopolymer, complex with 5′-cytidylic acid polymer with 5′-uridylic acid (1 : 1); (2) Poly[5′)-inosinyl-(3′→] duplex with poly[dodecakis[3′)-cytidylyl-(5′→]3′)-uridylyl-(5′→]. The molecular mass ranges from 400,000 to 1,750,000 daltons. *CAS-38640-92-5*. *Treatment of chronic fatigue syndrome; antiviral.* Ampligen (Hemispherx Biopharma).

Taspoglutide [*2008*] (tas′′ poe gloo′ tide). $C_{152}H_{232}N_{40}O_{45}$. 3339.71. (1) 7-36-Glucagon-like peptide I (human), 8-(2-methylalanine)-35-(2-methylalanine)-36-L-argininamide-; (2) [8-(2-Amino-2-methylpropanoic acid),35-(2-amino-2-methylpropanoic acid)]human glucagon-like peptide 1 (GLP-1)-(7-36)-peptidamide. *CAS-275371-94-3*. INN. *Treatment of type 2 diabetes.* ✧*RO5073031*

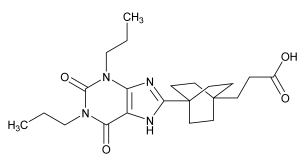
Tenifatecan [*2009*] (ten if′′ a tee′ kan). $C_{55}H_{72}N_2O_9$. 905.20. (1) Butanedioic acid, (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl (2*R*)-3,4-dihydro-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-6-yl ester; (2) (4*S*)-4,11-Diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl (2*R*)-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl butanedioate. *CAS-850728-18-6*. *Antineoplastic.* ✧*SN2310*



Tivozanib [*2009*] (tye voe′ za nib). $C_{22}H_{19}ClN_4O_5$. 454.90. (1) Urea, *N*-[2-chloro-4-[(6,7-dimethoxy-4-quinolinyl)oxy]-phenyl]-*N*′-(5-methyl-3-isoxazolyl)-; (2) *N*-{2-Chloro-4-[(6,7-dimethoxyquinolin-4-yl)oxy]phenyl}-*N*′-(5-methylisoxazol-3-yl)urea. *CAS-475108-18-0*. *Antineoplastic.* ✧*AV-951*

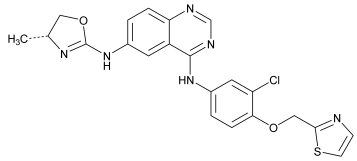


Tonapofylline [*2009*] (toe′′ na pof′ i lin). $C_{22}H_{32}N_4O_4$. 416.50. (1) Bicyclo[2.2.2]octane-1-propanoic acid, 4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-; (2) 3-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)bicyclo[2.2.2]oct-1-yl]propanoic acid. *CAS-340021-17-2*. *Treatment of heart failure.* ✧*BG9928*

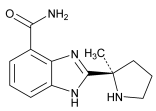


Nomenclature

Varlitinib [*2009*] (var li′ ti nib). $C_{22}H_{19}ClN_6O_2S$. 466.90. (1) 4,6-Quinazolinediamine, *N*⁴-[3-chloro-4-(2-thiazolylmethoxy)-phenyl]-*N*⁶-[(4*R*)-4,5-dihydro-4-methyl-2-oxazolyl]-; (2) *N*⁴-[3-Chloro-4-(thiazol-2-ylmethoxy)phenyl]-*N*⁶-[(4*R*)-4-methyl-4,5-dihydrooxazol-2-yl]quinazoline-4,6-diamine. *CAS-845272-21-1*. *Antineoplastic.* ✧*ARRY-334543*



Veliparib [*2009*] (ve lip′ a rib). $C_{13}H_{16}N_4O$. 244.30. (1) 1*H*-Benzimidazole-7-carboxamide, 2-[(2*R*)-2-methyl-2-pyrrolidinyl]-; (2) 2-[(2*R*)-2-Methylpyrrolidin-2-yl]-1*H*-benzimidazole-4-carboxamide. *CAS-912444-00-9*. *Antineoplastic enhancing agent.* ✧*ABT-888*



Yttrium Y 90 Clivatuzumab Tetraxetan [*2009*] (i′ tree um klye′′ va tooz′ oo mab te trax′ e tan). $C_{6496}H_{9952}N_{1716}O_{2014}S_{44}$. $(C_{16}H_{23}N_4O_7{}^{90}Y)_n$. 145.7 kilodaltons (antibody). (1) Immunoglobulin G1, anti-(human mucin MUC1) (human–mouse monoclonal hPAM4 heavy chain), disulfide with human–mouse monoclonal hPAM4 κ-chain, dimer, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid conjugate, yttrium-⁹⁰Y chelate; (2) Immunoglobulin G1, anti-(mucin-1 (MUC-1, PEMT, episialin, tumor-associated mucin, EMA, H23AG, PUM or CD227)); humanized mouse monoclonal hPAM4 γ1 heavy chain (222-215′)-disulfide with humanized mouse monoclonal hPAM4 κ light chain, dimer (228-228′′:231-231′′)-bisdisulfide, substituted (average of 2 to 5 substitutions) on *N*⁶ of lysyl residus by (4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclodec-1-yl)acetyl (substitutive tetraxetan), (90Y)yttrium(III) chelate. *CAS-943976-23-6*. *Treatment of pancreatic cancer.* hPAM4-Cide (Immunomedics). ✧*hPAM4-DOTA; IMMU-107;* ⁹⁰*hY-PAM4*

Nomenclature

INDEX

This is a cumulative directory for the content of all issues of *PF* beginning with *PF* 35(1).

[*Note—This index covers Vol. 35 No. 1, pp. 1–218; Vol. 35 No. 2, pp. 219–498; Vol. 35 No. 3, pp. 499–784; Vol. 35 No. 4, pp. 785–1050; Vol. 35 No. 5, pp. 1051–1408; Vol. 35 No. 6, pp. 1409–1638.*]

MONOGRAPHS

Index

EXCIPIENTS

GENERAL CHAPTERS

REAGENTS, INDICATORS, AND SOLUTIONS

Index

REFERENCE TABLES

GENERAL SUBJECTS

Index

CHROMATOGRAPHIC COLUMNS USED IN *USP–NF* AND *PHARMACOPEIAL FORUM*

This is an update based on the proposals published in this issue of *PF*.

Chromatographic Columns Used in *USP-NF* and *Pharmacopeial Forum*
Nov.–Dec. 2009

BENZALKONIUM CHLORIDE (DSD Mgh #7800)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(6)	L1	Nucleosil 100 C18	Limit of	Limit of benzyl alcohol, benzaldehyde, and (chloromethyl)benzene. 4.6 mm x 15 cm, 5 μm. Manufacturer: Macherey-Nagel
35(6)	L10	Nucleosil CN	Ratio of	Ratio of Alkyl Components. 4.6 mm x 25 cm, 10 μm. Manufacturer: Macherey-Nagel

BENZALKONIUM CHLORIDE SOLUTION (DSD Mgh #7830)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Nucleosil 5 C18	Limit of	Limit of Benzyl Alcohol, Benzaldehyde, and (Chloromethyl)Benzene. 4.6 mm x 15 cm, 5 μm. Manufacturer: Macherey-Nagel
35(6)	G43	DB-624	Alcohol Content	If added. 0.25 mm x 30 cm, 1.4 μm. Manufacturer: J & W Scientific
35(6)	L10	Nucleosil CN	Ratio of	Ratio of Alkyl Components. 4.6 mm x 25 cm, 10 μm. Manufacturer: Macherey-Nagel

BUTYLATED HYDROXYANISOLE (DSD Mgh #11060)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Symmetry C-18	Assay and Identification	4.6 mm x 7.5 cm, 3.5 μm. Manufacturer: Waters Corp.

CAPREOMYCIN FOR INJECTION (DSD Mgh #12362)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L10	Spherisorb CN	Content of	Content of Capreomycin I. 4.6 mm x 25 cm, 5 μm. Manufacturer: Waters Corp.

CAPREOMYCIN SULFATE (DSD Mgh #12358)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L10	Spherisorb CN	Content of	Content of Capreomycin I. 4.6 mm x 25 cm, 5 μm. Manufacturer: Waters Corp.

CARBOMER COPOLYMER (DSD Mgh #13025)

PF	LGS#	Reagent Brand	Type of Test	Comments
26(2)	L1	MicroBondapak C18	Limit of	Limit of Acrylic Acid. 8 mm x 10 cm, 10 μm. Catalog number WAT085721. Manufacturer: Waters Corp.

CETIRIZINE HYDROCHLORIDE AND PSEUDOEPHEDRINE HYDROCHLORIDE EXTENDED-RELEASE TABLETS (DSD Mgh #4576)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L9	ZORBAX 300 SCX	Dissolution	4.6 mm x 15 cm, 5 μm. Manufacturer: Agilent Technologies

CETIRIZINE HYDROCHLORIDE TABLETS (DSD Mgh #3171)

PF	LGS#	Reagent Brand	Type of Test	Comments
36(1)	L3	LiChrospher Si-100	Organic Impurities	4.0 mm x 25 cm, 5 μm. Manufacturer: Merck KGaA
36(1)	L1	Inertsil ODS-3	Dissolution	4.6 mm x 25 cm, 5 μm. Manufacturer: GL Sciences

CLOTRIMAZOLE CREAM (DSD Mgh #18890)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Purospher STAR RP18e	Assay	4.6 mm x 25 cm, 5 μm. Manufacturer: Merck KGaA

DIVALPROEX SODIUM DELAYED-RELEASE CAPSULES (DSD Mgh #27754)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Inertsil ODS-3	Assay and Dissolution	4.6 mm x 15 cm, 5 μm. Manufacturer: GL Sciences

DIVALPROEX SODIUM EXTENDED-RELEASE TABLETS (DSD Mgh #826)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L11	MicroBondapak Phenyl	Dissolution	3.9 mm x 15 cm, 10 μm. Manufacturer: Waters Corp.

FELODIPINE EXTENDED-RELEASE TABLETS (DSD Mgh #32660)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Symmetry C-18	Dissolution	Test 3. 4.6 mm x 15 cm, 5 μm. Alternative column: Ace C18, same dimensions. Manufacturer: Waters Corp.
32(1)	L1	Hypersil BDS C-18	Dissolution	Test 2. 4.6 mm x 15 cm. Manufacturer: Thermo Scientific

FEXOFENADINE HYDROCHLORIDE AND PSEUDOEPHEDRINE HYDROCHLORIDE EXTENDED-RELEASE TABLETS (DSD Mgh #33077)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Partisil 10 ODS	Dissolution	Test 3. 4.6 mm x 25 cm, 10 μm. Manufacturer: Whatman Inc.
0(0)	L7	Hypersil BDS C-8	Dissolution	Test 2. 4.6 mm x 25 cm, 5 μm. Manufacturer: Thermo Scientific

FEXOFENADINE HYDROCHLORIDE TABLETS (DSD Mgh #852)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	Spherisorb ODS-2	Dissolution	Test 3. 4.6 mm x 10 cm, 5 μm. Manufacturer: Waters Corp.

FLAVOXATE HYDROCHLORIDE (DSD Mgh #33185)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Inertsil ODS	Organic Impurities	4.6 mm x 15 cm, 5 μm. Manufacturer: GL Sciences

GLIPIZIDE AND METFORMIN HYDROCHLORIDE TABLETS (DSD Mgh #847)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L1	YMC 18 ODS-A	Dissolution	Test 2. 4.6 mm x 25 cm. Manufacturer: YMC Corp.

LEVETIRACETAM TABLETS (DSD Mgh #44660)

PF	LGS#	Reagent Brand	Type of Test	Comments
36(1)	L1	Nova-Pak C18	Assay and Organic Impurities	4.6 mm x 25 cm, 4 μm. Manufacturer: Waters Corp.
36(1)	L1	Inertsil ODS-3	Dissolution	4.6 mm x 15 cm, 5 μm. Manufacturer: GL Sciences

LEVOFLOXACIN (DSD Mgh #2861)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Inertsil ODS-3	Assay and Organic Impurities	4.6 mm x 25 cm, 5 μm. Alternative column: Prodigy ODS(3) in the same dimensions, manufactured by Phenomenex. Manufacturer: GL Sciences

METOLAZONE TABLETS (DSD Mgh #53470)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L7	Symmetry C8	Dissolution	4.6 mm x 15 cm, 5 μm. Manufacturer: Waters Corp.

MONTELUKAST SODIUM (DSD Mgh #54774)

PF	LGS#	Reagent Brand	Type of Test	Comments
36(1)	L41	Chiral AGP	Enantiomeric Purity	4.0 mm x 15 cm, 5 μm. Manufacturer: Chrom Tech AB
36(1)	L11	ZORBAX SB Phenyl	Assay and Organic Impurities	4.6 mm x 5 cm, 1.8 μm. Manufacturer: Agilent Technologies

ORLISTAT (DSD Mgh #58790)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L7	Superspher 60 RP-Select B	Limit of	Limit of Orlistat Related Compound D. 4.0 mm x 25 cm, 5 μm. Manufacturer: Merck KGaA

OXACILLIN SODIUM (DSD Mgh #59030)

PF	LGS#	Reagent Brand	Type of Test	Comments
20(1)	L11	MicroBondapak Phenyl	Assay	4 mm x 30 cm Manufacturer: Waters Corp

OXYBUTYNIN CHLORIDE EXTENDED-RELEASE TABLETS (DSD Mgh #59502)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L7	Symmetry C8	Dissolution	Test 4. 4.6 mm x 15 cm, 5 μm. Manufacturer: Waters Corp.
0(0)	L10	ZORBAX SB-CN	Dissolution	Test 3. 4.6 mm x 15 cm, 5 μm. Manufacturer: Agilent Technologies
33(4)	L7	Spherisorb C8	Dissolution	Test 2. 4.6 mm x 25 cm. Manufacturer: Waters Corp.

PILOCARPINE HYDROCHLORIDE (DSD Mgh #64990)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L11	Luna Phenyl-Hexyl	Assay and Organic Impurities	4.6 mm x 15 cm, 3 μm. Manufacturer: Phenomenex

RISEDRONATE SODIUM TABLETS (DSD Mgh #73736)

PF	LGS#	Reagent Brand	Type of Test	Comments
34(5)	L48	IonPac AG7	Dissolution	4.0 mm x 5 cm, 10 μm. Manufacturer: Dionex

RIVASTIGMINE TARTRATE (DSD Mgh #73772)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L41	Chiral AGP	Enantiomeric Purity	4.0 mm x 10 cm. Manufacturer: Chrom Tech AB

RIZATRIPTAN BENZOATE (DSD Mgh #73775)

PF	LGS#	Reagent Brand	Type of Test	Comments
36(1)	L11	ZORBAX SB Phenyl	Assay and Organic Impurities	4.6 mm x 25 cm, 5 μm. Manufacturer: Agilent Technologies

TERBINAFINE HYDROCHLORIDE (DSD Mgh #80845)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	Inertsil ODS-2	Assay	3.0 mm x 15 cm, 5 μm. Manufacturer: GL Sciences

TERBINAFINE TABLETS (DSD Mgh #2860)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L1	XTerra RP 18	Assay and Organic Impurities	Procedure 2. 4.6 mm x 15 cm, 5 μm. Manufacturer: Waters Corp.
36(1)	L7	Symmetry C8	Assay and Organic Impurities	Procedure 1. 3.9 mm x 15 cm, 5 μm. Manufacturer: Waters Corp.

TOBRAMYCIN INHALATION SOLUTION (DSD Mgh #83766)

PF	LGS#	Reagent Brand	Type of Test	Comments
0(0)	L11	Spherisorb P(Phenyl)	Assay and Related Compounds	4.6 mm x 25 cm, 5 μm. Manufacturer: Waters Corp.

TRICLOSAN (DSD Mgh #85150)

PF	LGS#	Reagent Brand	Type of Test	Comments
22(3)	G3	DB-17	Assay and Related Compounds	0.53 mm x 15 cm, 1 μm. Alternative column: AT-50 Manufacturer: J & W Scientific

VENLAFAXINE HYDROCHLORIDE (DSD Mgh #88080)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L7	Inertsil C8	Organic Impurities	4.6 mm x 25 cm, 5 μm. Manufacturer: GL Sciences
35(6)	L7	Kromasil C8	Assay	4.6 mm x 25 cm, 5 μm. Manufacturer: Eka Nobel

VENLAFAXINE TABLETS (DSD Mgh #88083)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(6)	L7	Inertsil C8-3	Organic Impurities	4.6 mm x 25 cm, 5 μm. Manufacturer: GL Sciences
35(6)	L1	ACE C18	Assay	4.6 mm x 25 cm, 5 μm. Manufacturer: Advanced Chrom. Technol.

ZIDOVUDINE ORAL SOLUTION (DSD Mgh #89518)

PF	LGS#	Reagent Brand	Type of Test	Comments
35(5)	L1	Hypersil BDS C-18	Organic Impurities	4.6 mm x 10 cm, 3 μm. Manufacturer: Thermo Scientific