Elemental Impurities: Standards-Setting Record

December 20, 2012

I. Purpose

Current official standards expressed in General Chapter <231> *Heavy Metals* were last updated in *USP 28*. This document summarizes the standards-setting activities relative to USP's new Elemental Impurities (EI) standards, which are designed to replace <231>. The document is divided into four sections: work done prior to 2000, and work in each of the three revision cycles of the 21st century.

II. Prior to 2000

Prior to 2000, there were a number of publications which appeared in *Pharmacopeial Forum* **(***PF*) relating to compendial testing for EI, some of which related to <231> and others of which related to the USP monograph for *Magnesium Stearate.* These are summarized below.

***PF Stimuli* article [1975] page 861(Attachment 1)**
This publication made the following recommendations with respect to <231>:

a. *It is recommended that all articles now tested by Method I be evaluated by the three-tube monitor procedure to confirm the suitability of the method (i.e., no complexation due to the sample, no interfering colors, and no precipitation) or use with each article. Note: It is hoped that each producer of the articles involved will look at his products and report directly to the appropriate Director of Revision (Dr. D. Banes for NF and USP articles; Mr. Duarward Dodgen for FCC articles) concerning the suitability, or lack of suitability, of the method for use with his products.*

b. *It is recommended that the three-tube monitor procedure, with or without the zirconium modification, be given consideration as a replacement for the current Method I procedure.*

c. *It is recommended that all future monographs specify use of Method II for Heavy metals determinations unless adequate evidence, including the results of recovery studies, is presented to support use of a different procedure.*

***PF* 17(5) [Sept.-Oct. 1991] page 2419 (Attachment 2)**
This *Stimuli* article recommended several changes to the USP *Magnesium Stearate* monograph, including the deletion of the current colorimetric *Lead* test and the addition of atomic absorption tests for cadmium, lead, and nickel. These recommendations were based on test results showing high levels of cadmium

and nickel in some magnesium stearate samples. Limits of 3 ppm, 10 ppm, and 5 ppm for cadmium, lead, and nickel, respectively, were suggested.

***PF* 18(4) [July-Aug. 1992] page 3591—(Attachment 3)**
Pharmacopeial Previews
This publication proposed the deletion of test colorimetric test for lead in the *Magnesium Stearate* monograph and suggested using graphite furnace atomic absorption spectrophotometers for testing cadmium, lead, and nickel. This proposal was based on the *PF* 17(5) *Stimuli* article referenced above and subsequently appeared as an in-process revision as described below.

***PF* 19(4) [July-Aug. 1993] page 5754—(Attachment 4)**
In-process Revision for the harmonized *Magnesium Stearate* monograph
This publication proposed the deletion of colorimetric test for testing lead in the USP *Magnesium Stearate* monograph and suggested using graphite furnace atomic absorption spectrophotometers for testing cadmium, lead, and nickel in Magnesium Stearate. This proposal was based on the *PF* 17(5) *Stimuli* article as referenced above and subsequently became official.

***PF* 21(1)[Jan-Feb. 1995] page 157—(Attachment 5)**
Magnesium Stearate**—**Proposed limits for cadmium, lead, and nickel. Zak T. Chowhan, Lee. T. Grady, and W. Larry Paul
Based on the comments received on *P*F 17(5) *Stimuli* article, the Committee of Revision (now the Council of Experts) decided to review this issue from a toxicity/safety viewpoint. USP estimated the maximum daily intake of cadmium, lead, and nickel in a worst-case setting, i.e., under conditions of maximum daily dosing of three currently marketed pharmaceutical products that are formulated with above average levels of magnesium stearate containing the maximum proposed levels of cadmium, lead, and nickel. Results of this study were presented in this *PF* publication. Although the results could have been used to justify more appropriate limits with better analytical procedure, further progress did not occur.

***PF* 21(6) [Nov-Dec.1995] page 1632—(Attachment 6)**
Harmonization of the USP, EP, and JP Heavy Metals Testing Procedures, by Katherine B. Blake, *IPEC HPMC Harmonization Task Force*

The study presented in this *PF* publication was conducted to harmonize heavy metals testing for HPMC across the three named pharmacopeias. Results indicated that approximately 50% of the metals may be lost during the ash process. The loss of metals is probably matrix-dependent, and because the procedures are very labor-intensive, recoveries can vary significantly among analysts. The report notes that mercury, which is one of the more toxic heavy metals, was not recovered from either set of samples. The differences among the pharmacopeias in the handling of the heavy metals test reference standard proved to be the basis for the different specifications for heavy metals seen in the monographs for many substances. Based on these findings, a USP limit of 10 ppm may be equivalent to the EP limit of 20 ppm. Because of the loss of metals during ignition, the validity of test results obtained with the current USP, JP, and

EP general test procedures is questionable. The data thus obtained should not be used to justify elimination of heavy metals requirements in monographs. Specific recommendations made included the following:

a. *USP Method III (wet digestion) should be added to the general test procedures in the EP and JP.*
b. *The Heavy Metals test procedures in which ignition is used, USP Method II, EP V.3.2.8 Method C, and JP Method 2, should be deleted, or revised to include a spiked control.*
c. *Spiked control samples should be taken through the general test procedures to validate the methodology for each monographs*
d. *Atomic absorption analytical techniques should be included as an option.*

***PF* 21(6) [Nov –Dec. 1995] page 1629—(Attachment 7)**
Excipient intake and heavy metals limits, by W.L. Paul.

USP surveyed all official USP correspondents to obtain maximum daily intake data for 28 frequently used excipients selected by the USP Subcommittee on Excipients. The publication presented results of survey responses reporting the highest daily intake for each of the selected excipients and discussed the proper way to apply daily excipient intake values to safety- and toxicity-based test limits in compendial monographs.

***PF* 24(4) [July-Aug. 1998] page 6460—(Attachment 8)**
In this *PF*, proposed revisions to General Chapter <231> revisions were published to address concerns over loss of metals during ashing. The revisions became official in *Supplement 1 to USP 28-NF 23*, p. 3295.

III. 2000-2005 Revision Cycle

The Pharmaceutical Analysis 6 Expert Committee (PA6 EC) discussed the EI topic continuously throughout this cycle. The PA6 EC formed a subcommittee to focus on the topic in 2004. The PA6 EC proposed to explore other techniques and initiated laboratory work to address a number of topics. PA6 EC focused their efforts on finding a temporary solution to the limitations of <231> and published various proposals in *PF*, summarized below. During this time they sought more general solutions that would result in a complete revision of the general chapter. The PA6 EC wanted to make use of the available advanced sample preparation and analytical techniques such as microwave digestion and ICP, respectively. During meeting #3 (November 13, 2003), the PA6 EC proposed to form a working group with representatives from the Committee itself as well as other key stakeholders (IPEC, ACS, EPA, FDA, EP, and JP). At this meeting, the PA6 EC also considered a letter from the FDA Center for Drug Evaluation and Research (CDER) that pertained to the harmonization proposal for General Chapter <231> *Heavy Metals* published in *PF* 28(5). In its response to the statement in the CDER letter that “the Method II test did not recover mercury,” the PA6 EC added a statement to <231> specifying that Method II did not recover mercury. Although the Expert Committee concluded that

<231> should be eliminated, this work did not progress in the cycle pending further laboratory studies.

***PF* 29(4) [July-Aug. 2003] page 1328—(Attachment 9)** An Atomic Spectroscopic Method as an Alternative to Both USP <231> Heavy Metals and <281> USP Residue on Ignition, by Tiebang Wang.

This *Stimuli* article demonstrated that a multi-element inductively coupled plasma–mass spectrometry (ICP–MS) method is a suitable alternative to both USP <231> Heavy Metals and USP<281> Residue on Ignition for drug substances, intermediates, and raw materials. The article stated that an ICP–MS method, combined with a direct-dissolution sample preparation procedure, is simpler, faster, more sensitive, and element specific. It consumes less sample and provides semi-quantitative to quantitative results covering all elements of pharmaceutical interest

***PF* 30(5) [Sept.-Oct 2004] page 1876—(Attachment 10)**
Changes to USP Heavy Metals <231> Test, by John Geary (PA6 EC)

In this *Stimuli* article, the PA6 EC proposed to withdraw *Method II* of General Chapter <231> due to a number of problems, with suggestions for continued use of *Methods I* and *III.* The article proposed an alternative procedure, Inductively Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES), to be approved for both *Method I* and *Method III*. This was the first time that a USP Council of Experts Expert Committee recommended abandonment of the existing <231>.

***PF* 30(6) [Nov-Dec. 2004] page 2271—(Attachment 11)**
Inductively coupled plasma – Optical emission spectroscopy as an alternative to the heavy metals test, by M. Schenkenberger, and N. Lewen.

This *Stimuli* article described the use of another spectroscopic technique, inductively coupled plasma–optical emission spectroscopy (ICP–OES), as an alternative to the compendial heavy metals test. ICP–OES offers many advantages over the compendial method. It is a rapid, multielement technique that can be used to assay for the following elements: antimony (Sb), arsenic (As), bismuth (Bi), cadmium (Cd), indium (In), lead (Pb), mercury (Hg), molybdenum (Mo), palladium (Pd), platinum (Pt), ruthenium (Ru), selenium (Se), silver (Ag), and tin (Sn). Other advantages to the use of this technique include the fact that only a small quantity of sample is required, and it provides element-specific results. Combined with ICP-MS, these two instrument techniques became the basis for a proposed new general chapter <233>. Pending a new general chapter, the following revisions to <231> occurred in this cycle.

***In Process* Revisions**

***PF* 29(5) [Sept.-Oct. 2003] page 1603–(Attachment 12)**
Chapter <231> was revised to:

- Allow the use of pH meter to adjust pH in Methods 1 and III
- Add monitor solution in Method II
- Increase the amount of sample needed for testing

***PF* 30(1) [Jan – Feb 2004] page 217—(Attachment 13)**
Chapter <231> was revised to add monitor preparation in *Method III.*

***PF* 30(2) [March - April 2004] page 614—(Attachment 14)**
Chapter <231> was revised to allow the option to use either pH meter or short-range pH indicator paper for pH adjustment.

***PF* 30(3) [May – June 2004] page 1004—(Attachment 15)**
Chapter <231> was revised to include a *Note* in *Method II* regarding the inability of this method to recover mercury.

***PF* 31(5) [Sept. – Oct 2005] page 1435—(Attachment 16)**
Method II was revised to adequately address the issues resulting from the official publication of the revised *Method II* in the *First Supplement to USP 28.* Committee also indicated that they are working on developing a more robust method and this proposed revision is not the final solution to the issues related to *Method II,* but this proposal may address the concerns of stakeholders at present.

Interim Revision Announcements

***USP 23-NF 18* 1995 IRA No. 20 page No. 4**

***USP 29-NF 24* 2006 IRA No. 3 page No. 747—(Attachment 17)**
USP responded to the comments received through this IRA and reverted to the Heavy Metals text that appeared in USP 28–NF 23 page 2300 for Heavy Metals Method II. This change appeared in the Third Interim Revision Announcement to *USP 29–NF 24,* which was published in *PF* 32(3) and became official on June 1, 2006. Committee indicated that they will keep searching for a more robust and practical method.

Harmonization

***PF* 27(3) [May-June 2001] page 2619—Harmonization Stage 3 proposal published.**

***PF* 28(5) [Sept.-Oct. 2002] page 1570—Harmonization Stage 4 proposal published.**

IV. 2005-2010 Revision Cycle

The EI work of the PA6 EC was assumed by the General Chapters Expert Committee (GC EC) during this revision cycle. The GC EC considered the EI topic at each of its 14 meetings. At the beginning of the cycle (2005), the Expert Committee formed the Heavy Metals Advisory Panel with expertise in analytical methodology and toxicology. The Advisory Panel met 15 times during this cycle. Advisory Panel members initiated work in their own laboratories to identify the best option for sample preparation as well as analytical measurement. While no standards were proposed, the GC EC emphasized the importance of improved EI testing based on extensive laboratory work. This work was summarized in the following Stimuli article:

***PF* 34(5) [Sept.—Oct. 2008] - General Chapter on Inorganic Impurities: Heavy Metals—(Attachment 18)**
This *Stimuli* article and allied comments became the basis for new general chapters numbered <232> and <233>. A digest of comments received on the *PF* 34(5) stimuli article was posted on the USP website and culminated in Attachment 20 below.

V. 2010-2015 Revision Cycle

In this cycle, the Chemical Analysis Expert Committee (CA EC) assumed responsibility for the EI topic and considered the topic in all six of its meetings in the cycle to date. The Elemental Impurities Expert Panel continued the work of the Advisory Panel in the prior cycle and has met twice thus far in this cycle. Publications emanating from the work of the CA EC and associated Expert Panel are as follows:

***PF* 36(1) [Jan-Feb. 2010]-Elemental Impurities—Information—(Attachment 19)**

***PF* 36(1) [Jan-Feb. 2010]-Elemental Impurities—Comments and Response—(Attachment 20)**

***PF* 36(1) [Jan-Feb. 2010]—(Attachments 21-22)**
Based on these publications and also the extensive prior work, the CA EC proposed three new chapters in this *PF*: <232> Elemental Impurities—Limits; <233>Elemental Impurities—Procedures, and <2232> Elemental Contaminants in Dietary Supplements. Revisions to the proposed new general chapters based on public comments appeared in the following *PF*.

***PF* 37(3) [May-June 2011]—(Attachments 23-24)**
Proposed revision to General Chapters <232> and <233>

No new information was provided in the comments received on these proposals. Therefore, the Expert Committee concluded two of the three general chapters (excluding <2232>, which was scheduled for balloting at a later date), which appeared with an official date of December 1, 2012 in:

***Second Supplement* to *USP 35-NF 30,* official date December 1, 2012—(Attachment 25)**

VI. ICH

At the request of manufacturers seeking international harmonization, ICH agreed to form an Expert Working Group (EWG) to consider EI elements and limits. The EWG was formed in October 2009. The activity thus promoted the work of USP, focusing on impurity limits for medicinal ingredients and products. At this time, a pre-step 2 document is available for review to the EWG (See Addendum 4 for ICH Q3D

proposed limits and USP limits). A Step 2 document is targeted for June 2013, and a Step 4 document is targeted for June 2014 in accordance with ICH procedures. Regional implementation will occur thereafter in accordance with national/regional procedures. USP staff observes the deliberations of the EWG. As the deliberations proceeded, USP made minor adjustments to all limits presented in <232> except for mercury.

See also the following Addenda:

- Addendum 1: List of Formal Expert Committee, Expert Panel, and Advisory Panel Discussions of Elemental Impurities Since 2005
- Addendum 2: List of Public Forums and Publications Related to Elemental Impurities[1]
- Addendum 3: Summary of Frequency and Location of Delivery of
- Pharmacopeial Education Course “Analysis of Elemental Impurities”
- Addendum 4: Tables of Permitted Daily Exposures and Concentrations for Metal Impurities and from ICH Q3D pre-Stage 2 Draft

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December 20, 2012

Addendum 1: List of Formal Expert Committee, Expert Panel, and Advisory Panel Discussions of Elemental Impurities Since 2005

February 2, 2005: Pharmaceutical Analysis 6 Expert Committee
August 23-24, 2005: 2005-2010 General Chapters Expert Committee (GC EC)
November 18, 2005: GC EC
February 3, 2006: Heavy Metals Advisory Panel (AP)
February 10, 2006: GC EC
June 13-14, 2006: GC EC
October 6, 2006: Heavy Metals AP
October 19, 2006: GC EC
March 8, 2007: Heavy Metals AP
March 8, 2007: GC EC
July 23-25, 2007: GC EC
November 6, 2007: GC EC
November 9, 2007: Heavy Metals AP
February 28, 2008: GC EC
July 23-24, 2008: GC EC
October 2, 2008: Heavy Metals AP
October 30, 2008: Metal Impurities AP
December 1, 2008: Metal Impurities AP
December 10, 2008: GC EC
March 11, 2009: Metal Impurities AP, Toxicology, Agenda
March 13, 2009: Metal Impurities AP
March 31, 2009: GC EC
April 29-30, 2009: Metal Impurities AP
June 18, 2009: Metal Impurities AP
July 10, 09: Metal Impurities AP
July 17, 2009: Metal Impurities AP
July 23, 2009: Metal Impurities AP
August 6, 2009: Metal Impurities AP
August 10-11, 2009: GC EC
December 9, 2009: GC EC
March 18, 2010: GC EC
August 18, 2010: Metal Impurities AP and Elemental Impurities Expert Panel (EI AP)
October 5-6, 2010: General Chapters - Chemical Analysis EC (GC-CA EC)
March 9, 2011: GC-CA EC
July 19, 2011: GC-CA EC
October 19-20, 2011: GC-CA EC
November 14, 2011: EI AP
January 18, 2012: EI AP
January 31, 2012: GC-CA EC
June 13, 2012: GC-CA EC
October 17-18, 2012: GC-CA EC

Addendum 2: List of Public Forums and Publications Related to Elemental Impurities[2]

May 24, 2005: Revision of Heavy Metals <231> discussed at Prescription/Non-Prescription Stakeholder Forum (PNP SF)

September 27, 2005: Proposals made to revise Heavy Metals <231> at PNP SF

July 10, 2006: Heavy Metals Project Team introduced at PNP SF

June 19, 2007: Discussion of Heavy Metals <231> at Dietary Supplements Stakeholder Forum (DS SF)

November 15, 2007: Heavy Metals General Chapter revision noted on work plan during PNP SF

February 20, 2008: Discussion of Heavy Metals <231> at Food Ingredients Stakeholder Forum (FI SF)

May 15, 2008: Heavy Metals Project Team status update at PNP SF

August 26-27, 2008: Institute of Medicine (IOM) hosted meeting to provide a non-biased view of the methodology and toxicology aspects (summary available at http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/key-issues/2008-MetalsWorkshopSummary.pdf).

September 25, 2008: Interactive General Session on Heavy Metals at USP Annual Science Meeting

November 21, 2008: Discussion of Heavy Metals <231> at PNP SF

December 8, 2008: Interview with USP Chief Science Officer on revisions of heavy metals standard published in *Pharmalot* (http://www.pharmalot.com/2008/12/heavy-metal-standards-usps-abernethy-explains/).

December 8, 2008: Article "Improving Metal Detection In Drugs" published in *Chemical and Engineering News.*

April 28-29, 2009: Workshop "Metals in Pharmaceuticals and Dietary Supplements" held at USP to gather input from stakeholders (presentations available at http://www.usp.org/sites/default/files/usp_pdf/EN/meetings/workshops/2009-04-29-MetalImpuritiesSlides.pdf and summary available at http://www.usp.org/sites/default/files/usp_pdf/EN/meetings/workshops/2009-04-29-MetalImpuritiesFinal.pdf). USP Advisory Panel Meeting immediately followed.

June 2, 2009: Advisory Panel Recommendations to the General Chapters Expert Committee announced via USP Compendial **Notice** (http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/metalAdvisoryPanelRec.pdf).

August 5, 2009: Discussion of Dietary Supplement chapter <2232> at DS SF

November 18-19, 2009: Discussion of Chapters <232> and <233> at PNP SF

March 18, 2010: Discussion of Chapters <232> <233>, and <2232 at PNP SF

May 2010: Article "Metal Impurities In Food and Drugs" published in *Pharmaceutical Research,* [May 2010, 27(5): 750-755]*.*

July 20, 2010: Elemental Impurities Hot Topic page posted to USP website to provide current information to stakeholders (http://www.usp.org/usp-nf/key-issues/elemental-impurities).

[2] This list primarily reflects USP-initiated activities and is not inclusive of all media coverage.

October 6, 2010: Revised General Chapters <232> and <233> posted to Hot Topic page for comment in advance of publication in *PF* 37(3) to allow additional comment time.

November 2-3, 2010: Discussion of Chapters <232> <233>, and <2232 at PNP SF

December 3, 2010: Discussion of Heavy Metals <231> at FI SF

May 19, 2011: Discussion of Chapters <232> and <233> at PNP SF

May 26, 2011: Informational update to Hot Topic page to describe Expert Panel intentions, in particular relative to ICH Q3D.

August 16, 2011: Discussion of Chapter <2232> at DS SF

April 27, 2012: Final General Chapters and implementation strategy appeared on Hot Topic page in advance of appearance in *Supplement 2 to USP 35-NF 30*

May 15, 2012: Discussion of Chapters <232> and <233> at PNP SF

May 24, 2012: Press release on "New Quality Standards Limiting Elemental Impurities in Medicines Announced" published in *Pharmaceutical Business Review*.

May 25, 2012: Article "USP Releases Overhaul of Impurities Testing Standards" in *Regulatory Focus* (http://www.raps.org/focus-online/news/news-article-view/article/1576/usp-releases-overhaul-of-impurities-testing-standards.aspx)

May 28, 2012: Article "New Quality Standards in Medicines" in MedIndia.net (http://www.medindia.net/news/new-quality-standards-in-medicines-101889-1.htm)

May 29, 2012: Article "USP Replaces 'Out of Date' Impurity Testing Standards" in In-PharmaTechnologist.com (http://www.in-pharmatechnologist.com/Regulatory-Safety/USP-replaces-out-of-date-impurity-testing-standards)

May 30, 2012: Press release on "New Quality Standards Limiting Elemental Impurities in Medicines Announced" published in MedicalXpress.com (http://medicalxpress.com/news/2012-05-quality-standards-limiting-elemental-impurities.html).

May 31, 2012: Blog in Pharmtech Talk, "USP Heavy Metal Chapter Revisions on the Way; New Screening Methods to Be Implemented" promoting Pharmaceutical Technologi webinar on Elemental Impurities (http://blog.pharmtech.com/2012/05/31/usp-heavy-metal-chapter-revisions-on-the-way-new-screening-methods-to-be-implemented/)

June 1, 2012: Press release on "New Quality Standards Limiting Elemental Impurities in Medicines Announced" published in Labonline.com.au (http://www.labonline.com.au/news/53568-New-quality-standards-limiting-elemental-impurities-in-medicines-announced-)

July 24, 2012: Interview with USP Senior Scientific Liaison Kahkashan Zaidi "Heavy Metals in Your Drugs? USP's Zaidi Explains" in *Pharmalot* (http://www.pharmalot.com/2012/07/heavy-metals-in-your-drugs-usps-zaidi-explains/)

August 1, 2012: Article "Pharma Gets More Time to Control Elemental Impurities" in *The Gold Sheet* (http://www.elsevierbi.com/publications/the-gold-sheet/46/8/pharma-gets-more-time-to-control-elemental-impurities)

October 1, 2012: Article "Elemental Impurities Control" in *Contract Pharma* (http://www.contractpharma.com/issues/2012-10/view_features/elemental-impurities-control/)

October 10, 2012: Article "The Impact of USP's New Elemental Impurities Standards on Manufacturers, Suppliers and Contract Laboratories" in *American Pharmaceutical Review* (online) and *Pharmaceutical Outsourcing* (online)

Addendum 3: Summary of Frequency and Location of Delivery of Pharmacopeial Education Course "Analysis of Elemental Impurities"

Frequency and Location of Delivery of PE Course "Analysis of Elemental Impurities" Offered:

	Americas/Far East	EMEA	India	China	Brazil	Total
FY2010	4	3				7
FY2011	5	5		2		12
FY2012	3	2	2	1		8
FY2013	1	4				5
Scheduled in FY2013	7	9		1		17
Total	20	23	2	4	0	49

Addendum 4:

Table A.2.1: Permitted Daily Exposures for Metal Impurities* from ICH Q3D pre-Stage 2 Draft

Metal	Class	Proposed Oral PDE, µg/day (50 kg bw)		Proposed Q3D Parenteral PDE, µg/day (50 kg bw)		Proposed Q3D Inhalation PDE, µg/day (50 kg bw)		USP Limits for LVP, µg/day
		ICH Q3D	USP	ICH Q3D	USP	ICH Q3D	USP	USP
As	1	15	1.5	15	1.5	15	1.5	0.15
Cd	1	5	25	5	2.5	5	1.5	0.25
Hg	1	50	15	5	1.5	5	1.5	0.15
Pb	1	5	5	5	5	5	5	0.5
Ag	2	3000	--	500	--	50	--	--
Au	2	10	--	10	--	5		--
Co	2	100	--	10	--	5		
Ir**	2	500	100	50	10	5	1.5	1.0
Mo	2	50	100	5	10	500	10	1.0
Ni	2	2000	500	100	50	5	1.5	5.0
Os**	2	500	100	50	10	5	1.5	1.0
Pd	2	100	100	10	10	5	1.5	1.0
Pt	2	500	100	50	10	5	1.5	1.0
Rh**	2	500	100	50	10	5	1.5	1.0
Ru**	2	500	100	50	10	5	1.5	1.0
Se	2	200	--	100	--	100	--	--
Tl	2	10	--	5	--	5	--	--
V	2	100	100	10	10	5	30	1.0
W	2	40000	--	20000		5000		
Al	3	50,000	--	Different regional regulations	--	5000	--	--
B	3	2000	--	2000		1000		
Ba	3	10000	--	1000		500		
Cr	3	10000	--	1000	--	10	25	--
Cu	3	1000	1000	100	100	15	100	10
Li	3	1000	--	500	--	25	--	--
Sb	3	1000	--	500	--	25	--	--
Sn	3	6000	--	500	--	50	--	--

*PDEs reported in this table are adjusted to a whole unit (µg/day). The calculated PDE is available in each respective safety assessment.

** Insufficient data to establish an appropriate PDE; the PDE was established based on platinum PDE.

-- not in USP chapter <232>

Table A2.2: Permitted concentrations of Metal Impurities from ICH Q3D pre-Stage 2 Draft

The values presented in this table represent permitted concentrations in micrograms per gram for metal impurities in drug products, drug substances and excipients. These concentration limits are intended to be used when Option 1 is selected to assess the metal impurity content in drug products with daily doses of not more than 10 grams per day.

Metal	Class	Proposed Option 1 Oral Concentration, µg/g		Proposed Option 1 Parenteral Concentration , µg/g		Proposed Option 1 Inhalation Concentration, µg/g	
		ICH	USP	ICH	USP	ICH	USP
As	1	1.5	0.15	1.5	0.15	1.5	0.15
Cd	1	0.5	2.5	0.5	0.25	0.5	0.15
Hg	1	5.0	1.5	0.5	0.15	0.5	0.15
Pb	1	0.5	0.5	0.5	0.5	0.5	0.5
Ag	2	300	--	50	--	5	--
Au	2	1.0	--	1.0	--	0.5	--
Co	2	10	--	1.0	--	0.5	--
Ir**	2	50	10	5.0	1.0	0.5	0.15
Mo	2	5.0	10	0.5	1.0	50	1.0
Ni	2	200	50	10	5.0	0.5	0.15
Os**	2	50	10	5.0	1.0	0.5	0.15
Pd	2	10	10	1.0	1.0	0.5	0.15
Pt	2	50	10	5.0	1.0	0.5	0.15
Rh**	2	50	10	5.0	1.0	0.5	0.15
Ru**	2	50	10	5.0	1.0	0.5	0.15
Se	2	20	--	10	--	10	--
Tl	2	1.0	--	0.5	--	0.5	--
V	2	10	10	1.0	1.0	0.5	3.0
W	2	4000		2000		500	
Al	3	5000	--	Different regional regulations	--	500	--
B	3	200	--	200	--	100	--
Ba	3	1000	--	100	--	50	--
Cr	3	1000	--	100	--	1.0	2.5
Cu	3	100	100	10	10	1.5	10
Li	3	100	--	50	--	2.5	--
Sb	3	100	--	50	--	2.5	--
Sn	3	600	--	50	--	5.0	--

** Insufficient data to establish an appropriate PDE; the PDE was established based on platinum PDE

-- Not in USP Chapter <232>

HEAVY METALS TEST CONSIDERATIONS

by Fred A. Morecombe
Chairman, Heavy Metals Task Force

The purposes of the collaborative study of the Heavy metals test were as follows:

1. To achieve greater uniformity in test methodology.
2. To reduce, if possible, the number of methods in current use.
3. To improve the precision, reproducibility, and methodology of the current procedures.
4. To eliminate the possibility of interference due to substances remaining from various manufacturing procedures (e.g., EDTA or similar complexing agents.)

Discussion by the Task Force members of the Method I procedure specified by the FCC, NF, and USP for those articles readily soluble in water indicated that there was concern about the possibility of chelating agents present in the sample leading to erroneous test results. Members of the Task Force were aware, for example, that EDTA is used in some manufacturing processes to remove iron and other metallic impurities, but it was not known whether traces of this or other chelating agents could be found in the final products. It was realized that if any chelating agent were present, by accident, design, or through ignorance, in the sample, a low result would be obtained when the Heavy metals Method I procedure was employed. A second concern was raised with respect to the possibility of the sample itself serving as a complexing agent. A final concern dealt with the possibility of precipitate or color formation taking place during the specified pH adjustment. In recognition of these concerns, the Task Force agreed to focus attention first upon Method I and to evaluate the suitability of the other methods in turn, after completing a study of Method I.

A three-tube monitor procedure was proposed by Grady (see elsewhere in this issue) as a simple way to determine interference due to chelation caused by either the sample itself or by added substances. It was recognized, however, that the proposed monitor procedure would not detect the presence of complexing agents present in amounts stoichiometrically equivalent or virtually equivalent to the heavy metals content, but it was felt that the possibility of such equivalence was sufficiently remote that it could be ignored at this time. Nevertheless, a variant of the three-tube procedure was developed (see next article) that was found to give satisfactory test results under these specific situations.

Collaborative study of the three-tube monitor procedure was approved by the Task Force, and members of the Task Force were requested to evaluate the three-tube monitor procedure on five articles of their choice. Four members tested only articles of their manufacture, one member tested only purchased articles, and one member tested both manufactured and purchased articles. In all, 44 separate articles were evaluated and, by coincidence, 22 were articles for which the manufacturing history was known, while 22 were articles purchased on the open market. In general, testing was limited to a single lot of each article, although in some cases, replicate lots were tested.

Results

A. The 33 articles listed below were found to be free of interference when tested by the three-tube monitor procedure:

Ascorbic Acid, USP
Aluminum Chloride, NF
Aminoacetic Acid, NF
Ammonium Citrate
Antipyrine, NF
Boric Acid, NF
Butylaminobenzoate, NF
Caffeine, USP
Calcium Chloride, USP
Calcium Pantothenate, USP
Calcium Propionate
Citric Acid, USP
Dicalcium Phosphate, Dihydrate, NF
Dextrose, Monohydrate, USP
Fructose, NF
Fumaric Acid
Magnesium Sulfate, USP
Mannitol, USP
Potassium Iodide, USP
Salicylic Acid, USP
Sodium Benzoate, USP
Sodium Borate, USP
Sodium Chloride, USP
Sodium Citrate, USP
Sodium Gluconate, FCC
Sodium Iodide, USP
Sodium Phosphate, Dried, NF
Sodium Acid Pyrophosphate, FCC
Sodium Tripolyphosphate, FCC
Sucrose, USP
Thenylene Fumarate, NF
Tyrosine * ?
Urea, USP

B. The 11 articles found to show interferences by the three-tube monitor procedure were as follows:

1. Glucose, USP
Twelve samples representing material from four producers were found to have interferences in that the monitor tube preparations showed little or no recovery of added lead. Four samples from a fifth producer showed no interferences. The presence of an added substance in the failing samples is suspected but not yet confirmed. No difficulties were encountered in carrying out USP Method II on some of the samples, and no test failures were noted using this method.

2. Glutamic Acid Hydrochloride, NF
The monitor tube preparation failed to show recovery of added lead, thus suggesting a complexation interference was present. In addition, a copious precipitate has been reported to develop after standing thirty minutes.

3. Nicotinamide, USP
Four samples representing material from two producers were found to have interferences in that the monitor tube preparations showed little or no recovery of added lead. A fifth sample from a third producer showed no interference. The significance of this difference in results has not been determined.

* Solubility problems have been reported.

4. Phenylpropanolamine Hydrochloride, NF
Five samples, all from the same producer, were tested and all showed interference, as evidenced by low recovery of added lead in the monitor preparations. The reason for the interference is unknown at this time. When tested by USP Method II, the samples all met the NF limit and there was excellent recovery of added lead.

5. Sodium and Potassium Citrates, USP-NF
A producer reported formation of a yellow color in potassium citrate sample preparation, and this color interfered with the test. Further studies on this article are needed. Another laboratory found one lot of sodium citrate to interfere with recovery of lead but not another lot.

6. Pyridoxine Hydrochloride, USP
The monitor tube preparation failed to show the presence of added lead, thus suggesting presence of a complexation interference.

7. Sodium Aluminum Phosphate, FCC
A producer reported that the specified pH adjustment resulted in precipitation of aluminum (presumably as the hydrous oxide) which appears to have scavenged the heavy metals from the solution, thus leading to low results when the solution is tested. Another laboratory, however, did not experience precipitation problems at the specified pH.

8. Sodium Cyclamate
A producer reported that five samples showed interference due, presumably, to internal complexing with the sample since it was known that complexing agents were not used in the manufacturing process. USP Method II was found to be a suitable alternate procedure and excellent recovery was obtained on samples containing known amounts of added lead.

9. Sodium Lauryl Sulfate, USP
Two samples from one producer showed a greenish color in solution, and this color interfered with the proper estimation of the sample turbidity. A single sample from a second producer showed no recovery of added lead in the monitor preparation.

10. Sodium Potassium Tartrate, NF
A precipitate was obtained when the pH was adjusted as specified, thus raising doubts as to the validity of the test procedure for this article.

11. Sorbitol Solution, USP
One out of five samples tested, all of which were from the same producer, failed to show recovery of added lead in the monitor preparation. Further studies are needed to confirm the suitability of the Method I procedure.

Discussion of Results

While it must be emphasized that the results reported herein are preliminary and subject to change as further testing is carried out and reported, they indicate

clearly that attention to the Method I uses is needed. If the 44 articles evaluated are truly representative of the 299 FCC, NF, and USP articles for which Method I is specified, there is an indication that revisions are needed for about 79 articles. At this time, it appears that the Method I difficulties fall into two categories: a misapplication of the method in that Method II would be a more appropriate selection, and a failure to appreciate the negative possibilities of metal complexation. Regardless of the category, however, it is clear that further studies are needed before any specific general conclusions can be drawn as to the need for revision of the official procedures. Admittedly, one solution would be to delete Method I entirely and substitute a method calling for preliminary ignition of all samples, but this would be a costly and inefficient solution that surely can be bettered if all concerned will work together to propose a realistic, and simpler, solution. On this premise, therefore, the following recommendations are proposed:

Recommendations

1. It is recommended that all articles now tested by Method I be evaluated by the three-tube monitor procedure to confirm the suitability of the method (i.e., no complexation due to the sample, no interfering colors, and no precipitation) for use with each article.

   Note: It is hoped that each producer of the articles involved will look at his products and report directly to the appropriate Director of Revision (Dr. D. Banes for NF and USP articles; Mr. Durward Dodgen for FCC articles) concerning the suitability, or lack of suitability, of the method for use with his products.

2. It is recommended that the three-tube monitor procedure, with or without the zirconium modification, be given consideration as a replacement for the current Method I procedure.

3. It is recommended that all future monographs specify use of Method II for Heavy metals determinations unless adequate evidence, including the results of recovery studies, is presented to support use of a different procedure.

* * *

Revision of the Magnesium Stearate Monograph and the Lactose Monograph

Zak T. Chowhan, *Chairman, Subcommittee on Excipients, USP Committee of Revision*

Introduction

The following revisions of the National Formulary monographs for Magnesium Stearate and Lactose are presented for comment. The revisions and comments presented in this article are based on discussions at the October 11–12, 1990, Magnesium Stearate/Lactose meeting at the USP headquarters and on discussions at the January 30–February 1, 1991, Joint Pharmacopeial Open Conference on International Harmonization of Excipient Standards in Orlando, Florida. Copies of these revised monographs and comments have been submitted to the European Pharmacopoeia (EP), British Pharmacopoeia (BP), and Japanese Pharmacopoeia (JP) for review. Comments for all interested parties regarding these monographs are welcomed by the Subcommittee on Excipients.

MAGNESIUM STEARATE

Octadecanoic acid, magnesium salt.
Magnesium stearate [*557-04-0*].

Magnesium Stearate consists mainly of magnesium salts of Stearic Acid ($C_{18}H_{36}O_2$), with substantially lesser amounts of magnesium salts of other fatty acids. The fatty acids are derived from edible sources. It contains not less than 4.1 percent and not more than 5.0 percent of Mg, calculated on the dried basis.

The limits for Mg in the BP/EP are 3.8% to 5.0%, while in the JP, the limits are 3.9% to 5.1%. The NF limits are expressed as MgO; this should be changed to Mg. The lower limit of Mg varies from 3.8% to 4.1%, and the upper limit varies from 5.0% to 5.1%. The BP/EP and the JP should consider tightening the limits to 4.1% to 5.0%.

Description and solubility: Very fine, light, white powder. Is unctuous and odorless or has a very faint odor of stearic acid. Insoluble in water, in absolute alcohol, and in ether.

Labeling—Label it to indicate particle size distribution using different screens and to indicate the specific surface area.

Particle size distribution and specific surface area are important parameters for consideration. The methods for the determination of particle size distribution and specific surface area need further discussion.

Identification—

A: Mix 25 g with 200 mL of hot water, add 60 mL of 2 *N* sulfuric acid, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Separate the aqueous layer, and retain it for *Identification test B*. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow to cool, and discard the water layer. Melt the acids, filter into a dry beaker while hot, and dry at 100° for 20 minutes: the solidification temperature of the fatty acids is not below 54°.

B: The aqueous layer obtained from the separated fatty acids in *Identification test A* responds to the test for *Magnesium* ⟨191⟩.

The identification tests in the NF, BP/EP, and JP are basically the same. The methodology can be harmonized, if so desired. The main difference is in the solidification temperature of the fatty acids, 53° vs. 54°. Since it is an indication of the amount of unsaturated fatty acids, for harmonization, a temperature of 54° rather than 53° should be considered. If the gas chromatographic method is adopted for the Fatty acid composition determination, Identification test A becomes redundant and should be deleted.

Microbial limits ⟨61⟩—The total bacterial count does not exceed 1000 per g, and the test for *Escherichia coli* is negative.

Only the NF requires a test for Microbial Limits. The rationale for this requirement is that the final step involves aqueous precipitation, which could be a source of microbial contamination.

Acidity or alkalinity—Mix 1 g with 20 mL of carbon dioxide–free water, boil for 1 minute, shaking continuously, cool, and filter. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS: not more than 0.05 mL of 0.1 *M* hydrochloric acid TS or 0.1 *M* sodium hydroxide TS is required to change the color of the solution.

This test is official only in the BP/EP. For harmonization, it is important to determine the importance of the test. If needed, the proposal is to include it in the NF and the JP; otherwise to delete it from the BP/EP.

Acid value of fatty acids ⟨401⟩: 195 to 210.

This limit test is required by the BP/EP only. This requirement should be considered only if a clear rationale can be found. Further discussions regarding the value of this requirement are needed for harmonization. If the gas chromatographic method is adopted for the Fatty acid composition determination, Identification test A becomes redundant and should be deleted.

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 6.0% of its weight. Products having more than 4.0% loss on drying must be labeled within ± 0.3%.

The loss on drying limit in the NF and the JP is not more than 4.0%, while in the BP/EP, the limit is 6.0%. The NF and the BP/EP specify drying temperatures of 105° or 100° to 105°, respectively, to a constant weight. The JP specifies drying at reduced pressure, over phosphorous pentoxide, for

4 hours, in order to protect magnesium stearate from melting at 105°. What is the effect of melting on loss on drying determination? If none, why use different conditions? There is a great deal of controversy dealing with the Loss on drying specification and the lubrication properties of Magnesium Stearate. The proposal of 6.0% loss on drying upper limit with a labeling requirement for products having more than 4.0% loss on drying should help the harmonization effort. The conditions for the determination of loss on drying should also be discussed and harmonized.

Solution A in the following tests for chloride and sulfate is prepared under Identification test A in the BP monograph for Magnesium Stearate, which reads as follows. "To 5.0 g add 50 mL of ether, 20 mL of 2M nitric acid and 20 mL of distilled water and heat under a reflux condenser until dissolution is complete. Allow to cool, separate the aqueous layer, and shake the ether layer with two 4-mL quantities of distilled water. Combine the aqueous layers, wash with 15 mL of ether, *and dilute to 50 mL with distilled water (Solution A)."*

Chloride ⟨221⟩—A 2-mL volume of *Solution A* diluted with water to 15 mL complies with the limit test for chlorides (250 ppm).

The limit test for chloride is official only in the BP/EP. A clear rationale is needed if this limit test is to be included in the other compendia.

Sulfate ⟨221⟩—A 0.3-mL volume of *Solution A* diluted with water to 15 mL complies with the limit test for sulfates (0.3%).

The limit test for sulfate is official only in the BP/EP. Sulfate is an atypical inorganic impurity originating from certain production processes. Most commercial grades pass the 0.1% limit on sulfate. A proposal has been made to limit the sulfate level to 0.3%.

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

The BP/EP and the JP require a test for heavy metals, while the NF requires a limit test for lead. It is important to limit the metallic impurities. A limit test for heavy metals would limit all heavy metals, not just lead. In addition, the test method for lead requires hazardous reagents, potassium cyanide and ammonium cyanide. Therefore, the limit test for lead should be deleted from the NF monograph. The limit test on heavy metals and the 20-ppm limit in the BP/EP and the JP should be adopted, thus harmonizing the requirements.

Cadmium, lead, and nickel—
Cadmium: not more than 3 ppm.
Lead: not more than 10 ppm.
Nickel: not more than 5 ppm.

Determine the amounts of cadmium, lead, and nickel present by flameless atomic absorption spectrophotometry, using the standard addition technique. Prepare the test solution by transferring about 40 mg of Magnesium Stearate, accurately weighed, into a teflon bomb, adding 0.5 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1). Allow to decompose for about 5 hours at about 170°, and then dissolve the residue in 5 mL of water. The following are the wavelengths, drying, ashing, and atomization conditions:

Equipment Parameter	Cadmium	Lead	Nickel
Wavelength (nm)	228.8	283.3	232.0
Drying (°C)	110	110	110
Ashing (°C)	450	450	1,000
Atomization (°C)	2,000	2,000	2,300

By far the largest amount of stearates is used for technical applications, where purity is of less importance. A clear differentiation between the technical and pharmaceutical grades is therefore essential. The results of tests on 49 samples obtained from worldwide sources reported a high content of nickel (up to 215 ppm) and cadmium (up to 108 ppm) in some samples. Nickel is used as a catalyst in the hydrogenation process, and cadmium content is a result of cross-contamination. The Heavy-metals test allows neither the detection of nickel nor the quantification of cadmium. Therefore, it is proposed that specific limits be established for these elements.

Packaging and storage—Preserve in well-closed containers.

Fatty acid composition—

System suitability preparation—Transfer about 50 mg each of USP Stearic Acid RS and USP Palmitic Acid RS, accurately weighed, to a small conical flask fitted with a suitable reflux attachment. Add 5.0 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL, swirl to mix, and reflux for 15 minutes until the solid is dissolved. Cool, transfer the reaction mixture to a 60-mL separator with the aid of chromatographic solvent hexane, and add 10 mL of water and 10 mL of saturated sodium chloride solution. Shake, allow the mixture to separate, and discard the lower, aqueous layer. Transfer the hexane layer to a suitable flask by passing through a funnel containing about 6 g of anhydrous sodium sulfate previously washed with chromatographic solvent hexane.

Test preparation—Prepare as directed for *System suitability preparation*, using a 100-mL portion of Magnesium Stearate in place of the Reference Standards.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a 254-nm detector and a 1.5-mm × 3-mm glass column that contains 15% phase G4 on support S1A. The column temperature is maintained at 165° and the injection port and detector are maintained at about 210°. Helium is used as the carrier gas at a flow rate of about 1.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for five replicate injections

is not more than 1.5%, and the resolution, R, between the methyl palmitate and methyl stearate peaks is not less than 2.0.

Procedure—Inject a volume (1 to 2 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Separately calculate the percentages of stearic acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$) in the portion of purified Magnesium Stearate taken by the same formula: $100(r_i/r_t)$, in which r_i is the response of the individual peak from methyl stearate or methyl palmitate, as appropriate, and r_t is the total of the peak responses of all of the fatty acid esters in the chromatogram. Magnesium Stearate contains not less than 40% stearate and a total of not less than 90% stearate and palmitate.

Gas chromatography is certainly the method of choice for reliable characterization of fatty acid composition. It makes the more time-consuming Acid value of fatty acids test redundant. Therefore, it is proposed that this test replace the Acid value of fatty acids test.

Reference standards—*USP Palmitic Acid Reference Standard*—Dry over silica gel for 4 hours before using. *USP Stearic Acid Reference Standard*—Dry over silica gel for 4 hours before using.

Assay—Boil about 1 g of Magnesium Stearate, accurately weighed, with 50 mL of 0.1 N sulfuric acid for about 30 minutes, or until the separated fatty acid layer is clear, adding water, if necessary, to maintain the original volume. Cool, filter, and wash the filter and the flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate with 1 N sodium hydroxide to litmus. While stirring, preferably with a magnetic stirrer, titrate with 0.05 M disodium ethylenediaminetetraacetate VS as follows. Add about 30 mL from a 50-mL buret, then add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and continue the titration to a blue endpoint. Each mL of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 1.2153 mg of Mg.

The Assay method is titrimetric in NF, BP/EP, and JP.

Lactose Monohydrate

Lactose Monohydrate is a natural disaccharide, obtained from milk, which consists of one glucose and one galactose moiety.

Description and solubility: White, odorless powder, freely but slowly soluble in water; practically insoluble in alcohol. It may be crystallized or sieved, ground or powdered.

Labeling—Label it to indicate particle size distribution.

Compendial methods for the determination of particle size distribution will be worked out. The three methods proposed by the suppliers are. Alpine Air Jet Sieve, Ro-Tap Sieve, and Laser instruments.

One method of particle size distribution determination may not be adequate for all grades of lactose or for all powders. If screening methodology is the method of choice, at least three size fractions should be examined. The Alpine Air Jet Sieve method should be required for a screen mesh size of less than 270.

Identification—

A: Add 5 mL of 1 N sodium hydroxide to 5 mL of a hot, saturated solution of Lactose Monohydrate, and gently warm the mixture: the liquid becomes yellow and finally brownish red. Cool to room temperature, and add a few drops of alkaline cupric tartrate TS: a red precipitate of cuprous oxide is formed.

Identification test A is currently official in the NF monograph for Lactose. Similar tests are official in the BP/EP and the JP.

B: Use the method for *Identification test A* in the BP monograph for *Lactose*, which reads as follows. "Carry out the method for thin-layer chromatography, Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *1,2-dichloroethane*, 25 volumes of *anhydrous glacial acetic acid*, 15 volumes of *methanol*, and 10 volumes of *water*, measured accurately, as the mobile phase. Apply separately to the chromatoplate 2 µL of each of three solutions in *methanol* (60%) containing (1) 0.05% w/v of the substance being examined, (2) 0.05% w/v of *lactose EPCRS* and (3) 0.05% w/v each of *fructose EPCRS*, *glucose EPCRS*, *lactose EPCRS* and *sucrose EPCRS*. Dry the points of application thoroughly before developing the chromatogram. After removal of the plate, dry it in a current of warm air and repeat the development after renewing the mobile phase. After removal of the plate, dry it in a current of warm air, spray with a 0.5% w/v solution of *thymol* in *ethanolic sulphuric acid* (5%) and heat at 130° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows four clearly separated principal spots."

Identification test B is official only in the BP/EP. This test eliminates the need for testing for glucose, sucrose, starch or dextrin, as required by the JP.

The differential scanning calorimetry test, which is a specific identification test for differentiating between alpha and beta lactose, is not included in this proposal because at the Joint Pharmacopeial Open Conference, the consensus was that in pharmaceutical applications the differentiation between alpha and beta lactose is not important.

A proposal to replace identification tests A and B with an infrared spectroscopy (potassium bromide disc) test has been made. The advantages of this proposal are (1) preference of the registration authorities for a more specific test, (2) one reference standard is used instead of four, and (3), no chlorinated solvents are needed for the test.

Clarity and color of solution—A solution of 1 g in 10 mL of boiling water is clear and odorless. Determine the ab-

sorbance of this solution at a wavelength of 400 nm in 1-cm cuvettes. The absorbance of a 10% (w/v) solution is not more than 0.04.

The NF, BP/EP, and JP use different concentrations of solution for this test. This can easily be harmonized. The other difference is visual or subjective examination of the color of the solution in NF and JP versus semiquantitative color matching with a reference standard solution in BP/EP. A quantitative determination of absorbance at 400 nm is definitely desirable and is proposed. The limit on absorbance is necessary to control the maximum color allowed. The sources of turbidity and color are remnants of proteinaceous matter, remnants of yeast and molds, insoluble burnt matter, residual riboflavin from whey, etc.

Acidity and alkalinity—Dissolve 30 g by heating in 100 mL of carbon dioxide–free water, and add 10 drops of phenolphthalein TS: the solution is colorless, and not more than 1.5 mL of 0.1 *N* sodium hydroxide are required to produce a red color.

The acidity and alkalinity requirement is designed to prevent production of lactose from sour whey and to limit the milk salts. The JP limit is too lenient. The NF limit is slightly more stringent than the BP/EP limit. It is proposed to use the NF limit for harmonization.

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

This test gives an idea of the purity of lactose. The limits in NF, BP/EP, and JP are the same. This may be a study case, where the terminology of the test method needs harmonization. The temperature of ignition should be harmonized. The ignition temperature of 600° is preferred due to the possible loss of cadmium at 800°.

Water, *Method I* ⟨921⟩: between 4.5% and 5.5%.

The method should be modified in view of the fact that methanol is a poor solvent for lactose. Formamide improves solubility of polar substances and is therefore preferred for the determination of water in lactose. Formamide is a better solvent and is less toxic than dimethylformamide.

Loss on drying ⟨731⟩—Dry it at 80° for two hours: it loses not more than 0.5% of its weight.

The water in lactose originates from the water of crystallization and free adsorbed water. The Karl Fischer titration is used for water of crystallization and loss on drying is used for free adsorbed water. The JP conditions of 80° and two hours are adequate to determine the free and adsorbed water.

In order to harmonize the differences between the NF, BP/EP, and the JP, it is proposed to include water and loss on drying limits.

Heavy metals ⟨231⟩—Dissolve 4 g in 20 mL of warm water, add 1 mL of 0.1 *N* hydrochloric acid, and dilute with water to 25 mL: the limit is 5 ppm.

It is important to limit the metallic impurities. The sources of these impurities are milk, whey, and the processing equipment. A limit test on heavy metals should limit all heavy metals (silver, arsenic, bismuth, cadmium, copper, mercury, lead, antimony, and tin). In addition, the test method for lead requires hazardous reagents: potassium cyanide and ammonium cyanide. Therefore, for the desired harmonization, it is proposed that the BP/EP change the requirements from lead to heavy metals and that the NF and the JP limits be used.

Specific optical rotation ⟨781⟩: between +54.8° and +55.5°, calculated on the anhydrous basis, determined at 20° in a solution containing 10 g of lactose and 0.2 mL of 6 *N* ammonium hydroxide in each 100 mL.

The terminology, specific optical rotation, optical rotation, and specific rotation, needs harmonization. It is proposed that the compendia adopt the term "Specific Optical Rotation."

In solution, lactose (alpha and beta) is subject to mutarotation. The equilibrium theoretical value reported on page C-395 in the CRC Handbook of Chemistry and Physics, 61st edition, for lactose monohydrate at 20° is +52.3°. This corresponds to +55.1° for anhydrous. Therefore, it is proposed that the compendia adopt a range of +55.1° to +55.8°. However, the data from the lactose producers and one of the users do not justify a tighter specification. Therefore, it is proposed that the current NF specifications be adopted.

Protein and light-absorbing impurities ⟨851⟩: Measure the *light absorption* of a 1% (w/v) solution in the range of 210 to 300 nm. The *absorbance* is not more than 0.25 in the range of 210 to 220 nm and is not more than 0.03 in the range of 270 to 300 nm.

Only the BP/EP has the limit test, which is an important purity test to limit carmelization products, proteins and proteinaceous matter, and hydroxymethylfurfural (degradation product of lactose), and turbidity. It is proposed that the NF and the JP adopt this test. It is also desirable to lower the maximum absorption limit to 0.03 (between 270 and 300 nm from 0.07 as official in the BP/EP).

Microbial limits ⟨61⟩—The total bacterial count does not exceed 100 per g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

This is an important test for lactose that is intended for pharmaceutical use. Since the test method described in International Standard ISO/DIS 6579 requires less time to perform and requires less expensive test media, it has been referred to the Microbiology Subcommittee for consideration. It is proposed that the JP and BP adopt microbial limits.

Packaging and storage—Preserve in well-closed containers.

Lactose Monohydrate Modified

Lactose Monohydrate Modified is obtained by a specified processing of Lactose Monohydrate. It may contain not more than 20 percent of amorphous lactose.

Description and solubility: White, odorless, free-flowing powder. Freely but slowly soluble in water; practically insoluble in alcohol.

Labeling—Label it to indicate the method of modification, particle size distribution, and specific surface area.

Compendial methods for the determination of particle size distribution need to be worked out. The three methods proposed by the suppliers are Alpine Air Jet Sieve, Ro-Tap Sieve, and Laser instruments.

One method of particle size distribution determination may not be adequate for all grades of lactose or for all powders. If screening methodology is the method of choice, at least three size fractions should be examined. The Alpine Air Jet Sieve method should be required for a screen mesh size of less than 270.

It was also proposed that a compendial method for the determination of specific surface area should be worked out. Since lactose monohydrate is modified by different methods to perform a specific function, it was proposed that the label indicate the specific surface area and the process of modification.

Identification—It responds to *Identification tests A* and *B* under *Lactose Monohydrate.*

Loss on drying ⟨731⟩—Dry it at 80° for two hours: it loses not more than 1.0% of its weight.

Specific surface area: within ±10% of the label claim.

Packaging and storage—Preserve in tight containers.

Other requirements—It meets the requirements for *Clarity and color of solution, Acidity and alkalinity, Residue on ignition, Water, Heavy metals, Specific optical rotation, Protein and light-absorbing impurities,* and *Microbial limits* under *Lactose Monohydrate.*

Anhydrous Lactose

Anhydrous Lactose is primarily alpha lactose, beta lactose, or a mixture of alpha and beta lactose.

Description and solubility: White, odorless powder. Freely but slowly soluble in water; practically insoluble in alcohol.

Identification—It responds to *Identification tests A* and *B* under *Lactose Monohydrate.*

Loss on drying ⟨731⟩—Dry it at 80° for 2 hours: it loses not more than 0.1% of its weight.

Other requirements—It meets the requirements for *Labeling, Clarity and color of solution, Acidity and alkalinity, Residue on ignition, Heavy metals, Specific optical rotation, Protein and light-absorbing impurities, Microbial limits,* and *Packaging and storage* under *Lactose Monohydrate.*

* * *

and make any necessary correction. Each mL of 0.1 *N* sodium hydroxide is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.

Description and Solubility

Add the following:

Ursodiol: White or almost white crystalline powder. Practically insoluble in water; freely soluble in alcohol and in glacial acetic acid; sparingly soluble in chloroform; slightly soluble in ether.

MONOGRAPHS (NF XVII)

Anhydrous Lactose—See briefing under *Lactose Monohydrate.*

7L00110 (EXC) RTS—10481-01

Add the following:

Anhydrous Lactose

» Anhydrous Lactose is primarily alpha lactose, beta lactose, or a mixture of alpha and beta lactose.

Description and solubility: White powder. Freely but slowly soluble in water; practically insoluble in alcohol.

Reference standard—*USP Anhydrous Lactose Reference Standard*—Dry at 80° for 2 hours before using.

Identification—The infrared absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Anhydrous Lactose RS.

Loss on drying ⟨731⟩—Dry it at 80° for 2 hours: it loses not more than 0.1% of its weight.

Water, *Method I* ⟨921⟩: not more than 1.0%.

Particle size distribution: within ±10% of the label claim.

Other requirements—It meets the requirements for *Packaging and storage, Labeling, Clarity and color of solution, Specific optical rotation* ⟨781⟩, *Microbial limits* ⟨61⟩, *Acidity and alkalinity, Residue on ignition* ⟨281⟩, *Heavy metals* ⟨231⟩, *Organic volatile impurities, Method V* ⟨467⟩, and *Protein and light-absorbing impurities* under *Lactose Monohydrate.*

Lactose Monohydrate; Lactose Monohydrate Modified, Anhydrous Lactose; Magnesium Stearate, NF XVII page 1945 and page 3414 of *PF* 18(3) [May–June 1992].

The addition of three new proposed monographs, *Lactose Monohydrate, Lactose Monohydrate Modified,* and *Anhydrous Lactose* and the revisions proposed for *Magnesium Stearate* are based on comments and data received in reponse to the Stimuli article published on pages 2419–2423 of *PF* 17(5) [Sept.–Oct. 1991]. Additional information is required before certain proposals, as indicated in this Briefing, can be adopted, and responses from interested parties are invited.

In the interest of international harmonization, a section on *Description and solubility* will be incorporated into each monograph after the Definition. This style change in the monograph format is subject to the approval of the DSD Executive Committee. All questions and comments concerning the inclusion of the *Description and solubility* section within the monographs are welcomed.

In the proposed *Lactose Monohydrate* monograph and by reference in the proposed *Anhydrous Lactose* monograph, the *Labeling* section has been revised from the one in the Stimuli article to indicate that the nominal value as well as the test method used to determine the particle size distribution is to be labeled. A compendial method for the de-

termination of particle size distribution needs to be finalized. The Stimuli *Identification* tests for these monographs have been replaced by an infrared (IR) spectroscopy test for the reasons stated in the Stimuli article. Verification that IR will distinguish the three types of lactose is required. The proposed Stimuli *Residue on ignition* test for *Lactose Monohydrate* and by reference in the proposed *Lactose Monohydrate Modified* and *Anhydrous Lactose* monographs remains unchanged. As written, a test temperature of 800° is required. The *EP* method specifies a 600° test temperature, thus possibly providing a more stringent test since the higher temperature used in the USP test could lead to the loss of volatile substances. Comments were received indicating that if 600° is employed as the ignition temperature, the residue is commonly greater than 0.1%. The Subcommittee felt that the 800° test temperature should be retained until the explanation for these different results is known. Reader comment on this topic is invited. The proposed Stimuli *Water* test remains unchanged. It was reported that methanol, not formamide, is the preferred solvent from a safety viewpoint for this test. A *Water* test has been added to the proposed *Anhydrous Lactose* monograph based on comments that the total water content value is needed in order to calculate the specific optical rotation on the anhydrous basis, and apparently some water is present that is not volatilized at 80° during the loss on drying procedure. The proposed test limit agrees with the water limit specified for anhydrous lactose in the current *Lactose* NF monograph. Comments were received regarding the lack of sensitivity and lack of specificity of the proposed Stimuli *Heavy metals* test. It was reported, however, that iron does react in the *Heavy metals* test, generating color equal to approximately one-fifth the corresponding amount of lead. For example, 50 ppm of iron will give the same amount of color as a 10 ppm lead standard. On this basis, the proposed *Heavy metals* test with a limit of 5 ppm should sufficiently control the amount of metallic impurities in lactose. The Subcommittee is aware also that the *Heavy metals* test has proven to be satisfactory for many years, and has approved the proposed *Heavy metals* test for these monographs. However, the Subcommittee acknowledges the need to study this topic further, and welcomes the submission of proposals involving other procedures, notably atomic absorption, for consideration for future revision. The proposed upper limit in the *Specific optical rotation* test was changed from ±55.5° to ±55.8° based on comments that the range of ±54.8° to ±55.5° in the current NF *Lactose* monograph is too restrictive. The *Organic volatile impurities* test was previously proposed on page 3414 of *PF* 18(3) [May–June 1992], scheduled for the Eighth Supplement, and is republished in this proposal for completeness of the monograph. In the proposed Stimuli *Protein and light-absorbing impurities* test, the absorbance limit in the range of 270 to 300 nm has been changed from 0.03 to 0.07. This change is based on data supporting the 0.07 maximum limit as being suitable for commercially available material. The proposed 0.07 maximum limit conforms with the limit in the current *EP* monograph for Lactose.

The *Labeling* section of the proposed *Lactose Monohydrate Modified* monograph has been revised to include labeling of the nominal test values, as well as the test methods used to obtain each of the indicated values. Compendial methods for the determination of particle size distribution and specific surface area need to be finalized. The Stimuli *Identification* tests in this proposed monograph have been changed to the preferred infrared spectroscopy test for the reasons stated in the Stimuli article. The proposed Definition specifies a limit of not more than 20 percent of amorphous lactose. However, a method for determining the amorphous lactose content has not been finalized by the Subcommittee. It has been suggested that a powder X-ray diffraction procedure may be best suited for this determination with lactose since this method detects crystal changes in a relatively noninvasive manner and is really the most readily available technique in most companies, compared with solution calorimetry, helium pycnometry, and differential scanning calorimetry. Differential scanning calorimetry would be a second choice, assuming that it is possible to get the amorphous lactose to recrystallize quantitatively as it is heated above the glass transition temperature. The Subcommittee welcomes comments and suggested test procedures on this topic.

In the *Magnesium Stearate* monograph, the proposal to revise the Definition was a necessary consequence of the proposal to change *Identification test B.* The proposed lower limit for Mg was changed from 4.1 percent to 4.0 percent, on the basis of data from samples of worldwide sources. The proposed Stimuli *Labeling* section has been revised to indicate that the nominal values as well as the test methods used to obtain the test values are to be labeled. The compendial methods for the determination of particle size distribution and specific surface area need to be finalized. The Stimuli *Identification tests* have been revised to include a gas chromatographic fatty acid composition test and to delete the solidification temperature test. Gas chromatography (GC) is reported to be the method of choice for the reliable and comprehensive characterization of the fatty acid fraction in magnesium stearate. As a consequence of this proposal to adopt the GC fatty acid composition test, the Stimuli *Identification test* for solidification temperature and the Stimuli *Acid value of fatty acids* test, which provide limited and nonspecific information about the distribution of fatty acids present in magnesium stearate, will not be proposed. The test for *Microbial limits* is revised editorially. As stated in the Stimuli article, this test is included in the revision proposal because the final step in the production of magnesium stearate may involve aqueous precipitation, which could be a source of microbial contamination. The *Acidity or alkalinity* test suggested in the Stimuli article will not be proposed based on comments that the test is misleading and unnecessary. The current *Loss on drying* limit remains unchanged. The 6.0% limit suggested in the Stimuli draft was an effort to harmonize with EP's current standard. EP now has agreed with the 4.0% limit. The proposed tests for *Chloride* and *Sulfate* in the Stimuli draft will not be proposed based on comments that these tests are unnecessary and test failures are not indicative of a poor production process. The Stimuli draft *Heavy metals* test will not be proposed because nickel, which is used as a catalyst in the hydrogenation process (a major contaminant found in technical grade), is not detected by the *Heavy metals* test using the hydrogen sulfide

method. In addition, other heavy metals, such as arsenic, cadmium, antimony, and tin cannot be quantified against a lead standard. For the reasons stated in the Stimuli article, it is proposed to delete the current *Lead* test and to replace it with the Stimuli test for *Cadmium, lead, and nickel.* It is noted that this proposed test specifies two different ashing temperatures. Clarification of this procedure, i.e., whether or not separate determinations are required, is needed. The *Fatty acid composition* test suggested in the Stimuli article will not be proposed. It has been replaced by the gas chromatographic *Identification test B* procedure in the current proposal. The referenced *Fatty Acid Composition* procedure in this test was proposed on pages 3432–3433 of *PF* 18(3) [May–June 1992]. The *Reference standards* section of the Stimuli draft will not be proposed since these reference standards are not needed in the current proposal. The proposed Stimuli *Assay* is revised to indicate that each mL of titrant is equivalent to 1.215 mg, not 1.2153 mg, of Mg. This change is consistent with the text in the current *Magnesium Salicylate* monograph assay.

The revisions presented in these proposals reflect the Committee of Revision's efforts to develop harmonized monographs for the subject articles. Issues requiring additional data and comments have been noted. The Subcommittee on Excipients invites comments regarding these revision proposals.

7L00120 (EXC) RTS—10479-01

Add the following:

Lactose Monohydrate

» Lactose Monohydrate is a natural disaccharide, obtained from milk, which consists of one glucose and one galactose moiety.

Description and solubility: White powder, freely but slowly soluble in water; practically insoluble in alcohol. It may be crystallized or sieved, ground or powdered.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate the nominal particle size distribution and the test method used to obtain this value.

Reference standard—*USP Lactose Monohydrate Reference Standard*—Dry at 80° for 2 hours before using.

Clarity and color of solution—A solution of 1 g in 10 mL of boiling water is clear and colorless or nearly colorless. Determine the absorbance of this solution at a wavelength of 400 nm in 1-cm cuvettes. The absorbance of a 10% (w/v) solution is not more than 0.04.

Identification—The infrared absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Lactose Monohydrate RS.

Specific optical rotation ⟨781⟩: between +54.8° and +55.8°, calculated on the anhydrous basis, determined at 20° in a solution containing 10 g of lactose and 0.2 mL of 6 *N* ammonium hydroxide in each 100 mL.

Microbial limits ⟨61⟩—The total bacterial count does not exceed 100 per g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

Acidity and alkalinity—Dissolve 30 g by heating in 100 mL of carbon dioxide–free water, and add 10 drops of phenolphthalein TS: the solution is colorless, and not more than 1.5 mL of 0.1 *N* sodium hydroxide are required to produce a red color.

Loss on drying ⟨731⟩—Dry it at 80° for 2 hours: it loses not more than 0.5% of its weight.

Water, *Method I* ⟨921⟩: between 4.5% and 5.5%.

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

Particle size distribution: within ±10% of the label claim.

Heavy metals ⟨231⟩—Dissolve 4 g in 20 mL of warm water, add 1 mL of 0.1 *N* hydrochloric acid, and dilute with water to 25 mL: the limit is 5 ppm.

Organic volatile impurities, *Method V* ⟨467⟩: meets the requirements.

Protein and light-absorbing impurities ⟨851⟩—Measure the light absorption of a 1% (w/v) solution in the range of 210 to 300 nm. The absorbance is not more than 0.25 in the range of 210 to 220 nm and is not more than 0.07 in the range of 270 to 300 nm.

Lactose Monohydrate Modified—See briefing under *Lactose Monohydrate.*

7L00130 (EXC) RTS—10480-01

Add the following:

Lactose Monohydrate Modified

» Lactose Monohydrate Modified is obtained by a specified processing of Lactose Monohydrate. It may contain not more than 20 percent of amorphous lactose.

Description and solubility: White, free-flowing powder. Freely but slowly soluble in water; practically insoluble in alcohol.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate the method of modification, the nominal particle size distribution, the nominal specific surface area, and the test methods used to obtain these values.

Reference standard—*USP Lactose Monohydrate Modified Reference Standard*—Dry at 80° for 2 hours before using.

Identification—The infrared absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Lactose Monohydrate Modified RS.

Loss on drying ⟨731⟩—Dry it at 80° for 2 hours: it loses not more than 1.0% of its weight.

Particle size distribution: within ±10% of the label claim.

Specific surface area: within ±10% of the label claim.

Other requirements—It meets the requirements for *Clarity and color of solution, Specific optical rotation* ⟨781⟩, *Microbial limits* ⟨61⟩, *Acidity and alkalinity, Water, Method I* ⟨921⟩, *Residue on ignition* ⟨281⟩, *Heavy metals* ⟨231⟩, *Organic volatile impurities, Method V* ⟨467⟩, and *Protein and light-absorbing impurities,* under *Lactose Monohydrate.*

Magnesium Stearate, NF XVII page 1945 and page 3414 of *PF* 18(3) [May–June 1992]—See briefing under *Lactose Monohydrate.*

7M00300 (EXC) RTS—10478-01

Change to read:

» Magnesium Stearate is a compound of magnesium with a mixture of solid organic acids, ~~obtained from fats, and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate. It contains the equivalent of not less than 6.8 percent and not more than 8.3 percent of MgO.~~

and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate. The fatty acids are derived from edible

sources. It contains not less than 4.0 percent and not more than 5.0 percent of Mg, calculated on the dried basis.

Add the following:

Description and solubility: Very fine, light, unctuous, white powder. Insoluble in water, in absolute alcohol, and in ether.

Add the following:

Labeling—Label it to indicate the nominal particle size distribution using different screens, and to indicate the nominal specific surface area. Also label it to indicate the test methods used to obtain these values.

Change to read:

Identification—

A: Mix 25 g with 200 mL of hot water, then add 60 mL of 2 *N* sulfuric acid, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Separate the aqueous layer: ~~and retain it for *Identification test B*. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow to cool, and discard the water layer. Then melt the acids, filter into a dry beaker while hot, and dry at 100° for 20 minutes: the solidification temperature of the fatty acids is not below 54°.~~

the aqueous layer responds to the test for *Magnesium* ⟨191⟩.

B: ~~The aqueous layer obtained from the separated fatty acids in *Identification test A* responds to the test for *Magnesium* ⟨191⟩.~~

Using about 10 g of the fatty acids separated in *Identification test A*, proceed as directed in the procedure for *Fatty Acid Composition* under *Fats and Fixed Oils* ⟨401⟩: the stearate peak comprises not less than 40%, and the sum of the stearate and palmitate peaks is not less than 90% of the total area of all peaks.

Change to read:

Microbial limits ⟨61⟩—The total ~~bacterial count does not exceed 1000 per g and the test for *Escherichia coli* is negative.~~

microbial count does not exceed 1000 per g, and it meets the requirements of the test for absence of *Escherichia coli*.

Add the following:

Particle size distribution: within ± 10% of the label claim.

Add the following:

Specific surface area: within ± 10% of the label claim.

Delete the following:

~~**Lead** ⟨251⟩—Ignite 0.50 g in a silica crucible in a muffle furnace at 475° to 500° for 15 to 20 minutes. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and again ignite at 475° to 500° for 30 minutes. Dissolve the residue in 1 mL of a mixture of equal parts by volume of nitric acid and water, and wash into a separator with several successive portions of water. Add 3 mL of *Ammonium citrate solution* and 0.5 mL of *Hydroxylamine hydrochloride solution*, and render alkaline to phenol red TS with ammonium hydroxide. Add 10 mL of *Potassium cyanide solution*. Immediately extract the solution with successive 5-mL portions of *Dithizone extraction solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 seconds with 20 mL of 0.2 *N* nitric acid, and discard the chloroform layer. Add to the acid solution 4.0 mL of the *Ammonia-cyanide solution* and 2 drops of *Hydroxylamine hydrochloride solution*. Add 10.0 mL of *Standard dithizone solution*, and shake the mixture for 30 seconds. Filter the chloroform layer through an acid-washed filter paper into a color-comparison tube, and compare the color with that of a standard solution prepared as follows. To 20 mL of 0.2 *N* nitric acid add 5 µg of lead, 4 mL of *Ammonia-cyanide solution* and 2 drops of *Hydroxylamine hydrochloride solution*, and shake with 10.0 mL of *Standard dithizone solution* for 30 seconds. Filter through an acid-washed filter paper into a color-comparison tube. The color of the sample solution does not exceed that in the control (0.001%).~~

Add the following:

Cadmium, lead, and nickel—

Cadmium: not more than 3 ppm.

Lead: Not more than 10 ppm.

Nickel: not more than 5 ppm.

Determine the amounts of cadmium, lead, and nickel present by flameless atomic absorption spectrophotometry using the standard addition technique (see *Spectrophotometry and Light-scattering* ⟨851⟩). Prepare the test solution transferring about 40 mg of Magnesium Stearate, accurately weighed, into a teflon bomb, and adding 0.5 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1). Allow to decompose for about 5 hours at about 170°, and then dissolve the

residue in 5 mL of water. The following are the wavelengths, drying, ashing, and atomization conditions:

Equipment Parameter	Cadmium	Lead	Nickel
Wavelength (nm)	228.8	283.3	232.0
Drying temperature	110°	110°	110°
Ashing temperature	450°	450°	1000°
Atomization temperature	2000°	2000°	2300°

Change to read:

Assay—Boil about 1 g of Magnesium Stearate, accurately weighed, with 50 mL of 0.1 *N* sulfuric acid for about 30 minutes, or until the separated fatty acid layer is clear, adding water, if necessary, to maintain the original volume. Cool, filter, and wash the filter and the flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate with 1 *N* sodium hydroxide to litmus. While stirring, preferably with a magnetic stirrer, titrate with 0.05 *M* disodium ethylenediaminetetraacetate VS as follows. Add about 30 mL from a 50-mL buret, then add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and continue the titration to a blue endpoint. Each mL of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to ~~2.015 mg of MgO.~~

1.215 mg of Mg.

GENERAL CHAPTERS

General Information

⟨1111⟩ Microbiological Attributes of Nonsterile Pharmaceutical Products, USP XXII page 1684. On the basis of discussions and recommendations made at the USP Open Conference on Microbiology and Sterilization Issues (Marco Island, Florida, Nov. 29–Dec. 1, 1990), the MCB Subcommittee proposes that this general informational chapter be modified to provide a uniform guideline for the development and implementation of microbiological attributes to raw materials, excipients, and nonsterile pharmaceutical products.

The proposal includes a 2-tier system with appropriate alert and action levels and guidelines for the assignment of microbial attributes based on the source of raw materials (synthetic or natural) and, for nonsterile pharmaceuticals, on the route of administration (inhalants, topical, vaginal, otic, nasal, rectal, or oral).

4M01300 (MCB) RTS—10345-01

Change to read:

⟨1111⟩ MICROBIOLOGICAL ATTRIBUTES OF ~~NON-STERILE PHARMACEUTICAL PRODUCTS~~ PHARMACEUTICAL RAW INGREDIENTS, EXCIPIENTS, DRUG SUBSTANCES, AND NONSTERILE DOSAGE FORMS

GENERAL CONSIDERATIONS

~~Few raw materials used in making pharmaceutical products are sterile as received, and special treatment may be required to render them microbiologically acceptable for use. Strict adherence to effective environmental control and sanitation, equipment cleaning practices, and good personal hygiene practices in pharmaceutical manufacture is vital in minimizing both the type and the number of microorganisms.~~

~~Monitoring, in the form of regular surveillance, should include an examination of the microbiological attributes of Pharmacopeial articles and a determination of compliance with such microbiological standards as are set forth in the individual monographs. It may be necessary also to monitor the early and intermediate stages of production, with emphasis being placed on raw materials, especially those of animal or botanical origin, or from natural mineral sources, which may harbor objectionable microorganisms not destroyed during subsequent processing. It is essential that ingredients and components be stored under conditions designed to deter microbial proliferation.~~

~~The nature and frequency of testing vary according to the product. Monographs for some articles require freedom from one or more species of selected indicator microorganisms such as *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. For some articles, a specific limit on the total aerobic count of viable microorganisms and/or the total combined molds and yeasts count is set forth in the individual monograph; in these cases a requirement for freedom from specified indicator microorganisms may also be included. The significance of microorganisms in nonsterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user. Also taken into account is the processing of the product in relation to an acceptable quality for pharmaceutical purposes.~~

~~It is suggested that certain categories of products should be tested routinely for total microbial count and for specified indicator microbial contaminants, e.g., natural, plant, animal, and some mineral products for *Salmonella* species; oral solutions and suspensions for *E. coli*; articles applied topically for *P. aeruginosa* and *S. aureus*; and articles intended for rectal, urethral, or vaginal administration for yeasts and molds.~~

~~Definitive microbial limits (stipulated microorganisms and/or counts) are incorporated into specific monographs on the basis of a major criterion, i.e., the potential of the stipulated microorganisms and/or counts, and of any others that they may reflect, to constitute a hazard in the end product. Such considerations also take into account the processing to which the product components are subjected, the current technology for testing, and the availability of desired quality material. Any of these may preclude the items from specific requirements under *Microbial Limit*~~

three-quarters of the plate. Remove the plate from the chamber, dry in a current of warm air, and redevelop the plate in fresh *Developing solvent*. Remove the plate from the chamber, mark the solvent front, and dry the plate in a current of warm air. Spray the plate evenly with a solution containing 0.5 g of thymol in a mixture of 95 mL of alcohol and 5 mL of sulfuric acid. Heat the plate at 130° for 10 minutes: the principal spot obtained from the *Test solution* corresponds in appearance and R_f value to that obtained from *Standard solution A*. The test is not valid unless the chromatogram obtained with *Standard solution B* shows four clearly separated spots.

C: Dissolve 250 mg in 5 mL of water. Add 3 mL of ammonium hydroxide, and heat in a water bath at 80° for 10 minutes: a red color develops.

Specific ~~optical~~ rotation ⟨781⟩: ~~between +54.8° and +55.8°, calculated on the anhydrous basis, determined at 20° in a solution containing 10 g of lactose and 0.2 mL of 6 *N* ammonium hydroxide in each 100 mL.~~ 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 minutes, and dilute with water to 100 mL: the specific rotation, calculated on the anhydrous basis, determined at 20°, is between +54.4° and +55.9°.

Microbial limits ⟨61⟩—The total ~~bacterial~~ aerobic microbial count does not exceed 100 per g, the total combined molds and yeasts count does not exceed 50 per g, and it meets the requirements of ~~and~~ the tests for absence of *Salmonella* species and *Escherichia coli*. ~~are negative~~.

Acidity ~~and~~ or alkalinity—Dissolve ~~30~~ 6 g by heating in ~~100~~ 25 mL of carbon dioxide–free water, cool, and add ~~10 drops~~ 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than ~~1.5~~ 0.4 mL of 0.1 *N* sodium hydroxide ~~are~~ is required to produce a red color.

Loss on drying ⟨731⟩—Dry it at 80° for 2 hours: ~~it~~ the monohydrate form loses not more than 0.5% of its weight, and the modified monohydrate form loses not more than 1.0% of its weight.

Water, *Method I* ⟨921⟩: between 4.5% and 5.5%, formamide being used as the solvent.

Residue on ignition ⟨281⟩: not more than 0.1%, determined on a specimen ignited at a temperature of 600 ± 25°.

~~**Particle size distribution:** within ±10% of the label claim.~~

Heavy metals ⟨231⟩—Dissolve 4 g in 20 mL of warm water, add 1 mL of 0.1 *N* hydrochloric acid, and dilute with water to 25 mL: the limit is 5 ppm.

Organic volatile impurities, *Method V* ⟨467⟩: meets the requirements.

Protein and light-absorbing impurities ⟨851⟩—Measure the light absorption of a 1% (w/v) solution in the range of 210 to 300 nm. The absorbance is not more than 0.25 in the range of 210 to 220 nm and is not more than 0.07 in the range of 270 to 300 nm.■9

(Official January 1, 1994)

Magnesium Stearate, *NF XVII* page 1945 and page 3594 of *PF* 18(4) [July–Aug. 1992]. The proposals presented in this *PF* reflect the Committee of Revision's efforts to develop a harmonized monograph for the subject article and are based on comments and data received in response to the proposals in *Pharmacopeial Previews*.

Because of major differences between these new proposals and those presented in *Pharmacopeial Previews*, the *Pharmacopeial Previews* will NOT be forwarded to *In-process Revision*. These new proposals include (1) removal of the *Description and solubility* section from the monograph; (2) revision of the *Packaging and storage* section to specify a tight container; (3) proposal of a new *Labeling* text; (4) replacement of the two previously proposed *Identification* tests with two new tests; (5) revision of the *Microbial limits* test to include specifications for total combined molds and yeasts and for absence of *Salmonella* species; (6) revision of the *Loss on drying* test to specify a 6.0% limit; (7) addition of new tests for *Acidity or alkalinity*, *Limit of chloride* and for *Limit of sulfate;* (8) deletion of the previously proposed tests for *Particle size distribution* and for *Specific surface area;* (9) inclusion of more detailed procedures for the proposed atomic absorption spectrophotometric tests for cadmium, lead, and nickel; (10) proposal of a new gas chromatographic test for *Relative content of stearic acid and palmitic acid*, based on analyses performed with the Stabilwax-DB brand of G16 column resulting in typical retention times for methyl palmitate and methyl stearate of about 24.5 and 28.5 minutes, respectively; and (11) replacement of the present *Assay* with a new procedure based on the *Assay* in the European Pharmacopoeia monograph.

Because of the wide international usage of this material, it is desirable to have an official harmonized monograph at the earliest possible date. These proposals are therefore designated for publication in the *Tenth Supplement* to *USP XXII–NF XVII*.

7M00300 (EXC) RTS—9947-04; 12157-01

Magnesium Stearate

Octadecanoic acid, magnesium salt.
Magnesium stearate [*557-04-0*].

Change to read:

» Magnesium Stearate is a compound of magnesium with a mixture of solid organic acids, ~~obtained from fats,~~

■ ■10

and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate. ~~It contains the equivalent of not less than 6.8 percent and not more than 8.3 percent of MgO.~~

■The fatty acids are derived from edible sources. It contains not less than 4.0 percent and not more than 5.0 percent of Mg, calculated on the dried basis.■10

Change to read:

Packaging and storage—Preserve in ~~well-closed~~

■tight■10
containers.

Add the following:

■**Labeling**—Where there is a labeling claim regarding surface area, the labeling indicates the specific surface area value and range. (See General Chapter ⟨ ⟩—To come.)■10

Add the following:

■**Reference standards**—*USP Stearic Acid Reference Standard*—Dry over silica gel for 4 hours before using. *USP Palmitic Acid Reference Standard*—Dry over silica gel for 4 hours before using.■10

Change to read:

Identification—

A: ~~Mix 25 g with 200 mL of hot water, then add 60 mL of 2 *N* sulfuric acid, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Separate the aqueous layer, and retain it for *Identification test B*. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow to cool, and discard the water layer. Then melt the acids, filter into a dry beaker while hot, and dry at 100° for 20 minutes: the solidification temperature of the fatty acids is not below 54°.~~

■Mix 5.0 g with 50 mL of peroxide-free ether, 20 mL of diluted nitric acid, and 20 mL of water in a round-bottom flask. Connect the flask to a reflux condenser, and reflux until dissolution is complete. Allow to cool, and transfer the contents of the flask to a separator. Shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the ether layer with two 4-mL portions of water, and add these aqueous extracts to the main aqueous extract. Wash the aqueous extract with 15 mL of peroxide-free ether, transfer the aqueous extract to a 50-mL volumetric flask, dilute with water to volume, and mix. Retain this solution for the *Chloride* and *Sulfate* tests. This solution responds to the test for *Magnesium* ⟨191⟩.■10

B: ~~The aqueous layer obtained from the separated fatty acids in *Identification test A* responds to the test for *Magnesium* ⟨191⟩.~~

■The retention times of the peaks corresponding to stearic acid and palmitic acid in the chromatogram of the *Test solution* correspond to those in the chromatogram of the *System suitability solution*, as obtained in the *Content of stearic acid and palmitic acid* test.■10

Change to read:

Microbial limits ⟨61⟩—The total ~~bacterial~~

■aerobic microbial■10
count does not exceed 1000 per g, ~~and the test for *Escherichia coli* is negative~~

■the total combined molds and yeasts count does not exceed 500 per g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.■10

Add the following:

■**Acidity or alkalinity**—Transfer 1.0 g to a 100-mL beaker, add 20 mL of carbon dioxide–free water, boil on a steam bath for 1 minute with continuous shaking, cool, and filter. Add 0.05 mL of bromothymol blue TS to 10 mL of the filtrate: not more than 0.05 mL of 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide is required to change the color of the indicator.■10

Change to read:

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than ~~4.0%~~

■6.0%■10
of its weight.

Add the following:

■**Limit of chloride** ⟨221⟩—A 10.0-mL portion of the aqueous solution obtained in *Identification* test *A* shows no more chloride than corresponds to 1.4 mL of 0.020 *N* hydrochloric acid (0.1%).■10

Add the following:

■**Limit of sulfate** ⟨221⟩—A 10.0 mL portion of the aqueous solution obtained in *Identification* test *A* shows no more sulfate than corresponds to 3.1 mL of 0.020 *N* sulfuric acid (0.3%).■10

Delete the following:

~~■**Lead** ⟨251⟩—Ignite 0.50 g in a silica crucible in a muffle furnace at 475° to 500° for 15 to 20 minutes. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and again ignite at 475° to 500° for 30 minutes. Dissolve the residue in 1 mL of a mixture of equal parts by volume of nitric acid and water, and wash into a separator with several successive portions of water. Add 3 mL of *Ammonium citrate solution* and 0.5 mL of *Hydroxylamine hydrochloride solution*, and render alkaline to phenol red TS with ammonium hydroxide. Add 10 mL of *Potassium cyanide solution*. Immediately extract the solution with successive 5-mL portions of *Dithizone extraction solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 seconds with 20 mL of 0.2 *N* nitric acid, and discard the chloroform layer. Add to the acid solution 4.0 mL of the *Ammonia cyanide solution* and 2 drops of *Hydroxylamine hydrochloride solution*. Add 10.0 mL of *Standard dithizone solution*, and shake the mixture for 30 seconds. Filter the chloroform layer through an acid-washed filter paper into a color comparison tube, and compare the color with that of a standard solution prepared as follows. To 20 mL of 0.2 *N* nitric acid add 5 µg of lead, 4 mL of *Ammonia cyanide solution* and 2 drops of *Hydroxylamine hydrochloride solution*, and shake with 10.0 mL of *Standard dithizone solution* for 30 seconds. Filter through an acid-washed filter paper into a color comparison tube. The color of the sample solution does not exceed that in the control (0.001%).■10~~

Add the following:

■**Limit of cadmium**—[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware 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, lead, and nickel as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 *N* nitric acid for 30 minutes and by rinsing with deionized water.]

Matrix modifier solution—Prepare a solution in water containing 2 g of monobasic ammonium phos-

phate and 100 mg of magnesium nitrate per 100 mL of solution.

Standard preparation—Transfer about 66 mg of cadmium nitrate, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Pipet 1 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, add 1.0 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1), dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.024 µg of Cd per mL.

Test preparation—Transfer about 80 mg of Magnesium Stearate, accurately weighed, to a suitable polytef-lined acid-digestion bomb, and add 1.0 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1). Close and seal the bomb according to the manufacturer's operating instructions. [*Caution—When using an acid-digestion bomb, be thoroughly familiar with the safety and operating instructions. Do not mix parts from different bombs, especially polytef lids and cups. Replace corrosion and rupture disks if they show signs of corrosion or wear. Before using a new polytef cup and cover, heat these parts in the bomb with a charge of pure water.*] Heat the bomb in an oven at 170° for 5 hours. Then let the oven cool to 70° or below for 30 minutes or open the oven and blow air on the bomb for 30 minutes. Remove the bomb from the oven. Transport the bomb to a hood, and air-cool the bomb to room temperature before opening. Open the bomb, and dilute the residue with water to 10.0 mL.

Blank—Transfer 10 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1) to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solutions—Prepare mixtures of the *Test preparation*, the *Standard preparation*, and the *Blank* with the following proportional compositions, by volume: 1.0:0:1.0, 1.0:0.5:0.5, and 1.0:1.0:0. Add 10 µL of *Matrix modifier solution* to each mixture, and mix. These *Test solutions* contain, respectively, 0, and about 0.006 and 0.012 µg per mL, of cadmium from the *Standard preparation*. [NOTE—Retain the remaining *Test preparation* for use in the tests for *Limit of lead* and *Limit of nickel*.]

Procedure—Concomitantly determine the absorbances of the *Test solutions* at the cadmium emission line at 228.8 nm, with a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* ⟨851⟩) equipped with a pyrolytic tube with platform and a cadmium hollow-cathode lamp, using the *Blank* to set the instrument to zero and maintaining the drying temperature of the furnace at 110° for 20 seconds after a 10-second ramp time, the ashing temperature at 800° for 30 seconds after a 10-second ramp time, and the atomization temperature at 1800° for 5 seconds. Plot the absorbances of the *Test solutions* versus their contents of cadmium, in µg per mL, as furnished by the *Standard preparation*, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis on the negative side. From the intercept determine the concentration, *C*, in µg per mL, of cadmium in the *Test solution* containing 0 mL of

the *Standard preparation.* Calculate the content, in ppm, of Cd in the specimen taken by the formula:

$$20{,}000(C/W),$$

in which W is the weight, in mg, of Magnesium Stearate taken to prepare the *Test preparation:* the limit is 3 ppm.■10

Add the following:

■**Limit of lead**—[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware 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, lead, and nickel as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 *N* nitric acid for 30 minutes and by rinsing with deionized water.]

Standard preparation—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, dilute with water to volume, and mix. Transfer 0.80 mL of this solution to a second 100-mL volumetric flask, add 10 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1), dilute with water to volume, and mix. This solution contains 0.08 μg of lead per mL.

Test preparation—Use a portion of the *Test preparation* retained from the test for *Limit of cadmium.*

Blank—Prepare as directed for *Blank* under *Limit of Cadmium.*

Test solutions—Prepare mixtures of the *Test preparation*, the *Standard preparation*, and the *Blank* with the following proportional compositions, by volume: 1.0:0:1.0, 1.0:0.5:0.5, and 1.0:1.0:0. These *Test solutions* contain, respectively, 0, 0.02, and 0.04 μg per mL, of lead from the *Standard preparation.*

Procedure—Concomitantly determine the absorbances of the *Test solutions* at the lead emission line at 283.3 nm, with a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* ⟨851⟩) equipped with a lead hollow-cathode lamp, using the *Blank* to set the instrument to zero and maintaining the drying temperature of the furnace at 110° for 20 seconds after a 10-second ramp time, the ashing temperature at 450° for 30 seconds after a 10-second ramp time, and the atomization temperature at 2000° for 5 seconds. Plot the absorbances of the *Test solutions* versus their contents of lead, in μg per mL, as furnished by the *Standard preparation*, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis on the negative side. From the intercept determine the concentration, C, in μg per mL, of lead in the *Test solution* containing 0 mL of the *Standard preparation.* Calculate the content, in ppm, of Pb in the specimen taken by the formula:

$$20{,}000(C/W),$$

in which W is the weight, in mg, of Magnesium Stearate taken to prepare the *Test preparation:* the limit is 10 ppm.■10

Add the following:

■**Limit of nickel**—[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware 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, lead, and nickel as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 *N* nitric acid for 30 minutes and by rinsing with deionized water.]

Standard preparation—Transfer about 162 mg of nickel chloride, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Pipet 1 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, add 1.0 mL of a mixture of 65% nitric acid and 30% hydrochloric acid (5:1), dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.04 µg of Ni per mL.

Test preparation—Use a portion of the *Test preparation* retained from the test for *Limit of cadmium.*

Blank—Prepare as directed for *Blank* under *Limit of cadmium.*

Test solutions—Prepare mixtures of the *Test preparation*, the *Standard preparation*, and the *Blank* with the following proportional compositions, by volume: 1.0:0:1.0, 1.0:0.5:0.5, and 1.0:1.0:0. These *Test solutions* contain, respectively, 0, and about 0.01 and 0.02 µg per mL, of nickel from the *Standard preparation.*

Procedure—Concomitantly determine the absorbances of the *Test solutions* at the nickel emission line at 232.0 nm, with a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* ⟨851⟩) equipped with a nickel hollow-cathode lamp, using the *Blank* to set the instrument to zero and maintaining the drying temperature of the furnace at 110° for 20 seconds after a 10-second ramp time, the ashing temperature at 1000° for 30 seconds after a 20-second ramp time, and the atomization temperature at 2300° for 5 seconds. Plot the absorbances of the *Test solutions* versus their contents of nickel, in µg per mL, as furnished by the *Standard preparation*, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis on the negative side. From the intercept determine the concentration, *C*, in µg per mL, of nickel in the *Test solution* containing 0 mL of the *Standard preparation.* Calculate the content, in ppm, of Ni in the specimen taken by the formula:

$$20{,}000(C/W),$$

in which *W* is the weight, in mg, of Magnesium Stearate taken to prepare the *Test preparation:* the limit is 5 ppm. ■10

Add the following:

■**Organic volatile impurities,** *Method IV* ⟨467⟩: meets the requirements. ■9

Add the following:

■**Relative content of stearic acid and palmitic acid**—

System suitability solution—Transfer about 50 mg each of USP Stearic Acid RS and USP Palmitic Acid RS to a small conical flask fitted with a suitable reflux condenser. Add 5.0 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL, swirl to mix, and reflux for 10 minutes until the solids have dissolved. Add 4 mL of chromatographic *n*-heptane through the condenser, and reflux for 10 minutes. Cool, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to sep-

arate. Pass the *n*-heptane layer through 0.1 g of anhydrous sodium sulfate (previously washed with chromatographic *n*-heptane) into a suitable flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with chromatographic *n*-heptane to volume, and mix.

Test solution—Transfer about 100 mg of Magnesium Stearate, accurately weighed, to a small conical flask fitted with a suitable reflux condenser, and proceed as directed for *System suitability solution*, beginning with "Add 5.0 mL of solution prepared by dissolving . . . ."

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, maintained at about 260°, a splitless injection system, and a 30-m × 0.32-mm fused silica capillary column bonded with a 0.5-μm layer of phase G16. The column temperature is maintained at 70° for about 2 minutes after injection, then programmed to increase at the rate of 5° per minute to 240° and to maintain this temperature for 5 minutes. The injection port temperature is maintained at about 220°. The carrier gas is helium with a linear velocity of about 50 cm per second.

Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure:* the relative retention times are about 0.86 for methyl palmitate and 1.0 for methyl stearate. The resolution, *R*, between the methyl palmitate and methyl stearate peaks is not less than 5.0. The relative standard deviation of the peak area responses for the palmitate and stearate peaks for replicate injections of the *System suitability solution* is not greater than 6.0%. The relative standard deviation of the peak area response ratio of the palmitate to stearate peaks from these replicate injections is not more than 1.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Inject about 1 μL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses for all of the fatty acid ester peaks in the chromatogram. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate taken by the formula:

$$100(A/B),$$

in which A is the area due to the methyl stearate peak, and B is the sum of the areas of all of the fatty acid ester peaks in the chromatogram. Similarly, calculate the percentage of palmitic acid in the portion of Magnesium Stearate taken. The stearate peak comprises not less than 40%, and the sum of the stearate and palmitate peaks is not less than 90% of the total area of all fatty acid ester peaks in the chromatogram. ■10

Change to read:

~~Assay—Boil about 1 g of Magnesium Stearate, accurately weighed, with 50 mL of 0.1 *N* sulfuric acid for about 30 minutes, or until the separated fatty acid layer is clear, adding water, if necessary, to maintain the original volume. Cool, filter, and wash the filter and the flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate with 1 *N* sodium hydroxide to litmus. While stirring, preferably with a magnetic stirrer, titrate with 0.05 *M* disodium ethylenediaminetetraacetate VS as follows. Add about 30 mL from a 50-mL buret, then add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and continue the titration to a blue endpoint. Each mL of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 2.015 mg of MgO.~~

■*Ammonium chloride pH 10 buffer solution*—Dissolve 5.4 g of ammonium chloride in water, add 21 mL of ammonium hydroxide, and dilute with water to 100 mL.

Procedure—Transfer about 500 mg of Magnesium Stearate, accurately weighed, to a 250-mL conical flask. Add 50 mL of a mixture of butyl alcohol and dehydrated alcohol (1:1), 5 mL of ammonium hy-

droxide, 3 mL of *Ammonium chloride pH 10 buffer solution*, 30.0 mL of 0.1 *M* edetate disodium VS, and 1 or 2 drops of eriochrome black TS, and mix. Heat at 45° to 50° until the solution is clear. Cool, and titrate the excess edetate disodium with 0.1 *M* zinc sulfate VS until the solution color changes from blue to violet (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.01 *M* edetate disodium is equivalent to 2.431 mg of Mg.■10

Oleyl Alcohol, *NF XVII* page 1954 and page 1674 of *PF* 17(2) [Mar.–Apr. 1991]—See briefing under *Dried Ferrous Sulfate.*

7O00300 (EXC) RTS—8851-04

Add the following:

■**Organic volatile impurities,** *Method ~~I~~ IV* ⟨467⟩: meets the requirements.■1

~~*Solvent*—Use dimethyl sulfoxide as the solvent.~~

Propylene Glycol Diacetate, *NF XVII* page 1973 and page 2967 of the *Sixth Supplement*—See briefing under *Chlordiazepoxide*, except the reference to the solvent, methanol, does not apply.

7P03600 (EXC) RTS—12042-07

Change to read:

■**Organic volatile impurities,** *Method ~~I~~*

■*IV*■1
⟨467⟩: meets the requirements. ■■6 ■5

Sorbitol, *NF XVII* page 1985, page 3385 of the *Eighth Supplement*, and page 3693 of *PF* 18(4) [July–Aug. 1992]—See briefing under *Chlordiazepoxide.*

7S02600 (CH4) RTS—12042-05

Add the following:

■**Organic volatile impurities,** *Method ~~I~~ IV* ⟨467⟩: meets the requirements.■1

~~*Solvent*—Use water as the solvent.~~

~~*Standard solution* and *Test preparation*—Prepare a *Test preparation* having a concentration of 20 mg per mL, and prepare a *Standard solution* having twice the stated concentration.~~

GENERAL CHAPTERS

General Tests and Assays

General Requirements for Tests and Assays

⟨1⟩ **Injections,** *USP XXII* page 1470, page 3118 of the *Seventh Supplement*, and page 4909 of *PF* 19(2) [Mar.–Apr. 1993]. A portion of a previously published revision proposal now is proposed for implementation via the *Tenth Supplement* to *USP XXII* and to *NF XVII.* There is continuing interest in the development of a standard to set forth general requirements for, and prohibition of, the use of black caps on vials and black bands on ampuls of compendial ar-

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Magnesium Stearate—Proposed Limits for Cadmium, Lead, and Nickel

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Suggested revisions for the international harmonization of the *National Formulary* monograph *Magnesium Stearate* were published as a Stimuli article in *Pharmacopeial Forum* 17(5) [Sept.–Oct. 1991]. Among these suggestions were the deletion of the current colorimetric *Lead* test and the addition of atomic absorption tests for cadmium, lead, and nickel. These recommendations were based on test results showing high levels of cadmium and nickel in some magnesium stearate samples from worldwide sources. Limits of 3 ppm, 10 ppm, and 5 ppm for cadmium, lead, and nickel, respectively, were suggested. These suggestions were subsequently proposed as a draft under *Pharmacopeial Previews* in *PF* 18(4) [July–Aug. 1992] and as a proposal under *In-process Revision* in *PF* 19(4) [July–Aug. 1993] for the harmonized *Magnesium Stearate* monograph.

Comments received in response to the proposed limit tests for cadmium, lead, and nickel focused on the significant capital costs of acquiring graphite furnace atomic absorption spectrophotometers, the added number of analyst hours required to perform these tests, the costs of having the tests performed by outside laboratories, and the need for these tests from a toxicity standpoint. One correspondent inquired as to whether or not any specific toxicity problems have been directly linked to magnesium stearate contaminated with these metals. Another correspondent wondered what problems would be resolved if these new tests were implemented, presuming that commercially available material could meet the proposed limits. Based on the comments received, it was decided to review this issue from a toxicity/safety viewpoint.

The review plan was to estimate the maximum daily intake of cadmium, lead, and nickel in a worst-case setting, i.e., under conditions of maximum daily dosing of three currently marketed pharmaceutical products that are formulated with above average levels of magnesium stearate containing the maximum proposed levels of cadmium, lead, and nickel. Based then on a comparison of these maximum daily intake values with literature toxicity values for cadmium, lead, and nickel, a decision can be made regarding the need, from a safety/toxicity viewpoint, for including these tests in the *Magnesium Stearate* monograph.

Literature toxicity values, provisional tolerable total intake levels (PTTILs), and no observed adverse effect levels (NOAELs) obtained from the *Federal Register* and the EPA Integrated Risk Information System (IRIS) database are as follows:

Lead (Pb): 75 μg per day (25 μg per day for pregnant women)[1]

Cadmium (Cd): 10 μg/kg per day (400 μg/day)[2]

Nickel (Ni): 20 μg/kg per day (800 μg/day)[2]

The review data are shown in Tables 1 and 2. These results indicate that the worst case Cd-Pb-Ni daily intake levels—Capsule A containing 4% magnesium stearate—are far below the reported PTTILs or NOAELs. The Pb PTTIL for adults is over 100 times greater than the maximum daily Pb intake level from Capsule A, and the Pb PTTIL for pregnant women is over 35 times greater than the maximum daily Pb intake level from Capsule A (see Table 2). Likewise, the Cd NOAEL is over 1900 times greater than the maximum daily Cd intake level, and the Ni NOAEL is almost 2300 times greater than the maximum daily nickel intake level.

The California Environmental Protection Agency, through the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), has adopted an acceptable daily intake level of 0.5 μg for Pb. This level represents the NOAEL for Pb divided by 1000. Based on a 69.9-mg maximum daily magnesium stearate intake with Capsule A (Table 1), the Proposition 65 daily Pb limit of 0.5 μg corresponds to 7.15 ppm (0.5 μg ÷ 0.0699 g) for Pb in magnesium stearate. A proposal to tighten the Pb limit in the *Magnesium Stearate* monograph from the current 10 ppm value to 7 ppm would, therefore, seem to be consistent with the Proposition 65 limit. It might also be appropriate to consider a more conservative limit of 5 ppm. In either case (7 ppm or 5 ppm), the current "wet chemistry" procedure under the USP general test chapter *Lead* ⟨251⟩ should be sufficiently sensitive to be applicable to magnesium stearate. Five *USP-NF* monographs have Pb limits of 5 ppm, and one *USP* monograph, *Calcium Carbonate*, has a Pb limit of 3 ppm, all determined by the procedure under *Lead* ⟨251⟩.

Reports of up to 108 ppm Cd and 215 ppm Ni in some magnesium stearate samples were submitted to the USP. The Cd content is reportedly a result of cross-contamination, and nickel reportedly may be present due to its use as a catalyst in the hydrogenation process. Even at these Cd and Ni levels, the Cd NOAEL is over 50 times greater than the 7.55 μg maximum daily Cd intake level (400 ÷ 7.55), and the nickel NOAEL is also over 50 times greater than the 15.03 μg maximum daily nickel intake level (800 ÷ 15.03) (Table 1).

[1] The Pb value is based on the PTTIL proposed by FDA in the 2/4/94 *Federal Register*.

[2] The Cd and Ni values in parentheses are values for a 40 kg "adult," to simulate a worst-case situation.

The data presented in this article do not support, from a safety/toxicity perspective, the need for inclusion of atomic absorption tests for Cd, Pb, or nickel in the *Magnesium Stearate* monograph. Also, this issue is not limited only to this monograph and to the work of the Subcommittee on Excipients. Certainly there should be scientifically sound rationale, perhaps based on toxicity, for all limit tests and specifications in the compendia. The Subcommittee on Excipients invites all interested parties to provide comments on this topic. Comments should be submitted to Dr. W. Larry Paul at the Drug Standards Division of the United States Pharmacopeia.

Table 1. Worst-case Daily Intake Levels of Cadmium, Lead, and Nickel.

		Capsule A	Capsule B	Capsule C
Capsule fill wt.		232.9 mg	170.5 mg	—
Magnesium stearate per capsule (1% level)		2.33 mg	1.70 mg	—
Magnesium stearate per capsule (4% level)		9.32 mg	6.82 mg	13.5 mg (5% level)
Ma ×. daily dose (number of capsules—*USP DI*)		6	4	4
Ma ×. daily dose +25% (worst case, number of capsules)		7.5	5	5
Magnesium stearate daily intake (1% level)		17.48 mg (2.33 × 7.5)	8.50 mg	—
Magnesium stearate daily intake (4% level)		69.9 mg (9.32 × 7.5)	34.10 mg	67.5 mg (13.5 × 5)
Ma ×. Pb Intake	1% level (10 ppm) →	0.18 μg (0.00001 × 17480 μg)	0.08 μg	—
	4% level (10 ppm) →	0.70 μg (0.00001 × 69900 μg)	0.34 μg	0.68 μg
Ma ×. Cd Intake	1% level (3 ppm) →	0.05 μg (0.000003 × 17480 μg)	0.03 μg	—
	4% level (3 ppm) →	0.21 μg	0.10 μg	0.20 μg
	4% (108 ppm) →	7.55 μg (0.000108 × 69900 μg)	—	7.29 μg
Ma ×. Ni Intake	1% (5 ppm) →	0.09 μg (0.000005 × 17480 μg)	0.04 μg	—
	4% (5 ppm) →	0.35 μg	0.17 μg	0.34 μg
	4% (215 ppm) →	15.03 μg (0.000215 × 69900 μg)	—	14.51 μg

Table 2. Maximum Daily Intake.

	Lead	Cadmium	Nickel
	Capsule A		
(Magnesium Stearate—1%)	0.18 μg	0.05 μg	0.09 μg
(Magnesium Stearate—4%)	0.70 μg	0.21 μg	0.35 μg
	Capsule B		
(Magnesium Stearate—1%)	0.08 μg	0.03 μg	0.04 μg
(Magnesium Stearate—4%)	0.34 μg	0.10 μg	0.17 μg
	Capsule C		
(Magnesium Stearate—5%)	0.68 μg	0.20 μg	0.34 μg

* * *

1632
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STIMULI TO THE REVISION PROCESS
Stimuli articles do not necessarily reflect the policies of the USPC or the USP Committee of Revision

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Harmonization of the USP, EP, and JP Heavy Metals Testing Procedures

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Introduction

One of the more complicated issues for those involved in the harmonization of the pharmacopeias is the resolution of the subtle differences in the general tests chapters. Some of these test procedures have been in use for decades and are required in the monographs for many different substances. It is easy to assume that the methods are equivalent and, therefore, the results are the same regardless of which pharmacopeia is used; but this is not always a valid assumption.

As the Merck & Co., Inc., representative on the International Pharmaceutical Excipients Council (IPEC) task force for Hydroxypropyl Methylcellulose (HPMC), I was assigned the harmonization of heavy metals testing for HPMC. All of the compendial monographs refer to their respective *Heavy Metals* general test procedures. Theoretically, the three pharmacopeias require testing for heavy metals by dissolving the metal salts and then precipitating them with a sulfide ion, which is compared colorimetrically against a lead standard, similarly treated. Initially, it was assumed that the major difference between the methods was the source of the sulfide ion. All of the pharmacopeias require ignition of HPMC as a step in the sample preparation. Since the temperature used for the ignition of the samples in the *Pharmacopoeia of Japan* (JP) and the *United States Pharmacopeia* (USP) was the same, the initial proposal was to determine the equivalency of the USP and *European Pharmacopoeia* (EP) procedures for sample preparation and to compare the three color development procedures, combining parts of each procedure to obtain a harmonized method. However, difficulties were encountered with the EP procedure, which uses as a reference standard a lead standard that has been taken through the ignition step. The color of the "ignited standard" solution was essentially the same intensity as the sample and the blank. A more thorough investigation of the test methodologies ensued.

Investigation

To verify that the problem was not due to the differences in the colorimetric portion of the general test procedures, samples of another compound (sodium bicarbonate), which does not require ignition, were tested according to USP *Heavy Metals Method I*, JP *Heavy Metals Method 1*, and EP V.3.2.8 *Heavy Metals Method A*. Equivalent results were obtained.

The American Society for Testing and Materials (ASTM procedure D2363-79, "Standard Test Methods for Hydroxypropyl Methylcellulose") recommends that the level of lead (heavy metals) in the final test solutions be 20 to 40 μg in 40 mL of solution for visual detection of the sulfide precipitate. The difficulties in determining the intensity of the EP reference standard indicated that the level of lead (Pb) in the EP standard solution was below this level of detection following ignition.

An experiment was initiated to confirm the loss of metals during the ignition step. Spiked samples of HPMC were taken through the EP and USP ash procedures and quantitatively analyzed by atomic absorption to determine the percent recovery of the metals. A Perkin Elmer PE40 Inductively Coupled Plasma (ICP) spectrometer equipped with a suitable PC and autosampler was used for analysis of the sample and standard preparations. Spiked samples were prepared by weighing 200-mg samples of HPMC and adding a 1-mL aliquot of 10-, 20-, and 50-ppm standard solutions containing the following metals: tin (Sn), arsenic (As), mercury (Hg), antimony (Sb), cadmium (Cd), lead (Pb), bismuth (Bi), and copper (Cu). All reagents used were reagent grade or better. The USP samples were ignited at 550°C and the EP samples were ignited at 750°C; the methodology was according to the respective general test procedures, except that ash residues were dissolved in nitric acid and diluted to a volume of 10 mL. Standards, sample blanks, and reagent blanks were injected into the ICP along with the samples. Experimental details are given in Appendix 1; Table 1 is a summary of the average recoveries.

Conclusions

It was concluded from this experiment that approximately 50% of the metals may be lost during the ash process. The loss of metals is probably matrix-dependent, and because the procedures are very labor-intensive, recoveries could vary significantly among analysts. Note that mercury, which is one of the more toxic heavy metals, was not recovered from either set of samples. The difference between the pharmacopeias in the handling of the reference standard in the general test methods is the basis for the different specifications for *Heavy metals* that are seen in the monographs for many substances. A USP limit of 10 ppm may be equivalent to the EP limit of 20 ppm.

Because of the loss of metals during ignition, the validity of test results obtained with the current USP, JP, and EP general test procedures is questionable. The data thus obtained should not be used to justify elimination of *Heavy metals* requirements in monographs.

Additional Information

The USP *Method III* wet digestion procedure should be able to detect some forms of mercury and provide better recovery of the other metals. Emmel and Nelson (*1*) re-

* Merck Research Laboratories, Sumneytown Pike, WP78-106, West Point, PA 19486.

ported validation of the wet digestion procedure. Consultation with several laboratories that routinely perform trace metal analysis confirms that a wet digestion procedure is preferred over ignition. Many laboratories are currently using microwave acid digestion techniques in sample preparation. Neither the EP nor the JP includes a wet digestion procedure at this time.

Alternatively, if the pharmacopeias decide to retain the general test methods with sample preparation by ignition, spiked control samples should be added. (Appendix 2 is a proposed revision of the USP *Method II.*) In the proposal, the final test solution volumes have been reduced to 25 mL in order to bring the lead concentration to the optimum level for visual detection of the sulfide precipitates, as recommended in the ASTM method, considering the approximately 50% loss of lead, which experiments indicate occurs during ignition. Appendix 3 summarizes the changes in the method presented in Appendix 2 as compared to the pharmacopeial methods.

It should be noted that improvements in testing may require that some of the *Heavy metals* limits be increased in the USP monographs that specify *Method II.* If proposals are made to tighten specifications to agree with the EP limits, manufacturers will need to conduct additional testing to confirm that they can meet tightened specifications before they are changed, because previous data would not be applicable.

Recommendations

(1) USP *Method III* (wet digestion) should be added to the general test procedures in the EP and JP.
(2) The *Heavy Metals* test procedures in which ignition is used, USP *Method II*, EP V.3.2.8 *Method C*, and JP *Method 2*, should be deleted, or revised to include a spiked control.
(3) Spiked control samples should be taken through the general test procedures to validate the methodology for each monograph.
(4) Atomic absorption analytical techniques should be included as an option.

APPENDIX 1
Heavy Metals Verification by ICP

Objective

Results of previous experiments indicated that lead and other "heavy metals" may be lost during the ignition step that is required for "heavy metals" testing of HPMC. Spiked samples of HPMC were taken through the EP and USP ash procedures and quantitatively analyzed by atomic absorption to determine the % recovery of the metals.

Experimental Details

Reagents (All reagents are reagent grade unless otherwise specified).
NITRIC ACID (Optima brand)
SULFURIC ACID (not less than 95% w/w)
MAGNESIUM SULFATE SOLUTION (25% w/v), prepared by dissolving 25 g of $MgSO_4$ in a mixture of 5.5 mL of H_2SO_4 in 50 mL of water, and diluting with water to 100 mL.
WATER: Purified water, deionized or distilled.
Instrument—PERKIN ELMER PE40 Inductively coupled plasma atomic absorption spectrophotometer equipped with a suitable PC and autosampler.
Standard Preparation—Standard solutions were prepared from Mercury, Lead, Antimony, Copper, Cadmium, Tin, Bismuth, and Arsenic Standards, 1000 μg/mL concentration, obtained from SPEX Plasma Standards.
Standard Solutions (2/15/94)—(NOTE—These standard solutions did not contain arsenic.) A standard stock solution containing 5 ppm each of Sn, Sb, Cd, Cu, Pb, Bi, and Hg was prepared by adding 10 mL of HNO_3 to a mixture containing 5 mL of each Standard and diluting to 1000 mL with water. Standard solutions containing 1, 0.5, and 0.1 ppm were prepared by diluting quantitatively as follows:

1-ppm Std.:	10 mL 5-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
0.5-ppm Std.:	5 mL 5-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
0.1ppm Std.:	5 mL 1-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water

Standard Solutions (2/16/94)—A standard stock solution containing 50 ppm each of Sn, Sb, Cd, Cu, Pb, Bi, Hg, and As was prepared by adding 10 mL of HNO_3 to a mixture containing 5 mL of each Standard and diluting to 100 mL with water. Standard solutions with concentrations of 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 ppm were prepared by diluting quantitatively as follows:

10-ppm Std.:	10 mL 50-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
5-ppm Std.:	5 mL 50-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
20-ppm Std.:	20 mL 50-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
1-ppm Std.:	5 mL 10-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
2-ppm Std.:	5 mL 20-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
0.5-ppm Std.:	5 mL 5-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
0.2-ppm Std.:	5 mL 2-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
0.1-ppm Std.:	5 mL 1-ppm Std. + 1 mL HNO_3 diluted to 50 mL with water
Std. Blank:	1 mL HNO_3 diluted to 50 mL with water

Sample Preparation—
(2/15/94) (Corresponding standard solutions used)

U:	200 mg HPMC + 2 mL H_2SO_4 [ash at 550°C]
U1:	200 mg HPMC + 1 mL 1-ppm std. + 2 mL H_2SO_4 [ash at 550°C]
U5:	200 mg HPMC + 1 mL 5-ppm std. + 2 mL H_2SO_4 [ash at 550°C]
E:	200 mg HPMC + 4 mL 25% $MgSO_4$ [ash at 800°C]
E1:	200 mg HPMC + 1 mL 1-ppm std. + 4 mL 25% $MgSO_4$ [ash at 800°C]
E5:	200 mg HPMC + 1 mL 5-ppm std. + 4 mL 25% $MgSO_4$ [ash at 800°C]

(2/16/94) (Corresponding standard solutions used)

U:	200 mg HPMC + 2 mL H_2SO_4 [ash at 550°C]
U10:	200 mg HPMC + 1 mL 10-ppm std. + 2 mL H_2SO_4 [ash at 550°C]
U20:	200 mg HPMC + 1 mL 20-ppm std. + 2 mL H_2SO_4 [ash at 550°C]
U50:	200 mg HPMC + 1 mL 50-ppm std. + 2 mL H_2SO_4 [ash at 550°C]
E:	200 mg HPMC + 4 mL 25% $MgSO_4$ [ash at 750°C]
E10:	200 mg HPMC + 1 mL 10-ppm std. + 4 mL 25% $MgSO_4$ [ash at 750°C]
E20:	200 mg HPMC + 1 mL 20-ppm std. + 4 mL 25% $MgSO_4$ [ash at 750°C]
E50:	200 mg HPMC + 1 mL 50-ppm std. + 4 mL 25% $MgSO_4$ [ash at 750°C]

Procedure (All operations are carried out in a hood.)

1. Prepare standard solutions and weigh HPMC samples.
2. Pipet standard solutions into crucibles containing HPMC samples as indicated in sample preparation and evaporate to dryness on a hot-plate at a very low setting.
3. Cool samples and add H_2SO_4 to USP samples and $MgSO_4$ solution to EP samples.
4. Heat gently on hot plate at a low temperature to avoid splattering. Increase heat as samples begin to char, emitting white fumes, then heat over a Bunsen burner for 5 to 10 minutes to drive off some carbon and the remaining fumes.
5. Place in a muffle furnace at the specified temperatures for approximately 1 hour. (Do not exceed 2 hours total ignition time.)
6. Cool, and add 2 mL of nitric acid (Optima grade) to each crucible, rinsing the insides of crucibles, and place on a steam bath for 5 minutes. Add approximately 2 mL of water, rinsing the sides, and heat for an additional 10 minutes. Quantitatively transfer the contents to labeled 15-mL plastic ICP tubes (calibrated in mL).
7. Repeat step 6 to ensure complete removal of all metals from crucible. ($MgSO_4$ in EP samples should also be completely dissolved.)
8. Dilute all samples to 10 mL with water.
9. Inject samples and standards in duplicate into ICP, using the appropriate program. Standardize the ICP, using the 1.0-ppm heavy metals standard prepared 2/16/94 and a blank of 1% nitric acid (Optima grade) in water.
10. Positions of vials in the autosampler:

(1)	0.1-ppm Std.	(7)	U	(13)	E20
(2)	0.2-ppm Std.	(8)	E	(14)	2-ppm Std.
(3)	0.5-ppm Std.	(9)	U10	(15)	U50
(4)	0.5-ppm Std. (2/15)	(10)	E10	(16)	E50
(5)	E5 (2/15)	(11)	1-ppm Std.	(17)	5-ppm Std.
(6)	U5 (2/15)	(12)	U20	(18)	1-ppm Std.

Results

(2/15/94)
The levels of the spiked samples were too close to the limit of detection, which is approximately 0.1 ppm. Also, the ICP was standardized with a standard that did not contain arsenic and thus gave erroneous readings due to noisy peaks and interference. The experiment was repeated the following day (2/16/94) with higher concentrations of standards.

(2/16/94)
The results of the duplicate injections were in good agreement. Standards placed early in the run read at the proper levels, but toward the end of the run they were reading higher (approximately 110% of the expected value). Therefore, the samples were calculated against the standard placed near them in the autosampler.

Summary of Recoveries, USP Samples:

	U5	U10	U20	U50	Average % Recovery
Sn	80.0	62.5	60.8	61.7	66
As	—	60.9	63.8	64.2	63
Hg	0	0	0	0	0
Sb	60.0	56.0	60.8	51.6	57
Cd	20.0	64.0	56.2	100.8	60
Pb	40.0	64.0	54.0	64.7	56
Bi	60.0	70.8	44.4	74.8	62
Cu	60.0	70.8	68.1	75.6	69

Summary of Recoveries, EP Samples:

	E5	E10	E20	E50	Average % Recovery
Sn	0	0	0	0	0
As	—	69.6	63.8	77.1	70
Hg	0	0	0	0	0
Sb	60.0	64.0	54.9	64.5	61
Cd	70.0	52.0	47.9	57.5	57
Pb	40.0	44.0	44.0	54.3	46
Bi	50.0	54.2	55.6	63.1	56
Cu	60.0	50.0	51.1	55.6	54

Observations

(1) The traditional definition of "heavy metals" is that they are metals that form black or dark sulfides. This group ordinarily includes Ag, Hg, Pb, and Cu. The program on the ICP was set up to test all water samples for the traditional qualitative analytical groups I and II. These groups include all of the heavy metals except silver, which is tested for separately by precipitation with chloride ion, and the additional metals, Sn, As, Sb, Cd, and Bi. The ICP program functions better with all of the ions included in the standard.

(2) The difference in recovery of the heavy metals is not significant, but recoveries are slightly better for most of the elements in spiked samples prepared according to the USP ash procedure.

(3) Mercury is lost in both procedures.

(4) Of the remaining heavy metals, lead exhibits the lowest recovery level and is thus a good choice for the standard.

(5) Tin (not a heavy metal) is retained in the USP ash, but lost in the EP preparation.
(6) This comparison was performed only once; the USP and EP procedures are both very labor-intensive.
(7) Another potential sample preparation is to use *Method II* of the USP general test for *Arsenic* ⟨211⟩.

Conclusion

Approximately 50% of the metals are lost during the ashing process; therefore, a spiked 200-mg sample should be taken through the same procedure as the sample, and used as the reference standard. The final concentration of the sample and standard solutions must be set at an optimum level for color development in order to facilitate the determination. According to ASTM D2363-79 "Standard Test Methods for Hydroxypropyl Methylcellulose," the optimum lead content in 40 mL should be between 20 and 40 μg for visual comparison.

APPENDIX 2

Changes Recommended for Heavy Metals ⟨231⟩, Method II

Reagents (All reagents are reagent grade).
SULFURIC ACID: contains not less than 95.0% (w/w) H_2SO_4.
PHENOLPHTHALEIN SOLUTION R: Dissolve 0.1 g in 80 mL of ethanol and dilute to 100 mL with water.
6 *N* HYDROCHLORIC ACID
CONCENTRATED AMMONIA R: contains not less than 25% w/w NH_3.
GLACIAL ACETIC ACID R: contains not less than 98.0% w/w $C_2H_4O_2$.
BUFFER SOLUTION pH 3.5 R: Dissolve 25.0 g of ammonium acetate in 25 mL of water and add 50.0 mL of 6 *N* hydrochloric acid (adjust, if necessary, with 6 *N* HCl or 6 *N* NH_4OH to a pH of 3.5) and dilute with water to 100 mL.
THIOACETAMIDE REAGENT R: Prepare the following for each tube: To 0.2 mL of a 4% (w/v) solution of thioacetamide, add 1 mL of a mixture of 4 mL of water, 15 mL of 1 *N* sodium hydroxide, and 20 mL of glycerin. Heat in a water bath for 20 seconds, and mix well. Prepare immediately before use.

Standard Preparation—Prepare 10 ppm (10 μg/mL) lead nitrate standard as directed in USP or EP.
Test Preparation—Weigh 2 g of the substance to be tested into a suitable crucible, that is large enough to accomodate the expansion of the substance.
Control Preparation—Pipet 2 mL or 4 mL of the 10-ppm lead standard (limit of 10 ppm or 20 ppm, respectively) into a suitable crucible containing a quantity of the substance to be tested equal to 1/10th the portion used in the *Test Preparation*. Evaporate to dryness on a steam bath or a hot plate at a low temperature.
Procedure—To the crucibles containing the *Test Preparation* and the *Control Preparation*, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucibles may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite both the test (T) and the control (C) at the same time, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off (no longer than 2 hours). (If carbon remains, allow residue to cool, add a few additional drops of sulfuric acid, evaporate, and ignite again.) Cool, add 5 mL of 6 *N* hydrochloric acid, cover, and heat on a low hot plate or steam bath for 10 minutes. Cool, and quantitatively transfer to matched color comparison tubes labeled T and C. Repeat, adding a second 5-mL portion of 6 *N* hydrochloric acid to each crucible.
Color Development—Pipet 2 mL or 4 mL of the 10-ppm lead standard (limit of 10 ppm or 20 ppm, respectively) into the apppropriately labeled test tube (S) and add 10 mL 6 *N* hydrochloric acid. Add 0.1 mL of phenolphthalein solution R to each tube (C, T, and S) (or use short-range pH indicator paper), then add concentrated ammonia R dropwise until a pink color is obtained. Cool, add glacial acetic acid R dropwise, until the solution is decolorized, and add 0.5 mL excess. Filter (if a precipitate forms, wash the filter), and dilute to 20 mL with water. To each tube, add 2 mL of buffer solution pH 3.5 R, mix, add 1.2 mL of thioacetamide reagent R, and dilute to 25 mL with distilled water. Mix immediately. Compare after 5 minutes: the color of the control (C) is not darker than the appropriate standard (S), and the color of the test (T) is not darker than the control (C).

APPENDIX 3

Recommended Changes from Current Heavy Metals General Test Procedures

USP Heavy Metals ⟨231⟩, Method II

- Does not include an ignited control.
- Does not specify a limit for ignition time.
- Does not use phenolphthalein as an indicator for pH adjustment.
- The final test solutions are more dilute.

EP V.3.2.8. Heavy Metals, Method C

- Sample and Standard are heated with 4 mL of 25% $MgSO_4$ solution and ignited in a muffle furnace at temperatures up to 800°C.
- In preparation of the control, the HPMC is added as a solution to the ignited standard, instead of being ignited with the standard.
- The final test solutions are more dilute.

JP Heavy Metals Limit Test, Method 2

- Does not include an ignited control.
- Does not specify a limit for ignition time.
- Does not use phenolphthalein as an indicator for pH adjustment.
- The final test solutions are more dilute.
- Uses sodium sulfide solution for color development.

Table 1. Average Recoveries of Individual Heavy Metals by USP Method II and EP Method C.

	USP Method II (ignited at 550°C)	EP V.3.2.8 Method C (ignited at 750°C)
Tin (Sn)	66%	0%
Arsenic (As)	63%	70%
Mercury (Hg)	0%	0%
Antimony (Sb)	57%	61%
Cadmium (Cd)	60%	57%
Lead (Pb)	56%	46%
Bismuth (Bi)	62%	56%
Copper (Cu)	69%	54%

Acknowledgement

The heavy metals verification experiment by ICP (Appendix 1) was designed to confirm observations made by Frank Huang, a 1993 Summer Intern at Merck Manufacturing Division Quality Control Operations Laboratory at West Point, Pennsylvania.

References

(1) Emmel HW, Nelson LD. Automation of the classical wet digestion via robotics. In: Advances in Laboratory Automation Robotics. Zymark, 1987.

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Excipient Intake and Heavy Metals Limits

W. Larry Paul, *USP Division of Standards Development**

Introduction

Recommendations for replacing the current colorimetric *Lead* test in the *National Formulary* monograph on *Magnesium Stearate* with atomic absorption tests for cadmium, lead, and nickel were discussed in a Stimuli article, *Magnesium Stearate—Proposed Limits for Cadmium, Lead, and Nickel*, in the January–February 1995 number of *Pharmacopeial Forum*. The article provided comparisons of literature toxicity values for these metals with maximum daily intake values based on maximum daily dosing of a currently marketed pharmaceutical product formulated with an above average level of magnesium stearate. These comparisons were then used to make a recommendation, from a safety and toxicity viewpoint, regarding the need to include atomic absorption tests for these metals in the *Magnesium Stearate* monograph.

To expand this review to other excipients, the USP initiated a survey of all official USP correspondents to obtain maximum daily intake data for 28 frequently used excipients selected by the USP Subcommittee on Excipients. The survey responses, representing the highest reported intake for each of the selected excipients, are presented in Table 1. During the review of the survey responses, concerns were raised about the proper way to apply the daily excipient intake values to the establishment of safety- and toxicity-based test limits in the compendial monographs. In the discussion that follows, a hypothetical product that would be possible based on the survey responses is used to illustrate two ways to approach this issue.

Individual Component Approach

The hypothetical product contains at least four ingredients in a formulation so that one dose unit weighs over 2 grams. Based on the maximum daily dose for this formulation and the current limits specified under *Heavy Metals* ⟨231⟩ or *Lead* ⟨251⟩ in the NF monographs for the four ingredients, the amount of lead provided in the worst case by each ingredient is as follows: ingredient A, 23.4 μg; ingredient B, 66.2 μg; ingredient C, 15.7 μg; and ingredient D, 3.2 μg (Table 2). These four ingredients *individually* do not provide lead levels that exceed the 75 μg per day provisional tolerable total intake level (PTTIL) for lead, as provided in the *Federal Register*.

In the individual component approach to the establishment of lead limits, the lead limit for ingredient A, for example, based on the 75 μg PTTIL and the maximum daily intake of ingredient A in the formulation, would be slightly less than 0.001%, as opposed to the current ingredient A monograph lead limit of 0.0003%. However, if ingredient A containing this maximum 0.001% lead level is used in the formulation, there must be no detectable lead in any other component of the formulation. In reality, this is probably never the case. The total daily intake of lead from this formulation, based only on the above ingredients, is 108.5 μg in the worst case, which exceeds the 75 μg limit by 33.5 μg. Data used to calculate these values are shown in Table 2.

Composite Component Approach

A more appropriate approach to the establishment of lead limits appears to be the composite component approach because this approach reflects the practical situation where components may not be completely lead-free; and it also reflects the total amount of lead being consumed when the product is ingested. This seems to be consistent with California Proposition 65, which specifies a total daily lead intake limit on a product-by-product basis. The composite component approach based on PTTIL levels requires the determination of the maximum amount of lead that each component may contribute to the formulation without causing the total lead level to exceed the limit of 75 μg per day. In the survey-derived formulation, this value would be 16.2 μg for ingredient A, based on the 75/108.5 ratio. This corresponds to a maximum allowable lead level of 0.0002% (2 ppm) for the ingredient A used in this formulation. This value is in good agreement with the lead limit of 0.0003% in the current ingredient A monograph. PTTIL-based values for the components of the survey-derived formulation are shown in Table 3.

The limit obtained by this composite component approach is dependent on the lead content in each component of the formulation. In the survey-derived formulation example, if totally lead-free ingredient B were used, for example, the resulting lead limit for the ingredient A component would increase from 0.0002% to 0.0005%; however, the 0.0002% limit represents the worst case, in which each of the four ingredients in the formulation contains the compendial limit of lead.

When based on the California Proposition 65 requirements, the limits obtained by the composite component approach are much lower. California Proposition 65 specifies a limit of not more than 0.5 μg of lead per day per product. Based on this limit, the resulting lead limit for the ingredient A component of the survey-derived formulation is 0.11 g or 0.000001% (10 ppb). This value is at or close to the lowest reasonably achievable detection limit with graphite furnace atomic absorption instruments. To readily achieve readings in this 10 ppb region, it is reportedly necessary to use an inductively coupled plasma spectrophotometric procedure. The California Proposition 65-based values for these four ingredients in the survey formulation are shown in Table 4.

* W. Larry Paul, Ph.D., is the staff liaison to the 1990–1995 USP Subcommittee on Excipients.

Discussion

Even if the hypothetical formulation referenced in this article provides more ingredient A daily than does any other pharmaceutical dosage form, it may not be appropriate to base the ingredient A monograph lead limit solely on the amount of ingredient A in this specific product because the total amount of lead provided by all components of the product must be considered. The survey formulation may contain more ingredient A than any other product, but, as shown in Table 2, the major source of lead in this product theoretically is ingredient B, not ingredient A. As mentioned previously, if totally lead-free ingredient B were used in the survey formulation, the allowable lead limit for the ingredient A component, based on PTTIL levels, would increase from 0.0002% to 0.0005%.

The composite component approach was not taken in the January–February 1995 Stimuli article. Other components in the formulation could contribute, significantly perhaps, to the total lead, cadmium, or nickel in the formulation. Also, the Stimuli article states that "a proposal to tighten the Pb limit in the *Magnesium Stearate* monograph from the current 10 ppm value to 7 ppm would, therefore, seem to be consistent with the Proposition 65 limit." This statement implies, as stated above in the discussion of the individual component approach, that other components of the dosage form do not contain any measurable amounts of lead.

The individual component approach and the composite component approach discussed in this article are primarily based on provisional tolerable total intake levels. If either approach is used to determine daily lead limits, for example, based on a single pharmaceutical dosage form, this would seem to require that an individual not consume any lead from any other source during the day. This may not be a realistic requirement. How meaningful is a total daily lead intake limit if the individual's lead intake from *all* individual lead-containing sources is not monitored, controlled, and limited; and how meaningful is a single-source lead intake limit, i.e., for a specific food item, dosage form, etc., if the individual's total daily lead intake is not monitored, controlled, and limited?

Another concern related to the presence of heavy metals in excipients is the potential effect of metallic impurities, notably iron, on the stability of a formulation. This physicochemical incompatibility concern is product formulation-specific. Company A, for example, is likely to have at least one product that is much more sensitive to iron contamination than the rest of the company's products. Also, company A's formulation for a given product may be more sensitive to iron contamination than company B's formulation of the "same" product. If there are no toxicity concerns associated with the presence of metallic impurities in a given article, should the control of the presence of these metallic impurities for physicochemical incompatibility reasons, perhaps by graphite furnace atomic absorption procedures, be within the purview of the compendia or be addressed through individual chemical supplier-pharmaceutical manufacturer purchase specifications?

The USP welcomes reader comments regarding the establishment of compendial heavy metals limit specifications, wet chemistry versus atomic absorption spectrophotometry test procedures, the California Proposition 65 approach, and related issues. Readers are invited to submit their comments to Dr. W. Larry Paul, Division of Standards Development, USP headquarters.

Table 1. Survey Response Summary

Pharmaceutic Ingredients	Maximum Daily Excipient Dose (mg) In Any Product
Alkalizing Agents	
Sodium Bicarbonate	13,200
Buffering Agents	
Citric Acid	9,760
Potassium Citrate	5,400
Sodium Citrate	1,200
Coating Agents	
Gelatin	4,300
Hydroxypropyl Cellulose	375
Methacrylic Acid Copolymer	560
Tablet Binders	
Povidone	2,240
Tablet and/or Capsule Diluent	
Calcium Carbonate	7,800
Calcium Phosphate, Dibasic	2,170
Calcium Phosphate, Tribasic	600
Calcium Sulfate	1,020
Cellulose, Microcrystalline	4,500
Cellulose, Powdered	1,480
Dextrose Excipient	3,360
Fructose	135
Kaolin	1.2
Lactose	16,000
Mannitol	9,000
Sorbitol	36,000
Starch	1,890
Starch, Pregelantinized	915
Sucrose	16,600
Sugar, Compressible	3,130
Tablet and/or Capsule Lubricant	
Magnesium Stearate	315
Polyethylene Glycol	3,780
Stearic Acid	1,500
Talc	700

Table 2. Daily Lead Intake from Survey Product.

Ingredient	Max. Daily Intake of Ingredient (mg)	Compendial Heavy Metals/ Lead Limit (%)	Max. Daily Intake Lead (μg)
Ingredient A	7803	0.0003	23.4
Ingredient B	6624	0.001	66.2
Ingredient C	3132	0.0005	15.7
Ingredient D	315	0.001	3.2
			total = 108.5 μg

Table 3. PTTIL-based Lead Limits.

Ingredient	Max. Daily Intake Lead Based on PTTIL (μg)	Max. Limit (%)
Ingredient A	16.2	0.0002
Ingredient B	45.8	0.0007
Ingredient C	10.8	0.0003
Ingredient D	2.2	0.0007
	total = 75.0 μg	

Table 4. Proposition 65-based Lead Limits.

Ingredient	Max. Daily Intake Lead Based on Calif. Proposition 65 (μg)	Max. Limit (%)
Ingredient A	0.11	0.000001
Ingredient B	0.30	0.000004
Ingredient C	0.07	0.000002
Ingredient D	0.02	0.000006
	total = 0.05 μg	

* * *

Change to read:

Tablet Binder
Acacia
Alginic Acid
Carboxymethylcellulose, Sodium
Cellulose, Microcrystalline

Copovidone
Dextrin
Ethylcellulose
Gelatin
Glucose, Liquid
Guar Gum
Hydroxypropyl Methylcellulose
Methylcellulose
Polyethylene Oxide
Povidone
Starch, Pregelatinized
Syrup

GENERAL CHAPTERS

Chemical Tests and Assays

LIMIT TESTS

〈**231**〉 **Heavy Metals,** *USP 23* page 1727 and page 4308 of the *Eighth Supplement*. The revisions to this general test chapter are based on the article *Harmonization of the USP, EP, and JP Heavy Metals Testing Procedures* published under *Stimuli to the Revision Process*, by Katherine B. Blake (see pages 1632–1637 of *PF* 21(6) [Nov.–Dec. 1995]). Blake had observed that the current procedure is deficient in that certain volatile metals are completely (mercury), or partially (lead), lost during the ashing procedure. The intent of the revisions is to limit or correct for the loss of metals by the use of a Monitor Preparation, which is taken through the ashing procedure, by limiting the time for ignition, and by increasing the concentration of heavy metals in both the Test Preparation and the Standard Preparation.

3L00600 (GEN) RTS—21282-01

Change to read:

Method II

■**pH 3.5 Acetate Buffer**—Prepare as directed under *Method I*.■3

Standard Preparation—~~Prepare as directed under *Method I*.~~

Pipet 4 mL of the *Standard Lead Solution* to a suitable test tube, and add 10 mL of 6 *N* hydrochloric acid.

Test Preparation—Use a quantity, in g, of the substance to be tested as calculated by the formula:

~~2.0/(1000*L*),~~

4.0/(1000*L*),

in which *L* is the *Heavy metals* limit, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off (no longer than 2 hours). If carbon remains, allow the residue to cool, add a few additional drops of sulfuric acid, evaporate, and ignite again.
Cool, add ~~4 mL~~

5 mL
of 6 *N* hydrochloric acid, cover,

and
digest on a steam bath for ~~15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 *N* ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 *N* acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.~~

10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 *N* hydrochloric acid, and transfer the rinsing to the test tube.

Monitor Preparation—Pipet 4 mL of the *Standard Lead Solution* to a crucible identical to that used in the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation*. Evaporate to dryness on a steam bath. Ignite at the same time, in the same muffle furnace, and under the same conditions as the

Test Preparation. Transfer the resulting residue to a test tube, and add 10 mL of 6 *N* hydrochloric acid.

Procedure ~~To~~

Adjust the solution in each of the tubes containing the *Standard Preparation*, ~~and~~ the *Test Preparation*, ■ ■7 ~~add~~ and the *Monitor Preparation*, cautiously and dropwise, with ammonium hydroxide to a pH of 9. Cool, and adjust dropwise with glacial acetic acid to a pH of 8, and then add 0.5 mL in excess. Filter, washing the filter with a few mL of water, into a 25-mL color comparison tube, and then dilute to 20 mL with water. Add ■ ■7 ■2 mL of *pH 3.5 Acetate Buffer*, ■then add 1.2 mL of thioacetamide–glycerin base TS, ■7 dilute with water to ~~50 mL,~~ ■3 25 mL, mix, allow to stand for ■2 ■7 minutes, and view downward over a white surface. • * •15 ~~the~~ The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation*.

[NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation*, proceed as directed for *Method III* for the substance being tested.]

• * In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface. •15

Residue on Ignition/Sulphated Ash Test. The following proposal from the *Japanese Pharmacopoeia* seeks to harmonize the requirements of the *JP*, the *EP*, and the *USP*. It should be noted that since November 1992 the *USP* has had a statement that the "Sulphated Ash" method of the *EP* is considered to be equivalent to the "*Residue on Ignition*" method of the *USP*, except where noted in the monograph. The temperature specified in the *JP* proposal is that used in the *EP* method. Given this history, it is not anticipated that significant problems would arise should this method become official in the *USP*. However, it is important that manufacturers inform us of specific items where problems might be anticipated, so that monographs for these items can be revised, if necessary, to accommodate the new conditions.

(GEN) RTS—20947-01

Proposal Stage (Stage 3)

Draft Text towards Harmonization of Residue on Ignition/Sulphated Ash Test (by JP)

The Japanese Pharmacopoeia is the lead pharmacopoeia for the international harmonization of this test. The following is the Proposal Stage Draft for Residue on Ignition/Sulphated Ash Test. We welcome comments from all interested parties concerned. Please send your comments in writing by the end of July, 1998 to: Secretariat of the Japanese Pharmacopoeia, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare, 1-2-2, Kasumigaseki, Chiyoda-ku, Tokyo, 100-8045, Japan.

Residue on Ignition/Sulphated Ash Test

The Residue on Ignition/Sulphated Ash Test is a method to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance, but in some cases, it is used for determining the amount of inorganic components in an organic substance or the amount of impurities in an inorganic substance volatilizable by heating.

Procedure

Ignite a suitable crucible of platinum, quartz or porcelain for 30 minutes at 600 ± 50°C, cool the crucible in a desiccator (silica gel) and weigh it accurately. Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of

An Atomic Spectroscopic Method as an Alternative to Both USP *Heavy Metals* ⟨231⟩ *and USP Residue on Ignition* ⟨281⟩

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ABSTRACT A multi-element inductively coupled plasma–mass spectrometry (ICP–MS) method has been demonstrated to be a suitable alternative to both USP *Heavy Metals* ⟨231⟩ and USP *Residue on Ignition* ⟨281⟩ for drug substances, intermediates, and raw materials. This ICP–MS method, combined with a direct-dissolution sample preparation procedure, is simpler, faster, more sensitive, and element specific. It consumes less sample and provides semiquantitative to quantitative results covering all elements of pharmaceutical interest and offers other advantages discussed herein.

INTRODUCTION

Metal or inorganic contamination of bulk drug substances, intermediates, and raw materials may be introduced in many ways, e.g., from reagents, solvents, electrodes, reaction vessels, plumbing and other equipment used in the synthesis, as well as via exposure to airborne particles or from container–closure systems. Most importantly, metals may be introduced by the utilization of catalysts at various steps during the synthesis. Because metals can catalyze decomposition and are potentially toxic, the metal content of process intermediates and final drug substances is widely monitored.

The United States Pharmacopoeia (USP) *Heavy Metals* ⟨231⟩ and similar tests provided in the European Pharmacopoeia (Ph. Eur.) and the Japanese Pharmacopoeia (JP) (*1–3*), consist of the precipitation of metal sulfides from an aqueous solution and the visual comparison of the color of that preparation to the color of a simultaneously and similarly treated standard lead solution. In order to obtain an aqueous solution for testing, ignition and combustion of the samples in a muffle furnace is often required in a preliminary step. In addition, after one adjusts the pH and adds either freshly prepared hydrogen sulfide or thioacetamide–glycerin base TS, the colors of the different metal sulfides range from white to yellow, orange, brown, and black (*4*), making the visual comparison with the dark brown–colored lead sulfide difficult. Furthermore, apart from the colors of the formed sulfides, there is no information about the identities of the metals that caused the positive result.

USP *Residue on Ignition (ROI)* ⟨281⟩ (*5*) and the similar sulfated ash limit test in Ph. Eur. also do not provide any information about the identity of inorganic impurities in the samples. These tests consist of heating 1 to 2 g of the sample in a suitable crucible that previously has been ignited, cooled, and weighed until the substance is thoroughly charred. The charred substance is then moistened by 1 mL of sulfuric acid, heated again until white fumes no longer are evolved, and ignited at 800 ±25° until the carbon is consumed. The residue is then cooled in a desiccator before it is weighed to determine the percentage of residue. Sometimes this procedure must be repeated in order to get a constant weight of the residue.

For USP *Heavy Metals* ⟨231⟩, Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu, and Mo typically will respond, but for USP *Residue on Ignition* ⟨281⟩, all elements that potentially react with sulfuric acid to form sulfated ash will respond. However, we observe that most of the time the alkaline and alkaline earth elements produce substantial amounts of sulfated ash.

Although both methods are still widely accepted and used in the pharmaceutical industry, they are nonspecific, insensitive, time-consuming, and labor intensive. USP *Heavy Metals* ⟨231⟩ has been shown to be suitable only for a few limited elements and has not been shown to be equally sensitive to all. In our laboratory it frequently yields either low recoveries or erroneous results. USP *ROI* ⟨281⟩ is not applicable to drug substances that are inorganic (e.g., sodium) salts. In addition, each test consumes a minimum of 1 to 2 g of sample, which can be a major problem when the quantities of the intermediates or the drug substances to be tested are limited.

Attempts have been made to improve these pharmacopeial methods to alleviate some of the limitations and shortcomings, but no major improvements have been achieved (*6–7*). We have recently published an ICP–MS method as an alternative to USP *Heavy Metals* ⟨231⟩ (*8*) and now wish to provide justification to expand its use to also cover USP *ROI* ⟨281⟩.

NEW TECHNOLOGY

Since 1980 ICP–MS has emerged as a major and powerful technique in elemental analysis (*9*), an area traditionally dominated by optical atomic spectrometry methods. In approximately 10 years, ICP–MS has progressed from a laboratory experiment to commercial development and wide-

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Pharmacopeial Forum
Vol. 29(4) [July–Aug. 2003]

spread analytical application (*10–16*). This growth is fueled primarily by the fact that ICP–MS offers extremely low detection limits that range from sub–part per billion (ppb) to sub–part per trillion (ppt) detection limits for most elements. In most cases, these detection limits are 100 to 1000 times superior to those that can be routinely achieved by Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP–AES). In addition, these detection limits are broadly achieved for almost all elements across the periodic table. Furthermore, the simpler mass spectra versus the much more complex optical spectra of the elements make this technique a quick tool for automated qualitative, semiquantitative, and quantitative elemental analysis.

In view of the superior and broad detection capability of ICP–MS and also because of the limitations inherent in USP *Heavy Metals* ⟨231⟩ and USP *Residue on Ignition* ⟨281⟩ prescribed by USP and Ph. Eur., a multi-element survey type ICP–MS method has been developed and employed at Merck as an alternative method. This method analyzes all sulfide-forming and sulfate-forming elements plus other elements with greatly enhanced specificity, sensitivity, speed, precision, and accuracy. In addition, because only part-per-million (ppm) levels of metals of pharmaceutical interest will be noted and reported, data processing and interpretation are also extremely simple. In most cases, a 5-second glance at the raw data will reveal the answers to the corresponding equivalent USP limit tests—that is, they pass or fail the limit tests for both heavy metals and *ROI* in a single run because more than 95% of the compounds tested contain no significant amount of inorganic impurities.

SAMPLE PREPARATION

In addition to the extreme sensitivity and specificity of the ICP–MS method, the sample preparation scheme for the simultaneous *Heavy Metals* and *ROI* tests is also simple and straightforward. Experience of handling thousands of pharmaceutical samples reveals that at least 99% of these samples are readily soluble in 80% (v/v) nitric acid solution. On some occasions, sonication may be needed to solubilize the samples or speed up their solubilization. In rare cases when samples do not dissolve, either a microwave digestion method can be used or the USP *Heavy Metals* and *ROI* tests can be carried out as usual. With the method proposed in this article, each sample can be prepared and analyzed in less than 15 minutes after initialization of the instrument, provided no microwave digestion is needed. By contrast, the USP *Heavy Metals* and *ROI* tests may each take several hours or longer to perform for one sample, particularly when sample combustion/digestion is involved. Another advantage of the proposed method is that only about 10 to 100 mg of sample is consumed for both the *Heavy Metals* and *ROI* tests using the ICP–MS method.

Furthermore, for potent compounds and other hazardous materials, the samples are denatured in 80% (v/v) nitric acid during the sample preparation procedure, and the dissolved samples can be analyzed safely using the ICP–MS method. In contrast, to perform the USP *Heavy Metals* test and particularly the USP *ROI* test, expensive and cumbersome containment facilities are required during sample preparation. In most laboratories, this would render the USP *Heavy Metals* and *ROI* tests impractical for potent and hazardous compounds.

METHOD PROCEDURE

Instrumentation—A Perkin-Elmer Elan 6000 Inductively Coupled Plasma–Mass Spectrometer (ICP–MS) equipped with an AS-91 auto-sampler was used throughout this study. The instrumental conditions and general method parameters are listed in *Table 1*.

Sample Analysis—After instrument warm-up (30–40 minutes), a two-point calibration of the ICP–MS is carried out by analyzing the calibration blank (80% nitric acid solution) and the mixed 69-element calibration standard (20 µg/L for all elements except Na, Si, P, K, Ca, and Fe, which are 1000 µg/L). All regulatory samples are spiked at a level equivalent to 10 mg/kg (10 ppm) of the metals that are sensitive to USP *Heavy Metals* ⟨231⟩ in solid sample (10 µg/L in solution). A spike recovery of 60–140% is required for each element. To monitor the drift of the instrument, the mixed 69-element standard (ICP–MS) is reanalyzed as a sample every 10 samples and at the end of the analysis.

REPORTING RESULTS

A) Heavy Metals Test—If those elements sensitive to the USP Heavy Metals test or the sum of these elements are found to have a concentration of higher than 10 ppm, the sample should be reanalyzed for those specific elements by a different atomic spectroscopic method such as ICP–AES and/or atomic absorption spectrometry.

B) ROI Test—Results from the ICP–MS analysis will be used for the conversions from ppm cations to the equivalent USP *ROI* ⟨281⟩ unless a % *ROI* result of higher than 0.05% is obtained. In this case, those elements with concentrations higher than their corresponding Limits of Quantitation or 10 ppm (whichever is larger) should be analyzed by a different atomic spectroscopic method (such as ICP–AES and/or atomic absorption spectrometry), and the results from the ICP–AES and/or atomic absorption spectrometry will be used for the conversions from ppm cations to the equivalent USP ⟨281⟩ *ROI.*

METHOD VALIDATION

Validation of the method for use as an alternative to the USP *Heavy Metals* ⟨231⟩ test was provided in our recent publication (8). That validation is also applicable for use of the method as an alternative for USP *ROI* ⟨281⟩, and the data will not be repeated here.

In summary, the analysis of National Institute of Standards and Technology (NIST) 1643d (Trace Elements in Water) standard reference material and the spiking experiments showed excellent to acceptable accuracy for a semi-quantitative method, with the exception of Fe (results shown in *Table 2*). The erroneously high data for Fe using both ^{54}Fe and ^{57}Fe result from spectral interferences mainly from $^{14}N^{40}Ar$, $^{16}O^{38}Ar$, and $^{40}Ar^{16}O^{1}H$, which cannot be circumvented with the current instrument capabilities. Spiked samples also demonstrated acceptable method precision, and spiked blanks provided Limit of Detection (LOD) and Limit of Quantitation (LOQ) values that were below part-per-million (ppm) levels for all elements of pharmaceutical interest. The LOD and LOQ values are provided in *Table 3*.

Matrix effects and spectral interference—Positive results at moderate levels for some elements in the presence of one or more other high-level elements should always be investigated further for spectral interferences or by confirming the results with an alternative method such as ICP–AES or Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). Matrix effects are minimized by running the calibration blank and calibration standard in the same matrix as in the samples and by dissolving a minimum amount of sample in the solution to be analyzed (0.1% total dissolved solids), taking advantage of the extremely high sensitivity of the ICP–MS. Spectral interferences are monitored by using more than one isotope for the same element whenever possible for some interference-prone low-mass elements.

EQUIVALENCE OF THE ICP–MS METHOD TO USP ⟨281⟩ *ROI*

A demonstration of the equivalence of the ICP–MS method and the USP ⟨281⟩ *ROI* procedure was provided by the analysis of six typical drug substances by both methods. The results are given in *Table 4* and *Table 5*. The agreements are excellent.

UTILIZATION OF THE METHOD

A survey method that permits simultaneous qualitative to quantitative detection (depending on the elements and the concentration levels) of up to 69 elements, including all those of pharmaceutical interest, in less than 15 minutes would be viewed by some as a giant leap forward compared to traditional USP methods. The use of such a method, which employs a very sophisticated and expensive instrument, as an alternative to a seemingly economical wet chemical test that has been in use for decades would be viewed by others as technological overkill.

We take a less extreme view and believe that because the equipment is already present in the laboratory to address other very challenging analytical problems, its application to more mundane uses is simply good resource management. We have found that the extensive use of the ICP–MS for this elemental survey analysis does not degrade its capability for even more challenging tasks.

CONCLUSION

The proposed method uses direct dissolution of the samples in 80% nitric acid solution combined with ICP–MS as the analytical tool. It is an attractive alternative method for both USP *Heavy Metals* ⟨231⟩ and USP *ROI* ⟨281⟩. The availability of the ICP–MS in the laboratory offers much more rapid, sensitive, precise, simple, and element-specific analysis. Furthermore, it consumes far less sample and is safely applicable to potent compounds and other hazardous materials.

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Table 1. Elan 6000 Instrumental Conditions and Method Parameters

RF power	1300 W
Coolant argon flow	15.0 L/min
Auxiliary argon flow	1 L/min
Nebulizer argon flow	0.86–1.06 L/min
Sample introduction system	Cross-flow nebulizer with Scott spray chamber
Operating frequency	40 MHz
Sample uptake rate	1.5 mL/min
Detector mode	Dual mode
Sampler/skimmer cones	Platinum
Scanning mode	Peak hopping
Number of points/peak	1
Dwell time	15 ms
Sweeps/reading	40
Number of replicates	2

Table 2. Calibration Verification with NIST 1643d in 80% HNO_3 Matrix

Element	Isotope	Measured Concentration (ppm)	Certified or Reference (*) Value by NIST (ppm)
Li	7	0.0168	0.01815 ±0.00064
Be	9	0.0115	0.01253 ±0.00028
B	11	0.127	0.1448 ±0.0052
Na	23	21.9	22.07 ±0.64
Mg	24	8.5	7.989 ±0.035
Al	27	0.115	0.1276 ±0.0035
Si	28	3.28	2.7*
P	31	<LOQ	No certified or reference value
K	39	2.37	2.356 ±0.035
Ca	44	33.7	31.04 ±0.64
Sc	45	<LOQ	No certified or reference value
Ti	48	<LOQ	No certified or reference value
Ti	49	<LOQ	No certified or reference value
V	51	0.0361	0.0351 ±0.0014
Cr	52	0.0185	0.01853 ±0.00020
Cr	53	0.0194	0.01853 ±0.00020
Mn	55	0.0389	0.03766 ±0.00083
Fe	54	0.9383	0.0912 ±0.0039
Fe	57	0.1596	0.0912 ±0.0039
Co	59	0.0255	0.02500 ±0.00059
Ni	58	0.0596	0.0581 ±0.0027
Ni	60	0.0612	0.0581 ±0.0027
Cu	63	0.0205	0.0205 ±0.0038
Cu	65	0.0217	0.0205 ±0.0038
Zn	64	0.085	0.07248 ±0.00065
Zn	66	0.0782	0.07248 ±0.00065
Ga	69	<LOQ	No certified or reference value
Ge	72	<LOQ	No certified or reference value

Table 2. Calibration Verification with NIST 1643d in 80% HNO_3 Matrix *(Continued)*

Element	Isotope	Measured Concentration (ppm)	Certified or Reference (*) Value by NIST (ppm)
As	75	0.053	0.05602 ±0.00073
Se	77	0.0107	0.01143 ±0.00017
Se	82	0.00944	0.01143 ±0.00017
Rb	85	0.0125	0.013*
Sr	88	0.349	0.2948 ±0.0034
Y	89	<LOQ	No certified or reference value
Zr	90	<LOQ	No certified or reference value
Nb	93	<LOQ	No certified or reference value
Mo	95	0.117	0.1129 ±0.0017
Ru	101	<LOQ	No certified or reference value
Rh	103	<LOQ	No certified or reference value
Pd	105	<LOQ	No certified or reference value
Ag	107	0.00135	0.001270 ±0.000057
Cd	111	0.00664	0.00647 ±0.00037
In	115	<LOQ	No certified or reference value
Sn	118	0.0038	No certified or reference value
Sn	120	0.00374	No certified or reference value
Sb	121	0.0524	0.0541 ±0.0011
Te	125	0.000991	0.001*
Cs	133	0.00432	No certified or reference value
Ba	137	0.507	0.5065 ±0.0089
La	139	<LOQ	No certified or reference value
Ce	140	<LOQ	No certified or reference value
Pr	141	<LOQ	No certified or reference value
Nd	146	<LOQ	No certified or reference value
Sm	147	<LOQ	No certified or reference value
Eu	153	<LOQ	No certified or reference value
Gd	157	<LOQ	No certified or reference value
Tb	159	<LOQ	No certified or reference value
Dy	163	<LOQ	No certified or reference value
Ho	165	<LOQ	No certified or reference value
Er	166	<LOQ	No certified or reference value
Tm	169	<LOQ	No certified or reference value
Yb	172	<LOQ	No certified or reference value
Lu	175	<LOQ	No certified or reference value
Hf	178	<LOQ	No certified or reference value
Ta	181	<LOQ	No certified or reference value
W	182	<LOQ	No certified or reference value
Re	185	<LOQ	No certified or reference value
Os	189	<LOQ	No certified or reference value
Ir	193	<LOQ	No certified or reference value
Pt	195	<LOQ	No certified or reference value
Au	197	<LOQ	No certified or reference value

Pharmacopeial Forum
Vol. 29(4) [July–Aug. 2003]

Table 2. Calibration Verification with NIST 1643d in 80% HNO_3 Matrix *(Continued)*

Element	Isotope	Measured Concentration (ppm)	Certified or Reference (*) Value by NIST (ppm)
Hg	202	<LOQ	No certified or reference value
Tl	205	0.00791	0.00728 ±0.00025
Pb	208	0.019	0.01815 ±0.00064
Bi	209	0.014	0.013*
Th	232	<LOQ	No certified or reference value
U	238	<LOQ	No certified or reference value

Table 3. LODs and LOQs

Element	Isotope	80% HNO_3 Solution	
		LOD (ppm)	LOQ (ppm)
Li	7	0.1	0.5
Be	9	0.08	0.3
B	11	6	22
Na	23	0.3	1
Mg	24	0.2	0.6
Al	27	5	16
Si	28	35	115
P	31	230	766
K	39	1	4
Ca	44	4	12
Sc	45	1	4
Ti	48	0.3	1
Ti	49	0.07	0.2
V	51	0.02	0.08
Cr	52	0.06	0.2
Cr	53	0.08	0.3
Mn	55	0.03	0.09
Fe	54	148	493
Fe	57	1	4
Co	59	0.01	0.05
Ni	58	3	8
Ni	60	2	5
Cu	63	0.05	0.2
Cu	65	0.09	0.3
Zn	64	6	20
Ga	69	0.01	0.05
Ge	72	0.03	0.1
As	75	0.06	0.2
Se	77	0.5	2
Se	82	0.8	3
Rb	85	0.01	0.03

Table 3. LODs and LOQs *(Continued)*

Element	Isotope	80% HNO_3 Solution	
Sr	88	0.01	0.03
Y	89	0.02	0.05
Zr	90	0.01	0.03
Nb	93	0.02	0.07
Mo	95	0.02	0.08
Ru	101	0.01	0.04
Rh	103	0.01	0.03
Pd	105	0.02	0.08
Ag	107	0.02	0.05
Cd	111	0.04	0.1
In	115	0.01	0.04
Sn	118	0.02	0.05
Sb	121	0.02	0.08
Te	125	0.2	0.6
Cs	133	0.01	0.02
Ba	137	0.02	0.06
La	139	0.01	0.03
Ce	140	0.01	0.02
Pr	141	0.01	0.03
Nd	146	0.01	0.05
Sm	147	0.02	0.05
Eu	153	0.01	0.02
Gd	157	0.02	0.06
Tb	159	0.01	0.03
Dy	163	0.01	0.04
Ho	165	0.01	0.03
Er	166	0.03	0.09
Tm	169	0.01	0.03
Yb	172	0.01	0.04
Lu	175	0.01	0.04
Hf	178	0.01	0.04
Ta	181	0.02	0.07
W	182	0.07	0.2
Re	185	0.01	0.05
Os	189	0.2	0.7
Ir	193	0.01	0.05
Pt	195	0.02	0.07
Au	197	0.04	0.1
Hg	202	0.05	0.2
Tl	205	0.01	0.03
Pb	208	0.01	0.04
Bi	209	0.01	0.04
Th	232	0.01	0.02
U	238	0.01	0.02

Pharmacopeial Forum
Vol. 29(4) [July–Aug. 2003]

Table 4. Comparison of USP ⟨281⟩ and ICP–MS *ROI* Results

Sample ID		Compound #1		Compound #2		Compound #3	
Elements	Sulfates Formed	ICP–MS Result (ppm)	Equivalent *ROI* (%)	ICP–MS Result (ppm)	Equivalent *ROI* (%)	ICP–MS Result (ppm)	Equivalent *ROI* (%)
Li	Li_2SO_4		0.000		0.000		0.000
Na	Na_2SO_4	463	0.138		0.000	262	0.081
Mg	$MgSO_4$		0.000		0.000		0.000
Al	$Al_2(SO_4)_3$		0.000		0.000		0.000
Si	SiO_2	22	0.005	24	0.005	17	0.004
K	K_2SO_4		0.000		0.000		0.000
Ca	$CaSO_4$	24	0.007		0.000	10	0.003
Ti	$Ti_2(SO_4)_3$		0.000		0.000		0.000
V	VSO_4		0.000		0.000		0.000
Cr	$Cr_2(SO_4)_3$		0.000		0.000		0.000
Mn	$MnSO_4$		0.000		0.000		0.000
Fe	$Fe_2(SO_4)_3$		0.000		0.000		0.000
Co	$CoSO_4$		0.000		0.000		0.000
Ni	$NiSO_4$		0.000		0.000		0.000
Cu	$CuSO_4$		0.000		0.000		0.000
Zn	$ZnSO_4$		0.000		0.000		0.000
Total *ROI* (%) by ICP–MS			**0.150**		**<0.05**		**0.088**
USP ⟨281⟩ *ROI* (%)			**0.12**		**<0.05**		**0.09**

Table 5. Comparison of USP ⟨281⟩ and ICP–MS *ROI* Results

Sample ID		Compound #4		Compound #5		Compound #6	
Elements	Sulfates Formed	ICP–MS Result (ppm)	Equivalent *ROI* (%)	ICP–MS Result (ppm)	Equivalent *ROI* (%)	ICP–MS Result (ppm)	ICP–MS Result (ppm)
Li	Li_2SO_4		0.000		0.000		0.000
Na	Na_2SO_4	10100	3.118		0.000		0.000
Mg	$MgSO_4$		0.000		0.000		0.000
Al	$Al_2(SO_4)_3$		0.000		0.000		0.000
Si	SiO_2	66	0.014		0.000		0.004
K	K_2SO_4		0.000		0.000		0.000
Ca	$CaSO_4$		0.00		0.000		0.000
Ti	$Ti_2(SO_4)_3$		0.000		0.000		0.000
V	VSO_4		0.000		0.000		0.000
Cr	$Cr_2(SO_4)_3$		0.000		0.000		0.000
Mn	$MnSO_4$		0.000		0.000		0.000
Fe	$Fe_2(SO_4)_3$		0.000		0.000		0.000
Co	$CoSO_4$		0.000		0.000		0.000
Ni	$NiSO_4$		0.000		0.000		0.000
Cu	$CuSO_4$		0.000		0.000		0.000
Zn	$ZnSO_4$		0.000		0.000		0.000
Total *ROI* (%) by ICP–MS			**3.132**		**<0.05**		**<0.05**
USP⟨281⟩ *ROI* (%)			**3.2**		**<0.05**		**<0.05**

Changes to USP General Chapter *Heavy Metals* ⟨231⟩

John T. Geary*

ABSTRACT USP General Chapter *Heavy Metals* ⟨231⟩ has been the subject of discussion within the industry for several years, and its deficiencies were discussed during a USP Open Conference that was convened in June, 2003 in Philadelphia, PA. This *Stimuli* article reviews a few of the problems with USP ⟨231⟩ and outlines the changes USP is considering for a future proposal.

USP General Chapter *Heavy Metals* ⟨231⟩ has been the subject of discussion within the industry for several years. Deficiencies of the method were recently the topic of the USP Open Conference on Analytical Methods and General USP Topics that was held in June 2003 in Philadelphia. The Basel Working Group on the Determination of Metal Traces presented a thorough discussion of the problems encountered using ⟨231⟩ for heavy metals screening. To summarize their paper, ''USP ⟨231⟩ was never intended to be a universal test for heavy metals as it is currently applied.''

Methods I, II, and *III* suffer to some degree from the lack of specificity using colorimetric detection. Not all heavy metals are detected by the procedure. The method is capable only of detecting metals precipitated by sulfide and those that produce a black or brown precipitate. The use of a 600 °C ignition temperature causes loss of analyte, especially mercury, and has been confirmed as a cause of false negative results.

USP is proposing the following changes to *Heavy Metals* ⟨231⟩: The *Method II* preparation using high-temperature ignition will be withdrawn. *Methods I* and *III* will be continued. Colorimetric detection for these preparations will be continued, but an alternative method of detection, Inductively Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES), will be approved for both *Method I* and *Method III,* which will permit the user to choose either method of detection. USP intends to seek additional alternatives to colorimetric detection, such as x-ray fluorescence, atomic absorption, and electroanalytical methods. These alternatives will be proposed when sufficient data are available to attest to their effectiveness.

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Pharmacopeial Forum
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Inductively Coupled Plasma–Optical Emission Spectroscopy as an Alternative to the Heavy Metals Test

Martha Schenkenberger* and Nancy Lewen

ABSTRACT There has been growing concern regarding the use of the United States Pharmacopoeia (USP) test for *Heavy Metals, Method II* ⟨231⟩. A recent publication (*3*) describes the use of inductively coupled plasma–mass spectrometry (ICP–MS) as an alternative to the compendial heavy metals test. USP recognizes that the current method utilizes dated technology and has proposed that modern spectroscopic techniques be used to perform the heavy metals testing of pharmaceutical ingredients. A general chapter on plasma spectrochemistry was proposed in *Pharmacopeial Forum* (*4*).

This article describes the use of another spectroscopic technique, inductively coupled plasma–optical emission spectroscopy (ICP–OES), as an alternative to the compendial heavy metals test. ICP–OES offers many advantages over the compendial method. It is a rapid, multielement technique that can be used to assay for the following elements: antimony (Sb), arsenic (As), bismuth (Bi), cadmium (Cd), indium (In), lead (Pb), mercury (Hg), molybdenum (Mo), palladium (Pd), platinum (Pt), ruthenium (Ru), selenium (Se), silver (Ag), and tin (Sn). Other advantages to the use of this technique include the fact that only a small quantity of sample is required, and it provides element-specific results.

INTRODUCTION

Recent publications (*1, 2, 3*) have proposed the use of inductively coupled plasma–mass spectrometry (ICP–MS) as an alternative to the USP test for *Heavy Metals, Method II* ⟨231⟩. Additionally, a general chapter on plasma spectrochemistry was proposed in *Pharmacopeial Forum* (*4*). As a result, the authors propose an inductively coupled plasma–optical emission spectroscopy (ICP–OES) method as a possible alternative to the compendial method in chapter ⟨231⟩ for heavy metals. This method would provide element-specific quantitative results for the following elements: arsenic (As), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), lead (Pb), bismuth (Bi), silver (Ag), mercury (Hg), ruthenium (Ru), and molybdenum (Mo). Platinum (Pt), palladium (Pd), and selenium (Se) may also be determined because these elements either are frequently used as catalysts or are sufficiently toxic to warrant examination.

EXPERIMENTAL PROCEDURE

Accurately prepare a 1% solution of sample dissolved in a suitable solvent in an acid-washed volumetric flask. (A suitable solvent is one that is capable of completely dissolving the solid sample and does not provide any analytical interferences when introduced into the ICP–OES instrument. Additionally, a suitable solvent should be relatively nonvolatile, such as deionized water, dilute nitric acid, dilute hydrochloric acid, or a solution of butoxyethanol and water [25:75].) If necessary, add an appropriate internal standard according to the guidelines detailed in the proposed general chapter on plasma spectrochemistry in *PF* (*4*). Prepare working standard solutions that contain 0.5 and 1.0 µg/mL each of As, Cd, In, Sb, Pb, Bi, Hg, Ru, Mo, Pt, Pd, and, if necessary, an appropriate internal standard according to the guidelines detailed in the proposed general chapter on plasma spectrochemistry (*4*). Dilute to volume with the same solvent used to dissolve and dilute the sample. Prepare a second set of working standard solutions that contain 0.5 and 1.0 µg/mL each of Se and Ag and, if necessary, an appropriate internal standard according to the guidelines detailed in the proposed general chapter on plasma spectrochemistry (*4*). Dilute to volume with the same solvent used to dissolve and dilute the sample.

Using either an axial or lateral ICP–OES instrument, select at least three wavelengths per analyte element. Follow the guidelines detailed in the proposed general chapter on plasma spectrochemistry in *PF* (*4*) for calibration and analysis.

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4. ⟨730⟩ *Plasma Spectrochemistry. In-Process Revision,* **2004,** *PF* 30(3):1022–1039.

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Attachment 12

BRIEFING

á231ñ Heavy Metals, *USP 26* page 2057 and page 1570 of *PF* 28(5) [Sept.–Oct. 2002]. On the basis of comments received, the use of a pH meter to adjust the pH in *Method I* and *Method III* is proposed. Situations in which the *Monitor Preparation* has a lighter color than the *Standard Preparation* have been reported. This problem could indicate that the pH is out of range, and external pH short-range paper indicators may not be accurate enough to detect this change. In addition to these changes, and to be consistent with *Methods I* and *II*, a formula to calculate the amount of substance to be tested is added in *Method III*.

Method II also reflects changes to the USP test appearing under the harmonization section (see page 1570 of *PF* 28(5) [Sept.–Oct 2002]). These changes are based on suggestions made in a *Stimuli* article by K. B. Blake entitled *Harmonization of the USP, EP, and JP Heavy Metals Testing Procedures* (see page 1632 of *PF* 21(6) [Nov.–Dec. 1995]). The Blake article concludes that the ashing process in *Method II* is responsible for a loss of as much as 100% of mercury content and up to 50% of the heavy metals present. Improvements in heavy metal limit determination by *Method II* are expected following the inclusion of a *Monitor Preparation,* an increase in the amount of sample, and a corresponding increase in the amount of lead in both the *Standard Preparation* and the *Monitor Preparation*. The *Monitor Preparation* is intended as a means of validating the use of *Method 1I,* with the instruction to proceed to *Method III* if the solution from the *Monitor Preparation* is less colored than the solution from the *Standard Preparation*.

Comments regarding this proposal are invited and should be submitted by March 15, 2004.

(PA6: H. Pappa) RTS—39947-1; 39948-1

á231ñ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* á851ñ) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I*, or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III*, a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

Special Reagents

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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Change to read:

Method I

pH 3.5 Acetate Buffer— Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation— Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with

water to 25 mL. ~~Adjust~~ ■Using a pH meter, adjust■ 2S (*USP27*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ■ ■ 2S (*USP27*)
dilute with water to 40 mL, and mix.

Test Preparation— Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, ~~in percentage. Adjust~~ ■as a percentage. Using a pH meter, adjust■ 2S (*USP27*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ■ ■ 2S (*USP27*)
dilute with water to 40 mL, and mix.

Monitor Preparation— Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* ~~Adjust~~ ■Using a pH meter, adjust■ 2S (*USP27*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ■ ■ 2S (*USP27*)
dilute with water to 40 mL, and mix.

Procedure— To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface~~*~~: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and ~~the intensity of~~ the color of the ■solution from the■ 2S (*USP27*)
Monitor Preparation is equal to or ~~greater~~ ■darker■ 2S (*USP27*)
than that of the ■solution from the■ 2S (*USP27*)
Standard Preparation. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Change to read:

Method II

pH 3.5 Acetate Buffer— Prepare as directed for *Method I.*

Standard Preparation— ~~Prepare as directed under *Method I.*~~ ■Pipet 4 mL of the *Standard Lead Solution* into a suitable test tube, and add 10 mL of 6 N hydrocloric acid.■ 2S (*USP27*)

Test Preparation— Use a quantity, in g, of the substance to be tested as calculated by the formula:

~~2.0/(1000*L*),~~

■4.0/(1000*L*),■ 2S (*USP27*)

in which *L* is the *Heavy metals* limit, ~~in~~ ■as a■ 2S (*USP27*)
percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off ■(no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate, and ignite again. ■ 2S (*USP27*)
Cool, add ~~4 mL~~ ■5 mL■ 2S (*USP27*)

of 6 N hydrochloric acid, cover,
and digest on a steam bath for ~~15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.~~
■10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

Monitor Preparation— Pipet 4 mL of the *Standard Lead Solution* into a crucible identical to that used for the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation*. Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, and under the same conditions used for the *Test Preparation*. Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.■2S (*USP27*)

Procedure— ~~To~~■Adjust the solution in■2S (*USP27*)
each of the tubes containing the *Standard Preparation,* ~~and~~ the *Test Preparation,* ■and the *Monitor Preparation* with ammonium hydroxide, added cautiously and dropwise, to a pH of 9. Cool, and adjust with glacial acetic acid, added dropwise, to a pH of 8, and then add 0.5 mL in excess. Filter, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL.■2S (*USP27*)
Add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* ■and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation*. [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation,* proceed as directed for *Method III* for the substance being tested.]
■2S (*USP27*)

Change to read:

Method III

pH 3.5 Acetate Buffer— Prepare as directed for *Method I.*

Standard Preparation— Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—

■Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which L is the *Heavy metals* limit, as a percentage.■2S (*USP27*)

If the substance is a solid— Transfer the ■weighed■2S (*USP27*)
quantity of the test substance ~~specified in the individual monograph~~■■2S (*USP27*)

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 additional 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 in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid— Transfer the ■weighed■ 2S (*USP27*) quantity of the test substance ~~specified in the individual monograph~~■ ■ 2S (*USP27*) 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 cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with “add additional portions of the same acid mixture.”

Procedure— Treat the *Test Preparation* and the *Standard Preparation* as follows: ~~Adjust~~ ■Using a pH meter, adjust■ 2S (*USP27*) the solution to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ■ ■ 2S (*USP27*) with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the *Test Preparation* is not darker than that of the *Standard Preparation.*

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* In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.

Attachment 13

BRIEFING

á231ñ Heavy Metals, *USP 27* page 2204 and page 1603 of *PF* 29(5) [Sept.–Oct. 2003]. It is proposed to add a *Monitor Preparation* to *Method III.*

(PA4: H. Pappa) RTS—40535-1

á231ñ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* á851ñ) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I,* unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I,* or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III,* a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

Special Reagents

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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Change to read:

Method I

pH 3.5 Acetate Buffer— Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation— Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. ~~Adjust~~▲Using a pH meter, adjust▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲▲*USP28*
dilute with water to 40 mL, and mix.

Test Preparation— Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, ~~in percentage. Adjust~~▲as a percentage. Using a pH meter, adjust▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲▲*USP28*
dilute with water to 40 mL, and mix.

Monitor Preparation— Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* ~~Adjust~~▲Using a pH meter, adjust▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*
dilute with water to 40 mL, and mix.

Procedure— To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and ~~the intensity of~~ the color of the ▲solution from the▲*USP28*
Monitor Preparation is equal to or ~~greater~~▲darker▲*USP28*
than that of the ▲solution from the▲*USP28*
Standard Preparation. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Change to read:

Method II

pH 3.5 Acetate Buffer— Prepare as directed for *Method I.*

Standard Preparation— ~~Prepare as directed under *Method I.*~~▲Pipet 4 mL of the *Standard Lead Solution* into a suitable test tube, and add 10 mL of 6 N hydrochloric acid.▲*USP28*

Test Preparation— Use a quantity, in g, of the substance to be tested as calculated by the formula:

~~2.0/(1000*L*),~~

▲4.0/(1000*L*),▲*USP28*

in which *L* is the *Heavy metals* limit, ~~in~~▲as a▲*USP28*
percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off ▲(no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate, and ignite again.▲*USP28*
Cool, add ~~4 mL~~▲5 mL▲*USP28*
of 6 N hydrochloric acid, cover, ▲ and▲*USP28*
digest on a steam bath for ~~15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.~~
▲10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

Monitor Preparation— Pipet 4 mL of the *Standard Lead Solution* into a crucible identical to that used for the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation.* Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, and under the same conditions used for the *Test Preparation.* Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.▲*USP28*

Procedure— ~~To~~ ▲Adjust the solution in ▲*USP28*
each of the tubes containing the *Standard Preparation,* ~~and~~ ▲ ▲*USP28*
the *Test Preparation,* ▲and the *Monitor Preparation* with ammonium hydroxide, added cautiously and dropwise, to a pH of 9. Cool, and adjust with glacial acetic acid, added dropwise, to a pH of 8, and then add 0.5 mL in excess. Filter, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL. ▲*USP28*
Add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* ▲and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation.* [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation,* proceed as directed for *Method III* for the substance being tested.] ▲*USP28*

Change to read:

Method III

pH 3.5 Acetate Buffer— Prepare as directed for *Method I.*

Standard Preparation— Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—

▲Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, as a percentage. ▲*USP28*

If the substance is a solid— Transfer the ▲weighed ▲*USP28*
quantity of the test substance ~~specified in the individual monograph~~ ▲ ▲*USP28*
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 additional 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 in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid— Transfer the ▲weighed ▲*USP28*

quantity of the test substance ~~specified in the individual monograph~~ ▲*USP28*
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 cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with "add additional portions of the same acid mixture."

▲Monitor Preparation— Proceed with the digestion using the same amount of sample and the same procedure as directed in the *Test Preparation* until the step "Cool, dilute cautiously with a few mL of water." Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix. ▲*USP28*

Procedure— Treat the *Test Preparation*, the *Standard Preparation* ▲and the *Monitor Preparation* ▲*USP28*
as follows: ~~Adjust~~ ▲Using a pH meter, adjust ▲*USP28*
the solution to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ▲ ▲*USP28*
with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface[*]: the color of the *Test Preparation* is not darker than that of the *Standard Preparation,* ▲and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation.* ▲*USP28*

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[*] In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.

Attachment 14

BRIEFING

á231ñ Heavy Metals, *USP 27* page 2204 and page 217 of *PF* 30(1) [Jan.–Feb. 2004]. On the basis of comments received, it is proposed that, when necessary, the adjustment of the pH can be made using a pH meter or short-range pH indicator paper.

(PA4: H. Pappa) RTS—40683-1; 40815-1

á231ñ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* á851ñ) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I,* unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I,* or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III,* a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

Special Reagents

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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Change to read:

Method I

pH 3.5 Acetate Buffer— Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation— Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. ~~Adjust~~▲Using a pH meter or short-range pH indicator paper as external indicator, adjust ▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*
dilute with water to 40 mL, and mix.

Test Preparation— Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, ~~in percentage. Adjust~~▲as a percentage. Using a pH meter or short-range pH indicator paper as external indicator, adjust▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*

dilute with water to 40 mL, and mix.

Monitor Preparation— Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* ~~Adjust~~▲Using a pH meter or short-range pH indicator paper as external indicator, adjust▲*USP28*

with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲▲*USP28*

dilute with water to 40 mL, and mix.

Procedure— To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and ~~the intensity of the color of the~~ ▲the color of the solution from the ▲*USP28*

Monitor Preparation is equal to or ~~greater~~▲darker▲*USP28*

than that of the▲solution from the▲*USP28*

Standard Preparation. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Change to read:

Method II

pH 3.5 Acetate Buffer— Prepare as directed under *Method I.*

Standard Preparation— ~~Prepare as directed under *Method I.*~~▲Pipet 4 mL of the *Standard Lead Solution* into a suitable test tube, and add 10 mL of 6 N hydrocloric acid.▲*USP28*

Test Preparation— Use a quantity, in g, of the substance to be tested as calculated by the formula:

~~2.0/(1000*L*),~~

▲4.0/(1000*L*),▲*USP28*

in which *L* is the *Heavy metals* limit, ~~in~~▲as a▲*USP28*

percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off▲(no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate, and ignite again.▲*USP28*

Cool, add ~~4 mL~~▲5 mL▲*USP28*

of 6 N hydrochloric acid, cover, ▲ and▲*USP28*

digest on a steam bath for ~~15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.~~

▲10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

Monitor Preparation— Pipet 4 mL of the *Standard Lead Solution* into a crucible identical to that used for the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation.* Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, and under the same conditions used for the *Test*

Preparation. Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.▲*USP28*

Procedure— ~~To~~▲Adjust the solution in▲*USP28*
each of the tubes containing the *Standard Preparation,* ~~and~~▲▲*USP28*
the *Test Preparation,*▲and the *Monitor Preparation* with ammonium hydroxide, added cautiously and dropwise, to a pH of 9. Cool, and adjust with glacial acetic acid, added dropwise, to a pH of 8, and then add 0.5 mL in excess. Using a pH meter or short-range pH indicator paper as external indicator, check the pH, and adjust, if necessary, with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Filter, if necessary, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL.▲*USP28*
Add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,*▲and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation.* [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation,* proceed as directed for *Method III* for the substance being tested.] ▲*USP28*

Change to read:

Method III

pH 3.5 Acetate Buffer— Prepare as directed under *Method I.*

Standard Preparation— Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—

▲Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, as a percentage.▲*USP28*

If the substance is a solid— Transfer the▲weighed▲*USP28*
quantity of the test substance ~~specified in the individual monograph~~▲▲*USP28*
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 additional 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 in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid— Transfer the▲ weighed *▲USP28* quantity of the test substance ~~specified in the individual monograph~~▲ *▲USP28* 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 cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with "add additional portions of the same acid mixture."

▲Monitor Preparation— Proceed with the digestion using the same amount of sample and the same procedure as directed in the *Test Preparation* until the step "Cool, dilute cautiously with a few mL of water." Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix. *▲USP28*

Procedure— Treat the *Test Preparation,* the *Standard Preparation*▲ and the *Monitor Preparation* *▲USP28* as follows: ~~Adjust~~▲ Using a pH meter or short-range pH indicator paper as external indicator, adjust *▲USP28* the solution to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ *▲USP28* with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface[*]: the color of the *Test Preparation* is not darker than that of the *Standard Preparation,*▲ and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation.* *▲USP28*

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[*] In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.

Attachment 15

BRIEFING

á231ñ Heavy Metals, *USP 27* page 2204 and page 614 of *PF* 30(2) [Mar.–Apr. 2004]. It is proposed to revise *Method II* to include a *Note* regarding the inability of this method to recover mercury.

(PA6: K. Zaidi) RTS—41212-1

á231ñ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* á851ñ) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I,* unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I,* or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III,* a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

SPECIAL REAGENTS

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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Change to read:

METHOD I

pH 3.5 Acetate Buffer— Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation— Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. ~~Adjust~~▲Using a pH meter or short-range pH indicator paper as external indicator, adjust ▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*
dilute with water to 40 mL, and mix.

Test Preparation— Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, ~~in percentage. Adjust~~▲as a percentage. Using a pH meter or short-range pH indicator paper as external indicator, adjust▲*USP28*
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*

dilute with water to 40 mL, and mix.

Monitor Preparation— Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* ~~Adjust~~▲Using a pH meter or short-range pH indicator paper as external indicator, adjust ▲*USP28*

with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~▲ ▲*USP28*

dilute with water to 40 mL, and mix.

Procedure— To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface *: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and ~~the intensity of the color of the~~ ▲the color of the solution from the ▲*USP28*

Monitor Preparation is equal to or ~~greater~~▲darker▲*USP28*

than that of the▲solution from the▲*USP28*

Standard Preparation. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Change to read:

METHOD II

■

NOTE—This method does not recover mercury.

■1S (*USP28*)

pH 3.5 Acetate Buffer— Prepare as directed under *Method I.*

Standard Preparation— ~~Prepare as directed under *Method I.*~~▲Pipet 4 mL of the *Standard Lead Solution* into a suitable test tube, and add 10 mL of 6 N hydrochloric acid.▲*USP28*

Test Preparation— Use a quantity, in g, of the substance to be tested as calculated by the formula:

~~2.0/(1000*L*),~~

▲4.0/(1000*L*),▲*USP28*

in which *L* is the *Heavy metals* limit, ~~in~~▲as a▲*USP28*

percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off▲(no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate, and ignite again.▲*USP28*

Cool, add ~~4 mL~~▲5 mL▲*USP28*

of 6 N hydrochloric acid, cover, ▲ and▲*USP28*

digest on a steam bath for ~~15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.~~

▲10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

Monitor Preparation— Pipet 4 mL of the *Standard Lead Solution* into a crucible identical to that used for the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation.* Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, and under the same conditions used for the *Test Preparation.* Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.▲*USP28*

Procedure— ~~To~~▲Adjust the solution in▲*USP28*
each of the tubes containing the *Standard Preparation,* ~~and~~▲▲*USP28*
the *Test Preparation,*▲and the *Monitor Preparation* with ammonium hydroxide, added cautiously and dropwise, to a pH of 9. Cool, and adjust with glacial acetic acid, added dropwise, to a pH of 8, and then add 0.5 mL in excess. Using a pH meter or short-range pH indicator paper as external indicator, check the pH, and adjust, if necessary, with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Filter, if necessary, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL.▲*USP28*

Add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,*▲and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation.* [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation,* proceed as directed for *Method III* for the substance being tested.]▲*USP28*

Change to read:

METHOD III

pH 3.5 Acetate Buffer— Prepare as directed under *Method I.*

Standard Preparation— Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—

▲Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, as a percentage.▲*USP28*

If the substance is a solid— Transfer the▲weighed▲*USP28*
quantity of the test substance ~~specified in the individual monograph~~▲▲*USP28*
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 additional 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 in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid— Transfer the ▲weighed ▲*USP28* quantity of the test substance ~~specified in the individual monograph~~ ▲ ▲*USP28* 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 cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with "add additional portions of the same acid mixture."

▲Monitor Preparation— Proceed with the digestion using the same amount of sample and the same procedure as directed in the *Test Preparation* until the step "Cool, dilute cautiously with a few mL of water." Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix. ▲*USP28*

Procedure— Treat the *Test Preparation,* the *Standard Preparation* ▲ and the *Monitor Preparation* ▲*USP28* as follows: ~~Adjust~~ ▲Using a pH meter or short-range pH indicator paper as external indicator, adjust ▲*USP28* the solution to a pH between 3.0 and 4.0, ~~using short-range pH indicator paper as external indicator,~~ ▲ ▲*USP28* with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface[*]: the color of the *Test Preparation* is not darker than that of the *Standard Preparation,* ▲ and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation.* ▲*USP28*

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[*] In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface

Attachment 16

BRIEFING

〈 231 〉 Heavy Metals, *USP 28* page 2299 and page 3295 of the *First Supplement.* On the basis of comments received, *Method II* is being revised to adequately address the issues resulting from the official publication of the revised *Method II* in the *First Supplement.* This proposed revision will not be the final solution to the issues related to *Method II,* but it will address the concerns of stakeholders at present. USP is working on developing a more robust method.

(PA6: K. Zaidi) RTS—42984-1; 42988-1

〈 231 〉 HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* 〈 851 〉) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I,* unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I,* or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III,* a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Change to read:

METHOD I

pH 3.5 Acetate Buffer—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. ■Using a pH meter or short-range pH indicator paper as external indicator, adjust■ 1S (*USP28*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ■■ 1S (*USP28*)
dilute with water to 40 mL, and mix.

Test Preparation—Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve in and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, ■as a percentage. Using a pH meter or short-range pH indicator paper as external indicator, adjust■ 1S (*USP28*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ■■ 1S (*USP28*)

dilute with water to 40 mL, and mix.

Monitor Preparation—Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* ■Using a pH meter or short-range pH indicator paper as external indicator, adjust ■1S (*USP28*)
with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, ■■1S (*USP28*)
dilute with water to 40 mL, and mix.

Procedure—To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface *: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and ■the color of the solution from the■1S (*USP28*)
Monitor Preparation is equal to or ■darker■1S (*USP28*)
than that of the ■solution from the■1S (*USP28*)
Standard Preparation. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Change to read:

METHOD II

■
NOTE—This method does not recover mercury.
■1S (*USP28*)

pH 3.5 Acetate Buffer—Prepare as directed for *Method I.*

Standard Preparation—■Pipet 4 mL of the *Standard Lead Solution* into a suitable test tube, and add 10 mL of 6 N hydrochloric acid.■1S (*USP28*)

Test Preparation—Use a quantity, in g, of the substance to be tested as calculated by the formula:

$$■4.0/(1000L)■$$ 1S (*USP28*)

in which L is the heavy metals limit, ■as a■1S (*USP28*)
percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off ■(no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate, and ignite again.
■1S (*USP28*)
Cool, add ■5 mL■1S (*USP28*)
of 6 N hydrochloric acid, cover, ■and■1S (*USP28*)
digest on a steam bath for
■10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

~~Monitor Preparation—Pipet 4 mL of the *Standard Lead Solution* into a crucible identical to that used for the *Test Preparation* and containing a quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation.* Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, and under the same conditions used for the *Test Preparation.* Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.■1S (*USP28*)~~

■■2S (*USP29*)

Procedure—■Adjust the solution in■1S (*USP28*)
each of the tubes containing the *Standard Preparation* ■■1S (*USP28*)
■and■2S (*USP29*)
the *Test Preparation* ■~~and the *Monitor Preparation* ~~■2S (*USP29*)
with ammonium hydroxide, added cautiously and dropwise, to a pH of 9. ■Thoroughly mix the solution after each addition of ammonium hydroxide.■2S (*USP29*)
Cool, and adjust with glacial acetic acid, added dropwise, to a pH of 8, then add 0.5 mL in excess. Using a pH meter, ~~or short-range pH indicator paper as external indicator, check the pH,and adjust,~~■check and adjust the pH,■2S (*USP29*)
if necessary, with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0 . Filter, if necessary, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL.■1S (*USP28*)
Add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation.* ■~~and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation.* [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation,* proceed as directed for *Method III* for the substance being tested.]~~
~~■1S (*USP28*)~~
■■2S (*USP29*)

Change to read:

METHOD III

pH 3.5 Acetate Buffer—Prepare as directed under *Method I.*

Standard Preparation—Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes; cool; cautiously add 10 mL of water; and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested. Boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—■Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which *L* is the *Heavy metals* limit, as a percentage.■1S (*USP28*)

If the substance is a solid—Transfer the ■weighed■1S (*USP28*)
quantity of the test substance ■■1S (*USP28*)
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, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid—Transfer the ■weighed■ 1S (*USP28*) quantity of the test substance ■■ 1S (*USP28*) 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 cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with “add portions of the same acid mixture.”

■Monitor Preparation—Proceed with the digestion, using the same amount of sample and the same procedure as directed in the subsection *If the substance is a solid* in the section *Test Preparation,* until the step “Cool, dilute cautiously with a few mL of water.” Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.■ 1S (*USP28*)

Procedure—Treat the *Test Preparation,* the *Standard Preparation,* ■and the *Monitor Preparation*■ 1S (*USP28*) as follows. ■Using a pH meter or short-range pH indicator paper as external indicator, adjust■ 1S (*USP28*) the solution to a pH between 3.0 and 4.0 ■■ 1S (*USP28*) with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the *Test Preparation* is not darker than that of the *Standard Preparation,* ■and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation.*■ 1S (*USP28*)

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* In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.

Notice of Revision to General Chapter Heavy Metals <231> Method II

Type of Posting
Interim Revision Announcement
Posting Date
14–Jun–2005

In response to comments from industry, USP is reverting back to the Heavy Metals text that appeared in USP 28–NF 23 page 2300 for Heavy Metals Method II. The USP 28–NF 23 test has been used in industry for some time. The search continues for a more robust and practical method. This change will appear in the Third Interim Revision Announcement to USP 29–NF 24 which will be published in Pharmacopeial Forum 32(3) and will become official on June 1, 2006.

Should you have any questions, please contact Kahkashan Zaidi, Ph.D., Senior Scientist, General Chapters (+1-301-816-8269 or kxz@usp.org).

General Chapter on Inorganic Impurities: Heavy Metals

USP Ad Hoc Advisory Panel on Inorganic Impurities and Heavy Metals and USP Staff[*]

ABSTRACT In the ICH Q3A *Impurities in Drug Substances* guidance, impurities are classified as organic, inorganic, and residual solvents. Within the inorganic impurities classification, the metals listed in *Table 1* are important to control in food, dietary supplements, and drug articles. Many toxic metal impurities found in pharmaceutical articles have been controlled for years by application of the Heavy Metals test described in *USP–NF* General Chapter *Heavy Metals* ⟨ 231 ⟩. However, the procedures and the methods contained in ⟨ 231 ⟩ lack the sensitivity, specificity, and recovery to monitor properly the levels of these metals. A number of additional chapters for the control of specific metals and other inorganic impurities are contained in *USP–NF*. This *Stimuli* article proposes a new *USP* General Chapter for the control of inorganic impurities in drug and dietary supplement articles intended for use in humans. For the purposes of this article, *inorganic impurity, metal,* and *element* all refer to those elements listed in *Table 1*. The proposed new General Chapter recommends procedures that rely on modern analytical technology and includes limits that are based on toxicity and exposure levels for the selected metals. The new General Chapter also introduces a performance-based approach for the selection of the appropriate technology. This chapter is proposed to replace ⟨ 231 ⟩ and may impact other General Chapters that control metals.

INTRODUCTION

Among the category of inorganic impurities, metal impurities have long been monitored in food and drug articles intended for consumption by humans and other animals. For purposes of this General Chapter, drug articles include: drug substances and products (including natural-source and rDNA biologics) and excipients. Dietary supplements and their ingredients are also included, but other foods and food ingredients will not be addressed. Some metals may pose no significant health hazard at sufficiently low exposure levels, when present as certain complexes, at certain oxidation states, or in organic combinations. This chapter should be considered a screening method to identify the presence of potentially hazardous elements. Where *speciation* of an element is important, further testing is necessary. In these cases, the monograph will include specific instructions for appropriate identification and control. The topic of speciation will not be covered further in this article.

Some inorganic impurities are toxic at low levels, and these impurities should be monitored to ensure safety. Sources of inorganic impurities include those that are deliberately added to the process (e.g., catalysts), those that are carried through a process that is conducted according to good manufacturing practices (e.g., undetected contaminants from starting materials or reagents), those coming from the process (e.g., leaching from pipes and other equipment), and those that occur naturally (e.g., from naturally derived plant or mineral sources). Regardless of source, the control of these impurities may be certified by a vendor, but purchasers also must corroborate the absence of impurities before using these materials in a manufactured article.

The General Chapters Expert Committee of the USP Council of Experts formed an Ad Hoc Advisory Panel on Inorganic Impurities and Heavy Metals to assist the Expert Committee in revision of General Chapter *Heavy Metals* ⟨ 231 ⟩. As drafted by this Ad Hoc Advisory Panel and revised by the Expert Committee, the proposed revision specifies that the level of each inorganic impurity should not exceed the limit defined in *Table 1* or otherwise specified in the individual monograph. This level is determined by concomitant comparison with a monitor solution and *USP Reference Standard* solution(s).

The selection of an instrumental technique and a procedure for the evaluation of the inorganic impurities specified in *Table 1* requires the evaluation of a large number of variables including, among others, sensitivity, precision, accuracy, compatibility, time, and cost. The method selected may include plasma spectrochemistry, atomic absorption spectroscopy, or any other method that displays requisite accuracy (trueness and uncertainty) and established sensitivity and specificity. Meeting this requirement must be demonstrated experimentally using the *USP Reference Standard(s)*. Any procedure that provides measurement values within ± 20% of the certified concentration for each element in the appropriate *USP Reference Standard(s)* is considered to be an acceptable procedure to demonstrate compliance. A guide for the selection of a procedure is presented in *Figure 1*. When a manufacturer does not have a preferred procedure, or when the preferred procedure does not meet criteria for performance described above, proceed as directed in the remainder of this General Chapter.

Procedure—Determine the levels of individual inorganic impurities by the test, unless the individual monograph specifies otherwise.

Reagents—All reagents used for the preparation of sample and standard solutions should be free of inorganic impurities in accordance with *Plasma Spectrochemistry* ⟨ 730 ⟩. Commercial, National Institute of Standards and Technology–traceable elemental stock standards, either single element or multi-element, containing Al, Sb, As, Be, B, Cd, Cr, Co, Cu, In, Ir, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ni, Os, Pd, Pt, Rh, Rb, Ru, Se, Sr, Tl, Sn, W, or Zn at a recommended concentration of 100 µg/mL or greater also are used as reagents.

Performance-based USP Reference Standards—

USP Inorganic Impurities Class 1 Reference Standard for test articles soluble in aqueous solutions.

USP Inorganic Impurities Class 2 Reference Standard for test articles soluble in organic solvents.

USP Inorganic Impurities Class 3 Reference Standard for closed-vessel microwave digestions.

Equipment—One of the following plasma spectrometers is required for an analyst to perform this multi-element analysis:

1. Inductively coupled plasma–atomic (optical) emission spectrometer.
2. Inductively coupled plasma–mass spectrometer.

In addition, a closed-vessel microwave digestion system may be required for the preparation of test materials (see *Figure 1*).

METHOD

Sample Preparation

Determine the means of sample preparation using the flow chart in *Figure 1*. The sample preparation scheme should provide sufficient sample loading to allow quantification of each element at the specified limit stated in the corresponding monograph or as stated in *Table 1*. For closed-vessel microwave digestions follow the manufacturer's recommended procedures to ensure safe usage. Use utmost caution if concentrated hydrofluoric acid (HF) is used for the preparation of test articles, and review or establish local procedures for safe handling, safe disposal, and HF-tolerant instrumental configurations. [NOTE—The specific details of the Sample preparation have not been included in this *Stimuli* article but have been developed by the Ad Hoc Advisory Panel. The decision to exclude the specific method details from the *Stimuli* article is based on the desire of the Ad Hoc Advisory Panel to receive feedback on the concepts proposed herein rather than on the specific method. Based on the feedback received, these details may be included in future chapter development.]

System Suitability Criteria—

Method reporting limit

The method reporting limit (MRL) is defined as the lowest element concentration of a solution prepared in the working calibration standard matrix that can be experimentally determined to within ± 30% of the prepared concentration. The sensitivity criterion for the method is that the MRL is 0.5 × the *USP* limit for each applicable element.

Recovery

The suitability of the sample preparation scheme must be demonstrated by preparation and analysis of a suitable *USP Reference Standard* and by spike recovery measurements of the specific test article according to ⟨ 730 ⟩. The spiked test article solution will be referred to as a *Monitor solution*. The experimental concentration results shall be ± 20% of the certified concentration for each required element in the analysis. The spike recovery results for the *Monitor solution* must be ± 20% of the spike concentration for each element. Analysis of a suitable *USP Reference Standard* shall be included with the analyses of test articles and must be within ± 20% of the certified concentration for each required element for the results to be considered acceptable.

Calibration

Prepare calibration standards in the same solution as used for preparation of the test articles. Analysts are encouraged to use internal standards according to ⟨ 730 ⟩ for preparation of test article and calibration standard solutions. Prepare 4 working standards plus a blank at element concentrations encompassing the required *USP* limits for the test article, the *USP Reference Standard,* and the *Monitor solution*. Standard curve acceptance criteria must be met according to ⟨ 730 ⟩. If the concentration of an element in the test article solution is determined to be greater than 110% of the highest calibration standard concentration, the test article solution should be appropriately diluted within the range of the standard curve and then re-analyzed.

Drift

To monitor instrument drift, analyze a working standard solution at an intermediate concentration of each element immediately following standardization, following the final test solution, and during the analysis at a frequency of one working standard solution analysis per not more than 10 sample analyses during the analytical run. The check standard results should agree to within ± 30% of the prepared concentration for each element. Reanalyze element results for test article solutions that are not bracketed with results within the tolerance for the check standard.

Analysis [NOTE—The specific details of the methods have not been included in this *Stimuli* article but have been developed by the Ad Hoc Advisory Panel. The decision to exclude the specific method details from the *Stimuli* article is based on the desire of the Ad Hoc Advisory Panel to receive feedback on the concepts proposed herein rather than on the specific method. Based on the feedback received, these details may be included in future chapter development.]

Calculations and Reporting—

Upon completion of the analysis, calculate the final concentration of a given element in the test article in µg/g from the solution element concentration in µg/mL as follows:

$$C = [(A \times V_1)/W] \times (V_2 / V_3)$$

where:

C = concentration of analyte in µg/g,
A = instrument reading in µg/mL,
V_1 = volume of initial test article preparation,
W = weight of test article preparation in g,
V_2 = total volume of any dilution performed in mL, and
V_3 = aliquot of initial test article preparation used in any dilution performed in mL.

Similarly, calculate the final concentration of a given element in the test article in µg/g from the solution element concentration in ng/mL as follows:

$$C = [(A \times V_1)/W] \times (1\ \mu g/1000\ ng)(V_2 / V_3)$$

where:

C = concentration of analyte in µg/g,
A = instrument reading in ng/mL,
V_1 = volume of initial test article preparation,
W = weight of test article preparation in g,
V_2 = total volume of any dilution performed in mL, and
V_3 = aliquot of initial test article preparation used in any dilution performed in mL.

Calculate the results for each analyte, and compare the values obtained for the test article to those provided in *Table 1*. The results should not exceed the values in the table.

CONCLUSION

The USP Ad Hoc Advisory Panel on Inorganic Impurities and Heavy Metals invites comments on the recommendations regarding the use of appropriate analytical instrumentation with limits that are based on toxicity and exposure levels for the metals and the new approach for the determination of an appropriate analytical procedure by the application of *USP Reference Standards* described in this *Stimuli* article. Please send detailed comments to: Kahkashan Zaidi, PhD, Senior Scientist, Documentary Standards Division, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel. 301.816.8269; e-mail kxz@usp.org.

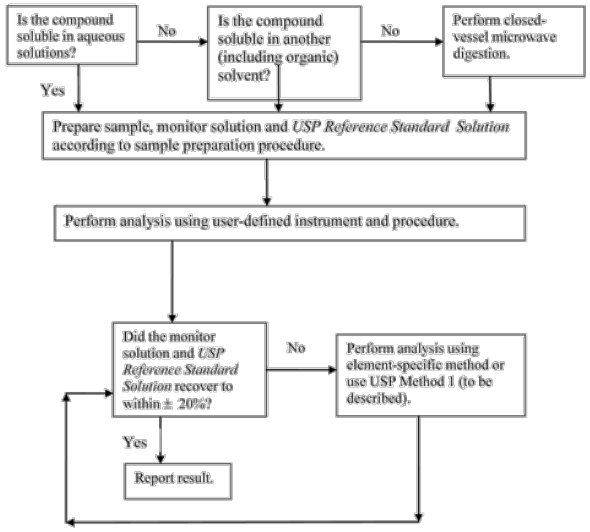

Figure 1. Inorganic impurity decision tree.

Table 1. Element limits for oral and parenteral materials. [**NOTE**—The contents of this table represent a first approximation by members of the Ad Hoc Advisory Panel and are under active discussion internationally.][a]

Element	Oral Permitted Daily Exposure for Dosage Forms, µg/day	USP Oral Limit, µg/g	USP Parenteral Limit, µg/g
Aluminum (Al)	50,000	5000	500
Antimony (Sb)	20	2	0.2
Arsenic (As)	15	1.5	0.15
Beryllium (Be)	100	10	1
Boron (B)	10,000	1000	100
Cadmium (Cd)	25	2.5	0.25
Chromium (Cr)	150	15	1.5
Cobalt (Co)	1000	100	10
Copper (Cu)	500	50	5
Indium (In)	100	10	1
Iridium (Ir)	100	10	1
Iron (Fe)	15,000	1500	150
Lead (Pb)	10[b]	1	0.1
Lithium (Li)	600	60	6
Magnesium (Mg)	[c]	[c]	[c]
Manganese (Mn)	7000	700	70

Mercury (Hg)	15	1.5	0.15
Molybdenum (Mo)	250	25	2.5
Nickel (Ni)	1000	100	10
Osmium (Os)	100	10	1
Palladium (Pd)	100	10	1
Platinum (Pt)	100	10	1
Rhodium (Rh)	100	10	1
Rubidium (Rb)	[c]	c	[c]
Ruthenium (Ru)	100	10	1
Selenium (Se)	250	25	2.5
Strontium (Sr)	30,000	3000	300
Thallium (Tl)	4	0.4	0.04
Tin (Sn)	30,000	3000	300
Tungsten (W)	375	37.5	3.8
Zinc (Zn)	15,000	1500	150

[a] Some of the limits in this table were calculated using the criteria given in the EMEA *Guideline on the Specification Limits for Residues of Metal Catalysts,* available at: http://www.emea.europa.eu/pdfs/human/swp/444600.pdf, accessed 25 March 2008.

[b] Limit for lead calculated from the FDA limit for bottled drinking water: 5 µg/L assuming consumption of 2 L/day.

[c] Under deliberation.

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Elemental Impurities—Information

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ABSTRACT This *Stimuli* article presents the toxicological and regulatory bases for the elemental impurities limits specified in a proposed new *USP–NF* General Chapter. The article focuses on four metallic elements of known toxicity: arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). The *Stimuli* article presents literature studies, along with the specific rationale for the proposed limits. This article also references the EMEA guidance on metal catalysts as a basis for certain other elemental impurities and presents specific considerations regarding dietary supplement products.

INTRODUCTION

The objective of this *Stimuli* article is to provide rationale in support of safe limits for certain elemental impurities in pharmaceuticals and dietary supplements. For pharmacopeial purposes, elemental impurities are defined as elements that are found in the environment or that are used or introduced in the manufacture of drug substances or excipients. The term *elemental impurities* is adopted here as an alternative to the ill-defined term *heavy metals*, and the highlighted elemental impurities include various transition metals and metalloids. In addition to catalysts or reagents normally used in chemical synthesis, sources of elemental impurities in pharmaceuticals could include minerals used in the manufacture of excipients, container–closure systems, and other product contact surfaces. Elemental impurities are those elements that are not completely removed by practical manufacturing techniques and should be evaluated relative to safety-based limits.

The permissible daily exposure (PDE) values provided for each highlighted elemental impurity have been adopted from published evaluations by regulatory bodies. USP has considered the rationale for reference doses (RfDs) published by the US Environmental Protection Agency (EPA) (*1*) as well as PDEs listed in the *Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents* (*2*). The EMEA guidance acknowledges that ''owing to wide variability of the nature, quality, and quantity of toxicological data amongst the metal elements of interest, it is not possible to employ a totally consistent approach.'' That is also the case for the PDE rationale highlighted below. To that end, USP will advise EMEA of inconsistencies, if any are found, in the data.

METHODS FOR ESTABLISHING EXPOSURE LIMITS

The PDEs (μg/day) were derived from the most sensitive toxicological endpoint using a standard set of assumptions for the risk assessment:

- 10 g/day dose for drug products for calculation of ppm limits
- 50-kg person for extrapolation from animal data on a body weight-basis
- 70-year lifetime
- 10% bioavailability for extrapolation from the oral PDE to the parenteral PDE.

Appropriate uncertainty factors were applied to the lowest no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL). The applied uncertainty factors span orders of magnitude and are considered adequate to account for the proportion of the total acceptable daily exposure attributable to drug product relative to other sources of exposure (i.e., food, water). Thus, adjustments for relative source contribution were not conducted.

CLASS 1 ELEMENTAL IMPURITIES

Arsenic (As)

Introduction

The natural abundance of arsenic in the Earth's crust is about 1.8 ppm. Arsenic will partly substitute for phosphorus in biochemical reactions. The largest worldwide production of arsenic occurs in China. Arsenic is used in glass production, light-emitting diodes, and many other places.

Toxicokinetics: Absorption, Disposition, Metabolism, and Excretion (ADME)

Water-soluble inorganic arsenic compounds are absorbed through the GI tract (> 90%) and lungs; are distributed primarily to the liver, kidney, lung, spleen, aorta, and skin; and as much as 80% of a dose is excreted mainly in the urine within 61 hours following oral dosing (*3–5*). Pentavalent arsenic is reduced to the trivalent form and then is methylated in the liver to less toxic methylarsinic acids (*4*).

Toxicological Effects

Acute oral toxicity is characterized by GI and neurological effects (*6*), and acute oral LD_{50} values range from about 10 to 300 mg/kg (*4, 7*). Low subchronic doses

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have resulted in immunosuppression, (*8*) and hepatorenal effects (*9–14*). Chronic exposures have resulted in mild hyperkeratosis and bile duct enlargement with hyperplasia, focal necrosis, and fibrosis (*15, 16*). Reduction in litter size, high male/female birth ratios, and fetotoxicity without significant fetal abnormalities have occurred following oral exposures (*17–19*). Parenteral dosing has resulted in exencephaly, encephaloceles, skeletal defects, and urogenital system abnormalities (*20–23*).

Human Toxicology

1. The data reported in these studies show an increased incidence of blackfoot disease that increases with age and dose. Blackfoot disease is a significant adverse effect. The prevalences (males and females combined) at the low dose are 4.6 per 1,000 for the 20–39 year group, 10.5 per 1,000 for the 40–59 year group, and 20.3 per 1000 for the > 60 year group. Moreover, the prevalence of blackfoot disease in each age group increases with increasing dose. However, a subsequent report indicates that the disease may not be due strictly to arsenic exposure (*27*). The data in Tseng et al. (*25*) also show increased incidences of hyperpigmentation and keratosis with age. The overall prevalences of hyperpigmentation and keratosis in the exposed groups are 184 and 71 per 1000, respectively. The text states that the incidence increases with dose, but data for the individual doses are not shown. These data show that the skin lesions are the more sensitive endpoint. The low dose in the study is considered a LOAEL.
2. The study by Cebrian et al. (*28*) shows an increase in skin lesions, 22% (64/296) at the high dose vs 2.2% (7/318) at the low dose in drinking water. The high dose was 410 μg/L and the low dose was 5–7 μg/L. For the dose estimates an average consumption of 3 L/day was used. No data are given regarding the arsenic exposure from food or the body weight of the participants. The 2.2% incidence of skin lesions in the low-dose group is higher than that reported in the Tseng et al. (*25*) control group, but the dose is lower (0.4 vs 0.8 μg/kg/day).
3. The study by Southwick et al. (*29*) shows a marginally increased incidence of a variety of skin lesions (palmar and plantar keratosis, diffuse palmar or plantar hyperkeratosis, diffuse pigmentation) and arterial insufficiency in individuals exposed to arsenic. Exposure times are not clearly defined but are > 5 years, and dose groups are ranges of exposure. The incidence of skin lesions in this group is about the same as in the low-dose group from the Cebrian et al. (*28*) study. The incidence of abnormal nerve conduction in the control group is higher than that from the low-dose group in the Hindmarsh et al. (*30*) study described below. The dosed group may or may not be a LOAEL because the study does not report statistically significant effects in the dosed group compared to the control group.
4. This study (*30*) shows an increased incidence of abnormal clinical findings and abnormal electromyographic findings with increasing dose of arsenic. However, the sample size is small. Percentages of abnormal clinical signs possibly attributed to As were 10%, 16%, and 40% at the low, middle, and high doses, respectively. Abnormal EMGs were 0%, 17%, and 53% in the same three groups. The average arsenic concentration of the low-dose wells was about 25 μg/L. The averages of the arsenic concentration in the middle- and high-dose wells were 70 and 680 μg/L, respectively.
5. Following is a summary of the defined doses in μg/kg/day from the principal and supporting studies:
   (1) Tseng (*24*): NOAEL = 0.8; LOAEL = 14
   (2) Cebrian et al. (*28*): NOAEL = 0.4; LOAEL = 22
   (3) Southwick et al. (*29*): NOAEL = 0.9; LOAEL = none (equivocal effects at 6)
   (4) Hindmarsh et al. (*30*): NOAEL = 0.7; LOAEL = 19 (equivocal effects at 2)

   An uncertainty factor of 3 is used to account for the lack of data to preclude reproductive toxicity as a critical effect and to determine whether the NOAEL of the critical study accounts for all sensitive individuals.

Regulatory Assessment

Both IARC and EPA classify inorganic arsenic as carcinogenic to humans. The EPA RfD for chronic oral exposures, 0.3 μg/kg/day, is based on a NOAEL of 0.8 μg/kg/day and a LOAEL of 14 μg/kg/day for hyperpigmentation, keratosis, and possible vascular complications in a human population consuming arsenic-contaminated drinking water. To address uncertainties in the data, EPA states that ''strong scientific arguments can be made for various values within a factor of 2 or 3 of the currently recommended RfD value.''

Conclusions

Based on the similarity of the findings, the oral RfD of arsenic is 0.3 μg/kg/day, and the recommended daily oral dose of 15 μg is based on a 50 kg person. Based on 10 g of drug product taken/day, a PDE of 1.5 μg/g (ppm) is derived. Under the assumption that the oral bioavailability is 10%, the parenteral PDE will be 1/10 of the oral PDE (i.e., 1.5 μg/day), resulting in an acceptable limit of 0.15 ppm.

Oral PDE: 0.3 μg/kg/day
Oral Daily Dose PDE: 15 μg/day
Oral Component Limit: 1.5 μg/g (ppm)
Parenteral Component Limit: 0.15 ppm.

Cadmium (Cd)

Introduction

Cadmium is a naturally occurring metal that is used in various chemical forms in metallurgical and other industrial processes and in the production of pigments. Environmental exposure can occur via the diet and drinking water (*31*). It has an abundance of approximately 0.15 ppm in the Earth's crust.

Toxicokinetics (ADME)

Cadmium is absorbed more efficiently by the lungs (30% to 60%) than by the GI tract, the latter being a saturable process (*32*). Cadmium is transported in the blood and is widely distributed in the body but accumulates primarily in the liver and kidneys (*33*). Cadmium burden (especially in the kidneys and liver) tends to increase in a linear fashion up to about 50 or 60 years of age, after which the body burden remains somewhat constant. Metabolic transformations of cadmium are limited to its binding to protein and nonprotein sulfhydryl groups and various macromolecules, such as metallothionein, which is especially important in the kidneys and liver (*31*). Cadmium is excreted primarily in the urine.

Toxicological Effects

Acute oral exposures of 20–30 g have caused fatalities in humans. Exposure to lower amounts may cause GI irritation, vomiting, abdominal pain, and diarrhea (*31*). An asymptomatic period of one-half to one hour may precede the onset of clinical signs. Oral LD_{50} values in animals range from 63 to 1125 mg/kg, depending on the cadmium compound (*34*). Longer term exposure to cadmium primarily affects the kidneys, resulting in tubular proteinosis, although other conditions such as ''itai-itai'' disease may involve the skeletal system. Cadmium involvement in hypertension is not fully understood (*33*).

Regulatory Assessment

A concentration of 200 μg Cd/g wet human renal cortex is the highest renal level not associated with significant proteinuria (*35*). A toxicokinetic model can help to determine the level of chronic human oral exposure (NOAEL) that results in 200 μg Cd/g wet human renal cortex. The model assumes that 0.01% day of the Cd body burden is eliminated per day (*35*). Assuming 2.5% absorption of Cd from food or 5% from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 5 and 10 μg/kg/day from water and food, respectively (i.e., levels that would result in 200 μg/g wet weight human renal cortex). Thus, based on an estimated NOAEL of 5 μg/kg/day for Cd in drinking water and an uncertainty factor (UF) of 10, an RfD of 0.5 μg/kg/day (water) was calculated. [NOTE: A UF of 10 is used to account for interhuman variability to the toxicity of this chemical in the absence of specific data about sensitive individuals.] An equivalent RfD for Cd in food is 1 μg/kg/day. Both values reflect incorporation of a UF of 10.

Using data from select environmental studies examining the relationship of urinary cadmium and the prevalence of elevated levels of biomarkers of renal function ATSDR issued the provisional Minimal Risk Level (MRL) for chronic cadmium exposure. The 95% lower confidence limit of urinary cadmium dose corresponding to the probability to exceed in 10% the risk of low molecular weight proteinuria has been estimated as 0.5 μg/g creatinine, assuming accumulation over a 55-year period. This value corresponds to an intake of 0.33 μg/kg/day in females, for which, applying a safety factor of 3 for human variability ATSDR has set the MRL to 0.1 μg/kg/day.

Conclusions

Using the ATSDR MRL as the Oral PDE:
Oral PDE: 0.1 μg/kg/day.
Oral Daily Dose PDE: 5 μg oral per day.
Oral Component Limit: 0.5 μg/g (ppm)
Parenteral Component Limit: 0.05 ppm.

Lead (Pb)

Introduction

Lead occurs naturally as a sulfide in galena. It is a soft, bluish-white, silvery gray, malleable metal with a melting point of 327.5°. Elemental lead reacts with hot boiling acids and is attacked by pure water. The solubility of lead salts in water varies from insoluble to soluble depending on the type of salt (*36–38*). Lead is a natural element that is persistent in water and soil. Most of the lead in the environment is from anthropogenic sources. The mean concentration is 3.9 μg/L in surface water and 0.005 μg/L in sea water. River sediments contain about 20,000 μg/g, and coastal sediments contain about 100,000 μg/g. Soil content varies with the location, ranging up to 30 μg/g in rural areas, 3,000 μg/g in urban areas, and 20,000 μg/g near point sources. Human exposure occurs primarily via diet, air, drinking water, and ingestion of dirt and paint chips (*39–41*).

Toxicokinetics (ADME)

The efficiency of lead absorption depends on the route of exposure, age, and nutritional status. Adult humans absorb about 10%–15% of ingested lead, but children may absorb up to 50%, depending on whether lead is in the diet, dirt, or paint chips. More than 90% of lead particles deposited in the respiratory tract are absorbed into systemic circulation. Inorganic lead is not efficiently absorbed through the skin, and consequently this route does not contribute considerably to the total body lead burden (*42*). Lead absorbed into the body is distributed to three major compartments: blood, soft tissue, and bone. The largest compartment is the bone, which contains about 95% of the total body lead burden in adults and about 73% in children. The half-life of bone lead is more than 20 years. The concentration of blood lead changes rapidly with exposure and has a half-life of only 25–28 days. Blood lead is in equilibrium with lead in bone and soft tissue. The soft tissues that take up lead are liver, kidneys, brain, and muscle. Lead is not metabolized in the body, but it may be conjugated with glutathione and excreted primarily in the urine (*40, 42, 43*). Exposure to lead is evidenced by elevated blood lead levels.

Toxicological Effects

The systemic toxic effects of lead in humans have been well documented by EPA (*42–48*) and ATSDR (*40*), who extensively reviewed and evaluated data reported in the literature up to 1991. The evidence shows that lead is a multitargeted toxicant, causing effects in the GI tract, he-

matopoietic system, cardiovascular system, central and peripheral nervous systems, kidneys, immune system, and reproductive system. Overt symptoms of subencephalopathic central nervous system (CNS) effects and peripheral nerve damage occur at blood lead levels of 40–60 μg/dL, and nonovert symptoms, such as peripheral nerve dysfunction, occur at levels of 30–50 μg/dL in adults. No clear threshold is evident. Cognitive and neuropsychological deficits are not usually the focus of studies in adults, but there is some evidence of neuropsychological impairment (*49*) and cognitive deficits in lead workers with blood levels of 41–80 μg/dL (*50*). Although similar effects occur in adults and children, children are more sensitive to lead exposure than are adults. Irreversible brain damage occurs at blood lead levels ≥ 100 μg/dL in adults and at 80–100 μg/dL in children. Death can occur at the same blood levels in children. Children who survive these high levels of exposure suffer permanent severe mental retardation.

Toxicology Studies

As discussed previously, neuropsychological impairment and cognitive (IQ) deficits are sensitive indicators of lead exposure. Both neuropsychological impairment and IQ deficits have been the subject of cross-sectional and longitudinal studies in children. One of the early studies reported IQ score deficits of four points at blood lead levels of 30–50 μg/dL and one to two points at levels of 15–30 μg/dL (*51*).

Detailed longitudinal studies have been conducted on children (starting at the time of birth) living in Port Pirie, Australia (*52–57*), Cincinnati, Ohio (*58–61*), and Boston, Massachusetts (*62–67*). Various measures of cognitive performance have been assessed in these children. Studies of the Port Pirie children up to 7 years of age revealed IQ deficits in 2-year-old children of 1.6 points for each 10-μg/dL increase in blood lead, deficits of 7.2 points in 4-year-old children, and deficits of 4.4 to 5.3 points in 7-year-old children as blood lead increased from 10 to 30 μg/dL. No significant neurobehavioral deficits were noted for children, 5 years or younger, who lived in the Cincinnati, Ohio, area. In 6.5-year-old children, performance IQ was reduced by seven points in children whose lifetime blood level exceeded 20 μg/dL. Because of the large database on subclinical neurotoxic effects of lead in children, only a few of the studies have been included. EPA (*42,48*) concluded that there is no clear threshold for neurotoxic effects of lead in children.

In adults, the cardiovascular system is a very sensitive target for lead. Hypertension (elevated blood pressure) is linked to lead exposure in occupationally exposed subjects and in the general population. Three large population-based studies have been conducted to study the relationship between blood lead levels and high blood pressure. The British Regional Heart Study (BRHS) (*68*), the NHANES II study (*48, 69–72*), and Welsh Heart Programme (*73, 74*) comprise the major studies for the general population. The BRHS study showed that systolic pressure greater than 160 mm Hg and diastolic pressure greater than 100 mm Hg were associated with blood lead levels greater than 37 μg/dL (*68*). An analysis of 9933 subjects in the NHANES study showed positive correlations between blood pressure and blood lead among 12–74-year-old males but not females (*69, 71*), 40–59-year-old white males with blood levels ranging from 7 to 34 μg/dL (*70*), and males and females greater than 20 years old (*75*). In addition, left ventricular hypertrophy was also positively associated with blood lead (*75*). The Welsh study did not show an association among men and women with blood lead of 12.4 and 9.6 μg/dL, respectively (*73, 74*). Other smaller studies showed both positive and negative results. EPA (*48*) concluded that increased blood pressure is positively correlated with blood lead levels in middle-aged men, possibly at concentrations as low as 7 μg/dL. In addition, EPA estimated that systolic pressure is increased by 1.5–3.0 mm Hg in males and 1.0–2.0 mm Hg in females for every doubling of blood lead concentration.

Regulatory Assessment

EPA has not developed an RfD for lead because it appears that lead is a nonthreshold toxicant, and it is not appropriate to develop RfDs for these types of toxicants. However, a maximum contaminant action level for lead of 15 μg/day was recommended for drinking water (40 CFR 141.80). In 2004, FDA set the maximum allowable level for lead in bottled water at 5 μg/L. Assuming an average water consumption of 2 L/day, the recommended RfD is 10 μg/day.

Conclusions

Assuming that the lead in the drug product will be absorbed in a manner similar to that from water, the RfD developed by FDA is used as the Oral Daily Dose PDE:

Oral PDE: 0.2 μg/kg/day
Oral Daily Dose PDE: 10 μg/day
Oral Component Limit: 1 μg/g (ppm)
Parenteral Component Limit: 0.1 ppm.

Mercury (Hg)

Introduction

Mercury is a naturally occurring element that exists in multiple forms and in various oxidation states. It is used in a wide variety of products and processes. In the environment, mercury may undergo transformations among its various forms and among its oxidation states. Exposure to mercury may occur in both occupational and environmental settings, the latter primarily involving dietary exposure (*76*).

Toxicokinetics (ADME)

Mercury's ADME depend on its form and oxidation state (*76, 77*). Organic mercurials are more readily absorbed than are inorganic forms. An oxidation–reduction cycle is involved in the metabolism of mercury and mercury compounds by both animals and humans (*76*). The urine and feces are the primary excretory routes. The elimination half-life is 35 to 90 days for elemental mercury and mercury vapor and about 40 days for inorganic salts (*77*).

Toxicological Effects

Ingestion of inorganic mercury salts may cause severe GI irritation, renal failure, and death with acute lethal doses in humans ranging from 1 to 4 g (*76*). Mercuric (divalent) salts are usually more toxic than are mercurous (monovalent) salts (*77*). Mercury is also known to induce hypersensitivity reactions such as contact dermatitis and acrodynia (pink disease) (*78*). Inhalation of mercury vapor may cause irritation of the respiratory tract, renal disorders, central nervous system effects characterized by neurobehavioral changes, peripheral nervous system toxicity, renal toxicity (immunologic glomerular disease), and death (*76*).

Toxicology Studies

Toxicity resulting from subchronic and chronic exposure to mercury and mercury compounds usually involves the kidneys and/or nervous system. The specific target and effect depend on the form of mercury (*76*). Organic mercury, especially methyl mercury, rapidly enters the central nervous system and results in behavioral and neuromotor disorders (*76, 77*). The developing central nervous system is especially sensitive to this effect, as documented by the epidemiologic studies in Japan and Iraq where ingestion of methyl mercury–contaminated food resulted in severe toxicity and death in adults and severe central nervous system effects in infants (*79–82*). Blood mercury levels of < 10 μg/dL and 300 μg/dL corresponded to mild effects and death, respectively (*79*). Teratogenic effects due to organic or inorganic mercury exposure do not appear to be well documented for humans or animals, although some evidence exists for mercury-induced menstrual cycle disturbances and spontaneous abortions (*76, 80, 83*).

A subchronic and chronic oral RfD of 0.1 μg/kg/day for methyl mercury is based on a benchmark dose of 1.1 μg/kg/day relative to neurologic developmental abnormalities in human infants (*1, 84*). A subchronic and chronic oral RfD of 0.3 μg/kg/day for mercuric chloride is based on immunologic glomerulonephritis (*1*). A LOAEL of 0.63 mg Hg/kg/day for mercuric chloride was identified (*85*). NOAELs were not available for oral exposure to inorganic mercury or methyl mercury.

Regulatory Assessment

EPA's existing RfD of 0.1 μg/kg/day is based on a poisoning episode in Iraq. Results for two large epidemiological studies in the Faroe Islands and Seychelles Islands have become available since the 1995 IRIS entry. The Faroe Islands study identified associations between in utero methyl mercury exposure and deficits on a number of endpoints, as did the New Zealand study. In contrast, the Seychelles Islands study found little or no evidence of impairment. These studies underwent a comprehensive review by the National Research Council (NRC) of the National Academy of Sciences, along with a smaller study from New Zealand. NRC performed benchmark dose (BMD) analyses of a number of neuropsychological endpoints from each study. In the assessment described here, EPA used the NRC analyses as the basis for the derivation of an RfD for methyl mercury. Based on BMD levels (lower limit on the BMD) for a number of endpoints from the Faroe Islands study, as well as an integrative analysis of all three studies, an RfD for 0.1 μg/kg/day was derived. This included a total uncertainty factor of 10 for interhuman toxicokinetic and toxicodynamic variables.

Conclusions

The presence of methyl mercury in drug products is unlikely. Therefore, the EPA-recommended RfD for mercuric chloride is used as the Oral PDE.

Oral PDE: 0.3 μg/day
Oral Daily Dose PDE: 15 μg/day
Oral Component Limit: 1.5 μg/g (ppm)
Parenteral Component Limit: 0.15 ppm.

CLASS 2 ELEMENTAL IMPURITIES

The limits for Class 2 elemental impurities are those of the EMEA *Guideline on the Specification Limits for Residual Metal Catalysts for Metal Reagents* (*2*).

Dietary Supplement-Specific Issues

Dietary supplements are composed of dietary ingredients (herbs or other botanicals, minerals, amino acids, vitamins, and other substances used by humans to supplement the diet) plus other inert components used in their composition. The dietary ingredients from natural sources are subject to contamination with elemental impurities from water, air pollution, or soil and other agricultural inputs. Processing procedures, such as extraction of plant parts and purification, can result in concentration or dilution of the elemental contaminants. Dietary substances of synthetic origin may also be contaminated with elemental impurities that are derived from their manufacturing processes (catalysts and residual reagents). Dietary ingredients of marine origin (such as fish oil or algal products) can accumulate methyl mercury chloride and cadmium, representing special cases. Limits for such exceptional dietary ingredients should be addressed in their specific monographs. Speciation of arsenic and mercury is another issue of relevance for dietary supplements.

Dietary supplements are regulated as a subset of foods and limits for contaminants set for food items are applicable. Major sources of exposure were considered at the time of setting limits for dietary supplements, and these sources include the environment, drinking water, and food. Recent surveys of dietary supplement intakes (*86*) were also taken in consideration to apply a safety factor related to the number of dietary supplements taken by a sizable portion of the population. Proposed limits for dietary supplements were derived from the Provisional Tolerable Weekly Intake (PTWI), which is recommended by FAO/WHO. Average daily exposures (μg/day) of each elemental contaminant from air, food, and drinking water were subtracted from the PTWI. From the remaining daily intake allowance, a range safety factor was used to account for multiple dietary supplement intakes was used to calculate the PDE for dietary supplements. With this approach, the recommended limits are consistent with the limits proposed in *Chapter* ⟨232⟩ for drugs and

with the limits set by other organizations. (WHO herbal drugs, EP herbal drugs, Health Canada for Natural Health Products, and the American Herbal Products Association-AHPA).

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APPENDIX

Members of the Advisory Panel are:

N Lewen (Chair); TL Shelbourn (Vice Chair); C Barton, PhD; CM Callis; SJ Dentali, Ph.D; AM Fan, PhD; R Frotschl, PhD; A Kazeminy, PhD; R Ko, PharmD, PhD; GC Turk, PhD; R Wiens; Government Liaisons: R Blosser; M De, PhD; BA Fowler, PhD; JF Kauffman, PhD; and JC Merrill, PhD.

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Attachment 20

Elemental Impurities—Comments and Responses

AJ DeStefano, K Zaidi,[a] TL Cecil, GI Giancaspro, and the USP Elemental Impurities Advisory Panel[b]

ABSTRACT In *Pharmacopeial Forum* (*PF*) 34(5) (September–October 2008) the Metal Impurities Advisory Panel of the USP General Chapters Expert Committee presented a *Stimuli to the Revision Process* article that proposed a new General Chapter to replace General Chapter *Heavy Metals* ⟨231⟩. The new Chapter presented a table of elements that could be limited and new approaches to evaluate those elements. Subsequently USPC initiated and participated in an Institute of Medicine workshop and hosted a separate Heavy Metals Testing Methodologies workshop. These public presentations and discussion forums yielded a large number of specific comments and suggestions from the pharmaceutical and excipient industries as well as the toxicological and regulatory fields. The comments can be broadly categorized into ten topics. This article presents a summary of these topics and the advisory panel's responses and approaches to incorporate the suggestions.

ELEMENTAL IMPURITIES RECOMMENDATIONS

Before presenting the comment topics and responses, we present the Advisory Panel's recommendations. These recommendations include the development of four new general chapters, two additional *Stimuli* articles, and an implementation strategy that involves a *General Notices* revision and a number of monograph revisions.

General Chapters

The General Chapter additions include: General Chapter *Elemental Impurities—Limits* ⟨232⟩, General Chapter *Elemental Impurities—Procedures* ⟨233⟩, General Chapter *Elemental Contaminants in Dietary Supplements* ⟨2232⟩, and General Information Chapter *Elemental Impurities—Other Elements* ⟨1232⟩ (the name of ⟨1232⟩ is subject to change). The first three chapters are included in this *PF*, and the General Informational Chapter will be developed over the course of the next few years.

General Chapter *Elemental Impurities—Limits* ⟨232⟩**:** The limits presented in this Chapter are based on in-depth review of the toxicological literature and discussions involving several experts in metals toxicology. These limits are based on documented toxicity and regulatory recommendations and focus on the four most toxic and well-understood metals (Pb, Hg, As, and Cd). The Chapter also provides limits of metal catalysts that can be added in the production of a drug substance or excipient. The metal catalyst limits are the same as those published by EMEA—with the exception of iron and zinc, which were not included due to their low toxicity. This Chapter also describes three separate options for determination of compliance to the limits. These options are similar to those presented in General Chapter *Residual Solvents* ⟨467⟩.

General Chapter *Elemental Impurities—Procedures* ⟨233⟩**:** The panel has determined that the procedures described in General Chapter *Heavy Metals* ⟨231⟩ are inadequate to provide the basis for control of the elements in ⟨232⟩ at their proposed limits. Instead, this chapter details two procedures and provides criteria for the approval of alternative procedures for the measurement of elemental impurities. The referee procedures, ICP-OES and ICP-MS with closed-vessel microwave digestion, are described. The choice of procedure, including the sample preparation and instrument parameters, are the responsibility of the user. The performance criteria necessary to demonstrate that an alternative procedure is equivalent to the referee procedures for quantitative determinations are described.

General Chapter *Elemental Contaminants in Dietary Supplements* ⟨2232⟩**:** The limits presented in this Chapter are based on in-depth review of the toxicological literature of specific interest and impact to Dietary Supplements. These limits are based on documented toxicity and regulatory recommendations, and focus on the four most toxic and well-understood metals (Pb, Hg, As, and Cd). This Chapter also describes three separate options for determination of compliance to the limits. These oral limits and their described options for compliance are similar to those presented in General Chapter ⟨232⟩. Finally, this Chapter presents several procedures for speciation of specific elements of particular concern for dietary supplements.

General Notices

A revision is proposed to the *General Notices* to indicate that General Chapters ⟨232⟩ and ⟨233⟩ will apply to all oral and parenteral articles in *USP–NF*. This revision is similar in content to that describing the residual solvents requirements.

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[b] For a list of the members of the Advisory Panel please see the Appendix.

Stimuli Articles

The Advisory Panel also recommended the development of two *Stimuli* articles. The first (this article) discusses the comments and responses of the panel, and the second, also in this volume of *PF*, presents the toxicological rationale for the limits presented in ⟨232⟩.

Implementation (General Notices and Monographs): The Advisory Panel recommends a staged approach to the implementation of the new General Chapters.

Stage 1: After the initial presentation of the standards in this *PF*, the Panel recommends consideration by the expert committee of a standard implementation period for the General Chapters.

Stage 2: The Advisory Panel recommends the adoption of the *General Notices* revision, also in *PF*, with an extended implementation date. They recommend that the committee consider an official date that coincides with the official date of the EMEA Metal Catalyst guideline (Sept. 2013).

Stage 3: The Advisory Panel recommends that all of the references to General Chapter ⟨231⟩ *Heavy Metals* be removed from *USP–NF* monographs in a manner to coincide with the official date approved for the *General Notices* revision.

COMMENTS AND RESPONSES

After reviewing all of the comments received to date, the authors have identified ten topics that encompass those comments. These topics include:

Topic 1: Instrumental Details
Topic 2: Implementation
Topic 3: Specific Metals and Limits
Topic 4: Using Residual Solvent Concepts
Topic 5: Scope (Dosage Forms, Foods, Dietary Supplements)
Topic 6: Reference Standards
Topic 7: Imminent Threat ⟨231⟩
Topic 8: Harmonization (EDQM, EMEA, MHLW)
Topic 9: GMPs and USP
Topic 10: Other Comments.

In many cases, comments received from several sources are similar in nature. Therefore, individual comments are not specifically identified in this section. In addition to the comments received in response to the *Stimuli* article, the comments received at the two workshops will also be addressed in this section.

Topic 1: Instrumental Details

Comment Summary 1.1: The instruments necessary to meet the limits described can be complicated, expensive, and application dependant. Some preparations may also be dangerous. Defining a single procedure, including reagents, will not work for all applications.

Number of Commenters: 22

Response: General Chapter ⟨233⟩ specifically indicates that any procedure that is capable of meeting the critical validation parameters can be used. The choice of procedure, including sample preparation, instrument type and configuration, and reagents used are at the discretion of the user. The standard assumes that the user has evaluated the risk–benefit ratios of the available options and has selected the most appropriate procedure for the user's application. The referee procedures have been validated using a number of samples and the risk–benefit ratios have been evaluated. Because these procedures will be used for substances and products that have not been evaluated by the advisory panel, verification is indicated and steps that may pose health hazards have been noted. The use of multiple procedures is within the scope of a user's application of the standard.

Comment Summary 1.2: The instrumental requirements should be linked to the critical validation requirements and should be clearly defined.

Number of Commenters: 6

Response: The critical validation criteria necessary to define an acceptable procedure are included in General Chapter ⟨233⟩ and are based on the requirements for validation of a limit test and quantitative impurity procedure as described in General Chapter *Validation of Compendial Procedures* ⟨1225⟩.

Comment Summary 1.3: Several of the terms used in the proposal are confusing or are not well defined in the text. There are also specific contradictions in the text regarding precision.

Number of Commenters: 5

Response: The Advisory Panel has incorporated all of these comments in the draft Chapters. The confusing terminology has been removed, or terms have been better defined. The contradictions in the text have been resolved, and the presentation has been refined to aid in understanding.

Comment Summary 1.4: Clarification of the expectations for validation, ongoing verification, check standards, and spike and recovery details are requested.

Number of Commenters: 3

Response: General Chapter ⟨233⟩ has added clarifications that incorporate each of these suggested improvements.

Topic 2: Implementation

Comment Summary 2.1: A change that affects many monographs—as this change will—should have an extended implementation date.

Number of Commenters: 2

Response: The details of the proposed implementation approach are provided above. The Advisory Panel has recommended an extended implementation period.

Comment Summary 2.2: The development of this standard should be as transparent as possible, and updates should be posted on the USP Web site.

Number of Commenters: 5

Response: USP has added the progress of the development of this standard on the USP Web site in the Hot Topics section. The standard has been discussed at several open forums, and the development has been as open as possible.

Comment Summary 2.3: The new standard should be a screening procedure that should not quantify individual elemental impurities.

Number of Commenters: 2

Response: Although this standard may be used as a screen for impurities, it is designed to encompass the quantification of these impurities. Because of the wide range of elements and acceptance criteria, the use of a true screening procedure is not practical.

Comment Summary 2.4: The new standard should focus on the big four with the addition of other elemental impurities at a later date. The new standard should be limited to those metals that are expected to be present or that were added as part of the process.

Number of Commenters: 3

Response: The proposed chapters will focus on the big four and the metal catalysts defined in the EMEA guidance, except for zinc and iron. General Chapter ⟨232⟩ clearly differentiates between the big four and the other elemental impurities in such a way that both can exist in a single Chapter.

Comment Summary 2.5: Clarification of the expectations for calculations and units for calculations are requested.

Number of Commenters: 3

Response: General Chapter ⟨232⟩ has incorporated clarifications to each of these suggested improvements.

Topic 3: Specific Metals and Limits

Comment Summary 3.1: The limits should be based on toxicology and should include only those elements that have a likelihood of being present. The rationale for the limits should be developed transparently and presented as a basis for the standard.

Number of Commenters: 12

Response: The limits have been developed by a team of toxicologists from industry, academia, ATSDR, BfArM, and FDA. The rationale for the limits for the big four is included in a separate *Stimuli to the Revision Process* article elsewhere in this number of *PF*.

Topic 4: Using Residual Solvent Concepts

Comment Summary 4.1: A risk-based strategy like the one presented in General Chapter ⟨467⟩ is recommended. The Chapter should be referenced in the *General Notices* and should not be added to the monographs.

Number of Commenters: 3

Response: General Chapter ⟨232⟩ applies a risk-based approach like that of EMEA for Class 2 elements. The control of Class 1 impurities is required, but the extent of testing and the timing of that testing are the responsibility of the manufacturer. Although the Chapter does not require testing, it does require compliance for Class 1 impurities, regardless of source.

Comment Summary 4.2: Multiple options for the calculation of amount of impurity present like that in General Chapter ⟨467⟩ *Residual Solvents* should be used.

Number of Commenters: 5

Response: Three options for the calculation of measured impurities and assessment of their compliance to the limits for a drug product are included in General Chapter ⟨232⟩.

Topic 5: Scope (Dosage Forms, Foods, Dietary Supplements)

Comment Summary 5.1: To which articles do these standards apply? How about ophthalmics, food, preclinical supplies?

Number of Commenters: 6

Response: General Chapter ⟨232⟩ applies to drug substances and products including natural-source and rDNA biologics, ophthalmics, parenteral nutrients, and excipients. It does not apply to food or dietary supplements. General Chapter ⟨2232⟩ covers dietary supplements and dietary ingredients. Preclinical supplies are not covered by a *USP* monograph and are not within the scope of this standard.

Comment Summary 5.2: This standard should replace all of the other procedures for inorganic impurities in *USP–NF*, such as *Residue on Ignition*, *Lead*, *Aluminum*, *Sodium*, *Calcium*, and others.

Number of Commenters: 2

Response: Although the Advisory Panel considered the change, they determined that it was not within the scope of this revision or Advisory Panel to make a recommendation. USP will consider this proposal further.

Topic 6: Reference Standards (RS)

Comment Summary 6.1: Commenters presented strong arguments both for and against the development of USP RS materials.

Number of Commenters: 7

Response: USP plans to develop standard mixtures that can be used in validation studies and for system suitability testing. USP currently has no plans to develop individual elemental impurity standards, but if a need is identified USP will consider developing such standards. When a USP standard is not available, a suitable NIST or NIST-traceable standard is recommended.

Topic 7: Imminent Threat ⟨231⟩

Comment Summary 7.1: There is no need to improve this standard. It has worked for a long time, there is significant uncontrolled environmental exposure, and the toxicity of these materials has not changed in the past 100 years.

Number of Commenters: 5

Response: The Advisory Panel disagrees with these comments. The current procedures in General Chapter ⟨231⟩ no longer represent the state of the industry, and the limits in the individual monographs are inconsistent with the recommendations of US and international regulatory authorities. The current lack of protection from environmental exposure increases the need to control those toxicants that are added with the intent of treating a medical condition or supplementing the diet. Although the toxicity of the elements has not changed in the past 100 years, our understanding of the detrimental effects of some of these impurities has increased manyfold.

Comment Summary 7.2: General Chapter ⟨231⟩ *Procedures I* and *III* are still usable procedures. Allow their use as a screening test.

Number of Commenters: 2

Response: Although the advisory panel considered these procedures of little value or to be an ineffective approach to the evaluation of the Class 1 impurities, provisions to allow their use have been included in General Chapter ⟨233⟩. Where one of the procedures has been successfully validated as described in the Chapter, then the procedure may be used for that application.

Topic 8: Harmonization (EDQM, EMEA, MHLW)

Comment Summary 8.1: The limits and procedures should be harmonized with EMEA, EP, BP, and JP.

Number of Commenters: 5

Response: General Chapter ⟨232⟩ incorporates the limits described in the EMEA guidance as Class 2 impurities. A representative of BfArM has participated on the Advisory Panel to ensure that the standards are kept in harmony, and representatives from both EP and JP attended the USP workshop on this topic. USP is discussing these chapters with EP and JP as part of the PDG harmonization effort. The development of two chapters to replace ⟨231⟩ was executed on the advice of EP to allow an easier route to accomplish a harmonized standard.

Topic 9: GMPs and USP

Comment Summary 9.1: The control of these elemental impurities is maintained by cGMP compliance, so testing is unnecessary.

Number of Commenters: 4

Response: The limits presented in General Chapters ⟨232⟩ and ⟨2232⟩ are the maximum amount of elemental impurities that may be present in a product or ingredient (depending upon application). The periodicity for testing, the extent of testing, and the elements included in the testing are established at the discretion of the drug product or dietary supplement manufacturer. All products are expected to comply with the standard.

Topic 10: Other Comments

Comment Summary 10.1: We support the improvements proposed by USP.

Number of Commenters: 2

Response: The Panel appreciates the support of the commenters. Thank you.

Comment Summary 10.2: Several specific wording changes should be incorporated.

Number of Commenters: 5

Response: The changes have been incorporated.

Comment Summary 10.3: USP and FDA should work closely on this topic.

Number of Commenters: 2

Response: FDA toxicologists and reviewers are members of the Advisory Panel. USP staff have discussed the revisions with FDA, and copies of the proposed text have been provided to FDA before publication in *PF*.

APPENDIX

Members of the Advisory Panel are:

N Lewen (Chair); TL Shelbourn (Vice Chair); C Barton, PhD; CM Callis; SJ Dentali, PhD; AM Fan, PhD; R Frotschl, PhD; A Kazeminy, PhD; R Ko, PharmD, PhD; GC Turk, PhD; R Wiens; Government Liaisons: R Blosser; M De, PhD; BA Fowler, PhD; JF Kauffman, PhD; and JC Merrill, PhD.

■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*)

LIMIT TESTS

BRIEFING

⟨232⟩ Elemental Impurities—Limits. This proposed new general test chapter is the first of two being developed to replace the general test chapter *Heavy Metals* ⟨231⟩; the second chapter is *Elemental Impurities—Limits* ⟨233⟩. The term *elemental impurities* is used here as an alternative to the term *heavy metals*. The limits presented in this chapter are based on in-depth review of the toxicological literature and discussions involving several experts in metal toxicology. These limits, based on documented toxicity and regulatory recommendations, focus on the four most toxic and best-understood metals: lead, mercury, arsenic, and cadmium. The chapter also provides limits of metal catalysts that can be added in the production of a drug substance or excipient. The metal catalyst limits are the same as those published by the European Medicines Agency (EMEA),* with the exception of iron and zinc, which because of their low toxicity were not included. The chapter also describes three separate options for determination of compliance with limits. These options are similar to those presented in the chapter *Residual Solvents* ⟨467⟩.

(GC: K. Zaidi.) RTS—C79497

* See page 6 at http://www.emea.europa.eu/pdfs/human/swp/444600enfin.pdf.

Add the following:

▲⟨232⟩ ELEMENTAL IMPURITIES—LIMITS

INTRODUCTION

The objective of this chapter is to set limits on the amounts of elemental impurities in pharmaceuticals. The chapter applies to drug substances, drug products (including natural-source and rDNA biologics), and excipients. Dietary supplements and their ingredients are addressed in chapter *Elemental Impurities in Dietary Supplements* ⟨2232⟩. For articles that are designated ''For Veterinary Use Only,'' higher or lower levels for the permissible daily exposure and concentration limit may be justified in exceptional cases, based on the actual daily dose, actual target species, relevant toxicological data, and consumer safety considerations.

Elemental impurities addressed in this chapter are classified as shown in *Table 1*.

Table 1. Elemental Impurity Classes

Class	Assessment
Class 1	Elements should be essentially absent
	Known or strongly suspected human toxicants
	Environmental hazards
Class 2	Elements should be limited
	Elements with less toxicity than Class 1
	Elements deliberately added to an article

Class 1 Elemental Impurities

Compliance with the limits specified for Class 1 elemental impurities is required for all drug products, regardless of the likelihood of the presence of impurities. The presence of unexpected elemental contaminants,

In-Process Revision

as well as that of impurities likely to be present, should be considered in determining compliance and planning the risk-based extent of testing.

Class 2 Elemental Impurities

In general, for Class 2 elemental impurities, the testing of drug substances, excipients, and drug products for elemental impurities need be conducted only when these elements are added during the manufacture of the article.

LIMITS OF ELEMENTAL IMPURITIES

Class 1

Class 1 elemental impurities (*Table 2*), because of their unacceptable toxicities or deleterious environmental effects, should not be present in a drug substance, excipient, or drug product. However, if their presence is unavoidable, their levels should be restricted as shown in *Table 2*, unless otherwise stated in the individual monograph.

Class 2

Class 2 elemental impurities (*Table 3*) should be limited in drug substances, excipients, and drug products because of their inherent toxicities.

Table 2. Class 1 Elemental Impurities

Element	Component Limit (μg/g)	Oral Daily Dose PDE* (μg/day)	Parenteral Component Limit (μg/g)	Parenteral Daily Dose PDE (μg/day)
Arsenic	1.5	15	0.15	1.5
Cadmium	0.5	5	0.05	0.5
Lead	1	10	0.1	1
Mercury	1.5	15	0.15	1.5

* Permitted daily exposure.

Table 3. Class 2 Elemental Impurities

Element	Component Limit (μg/g)	Oral Daily Dose PDE* (μg/day)	Parenteral Component Limit (μg/g)	Parenteral Daily Dose PDE (μg/day)
Chromium	25	250	2.5	25
Copper	250	2500	25	250
Manganese	250	2500	25	250
Molybdenum	25	250	2.5	25
Nickel	25	250	2.5	25
Palladium	10	100	1.0	10
Platinum	10	100	1.0	10

Table 3. Class 2 Elemental Impurities *(Continued)*

Element	Component Limit (μg/g)	Oral Daily Dose PDE* (μg/day)	Parenteral Component Limit (μg/g)	Parenteral Daily Dose PDE (μg/day)
Vanadium	25	250	2.5	25
Osmium	10 (combination not to exceed)	100 (combination not to exceed)	1.0 (combination not to exceed)	10 (combination not to exceed)
Rhodium				
Ruthenium				
Iridium				

* Permitted daily exposure.

OPTIONS FOR DESCRIBING LIMITS OF ELEMENTAL IMPURITIES

Three options are available when applying limits of elemental impurities for orally dosed products. Parenteral products are covered separately (see *Parenteral Products* section below).

Drug Product Analysis Option

This option is generally applicable. The results obtained from the analysis of a typical dosage unit, scaled to a maximum daily dose, are compared to the Daily Dose PDE, as shown in *Table 2* and *Table 3*.

Daily Dose PDE $\geq$ measured value x (maximum daily dose)

Individual Component Option

For drug products with a maximum daily dose of NMT 10 g, the product meets the requirements when each drug substance and excipient meets the limits provided in the Component Limit column (*Table 2* and *Table 3*). If all drug substances and excipients in a formulation meet the limits shown in the Component Limit, these components may be used in any proportion. No further calculation is necessary.

Summation Option

This option can be used for drug products that are administered in doses other than 10 g/day or products in which any component of a product exceeds the applicable Component Limit. The Daily Dose PDE, as shown in *Table 2* and *Table 3*, can be used to calculate the concentration of elemental impurities allowed in a drug product. Apply this option by separately adding the amounts of each elemental impurity (in μg/day) present in each of the components of the drug product, using the following equation:

$$\text{Result} = \Sigma^{1}{}_{m}(C_M \times W_M)$$

m = each ingredient used to manufacture the dosage form

C_M = element concentration in that component (μg/g)

W_M = weight of component in a dosage form (g)

The sum of the quantities of each element/day should be less than that shown by the Daily Dose PDE in *Table 2* and *Table 3* for that element.

Examples

Consider an example of the application of the *Individual Component Option* and the *Summation Option* to the arsenic concentration in a drug product. The Daily Dose PDE is 15 μg/day, and the Component Limit is 1.5 μg/g (ppm). The maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of arsenic are shown in *Table 4*.

Table 4

Component	Amount in Formulation (g)	Arsenic Content (μg/g)	Daily Exposure (μg/day)
Drug substance	0.3	3.0	0.9
Excipient 1	0.9	1.0	0.9
Excipient 2	3.8	2.0	7.6
Drug product	5.0	—	9.4

Excipient 1 and the drug substance meet the Component Limit, but Excipient 2 does not. Thus, the *Individual Component Option* cannot be used. However, under the *Summation Option*, the drug product meets the Daily Dose PDE limit of 15 μg/day and thus conforms to the acceptance criteria in this chapter.

Consider another example where the maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of arsenic are shown in *Table 5*.

Table 5

Component	Amount in Formulation (g)	Arsenic Content (μg/g)	Daily Exposure (μg/day)
Drug substance	0.3	5.0	1.5
Excipient 1	0.9	5.0	4.5
Excipient 2	3.8	5.0	19.0
Drug product	5.0	—	25.0

In this example, the drug product exceeds the limits in *Table 2*, using both the *Individual Component Option* and the *Summation Option*. The manufacturer can test the drug product by using the *Drug Product Analysis Option*. If the level of arsenic in the formulation exceeds the Daily Dose PDE, the product fails to meet the impurity limits as described in this chapter.

Parenteral Products

Because of the presumption of 100% bioavailability of the elemental impurity during parenteral administration, versus the presumed 10% bioavailability via the oral route, the Parenteral Component Limit and the Parenteral Daily Dose PDE (*Table 2* and *Table 3*) are 10% of those for the oral route of introduction. To evaluate the limits for elemental impurities, one can apply the three options described above, using the Parenteral Component Limit instead of the Component Limit, and using the Parenteral Daily Dose PDE instead of the Oral Daily Dose PDE.

ANALYTICAL PROCEDURES

For a presentation of the alternatives for testing, see the chapter *Elemental Impurities—Procedures* ⟨233⟩. The validation necessary will vary depending on the situation. For all three options described in Chapter ⟨232⟩ in the section *Options for Describing Limits of Elemental Impuri-*

ties, it may be appropriate to use the section *Limit Procedure Validation* in Chapter ⟨233⟩. However, for the *Summation Option* in Chapter ⟨232⟩, acceptable levels of validation must be determined on a case-by-case basis. Validation of a procedure using the *Quantitative Procedure Validation* in Chapter ⟨233⟩ is acceptable for all options under all circumstances, and it is generally preferred. The determination of the level of validation necessary is at the discretion of the manufacturer and the competent regulatory authority.▲*USP34*

BRIEFING

⟨**233**⟩ **Elemental Impurities—Procedures.** This proposed new general test chapter is the second of two being developed to replace the general test chapter *Heavy Metals* ⟨231⟩; the first chapter is *Elemental Impurities—Limits* ⟨232⟩. The procedures described in Chapter ⟨231⟩ are inadequate to provide the basis for control of the elements in Chapter ⟨232⟩ at their proposed limits.

This chapter describes the validation of two types of procedures, limit and quantitative, for the measurement of elemental impurities and provides criteria for the approval of alternative procedures. The chapter also describes two referee procedures, inductively coupled plasma–atomic (optical) emission spectroscopy (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS), both using closed vessel microwave digestion.

The choice of procedure, including the sample preparation and the instrument parameters, is the responsibility of the user.

(GC: K.Zaidi) RTS—C79498

Add the following:

▲⟨233⟩ ELEMENTAL IMPURITIES—PROCEDURES

INTRODUCTION

This chapter describes analytical procedures for the evaluation of elemental impurities in *USP* for drug substances and drug products (including natural-source and rDNA biologics); in *NF* for excipients; and in the *USP Dietary Supplements Compendium* for dietary supplements and dietary ingredients (all drug articles). Two referee procedures are described. Criteria for the approval of alternative procedures are also described. An alternative procedure will require complete validation for each element of interest. In addition, a system suitability evaluation using a USP Reference Standard or its equivalent should be demonstrated on the day of analysis. Alternative procedures that meet the validation requirements described herein are considered to be equivalent to Procedures 1 and 2. A decision-tree that can be used to guide a user to an appropriate alternative procedure is presented in *Figure 1*. The test requirement is specified in *General Notices* or the individual monograph.

Speciation

When elements are present in certain complexes, oxidation states, or organic combinations, they may show more significant toxicity than in other forms and may require further testing and control. The determination of the oxidation state or organic complex or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter.

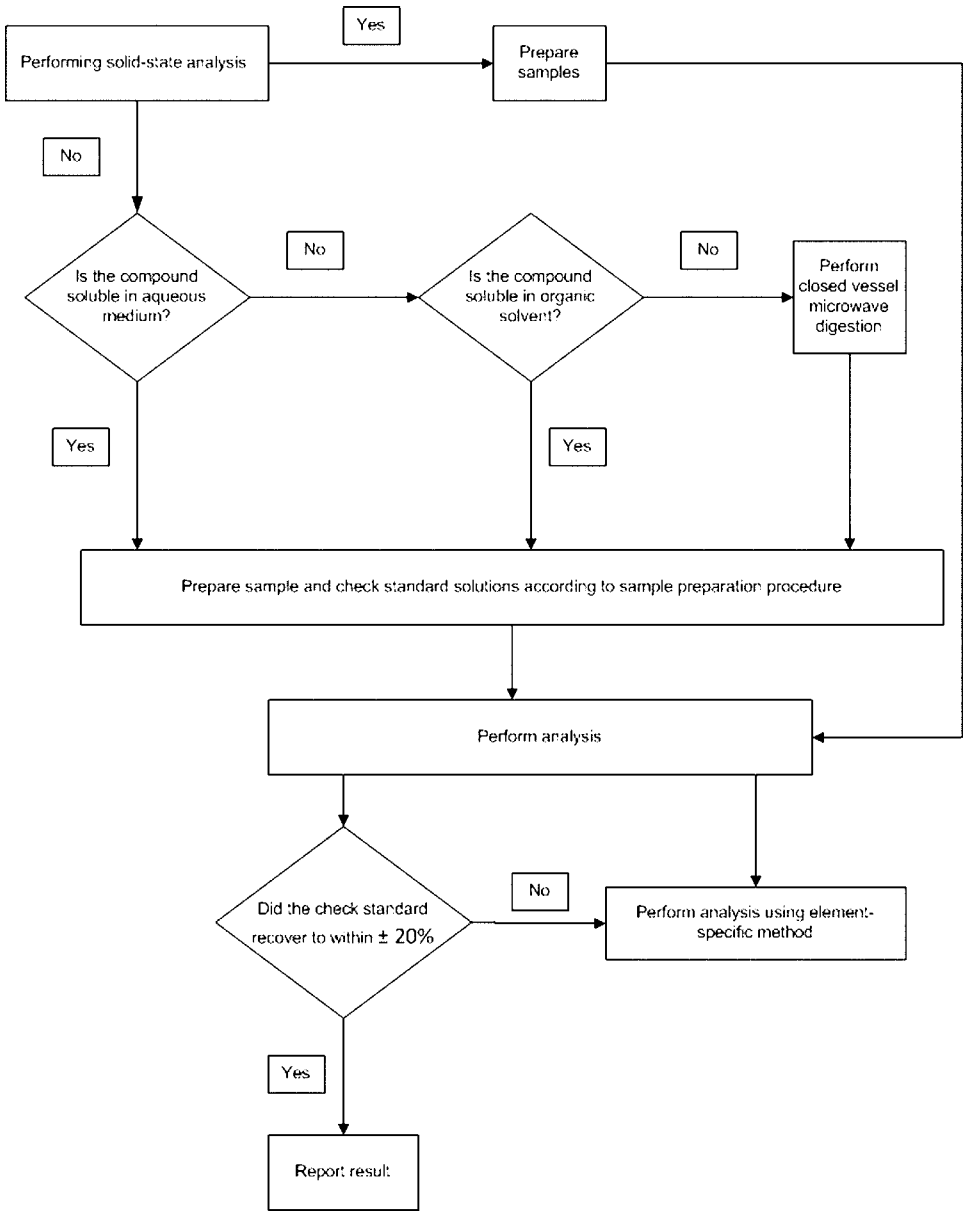

Figure 1. Elemental impurities decision tree.

ALTERNATIVE PROCEDURE VALIDATION REQUIREMENTS

The level of validation necessary to ensure that a procedure is appropriate for its intended purpose—that is, that it is acceptable—will differ, depending on whether a limit test or a quantitative determination is necessary. The requirements for validation of an elemental impurities procedure for either type of determination are described below.

VALIDATION OF LIMIT PROCEDURES

For elemental impurities, validation of a limit procedure should include accuracy, precision, and specificity. Following are acceptable validation parameters that allow a procedure to be deemed appropriate as a limit procedure:

Accuracy

Control Sample—A preparation of certified reference materials for the element of interest at the indicated level

Test Sample—A sample of material under test, spiked with certified reference materials for the element of interest at the indicated level, prepared in triplicate

Acceptance Criteria—Each *Test sample* provides a signal of intensity or value equivalent to or greater than that of the *Control sample*. [NOTE—The signal obtained must show a change from the value obtained compared to a blank determination.] The accuracy of the method must be determined by conducting studies with test materials supplemented with known concentrations of each element at the appropriate acceptance limit concentration. The test materials must be spiked before any sample preparation steps are taken. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked before the digestion procedure.

Precision for Instrumental Methods (Repeatability)

[NOTE—Noninstrumental precision is demonstrated by meeting the *Accuracy* requirement above.]

Test Samples: Six independent samples of the material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria: Relative standard deviation, NMT 20%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained for that element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

VALIDATION OF QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of a quantitative procedure. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference material.

Accuracy

Control Sample 1: 0.5*J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Control Sample 2: *J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Control Sample 3: 1.5*J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Test Sample 1: Sample of material under test, spiked with certified reference materials for the element of interest at 0.5*J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 2: Sample of material under test, spiked with certified reference materials for the element of interest at *J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 3: Sample of material under test, spiked with certified reference materials for the element of interest at 1.5*J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Acceptance Criteria: Spike recovery: 80%–150% for the mean of three replicate preparations at each concentration. The test materials must be supplemented before any sample preparation steps. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked at the beginning of the digestion procedure.

Precision

REPEATABILITY

Test Samples: Six independent samples of material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria: Relative standard deviation, NMT 20%

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the method must be established. Acceptable experiments for establishing intermediate precision include performing the *Repeatability* analysis

1. On different days,
2. With different instrumentation, or
3. With different analysts.

Note that executing only one of the three experiments listed is required in order to demonstrate intermediate precision.

Acceptance Criteria: Relative standard deviation, NMT 25%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained for that element in the presence of other elements that may interfere with the evaluation, at their indicated limits.

Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

Limit of Quantitation (Sensitivity)—Demonstrated by meeting the *Accuracy* requirement.

REFEREE PROCEDURES 1 AND 2

Procedure and Detection Technique

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP-OES). Procedure 2 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP-MS).

Verification

Before the initial use of a referee procedure, the analyst should ensure that the procedure is appropriate for the instrument and sample used. This is accomplished by procedure verification, as described in *Verification of Compendial Procedures* ⟨1226⟩.

Sample Preparation

Sample preparation is critical to the successful completion of the evaluation. Use the flow chart in *Figure 1* to determine the means of sample preparation. The sample

preparation scheme should yield sufficient sample to allow quantification of each element at the specified limit stated in the corresponding monograph or chapter. [NOTE—All liquid samples should be weighed.]

Closed Vessel Microwave Digestion—This sample preparation procedure is designed for samples that must be digested. The procedure also applies to samples that are not soluble in nitric acid. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the microwave digestion apparatus used, if proportions remain constant.]

Sample Preparation—Dehydrate and predigest 0.5 g of sample in 5 mL of freshly prepared aqua regia.[1] Sulfuric acid may also be used as a last resort.[2] Allow the sample to sit loosely covered for 30 min in a fume hood. Add 10 mL more of aqua regia, and digest, using a closed vessel microwave technique. Microwave until digestion or extraction is complete. Repeat if necessary by adding 5 mL more of aqua regia. [NOTE—Where closed vessel microwave digestion is necessary, follow the manufacturer's recommended procedures to ensure safe usage.][NOTE—In closed vessel microwave digestion, the use of concentrated hydrofluoric acid (HF) is not recommended. However, when its use is necessary, practice the utmost caution in the preparation of test articles, and review or establish local procedures for safe handling, safe disposal, and HF-tolerant instrumental configurations.]

Reagents—All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* ⟨730⟩. Reagents should be commercial elemental stock standards that are National Institute of Standards and Technology (NIST)–traceable, at a recommended concentration of 100 µg/mL or greater; or appropriate USP Reference Standards, as either single element or multielement.

[1] Ultra pure nitric acid/hydrochloric acid (1 : 3) prepared as needed. (A 1%–5% solution of aqua regia is used as a rinsing solution between analyses and as calibration blanks.)

[2] Sulfuric acid should be used only when absolutely needed, for the following reasons:
Upon addition of sulfuric acid, elements may be lost as a result of extreme exothermic reaction.
The viscosity of sulfuric acid is higher than that of other acids, which affects the overall flow of solution.

Procedure 1: ICP-OES

Sample Solution: Proceed as directed in *Sample preparation* above. When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool (add an appropriate stabilizer, such as gold at about 0.1 ppm, for mercury measurement), and dilute with *Purified Water* to 50.0 mL.

Calibration Solution 1: *2J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2: 0.1*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Check Standard Solution: 1 ppm of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*) [NOTE—Multiple elements of interest may be included in this solution at 1 ppm each. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank: Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* ⟨730⟩)

Mode: ICP

Detector: Optical emission spectroscopy

Rinse: 5% aqua regia

Calibration: Two-point, using *Calibration solution 1, Calibration solution 2,* and *Blank*

System Suitability

Sample: *Check Standard Solution*

Suitability requirements—

Drift: differs from actual concentration by NMT 20%. [NOTE—If samples are high in mineral content, to minimize sample carryover, rinse system well (60 sec) before introducing *Check Standard Solution*.]

Analysis: Analyze according to manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size.

Procedure 2: ICP-MS

Sample Solution: Proceed as directed in *Sample preparation* above, and add appropriate internal standards at appropriate concentrations.

When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool, add appropriate internal standards at appropriate concentrations (gold should be one of the internal standards for mercury measurement), and dilute with *Purified water* to 50.0 mL.

Calibration Solution 1: *2J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2: 0.1*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank: Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* ⟨730⟩)

Mode: ICP [NOTE—An instrument with a cooled spray chamber is recommended.]

Detector: Mass spectrometer

Rinse: 5% aqua regia

Calibration: *Calibration solution 1, Calibration solution 2,* and *Blank*

System Suitability

Sample: *Calibration solution 1*

Suitability requirements—

Drift: differs from actual concentration by NMT 20%. [NOTE—If samples are high in mineral content, rinse system well (60 sec) before introducing *Check Standard Solution* to minimize sample carryover.]

Analysis: Analyze per manufacturer's suggestions for program and m/z. Calculate and report results based on the original sample size. [NOTE: Arsenic is subject to interference from argon chloride. Appropriate measures, including a sample preparation without aqua regia, must be taken to correct for the interference, depending on instrumental capabilities.]

CALCULATIONS AND REPORTING

Upon completion of the analysis, calculate the final concentration of a given element in the test article (μg/g) from the solution element concentration (μg/mL) as follows:

$$C = [(A \times V_1) / W] \times (V_2 / V_3)$$

where

C = concentration of analyte (μg/g)

A = instrument reading (μg/mL)

V_1 = volume of initial test article preparation (mL)

W = weight of test article preparation (g)

V_2 = total volume of any dilution performed (mL)

V_3 = aliquot of initial test article preparation used in any dilution performed (mL)

Similarly, calculate the final concentration of a given element in the test article (μg/g) from the solution element concentration (ng/mL) as follows:

$$C = [(A \times V_1) / W] \times (1\ \mu g / 1000\ ng)(V_2 / V_3)$$

C = concentration of analyte (μg/g)

A = instrument reading (ng/mL)

V_1 = volume of initial test article preparation (mL)

W = weight of test article preparation (g)

V_2 = total volume of any dilution performed (mL)

V_3 = aliquot of initial test article preparation used in any dilution performed (mL)▲*USP34*

OTHER TESTS AND ASSAYS

BRIEFING

⟨**561**⟩ **Articles of Botanical Origin,** *USP 32* page 182. The Dietary Supplement General Chapters Committee recommends updating the *Test for Aflatoxins* through a revision of the thin-layer chromatography method and introducton of an HPLC procedure (AOAC Official Method 2008.2). Aflatoxins are difuranocoumarin toxic metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* fungi, which are naturally occurring contaminants of food and dietary supplements. At least 13 naturally occurring highly toxic and carcinogenic aflatoxins have been isolated. Of these, aflatoxin B_1 is recognized as the most toxic, and its contamination of articles of botanical origin should be minimized. The acceptance limits of each aflatoxin are also indicated. In addition, compendial requirements for residual pesticide suspected in botanical dietary supplements are addressed in *General Method for Pesticide Residues Analysis*. The residual pesticide limits for crude drugs are harmonized with the *European Pharmacopoeia, 6.6 Edition*.

(DS-GC: Y. Tokiwa) RTS—C76308

Change to read:

SAMPLING

In order to reduce the effect of sampling bias in qualitative and quantitative results, it is necessary to ensure that the composition of the sample used be representative of the batch of drugs being examined. The following sampling procedures are the minimum considered applicable to vegetable drugs. Some articles, or some tests, may require more rigorous procedures involving more containers being sampled or more samples per container.

Gross Sample

Where external examination of containers, markings, and labels indicates that the batch can be considered to be homogeneous, take individual samples from the number of randomly selected containers indicated below. Where the batch cannot be considered to be homogeneous, divide it into sub-batches that are as homogeneous as possible, then sample each one as a homogeneous batch. It is recommended to include samples from the first, middle, and last containers where the *No. of Containers in Batch* (*N*) is 11 or more and each container in the batch is numbered or lettered in order.

No. of Containers in Batch (*N*)	No. of Containers to be Sampled (*n*)
1 to 10	all
11 to 19	11
>19	$n = 10 + (N/10)$

(Round calculated ''*n*'' to next highest whole number.)

Samples are taken from the upper, middle, and lower sections of each container. If the crude material consists of component parts which are 1 cm or less in any dimension, and in the case of all powdered or ground materials, withdraw the sample by means of a sampling device that removes a core from the top to the bottom of the container, not less than two cores being taken ~~in opposite directions~~

▲from different angles.▲*USP34*

For materials with component parts over 1 cm in any dimension, withdraw samples by hand. In the case of large bales or packs, samples should be taken from a depth of 10 cm because the moisture content of the surface layer may be different from that of the inner layers.

Prepare the gross sample by combining and mixing the individual samples taken from each opened container, taking care not to increase the degree of fragmentation or significantly affect the moisture content.

▲For articles in containers holding less than 1 kg, mix the contents, and withdraw a quantity sufficient for the tests. For articles in containers holding between 1 and 5 kg, withdraw equal portions from the upper, middle, and lower parts of the container, each of the samples be-

Attachment 23

BRIEFING

〈 232 〉 **Elemental Impurities—Limits,** page 197 of *PF* 36(1) [Jan–Feb 2010]. This revision to general chapter *Elemental Impurities* 〈 232 〉 is based on comments received during the public comment period. The Expert Panel on elemental impurities has reviewed these comments and is proposing revisions both to 〈 232 〉 and its accompanying general chapter *Elemental Impurities—Procedures* 〈 233 〉. Although these proposed changes do not materially impact the scientific content of these chapters, they are being published in *PF* to assure that the chapter requirements are clear to all users and to seek any final input.

The Permissible Daily Exposure (PDE) limits presented in the proposed new general chapter 〈 232 〉 are consistent with the current early deliberations of the International Congress on Harmonization (ICH) Q3D expert working party on metal impurities. Changes by ICH Q3D to the PDE limits for the elements contained in this chapter will be managed as proposed changes to the chapter via existing USP revision processes, with corresponding changes to the implementation times if the limits decrease. The addition of elements to this chapter based on additions made by ICH Q3D will be managed similarly. However, any elements (and their accompanying PDE) included in the final ICH document that are less toxic than those included in chapter 〈 232 〉 will be incorporated in a future informational general chapter rather than in 〈 232 〉.

The previously published *PF* 36(1) revision to the *General Notices and Requirements* pertaining to the *Elemental Impurities* chapters (section 5.60.30) was deferred from USP 34–NF 29. Any change in the implementation date will be reflected in a *General Notices* revision. This proposal will be included on the official ballot when chapters 〈 232 〉 and 〈 233 〉 are considered for approval by the Expert Committee.

(GCCA: K. Zaidi.)
Correspondence Number—C89972

Comment deadline: July 31, 2011

Add the following:

■〈 232 〉 ELEMENTAL IMPURITIES—LIMITS

~~INTRODUCTION~~

~~The objective of this chapter is to set limits on the amounts of elemental impurities in pharmaceuticals. The chapter applies to drug substances, drug products (including natural-source and rDNA biologics), and excipients. Dietary supplements and their ingredients are addressed in chapter *Elemental Impurities in Dietary Supplements* 〈2232〉. For articles that are designated "For Veterinary Use Only," higher or lower levels for the permissible daily exposure and concentration limit may be justified in exceptional cases, based on the actual daily dose, actual target species, relevant toxicological data, and consumer safety considerations.~~

~~Elemental impurities addressed in this chapter are classified as shown in *Table 1*.~~

~~Table 1. Elemental Impurity Classes~~

~~Class~~	~~Assessment~~
~~Class 1~~	~~Elements should be essentially absent~~
	~~Known or strongly suspected human toxicants~~
	~~Environmental hazards~~

Class	Assessment
Class 2	Elements should be limited
	Elements with less toxicity than Class 1
	Elements deliberately added to an article

Class 1 Elemental Impurities

Compliance with the limits specified for Class 1 elemental impurities is required for all drug products, regardless of the likelihood of the presence of impurities. The presence of unexpected elemental contaminants, as well as that of impurities likely to be present, should be considered in determining compliance and planning the risk-based extent of testing.

Class 2 Elemental Impurities

In general, for Class 2 elemental impurities, the testing of drug substances, excipients, and drug products for elemental impurities need be conducted only when these elements are added during the manufacture of the article.

LIMITS OF ELEMENTAL IMPURITIES

Class 1

Class 1 elemental impurities (*Table 2*), because of their unacceptable toxicities or deleterious environmental effects, should not be present in a drug substance, excipient, or drug product. However, if their presence is unavoidable, their levels should be restricted as shown in *Table 2*, unless otherwise stated in the individual monograph.

Class 2

Class 2 elemental impurities (*Table 3*) should be limited in drug substances, excipients, and drug products because of their inherent toxicities.

Table 2. Class 1 Elemental Impurities

Element	Component Limit (µg/g)	Oral Daily Dose PDE* (µg/day)	Parenteral Component Limit (µg/g)	Parenteral Daily Dose PDE (µg/day)
Arsenic	1.5	15	0.15	1.5
Cadmium	0.5	5	0.05	0.5
Lead	1	10	0.1	1
Mercury	1.5	15	0.15	1.5

* Permitted daily exposure.

Table 3. Class 2 Elemental Impurities

Element	Component Limit (µg/g)	Oral Daily Dose PDE* (µg/day)	Parenteral Component Limit (µg/g)	Parenteral Daily Dose PDE (µg/day)
Chromium	25	250	2.5	25
Copper	250	2500	25	250
Manganese	250	2500	25	250

* Permitted daily exposure.

Element	Component Limit (µg/g)	Oral Daily Dose PDE* (µg/day)	Parenteral Component Limit (µg/g)	Parenteral Daily Dose PDE (µg/day)
Molybdenum	25	250	2.5	25
Nickel	25	250	2.5	25
Palladium	10	100	1.0	10
Platinum	10	100	1.0	10
Vanadium	25	250	2.5	25
Osmium	10 (combination not to exceed)	100 (combination not to exceed)	1.0 (combination not to exceed)	10 (combination not to exceed)
Rhodium				
Ruthenium				
Iridium				

* Permitted daily exposure.

OPTIONS FOR DESCRIBING LIMITS OF ELEMENTAL IMPURITIES

Three options are available when applying limits of elemental impurities for orally dosed products. Parenteral products are covered separately (see *Parenteral Products* section below).

Drug Product Analysis Option

This option is generally applicable. The results obtained from the analysis of a typical dosage unit, scaled to a maximum daily dose, are compared to the Daily Dose PDE, as shown in *Table 2* and *Table 3*.

$$\text{Daily Dose PDE} \geq \text{measured value} \times (\text{maximum daily dose})$$

Individual Component Option

For drug products with a maximum daily dose of NMT 10 g, the product meets the requirements when each drug substance and excipient meets the limits provided in the Component Limit column (*Table 2* and *Table 3*). If all drug substances and excipients in a formulation meet the limits shown in the Component Limit, these components may be used in any proportion. No further calculation is necessary.

Summation Option

This option can be used for drug products that are administered in doses other than 10 g/day or products in which any component of a product exceeds the applicable Component Limit. The Daily Dose PDE, as shown in *Table 2* and *Table 3*, can be used to calculate the concentration of elemental impurities allowed in a drug product. Apply this option by separately adding the amounts of each elemental impurity (in µg/day) present in each of the components of the drug product, using the following equation:

$$\text{Result} = \sum_{m}(C_M \times W_M)$$

m = each ingredient used to manufacture the dosage form

C_M = element concentration in that component (µg/g)

W_M = weight of component in a dosage form (g)

The sum of the quantities of each element/day should be less than that shown by the Daily Dose PDE in *Table 2* and *Table 3* for that element.

Examples

Consider an example of the application of the *Individual Component Option* and the *Summation Option* to the arsenic concentration in a drug product. The Daily Dose PDE is 15 µg/day, and the Component Limit is 1.5 µg/g (ppm). The maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of arsenic are shown in *Table 4*.

Table 4

Component	Amount in Formulation (g)	Arsenic Content (µg/g)	Daily Exposure (µg/day)
Drug substance	0.3	3.0	0.9
Excipient 1	0.9	1.0	0.9
Excipient 2	3.8	2.0	7.6
Drug product	5.0	—	9.4

Excipient 2 and the drug substance do not meet the Component Limit, but Excipient 1 does. Thus, the *Individual Component Option* cannot be used. However, under the *Summation Option*, the drug product meets the Daily Dose PDE limit of 15 µg/day and thus conforms to the acceptance criteria in this chapter.

Consider another example where the maximum administered daily weight of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of arsenic are shown in *Table 5*.

Table 5

Component	Amount in Formulation (g)	Arsenic Content (µg/g)	Daily Exposure (µg/day)
Drug substance	0.3	5.0	1.5
Excipient 1	0.9	5.0	4.5
Excipient 2	3.8	5.0	19.0
Drug product	5.0	—	25.0

In this example, the drug product exceeds the limits in *Table 2*, using both the *Individual Component Option* and the *Summation Option*. The manufacturer can test the drug product by using the *Drug Product Analysis Option*. If the level of arsenic in the formulation exceeds the Daily Dose PDE, the product fails to meet the impurity limits as described in this chapter.

Parenteral Products

Because of the presumption of 100% bioavailability of the elemental impurity during parenteral administration, versus the presumed 10% bioavailability via the oral route, the Parenteral Component Limit and the Parenteral Daily Dose PDE (*Table 2* and *Table 3*) are 10% of those for the oral route of introduction. To evaluate the limits for elemental impurities, one can apply the three options described above, using the Parenteral Component Limit instead of the Component Limit, and using the Parenteral Daily Dose PDE instead of the Oral Daily Dose PDE.

~~ANALYTICAL PROCEDURES~~

~~For a presentation of the alternatives for testing, see the chapter *Elemental Impurities—Procedures* 〈233〉. The validation necessary will vary depending on the situation. For all three options described in Chapter <232> in the section *Options for Describing Limits of Elemental Impurities*, it may be appropriate to use the section *Limit Procedure Validation* in Chapter <233>. However, for the *Summation Option* in Chapter <232>, acceptable levels of validation must be determined on a case-by-case basis. Validation of a procedure using the *Quantitative Procedure Validation* in Chapter <233> is acceptable for all options under all circumstances, and it is generally preferred. The determination of the level of validation necessary is at the discretion of the manufacturer and the competent regulatory authority.~~

INTRODUCTION

This general chapter specifies limits for the amounts of elemental impurities in drug products. Elemental impurities include catalysts and environmental contaminants that may be present in drug substances, excipients, or drug products. These impurities may occur naturally, be added intentionally, or be introduced inadvertently (e.g., by interactions with processing equipment). When elemental impurities are known to be present, have been added, or have the potential for introduction, assurance of compliance to the specified levels is required. A risk-based control strategy may be appropriate when analysts determine how to assure compliance with this standard. Regardless of the approach used, compliance with the limits specified is required for all drug products.

The limits presented in this chapter do not apply to excipients and drug substances, except where specified in this chapter or in the individual monographs. However, elemental impurity levels present in drug substances and excipients must be known and reported.

Dietary supplements and their ingredients are addressed in *Elemental Contaminants in Dietary Supplements* 〈2232〉. For articles that are designated “For Veterinary Use Only”, or for which veterinary administration is intended, the permissible daily exposures (PDE) presented in this chapter are applicable. However, higher or lower PDE and concentration limits may be appropriate based on the daily dose, target species, relevant toxicological data, or consumer safety impact.

SPECIATION

The determination of the oxidation state, organic complex, or combination is termed speciation. Each of the elemental impurities has the potential to be present in differing oxidation or complexation states. However, arsenic and mercury are of particular concern because of the differing toxicities of their inorganic and complexed organic forms.

The arsenic limits are based on the inorganic (most toxic) form. Arsenic can be measured using a total-arsenic procedure under the assumption that all arsenic contained in the material under test is in the inorganic form. Where the limit is exceeded using a total arsenic procedure, it may be possible to show via a procedure that quantifies the different forms that the inorganic form meets the specification.

The mercury limits are based upon the inorganic (2^{+}) oxidation state. The methyl mercury form (most toxic) is rarely an issue for pharmaceuticals. Thus, the limit was established assuming the most common (mercuric) inorganic form. Limits for articles that have the potential to contain methyl mercury (e.g., materials derived from fish) are to be provided in the monograph.

ROUTES OF EXPOSURE

The toxicity of an elemental impurity is related to its extent of exposure (bioavailability). The *Exposure Factor* in *Table 1* is used to modify the PDEs presented in *Table 2*, column 2, based on the route of administration, assuming 100% bioavailability for the parenteral and inhalational routes. These limits are based on chronic exposure but exclude potential genotoxic effects. When carcinogenicity is suspected (e.g., arsenic in inhalation products), the limits should be modified. [NOTE—The routes of administration of drug products are defined in general chapter *Pharmaceutical Dosage Forms* 〈 1151 〉.]

Table 1. Exposure Factor

Route of Administration	Exposure Factor
Oral (solids and liquids)	1
Parenteral (Injectables, implants, and ophthalmics)	0.1
Topicals and Dermal	1
Mucosal (nasal, otic, rectal, vaginal, urethral, others)	1
Inhalational (aerosols, inhalers, and gases)	0.1

DRUG PRODUCTS

The limits described in the second column of *Table 2* are the base daily dose PDEs of the elemental impurities of interest for a drug product taken by an adult patient according to indicated routes of administration. Exceptions for pediatric or other special populations that lower the PDE should be reflected in the limits in the appropriate monographs. Parenterals with an intended maximum dose of greater than 10 mL and not more than 100 mL must use the *Summation Option* described below.

Large Volume Parenterals

The amount of elemental impurities present in a Large Volume Parenteral (LVP – daily dose greater than 100 mL) drug product must be controlled through the individual components used to produce the product. The amounts of elemental impurities present in each component used in an LVP are less than the values included in the third column of *Table 2*.

Table 2. Elemental Impurities for Drug Products

Element	Daily Dose PDE[a] (µg/day)	LVP Component Limit (µg/g)
Cadmium	5	0.05
Lead	10	0.1
Inorganic arsenic[b]	15	0.15
Inorganic Mercury[b]	15	0.15
Iridium	100	1.0
Osmium	100	1.0
Palladium	100	1.0
Platinum	100	1.0

a PDE = Permissible Daily Exposure based on a 50Kg person.

b See *Speciation* section.

Element	Daily Dose PDE[a] (µg/day)	LVP Component Limit (µg/g)
Rhodium	100	1.0
Ruthenium	100	1.0
Chromium	250	2.5
Molybdenum	250	2.5
Nickel	250	2.5
Vanadium	250	2.5
Copper	2500	25
Manganese	2500	25

a PDE = Permissible Daily Exposure based on a 50Kg person.

b See *Speciation* section.

Modified Daily Dose PDE

The *Modified Daily Dose PDE* is the maximum exposure to an impurity that a patient should experience from the maximum daily dose of a drug product. The *Modified Daily Dose PDE* is calculated by multiplying the *Daily Dose PDE* values in *Table 2* by the *Exposure Factor* from *Table 1* for the elements in question.

$$\textit{Modified Daily Dose PDE} = \textit{Daily Dose PDE} \times \textit{Exposure Factor}$$

Options for Demonstrating Compliance

DRUG PRODUCT ANALYSIS OPTION

The results obtained from the analysis of a typical dosage unit, scaled to a maximum daily dose, are compared to the *Modified Daily Dose PDE.*

$$\textit{Modified Daily Dose PDE} \geq \text{measured value (µg/g)} \times \text{maximum daily dose (g/day)}$$

The measured amount of each impurity is NMT the *Modified Daily Dose PDE,* unless otherwise stated in the individual monograph.

SUMMATION OPTION

Separately add the amounts of each elemental impurity (in µg/g) present in each of the components of the drug product using the following equation:

$$\textit{Modified Daily Dose PDE} \geq [S^{M}_{1}(C_M \times W_M)] \times D_D$$

where

M = each ingredient used to manufacture a dosage unit

C_M = element concentration in component (drug substance or excipient) (µg/g)

W_M = weight of component in a dosage unit (g/unit). [NOTE—unit = dosage unit.]

D_D = number of units in the maximum daily dose (unit/day)

The result of the summation of each impurity is NMT the *Modified Daily Dose PDE,* unless otherwise stated in the individual monograph. Before products can be evaluated using this option, the manufacturer must validate that additional elemental impurities cannot be inadvertently added through the manufacturing process.

DRUG SUBSTANCE AND EXCIPIENTS

The presence of elemental impurities in drug substances and excipients must be controlled and, where present, reported. The acceptable levels for these impurities depend on the material's ultimate use. Therefore, drug product manufacturers must determine the acceptable level of elemental impurities in the drug substances and excipients used to produce their products.

The values provided in *Table 3* represent concentration limits for components (drug substances and excipients) of drug products dosed at a maximum daily dose of ≤10 g/day. These values serve as default concentration limits to aid discussions between drug product manufacturers and the suppliers of the components of their drug products. [NOTE—Individual components may need to be limited at levels different from those in the table depending on monograph-specific mitigating factors.]

Table 3. Default concentration limits for drug substances and excipients

Element	Concentration limits (µg/g) for Table 1 Exposure Factor 1 Drug Products with a Maximum Daily dose of ≤ 10 g/day	Concentration limits (µg/g) for Table 1 Exposure Factor 0.1 Drug Products with a Maximum Daily dose of ≤ 10 g/day
Cadmium	0.5	0.05
Lead	1	0.1
Inorganic Arsenic	1.5	0.15
Inorganic Mercury	1.5	0.15
Iridium	10	1
Osmium	10	1
Palladium	10	1
Platinum	10	1
Rhodium	10	1
Ruthenium	10	1
Chromium	25	2.5
Molybdenum	25	2.5
Nickel	25	2.5
Vanadium	25	2.5
Copper	250	25
Manganese	250	25

ANALYTICAL TESTING

If, by validated processes and supply-chain control, manufacturers can demonstrate the absence of impurities, then further testing is not needed. If testing is done to demonstrate compliance, see general chapter *Elemental Impurities*

—Procedures 〈 233 〉 .

■1S (*USP35*)

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Attachment 24

BRIEFING

〈 **233** 〉 **Elemental Impurities—Procedures,** page 201 of *PF* 36(1) [Jan.–Feb. 2010]. This revision to the general test chapter, *Elemental Impurities—Procedures* 〈 233 〉 is based on comments received during the public comment period. The Expert Committee on elemental impurities has reviewed these comments and is proposing this revision to provide additional clarity and flexibility. Although these proposed changes do not materially impact the scientific content of the chapter, they are being published in *PF* to assure that the chapter requirements are clear to all users and also to seek any final input. This chapter is expected to be included on the official ballot along with general chapter *Elemental Impurities—Limits* 〈 232 〉 for approval by the Expert Committee.

(GCCA : K.Zaidi.)
Correspondence Number—C89972

Comment deadline: July 31, 2011

Add the following:

〈 ■233 〉 ELEMENTAL IMPURITIES—PROCEDURES

~~INTRODUCTION~~

~~This chapter describes analytical procedures for the evaluation of elemental impurities in *USP* for drug substances and drug products (including natural-source and rDNA biologics); in *NF* for excipients; and in the *USP Dietary Supplements Compendium* for dietary supplements and dietary ingredients (all drug articles). Two referee procedures are described. Criteria for the approval of alternative procedures are also described. An alternative procedure will require complete validation for each element of interest. In addition, a system suitability evaluation using a USP Reference Standard or its equivalent should be demonstrated on the day of analysis. Alternative procedures that meet the validation requirements described herein are considered to be equivalent to Procedures 1 and 2. A decision-tree that can be used to guide a user to an appropriate alternative procedure is presented in *Figure 1*. The test requirement is specified in *General Notices* or the individual monograph.~~

~~Speciation~~

~~When elements are present in certain complexes, oxidation states, or organic combinations, they may show more significant toxicity than in other forms and may require further testing and control. The determination of the oxidation state or organic complex or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter.~~

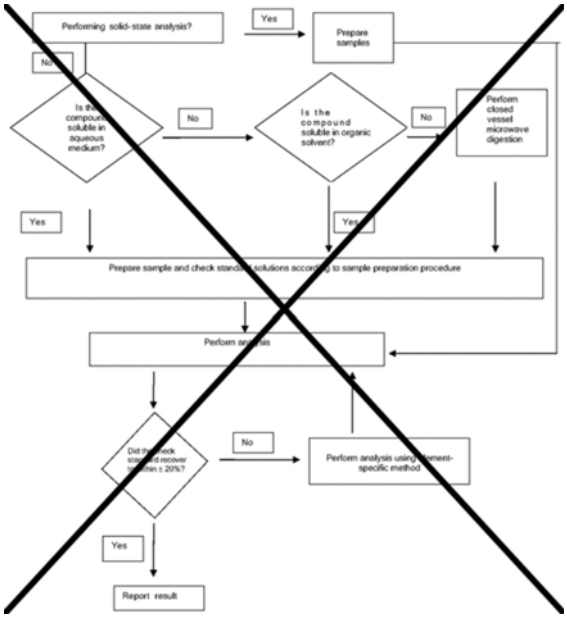

Figure 1. Elemental impurities decision tree.

ALTERNATIVE PROCEDURE VALIDATION REQUIREMENTS

The level of validation necessary to ensure that a procedure is appropriate for its intended purpose—that is, that it is acceptable—will differ, depending on whether a limit test or a quantitative determination is necessary. The requirements for validation of an elemental impurities procedure for either type of determination are described below.

VALIDATION OF LIMIT PROCEDURES

For elemental impurities, validation of a limit procedure should include accuracy, precision, and specificity. Following are acceptable validation parameters that allow a procedure to be deemed appropriate as a limit procedure:

Accuracy

Control Sample—A preparation of certified reference materials for the element of interest at the indicated level

Test Sample—A sample of material under test, spiked with certified reference materials for the element of interest at the indicated level, prepared in triplicate

Acceptance Criteria—Each *Test sample* provides a signal of intensity or value equivalent to or greater than that of the *Control sample*. [NOTE—The signal obtained must show a change from the value obtained compared to a blank determination.] The accuracy of the method must be determined by conducting studies with test materials supplemented with known concentrations of each element at the appropriate acceptance limit concentration. The test materials must be spiked before any sample preparation steps are taken. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked before the digestion procedure.

Precision for Instrumental Methods (Repeatability)

[NOTE—Noninstrumental precision is demonstrated by meeting the *Accuracy* requirement above.]

Test Samples: Six independent samples of the material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria: Relative standard deviation, NMT 20%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained for that element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.
Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

VALIDATION OF QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of a quantitative procedure. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference material.

Accuracy

Control Sample 1:0.5*J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Control Sample 2:*J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Control Sample 3:1.5*J*, of the certified reference materials for the element of interest, where *J* is the indicated limit

Test Sample 1:Sample of material under test, spiked with certified reference materials for the element of interest at 0.5*J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 2:Sample of material under test, spiked with certified reference materials for the element of interest at *J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 3:Sample of material under test, spiked with certified reference materials for the element of interest at 1.5*J*, where *J* is the indicated limit [NOTE—Prepare in triplicate.]

Acceptance Criteria:Spike recovery: 80%–150% for the mean of three replicate preparations at each concentration. The test materials must be supplemented before any sample preparation steps. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked at the beginning of the digestion procedure.

Precision

REPEATABILITY

Test Samples:Six independent samples of material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria:Relative standard deviation, NMT 20%

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the method must be established. Acceptable experiments for establishing intermediate precision include performing the *Repeatability* analysis

1. On different days,
2. With different instrumentation, or

3. With different analysts.

Note that executing only one of the three experiments listed is required in order to demonstrate intermediate precision.

Acceptance Criteria:Relative standard deviation, NMT 25%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained for that element in the presence of other elements that may interfere with the evaluation, at their indicated limits.
Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

Limit of Quantitation (Sensitivity)—Demonstrated by meeting the *Accuracy* requirement.

REFEREE PROCEDURES 1 AND 2

Procedure and Detection Technique

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP-OES). Procedure 2 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP-MS).

Verification

Before the initial use of a referee procedure, the analyst should ensure that the procedure is appropriate for the instrument and sample used. This is accomplished by procedure verification, as described in *Verification of Compendial Procedures* ⟨1226⟩.

Sample Preparation

Sample preparation is critical to the successful completion of the evaluation. Use the flow chart in *Figure 1* to determine the means of sample preparation. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the specified limit stated in the corresponding monograph or chapter. [NOTE—All liquid samples should be weighed.]

Closed Vessel Microwave Digestion—This sample preparation procedure is designed for samples that must be digested. The procedure also applies to samples that are not soluble in nitric acid. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the microwave digestion apparatus used, if proportions remain constant.]

Sample Preparation—Dehydrate and predigest 0.5 g of sample in 5 mL of freshly prepared aqua regia.[1] Sulfuric acid may also be used as a last resort.[2] Allow the sample to sit loosely covered for 30 min in a fume hood. Add 10 mL more of aqua regia, and digest, using a closed vessel microwave technique. Microwave until digestion or extraction is complete. Repeat if necessary by adding 5 mL more of aqua regia. [NOTE—Where closed vessel microwave digestion is necessary, follow the manufacturer's recommended procedures to ensure safe usage.] [NOTE—In closed vessel microwave digestion, the use of concentrated hydrofluoric acid (HF) is not recommended. However, when its use is

necessary, practice the utmost caution in the preparation of test articles, and review or establish local procedures for safe handling, safe disposal, and HF-tolerant instrumental configurations.]

Reagents—All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* ‹730›. Reagents should be commercial elemental stock standards that are National Institute of Standards and Technology (NIST)–traceable, at a recommended concentration of 100 µg/mL or greater; or appropriate USP Reference Standards, as either single element or multielement.

Procedure 1: ICP-OES

Sample Solution:Proceed as directed in *Sample preparation* above. When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool (add an appropriate stabilizer, such as gold at about 0.1 ppm, for mercury measurement), and dilute with *Purified Water* to 50.0 mL.

Calibration Solution 1:2*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2:0.1*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Check Standard Solution:1 ppm of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*) [NOTE—Multiple elements of interest may be included in this solution at 1 ppm each. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank:Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* ‹730›)

Mode:ICP

Detector: Optical emission spectroscopy

Rinse:5% aqua regia

Calibration: Two-point, using *Calibration solution 1, Calibration solution 2,* and *Blank*

System Suitability

Sample:*Check Standard Solution*

Suitability requirements—

Drift: differs from actual concentration by NMT 20%. [NOTE—If samples are high in mineral content, to minimize sample carryover, rinse system well (60 sec) before introducing *Check Standard Solution*.]

Analysis:Analyze according to manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size.

Procedure 2: ICP-MS

Sample Solution:Proceed as directed in *Sample preparation* above, and add appropriate internal standards at appropriate concentrations.
When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool, add

appropriate internal standards at appropriate concentrations (gold should be one of the internal standards for mercury measurement), and dilute with *Purified water* to 50.0 mL.

Calibration Solution 1:2*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2:0.1*J* of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where *J* is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank:Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* ‹730›)

Mode:ICP [NOTE—An instrument with a cooled spray chamber is recommended.]

Detector: Mass spectrometer

Rinse: 5% aqua regia

Calibration:*Calibration solution 1*, *Calibration solution 2*, and *Blank*

System Suitability

Sample:*Calibration solution 1*

Suitability requirements—

*Drift:*differs from actual concentration by NMT 20%. [NOTE—If samples are high in mineral content, rinse system well (60 sec) before introducing *Check Standard Solution* to minimize sample carryover.]

Analysis:Analyze per manufacturer's suggestions for program and m/z. Calculate and report results based on the original sample size. [NOTE—Arsenic is subject to interference from argon chloride. Appropriate measures, including a sample preparation without aqua regia, must be taken to correct for the interference, depending on instrumental capabilities.]

CALCULATIONS AND REPORTING

Upon completion of the analysis, calculate the final concentration of a given element in the test article (µg/g) from the solution element concentration (µg/mL) as follows:

$$C = [(A \times V_1) / W] \times (V_2 / V_3)$$

where

C = concentration of analyte (µg/g)
A = instrument reading (µg/mL)
V_1 = volume of initial test article preparation (mL)
W = weight of test article preparation (g)
V_2 = total volume of any dilution performed (mL)
V_3 = aliquot of initial test article preparation used in any dilution performed (mL)

~~Similarly, calculate the final concentration of a given element in the test article (µg/g) from the solution element concentration (ng/mL) as follows:~~

$$~~C = [(A \times V_1) / W] \times (1\ \mu g / 1000\ ng)(V_2 / V_3)~~$$

~~C = concentration of analyte (µg/g)~~
~~A = instrument reading (ng/mL)~~
~~V_1 = volume of initial test article preparation (mL)~~
~~W = weight of test article preparation (g)~~
~~V_2 = total volume of any dilution performed (mL)~~
~~V_3 = aliquot of initial test article preparation used in any dilution performed (mL)~~

INTRODUCTION

This chapter describes two analytical procedures (*Procedures 1* and *2*) for the evaluation of the levels of the elemental impurities that are described in *Elemental Impurities—Limits* ⟨ 232 ⟩ and *Elemental Contaminants in Dietary Supplements* ⟨ 2232 ⟩. The chapter also describes criteria for acceptable alternative procedures. Alternative procedures that meet the validation requirements described herein may be considered equivalent to *Procedures 1* and *2* for the purposes of this test. In addition, system standardization and suitability evaluation using applicable reference materials should be performed on the day of analysis. The requirement for an elemental impurity test is specified in *General Notices and Requirements* or in the individual monograph. By means of verification studies, analysts will confirm that the analytical procedures described herein, as well as alternative analytical procedures, are suitable for use on specified material.

Speciation

The determination of the oxidation state, organic complex or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter but examples may be found elsewhere in the *USP–NF* and in the literature.

Definitions

Concentrated Acid: Concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or Aqua Regia
Matched Matrix: Solutions having the same solvent composition as the *Sample solution.* In the case of aqueous solution, *matched matrix* would indicate that the same acids, acid concentrations, and mercury stabilizer are used in both preparations.
Target Elements: Elements with the potential of being present in the material under test. *Target Elements* must include lead, mercury, arsenic, and cadmium and should include any of those remaining elemental impurities presented in general chapter *Elemental Impurities—Limits* ⟨ 232 ⟩ that are used in production of the material under test or the components therein. *Target Elements* should also include any other elements that may be added through material processing or storage or any elements whose presence may interfere with the operation of the analytical procedures. [NOTE—Exclusion of elements from the list does not exempt the user from compliance with the requirements described in *Elemental Impurities—Limits* ⟨ 232 ⟩ or in this chapter.]
Target Limit or Target Concentration: The uppermost acceptance value for the elemental impurity being evaluated. Exceeding the target limit indicates that a material under test exceeds the acceptable value. The determination of

compliance is addressed in other chapters. [NOTE—*Target Limits* can be approximated by dividing the *Modified Daily Dose PDEs* by the maximum daily dose for the *Drug Product Analysis Option* in ⟨ 232 ⟩ or the *Daily Serving PDE* divided by the maximum daily serving size in ⟨ 2232 ⟩] (see *Elemental Impurities—Limits* ⟨ 232 ⟩ or *Elemental Contaminants in Dietary Supplements* ⟨ 2232 ⟩).

J: The concentration (w/w) of the element(s) of interest at the *Target Limit*, appropriately diluted to the working range of the instrument.

Appropriate Reference Materials: Where *Appropriate Reference Materials* are specified in the chapter, certified reference materials (CRM) from a national metrology institute (NMI, e.g., the National Institute of Standards and Technology in the United States) or reference materials that are traceable to the CRM of a NMI should be used.

COMPENDIAL PROCEDURES 1 AND 2

Procedure and Detection Technique

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP–AES or ICP–OES). *Procedure 2* can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP–MS). Before initial use, the analyst should verify that the procedure is appropriate for the instrument and sample used (procedural verification) by meeting the *Alternative Procedure Validation* requirements below.

Sample Preparation

Forms of sample preparation include *Neat*, *Direct Aqueous Solution*, *Direct Organic Solution*, and *Indirect Solution*. The selection of the appropriate sample preparation depends on the material under test and is the responsibility of the analyst. When a sample preparation is not indicated in the monograph, an analyst may use any of the following appropriately verified preparation procedures. Samples and blanks may be spiked with *Target Elements* where an analyte has limited solubility in the solvent system of choice. Standard solutions may contain multiple *Target Elements.* [NOTE—All liquid samples should be weighed.]

Neat: Used for liquids or alternative procedures that allow the examination of unsolvated samples.

Direct Aqueous Solution: Used when the sample is soluble in an aqueous solvent.

Direct Organic Solution: Used where the sample is soluble in an organic solvent.

Indirect Solution: Used when a material is not directly soluble in aqueous or organic solvents. Digest the sample using a closed-vessel digestion procedure, similar to the procedure provided below. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the limit specified in the corresponding monograph or chapter.

Closed Vessel Digestion: This sample-preparation procedure is designed for samples that must be digested in a *Concentrated Acid* using a closed-vessel digestion apparatus. Closed-vessel digestion minimizes the loss of volatile impurities. The choice of a *Concentrated Acid* depends on the sample matrix. The use of any of the *Concentrated Acids* may be appropriate, but each introduces inherent safety risks. Therefore, appropriate safety precautions should be employed at all times. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the digestion apparatus used.]

An example procedure that has been shown to have broad applicability is the following:

Dehydrate and predigest 0.5 g of primary sample in 5 mL of freshly prepared *Concentrated Acid*. Allow to sit loosely covered for 30 minutes in a fume hood. Add an additional 10 mL of *Concentrated Acid*, and digest, using a closed vessel technique, until digestion or extraction is complete. Repeat if necessary by adding an additional 5 mL of

Concentrated Acid. [NOTE—Where closed vessel digestion is necessary, follow the manufacturer's recommended procedures to ensure safe use.]

Reagents: All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* ⟨ 730 ⟩ .

Procedure 1: ICP-AES

Standardization Solution 1: 2*J* of the *Target Element(s)* in a *Matched Matrix*

Standardization Solution 2: 0.5*J* of the *Target Element(s)* in a *Matched Matrix*

Sample Stock Solution: Proceed as directed in *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample Solution: Dilute the *Sample Stock Solution* with an appropriate solvent to obtain a final concentration of the *Target Elements* at NMT 2*J.*

Blank: *Matched Matrix*

Elemental Spectrometric System
(See *Plasma Spectrochemistry* ⟨ 730 ⟩ .)

Mode: ICP

Detector: Optical detection system

Rinse: Diluent used

Standardization: *Standardization Solution 1*, *Standardization Solution 2*, and *Blank*

System Suitability

Sample: *Standardization Solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization Solution 1* before and after the analyis of the *Sample Solutions*.

Suitability criteria: NMT 20% for each *Target Element*. [NOTE—If samples are high in mineral content, rinse system well (60 seconds) before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size.

Procedure 2: ICP-MS

Standardization Solution 1: 2*J* of the *Target Element(s)* in a *Matched Matrix*

Standardization Solution 2: 0.5*J* of the *Target Element(s)* in a *Matched Matrix*

Sample Stock Solution: Proceed as directed for *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample Solution: Dilute the *Sample Stock Solution* with an appropriate solvent to obtain a final concentration of the *Target Elements* at NMT 2*J.*

Blank: *Matched Matrix*

Elemental Spectrometric System

(See *Plasma Spectrochemistry* ⟨ 730 ⟩.)

Mode: ICP. [NOTE—An instrument with a cooled spray chamber is recommended.]

Detector: Mass spectrometer

Rinse: Diluent used

Standardization: *Standardization Solution 1*, *Standardization Solution 2*, and *Blank*

System Suitability

Sample: *Standardization Solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization Solution 1* before and after the analyis of the *Sample Solutions*.

Suitability criteria: NMT 20% for each *Target Element*. [NOTE—If samples are high in mineral content, rinse system well (60 seconds) before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and *m*/*z*. Calculate and report results based on the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences (e.g., argon chloride interference with arsenic determinations.]

ALTERNATE PROCEDURE VALIDATION

If a specified compendial procedure does not meet the needs of a specific application, an alternative procedure may be used (see *General Notices* 6.30). Alternative procedures must be validated and must be acceptable and therefore equivalent to the compendial procedures for the purposes of the test. The principles of validation are provided in general chapter *Validation of Compendial Procedures* ⟨ 1225 ⟩. The level of validation necessary to ensure that an alternative procedure is acceptable depends on whether a limit test or a quantitative determination is necessary. The requirements for validation of an elemental impurities procedure for either type of determination are described below. Where this information differs from that presented in *Validation of Compendial Procedures* ⟨ 1225 ⟩, the parameters and acceptance criteria presented in this chapter take precedence. Any alternative procedure that has been validated and meets the acceptance criteria that follow is considered to be equivalent to the compendial procedures for the purposes of this test.

LIMIT PROCEDURES

The following section defines the validation parameters for the acceptability of alternative limit procedures. Meeting these requirements must be demonstrated experimentally using an appropriate system suitability procedure and reference material. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure as a limit procedure for the *Target Element*.
The suitability of the method must be determined by conducting studies with test materials supplemented with known concentrations of each *Target Element* of interest at the appropriate acceptance limit concentration. The test materials must be spiked before any sample preparation steps are performed.

Detectability

Standard Solution: A preparation of reference materials for the *Target Element(s)* at the *Target Concentrations*.

Spiked Sample Solution 1: Prepare a solution of sample under test, spiked with appropriate reference materials for the *Target Elements* at the *Target Concentration*, solubilized or digested as described in *Sample Preparation*.

Spiked Sample Solution 2: Prepare a solution of the sample under test, spiked with appropriate reference materials at 80% of the *Target Concentration* for the *Target Elements*, solubilized or digested as described in *Sample Preparation*.

Blank solution: A sample of material under test, solubilized or digested in the same manner as the *Sample Solutions*.

Acceptance Criteria

Non-Instrumental Procedures: *Spiked Sample Solution 1* provides a signal or intensity equivalent to or greater than that of the *Standard Solution. Spiked Sample Solution 2* must provide a signal or intensity less than that of the *Spiked Solution 1.* [NOTE—The signal from each *Spiked Sample* is NLT the blank determination.]

Instrumental Procedures: The average value of the replicate measurements of *Spiked Sample Solution 1* is equivalent to (±10%) or greater than that of the average value obtained for the replicate measurements of the *Standard Solution*. The average value of the replicate measurements of *Spiked Sample Solution 2* must provide a signal intensity or value less than that of the *Standard Solution*. [NOTE—Correct the values obtained for each of the spiked solutions using the *Blank Solution.*]

Precision for Instrumental Methods (Repeatability)

[NOTE—Non-instrumental precision is demonstrated by meeting the *Limit of Detection* requirement above.]

Sample Solutions: Six independent samples of the material under test, spiked with appropriate reference materials for the *Target Elements* at the indicated levels.

Acceptance Criteria

Relative standard deviation: NMT 20% for each *Target Element.*

Specificity

The procedure must be able to unequivocally assess each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of alternative quantitative procedures. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference material. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure for the purpose of quantifying the *Target Elements.*

Accuracy

Standard Solutions: Prepare solutions containing the *Target Elements* at concentrations ranging from 50% to 150% of *J,* using appropriate reference materials.

Test Samples: Prepare samples of the material under test spiked with appropriate reference materials before any sample preparation steps (digestion or solubilization) at concentrations ranging from 50% to 150% of *J* for each *Target Element*.

Acceptance Criteria

Spike recovery: 70%–150% for the mean of three replicate preparations at each concentration.

Precision

REPEATABILITY

Test Samples: Six independent samples of material under test [NOTE—Taken from the same lot] spiked with appropriate reference materials for the *Target Element(s)* at the indicated level.

Acceptance Criteria

Relative standard deviation: NMT 20% for each *Target Element.*

RUGGEDNESS

Perform the *Repeatability* analysis

1. on different days, or
2. with different instrumentation, or
3. with different analysts.

Executing only one of the three experiments listed is required to demonstrate ruggedness.

Acceptance Criteria

Relative standard deviation: NMT 20% for each *Target Element.*

Specificity

The procedure must be able to unequivocally assess each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

Limit of Quantitation, Range, and Linearity: Demonstrated by meeting the *Accuracy* requirement.

■1S (*USP35*)

[1] ~~Ultra pure nitric acid/hydrochloric acid (1:3) prepared as needed. (A 1%–5% solution of aqua regia is used as a rinsing solution between analyses and as calibration blanks.)~~

[2] ~~Sulfuric acid should be used only when absolutely needed, for the following reasons:~~

~~Upon addition of sulfuric acid, elements may be lost as a result of extreme exothermic reaction.~~

~~The viscosity of sulfuric acid is higher than that of other acids, which affects the overall flow of solution.~~

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Chemical Tests and Assays

LIMIT TESTS

Add the following:

■⟨232⟩ ELEMENTAL IMPURITIES—LIMITS

INTRODUCTION

This general chapter specifies limits for the amounts of elemental impurities in drug products. Elemental impurities include catalysts and environmental contaminants that may be present in drug substances, excipients, or drug products. These impurities may occur naturally, be added intentionally, or be introduced inadvertently (e.g., by interactions with processing equipment). When elemental impurities are known to be present, have been added, or have the potential for introduction, assurance of compliance to the specified levels is required. A risk-based control strategy may be appropriate when analysts determine how to assure compliance with this standard. Due to the ubiquitous nature of As, Cd, Pb, and Hg, they (at the minimum) must be considered in the risk-based control strategy. Regardless of the approach used, compliance with the limits specified is required for all drug products.

The limits presented in this chapter do not apply to excipients and drug substances, except where specified in this chapter or in the individual monographs. However, elemental impurity levels present in drug substances and excipients must be known and reported.

The limits indicated in this chapter are not required for articles intended only for veterinary use and conventional vaccines. Dietary supplements and their ingredients are addressed in *Elemental Contaminants in Dietary Supplements* ⟨2232⟩.[1]

SPECIATION

The determination of the oxidation state, organic complex, or combination is termed speciation. Each of the elemental impurities has the potential to be present in differing oxidation or complexation states. However, arsenic and mercury are of particular concern because of the differing toxicities of their inorganic and complexed organic forms.

The arsenic limits are based on the inorganic (most toxic) form. Arsenic can be measured using a total-arsenic procedure under the assumption that all arsenic contained in the material under test is in the inorganic form. Where the limit is exceeded using a total arsenic procedure, it may be possible to show via a procedure that quantifies the different forms that the inorganic form meets the specification.

The mercury limits are based upon the inorganic (2^+) oxidation state. The methyl mercury form (most toxic) is rarely an issue for pharmaceuticals. Thus, the limit was established assuming the most common (mercuric) inorganic form. Limits for articles that have the potential to contain methyl mercury (e.g., materials derived from fish) are to be provided in the monograph.

ROUTES OF EXPOSURE

The toxicity of an elemental impurity is related to its extent of exposure (bioavailability). The extent of exposure has been determined for each of the elemental impurities of interest for three routes of administration: oral, parenteral, and inhalational. These limits are based on chronic exposure. The other two routes of administration, mucosal and topical, are considered to be the same as oral for the purpose of this standard, and the PDEs described in *Table 1* would apply to these products. [NOTE—The routes of administration of drug products are defined in general chapter *Pharmaceutical Dosage Forms* ⟨1151⟩.]

DRUG PRODUCTS

The limits described in the second through fourth columns of *Table 1* are the base daily dose PDEs of the elemental impurities of interest for a drug product taken by the patient according to indicated routes of administration. Parenterals with an intended maximum dose of greater than 10 mL and not more than 100 mL must use the *Summation Option* described below.

Large Volume Parenterals

When the daily dose of an injection is greater than 100 mL (large volume parenteral (LVP)), the amount of elemental impurities present in the drug product must be controlled through the individual components used to produce the product. The amounts of elemental impurities present in each component used in an LVP are less than the values included in the fifth column of *Table 1*.

Table 1. Elemental Impurities for Drug Products

Element	Oral Daily Dose PDE[a] (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalational Daily Dose PDE (µg/day)	LVP Component Limit (µg/g)
Cadmium	25	2.5	1.5	0.25
Lead	5	5	5	0.5
Inorganic arsenic[b]	1.5	1.5	1.5	0.15
Inorganic mercury[b]	15	1.5	1.5	0.15
Iridium	100	10	1.5	1.0
Osmium	100	10	1.5	1.0
Palladium	100	10	1.5	1.0
Platinum	100	10	1.5	1.0
Rhodium	100	10	1.5	1.0
Ruthenium	100	10	1.5	1.0
Chromium	*	*	25	*
Molybdenum	100	10	250	1.0

[a] PDE = Permissible daily exposure based on a 50-kg person.
[b] See *Speciation* section.
* Not a safety concern.

[1] This dietary supplement chapter is still under revision and will appear online in *PF* 38(3) [May–June 2012].

Table 1. Elemental Impurities for Drug Products *(Continued)*

Element	Oral Daily Dose PDE[a] (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalational Daily Dose PDE (µg/day)	LVP Component Limit (µg/g)
Nickel	500	50	1.5	5.0
Vanadium	100	10	30	1.0
Copper	1000	100	70	25

[a] PDE = Permissible daily exposure based on a 50-kg person.
[b] See *Speciation* section.
* Not a safety concern.

Options for Demonstrating Compliance

DRUG PRODUCT ANALYSIS OPTION

The results obtained from the analysis of a typical dosage unit, scaled to a maximum daily dose, are compared to the *Daily Dose PDE*.

Daily Dose PDE ≥ measured value (µg/g) × maximum daily dose (g/day)

The measured amount of each impurity is NMT the *Daily Dose PDE*, unless otherwise stated in the individual monograph.

SUMMATION OPTION

Separately add the amounts of each elemental impurity (in µg/g) present in each of the components of the drug product using the following equation:

$$\textit{Daily Dose PDE} \geq [\Sigma^{M}_{1}(C_M \times W_M)] \times D_D$$

where
M = each ingredient used to manufacture a dosage unit
C_M = element concentration in component (drug substance or excipient) (µg/g)
W_M = weight of component in a dosage unit (g/dosage unit)
D_D = number of units in the maximum daily dose (unit/day)

The result of the summation of each impurity is NMT the *Daily Dose PDE*, unless otherwise stated in the individual monograph. Before products can be evaluated using this option, the manufacturer must validate that additional elemental impurities cannot be inadvertently added through the manufacturing process.

DRUG SUBSTANCE AND EXCIPIENTS

The presence of elemental impurities in drug substances and excipients must be controlled and, where present, reported. The acceptable levels for these impurities depend on the material's ultimate use. Therefore, drug product manufacturers must determine the acceptable level of elemental impurities in the drug substances and excipients used to produce their products.

The values provided in *Table 2* represent concentration limits for components (drug substances and excipients) of drug products dosed at a maximum daily dose of ≤ 10 g/day. These values serve as default concentration limits to aid discussions between drug product manufacturers and the suppliers of the components of their drug products. [NOTE—Individual components may need to be limited at levels different from those in the table depending on monograph-specific mitigating factors.]

Table 2. Default Concentration Limits for Drug Substances and Excipients

Element	Concentration Limits (µg/g) for Oral Drug Products with a Maximum Daily Dose of ≤10 g/day	Concentration Limits (µg/g) for Parenteral Drug Products with a Maximum Daily Dose of ≤10 g/day	Concentration Limits (µg/g) for Inhalational Drug Products with a Maximum Daily Dose of ≤10 g/day
Cadmium	2.5	0.25	0.15
Lead	0.5	0.5	0.5
Inorganic arsenic	0.15	0.15	0.15
Inorganic mercury	1.5	0.15	0.15
Iridium	10	1.0	0.15
Osmium	10	1.0	0.15
Palladium	10	1.0	0.15
Platinum	10	1.0	0.15
Rhodium	10	1.0	0.15
Ruthenium	100	10	1.5
Chromium	*	*	2.5
Molybdenum	10	1.0	25
Nickel	50	5.0	0.15
Vanadium	100	10	30
Copper	100	10	7

* Not a safety concern.

ANALYTICAL TESTING

If, by validated processes and supply-chain control, manufacturers can demonstrate the absence of impurities, then further testing is not needed. When testing is done to demonstrate compliance, proceed as directed in general chapter *Elemental Impurities—Procedures* ⟨233⟩, and minimally include As, Cd, Pd, and Hg in the *Target Element* evaluation.■2S (*USP35*)

Add the following:

■⟨233⟩ ELEMENTAL IMPURITIES—PROCEDURES

INTRODUCTION

This chapter describes two analytical procedures (*Procedures 1* and *2*) for the evaluation of the levels of the elemental impurities. The chapter also describes criteria for acceptable alternative procedures. Alternative procedures that meet the validation requirements described herein may be considered equivalent to *Procedures 1* and *2* for the purposes of this test. In addition, system standardization and

suitability evaluation using applicable reference materials should be performed on the day of analysis. The requirement for an elemental impurity test is specified in *General Notices and Requirements* or in the individual monograph. By means of verification studies, analysts will confirm that the analytical procedures described herein, as well as alternative analytical procedures, are suitable for use on specified material.

Speciation

The determination of the oxidation state, organic complex or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter but examples may be found elsewhere in the *USP–NF* and in the literature.

Definitions

Concentrated Acid: Concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or *Aqua Regia*.

Aqua Regia: Aqua regia is a mixture of concentrated hydrochloric and nitric acids, typically at ratios of 3:1 or 4:1, respectively.

Matched Matrix: Solutions having the same solvent composition as the *Sample solution*. In the case of an aqueous solution, *Matched Matrix* would indicate that the same acids, acid concentrations, and mercury stabilizer are used in both preparations.

Target Elements: Elements with the potential of being present in the material under test. Include As, Cd, Pd, and Hg in the target element evaluation when testing is done to demonstrate compliance. Target elements should also include any elements that may be added through material processing or storage, and any elements whose presence may interfere with the operation of the analytical procedures.

Target Limit or Target Concentration: The acceptance value for the elemental impurity being evaluated. Exceeding the target limit indicates that a material under test exceeds the acceptable value. The determination of compliance is addressed in other chapters. [NOTE—When applying this chapter to *Elemental Impurities—Limits* ⟨232⟩ and *Elemental Contaminants in Dietary Supplements* ⟨2232⟩,[1] *Target Limits* can be approximated by dividing the *Daily Dose PDEs* by the maximum daily dose for the *Drug Product Analysis Option* in ⟨232⟩ or the *Daily Serving PDE* divided by the maximum daily serving size in ⟨2232⟩]

J: The concentration (w/w) of the element(s) of interest at the *Target Limit*, appropriately diluted to the working range of the instrument. For example, if the target elements are Pb and As for an analysis of an oral solid drug product with a daily dose of 10 g/day using an inductively coupled plasma–mass spectrometry (ICP-MS). The target limit for these elements would be 0.5 µg/g and 0.15 µg/g (see *Table 2* in chapter ⟨232⟩). However, in this case, the linear dynamic range of the ICP-MS is known to extend from 0.01 ng/mL to 0.1 µg/mL for these elements. Therefore, a dilution factor of at least 1:10 is required to ensure that the analysis occurs in the linear dynamic range of the instrument. *J* would thus equal 0.05 µg/mL and 0.015 µg/mL for Pb and As, respectively, when the dilution factor is added.

Appropriate Reference Materials: Where *Appropriate Reference Materials* are specified in the chapter, certified reference materials (CRM) from a national metrology institute (NMI), or reference materials that are traceable to the CRM of a NMI should be used. An example of a NMI in the United States is the National Institute of Standards and Technology.

[1] This dietary supplement chapter is still under revision and will appear online in *PF* 38(3) [May–June 2012].

COMPENDIAL PROCEDURES 1 AND 2

Procedure and Detection Technique

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP–AES or ICP–OES). *Procedure 2* can be used for elemental impurities generally amenable to detection by ICP–MS. Before initial use, the analyst should verify that the procedure is appropriate for the instrument and sample used (procedural verification) by meeting the *Alternative Procedure Validation* requirements below.

Sample Preparation

Forms of sample preparation include *Neat*, *Direct Aqueous Solution*, *Direct Organic Solution*, and *Indirect Solution*. The selection of the appropriate sample preparation depends on the material under test and is the responsibility of the analyst. When a sample preparation is not indicated in the monograph, an analyst may use any of the following appropriately verified preparation procedures. In cases where spiking of a material under test is necessary to provide an acceptable signal intensity, the blank should be spiked with the same *Target Elements*, and where possible, using the same spiking solution. Standard solutions may contain multiple *Target Elements*. [NOTE—All liquid samples should be weighed.]

Neat: Used for liquids or alternative procedures that allows the examination of unsolvated samples.

Direct Aqueous Solution: Used when the sample is soluble in an aqueous solvent.

Direct Organic Solution: Used where the sample is soluble in an organic solvent.

Indirect Solution: Used when a material is not directly soluble in aqueous or organic solvents. Digest the sample using a closed-vessel digestion procedure, similar to the procedure provided below. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the limit specified in the corresponding monograph or chapter.

Closed Vessel Digestion: This sample-preparation procedure is designed for samples that must be digested in a *Concentrated Acid* using a closed-vessel digestion apparatus. Closed-vessel digestion minimizes the loss of volatile impurities. The choice of a *Concentrated Acid* depends on the sample matrix. The use of any of the *Concentrated Acids* may be appropriate, but each introduces inherent safety risks. Therefore, appropriate safety precautions should be used at all times. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the digestion apparatus used.]

An example procedure that has been shown to have broad applicability is the following. Dehydrate and predigest 0.5 g of primary sample in 5 mL of freshly prepared *Concentrated Acid*. Allow to sit loosely covered for 30 minutes in a fume hood. Add an additional 10 mL of *Concentrated Acid*, and digest, using a closed vessel technique, until digestion or extraction is complete. Repeat if necessary by adding an additional 5 mL of *Concentrated Acid*. [NOTE—Where closed vessel digestion is necessary, follow the manufacturer's recommended procedures to ensure safe use.]

Reagents: All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* ⟨730⟩.

Procedure 1: ICP-AES

Standardization solution 1: 2*J* of the *Target Element(s)* in a *Matched Matrix*

Standardization solution 2: 0.5*J* of the *Target Element(s)* in a *Matched Matrix*

Sample stock solution: Proceed as directed in *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample solution: Dilute the *Sample Stock Solution* with an appropriate solvent to obtain a final concentration of the *Target Elements* at NMT 2*J*.

Blank: *Matched Matrix*

Elemental spectrometric system
(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: ICP

Detector: Optical detection system

Rinse: Diluent used

Standardization: *Standardization solution 1*, *Standardization solution 2*, and *Blank*

System suitability

Sample: *Standardization solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization solution 1* before and after the analyis of the *Sample solutions*.

Suitability criteria: NMT 20% for each *Target Element*. [NOTE—If samples are high in mineral content, rinse system well (60 seconds) before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences (e.g., Wavelength overlaps).]

Procedure 2: ICP-MS

Standardization solution 1: 2*J* of the *Target Element(s)* in a *Matched Matrix*

Standardization solution 2: 0.5*J* of the *Target Element(s)* in a *Matched Matrix*

Sample stock solution: Proceed as directed for *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample solution: Dilute the *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target Elements* at NMT 2*J*.

Blank: *Matched Matrix*

Elemental spectrometric system
(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: ICP. [NOTE—An instrument with a cooled spray chamber is recommended. (A collision cell or reaction cell may also be beneficial.)]

Detector: Mass spectrometer

Rinse: Diluent used

Standardization: *Standardization solution 1*, *Standardization solution 2*, and *Blank*

System suitability

Sample: *Standardization solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization solution 1* before and after the analysis of the *Sample solutions*.

Suitability criteria: *Drift* NMT 20% for each *Target Element*. [NOTE—If samples are high in mineral content, rinse system well (60 seconds) before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and *m*/*z*. Calculate and report results based on the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences (e.g., argon chloride interference with arsenic determinations.]

ALTERNATE PROCEDURE VALIDATION

If a specified compendial procedure does not meet the needs of a specific application, an alternative procedure may be used (see *General Notices* 6.30). Alternative procedures must be validated and must be acceptable and therefore equivalent to the compendial procedures for the purposes of the test. The principles of validation are provided in general chapter *Validation of Compendial Procedures* ⟨1225⟩. The level of validation necessary to ensure that an alternative procedure is acceptable depends on whether a limit test or a quantitative determination is necessary. The requirements for validation of an elemental impurities procedure for either type of determination are described below. Where this information differs from that presented in *Validation of Compendial Procedures* ⟨1225⟩, the parameters and acceptance criteria presented in this chapter take precedence. Any alternative procedure that has been validated and meets the acceptance criteria that follow is considered to be equivalent to the compendial procedures for the purposes of this test.

LIMIT PROCEDURES

The following section defines the validation parameters for the acceptability of alternative limit procedures. Meeting these requirements must be demonstrated experimentally using an appropriate system suitability procedure and reference material. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure as a limit procedure for the *Target Element*.

The suitability of the method must be determined by conducting studies with material or mixture under test supplemented with known concentrations of each *Target Element* of interest at the appropriate acceptance limit concentration. The material or mixture under test must be spiked before any sample preparation steps are performed.

Detectability

Standard solution: A preparation of reference materials for the *Target Element(s)* at the *Target Concentrations*.

Spiked sample solution 1: Prepare a solution of sample under test, spiked with appropriate reference materials for the *Target Elements* at the *Target Concentration*, solubilized or digested as described in *Sample Preparation*.

Spiked sample solution 2: Prepare a solution of the sample under test, spiked with appropriate reference materials at 80% of the *Target Concentration* for the *Target Elements*, solubilized or digested as described in *Sample Preparation*.

Unspiked sample solution: A sample of material under test, solubilized or digested in the same manner as the *Sample solutions*.

Acceptance criteria

Non-instrumental procedures: *Spiked sample solution 1* provides a signal or intensity equivalent to or greater than that of the *Standard Solution*. *Spiked sample solution 2* must provide a signal or intensity less than that of the *Spiked sample solution 1*. [NOTE—The signal from each *Spiked sample solution* is NLT the *Unspiked sample solution* determination.]

Instrumental procedures: The average value of the three replicate measurements of *Spiked sample solution 1* is

within (±15%) of the average value obtained for the replicate measurements of the *Standard solution*. The average value of the replicate measurements of *Spiked sample solution 2* must provide a signal intensity or value less than that of the *Standard solution*. [NOTE—Correct the values obtained for each of the spiked solutions using the *Unspiked sample solution*.]

Precision for Instrumental Methods (Repeatability)

[NOTE—Non-instrumental precision is demonstrated by meeting the *Detectability* requirement above.]

Sample solutions: Six independent samples of the material under test, spiked with appropriate reference materials for the *Target Elements* at the *Target Concentration*.

Acceptance criteria

Relative standard deviation: NMT 20% for each *Target Element*.

Specificity

The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of alternative quantitative procedures. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference materials. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure for the purpose of quantifying the *Target Elements*.

Accuracy

Standard solutions: Prepare solutions containing the *Target Elements* at concentrations ranging from 50% to 150% of *J*, using appropriate reference materials.

Test samples: Prepare samples of the material under test spiked with appropriate reference materials before any sample preparation steps (digestion or solubilization) at concentrations ranging from 50% to 150% of *J* for each *Target Element*.

Acceptance criteria

Spike recovery: 70%–150% for the mean of three replicate preparations at each concentration

Precision

REPEATABILITY

Test samples: Six independent samples of material under test (taken from the same lot) spiked with appropriate reference materials for the *Target Element(s)* at the indicated level.

Acceptance criteria

Relative standard deviation: NMT 20% for each *Target Element*

RUGGEDNESS

Perform the *Repeatability* analysis over three independent events using the following events or combinations thereof:

1. on different days, or
2. with different instrumentation, or
3. with different analysts.

Acceptance criteria

Relative standard deviation: NMT 25% for each *Target Element*

Specificity

The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

Limit of Quantitation, Range, and Linearity

Demonstrated by meeting the *Accuracy* requirement. ■2S *(USP35)*

Physical Tests and Determinations

⟨616⟩ BULK DENSITY AND TAPPED DENSITY OF POWDERS

Change to read:

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 kilograms 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 specify 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 ■sieve■2S *(USP35)*, into a graduated cylinder (*Method I*), or by