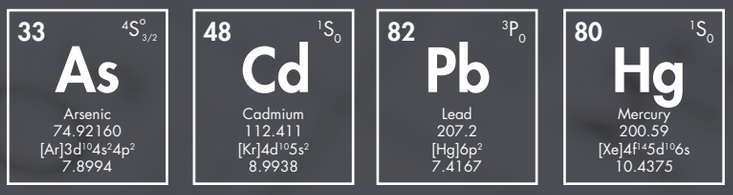

FEELING OUT OF YOUR ELEMENT?

USP <233>

ELEMENTAL IMPURITIES

TOOL KIT

Irvine Pharmaceutical Services

Irvine Pharmaceutical Services

A Pharmaceutical Development Company.

ELEMENTAL IMPURITIES TOOL KIT
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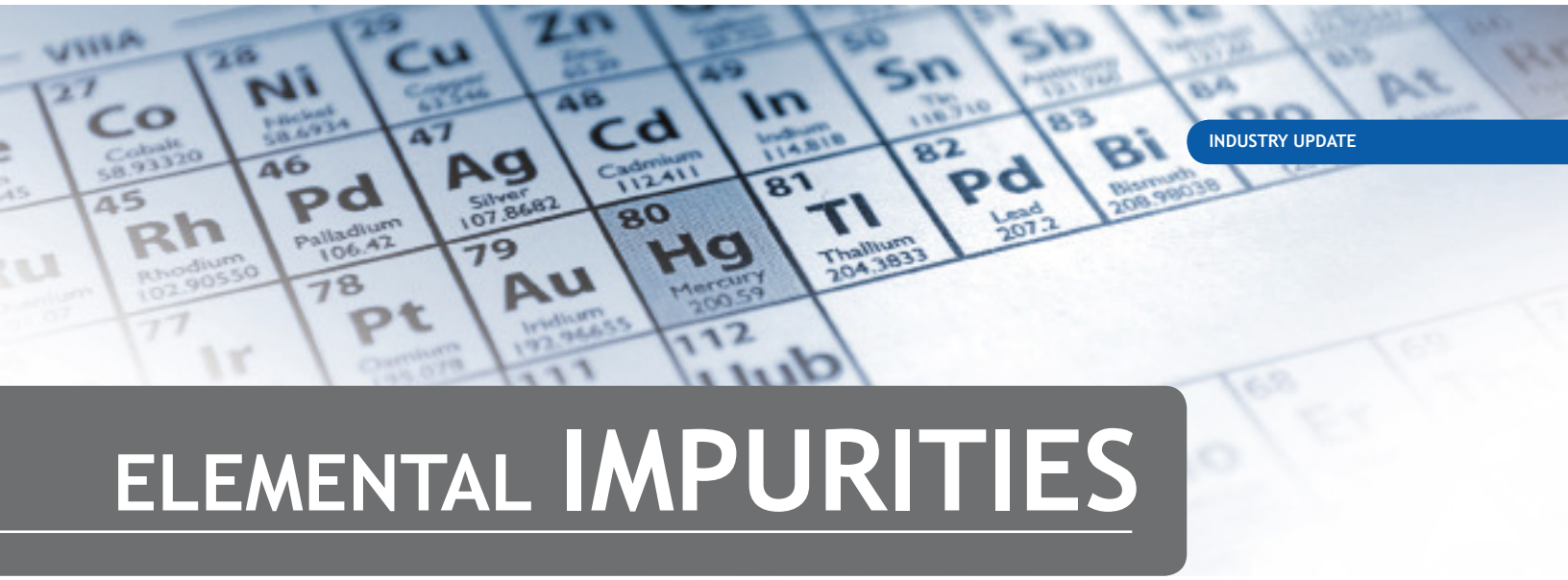
Irvine/USP Educational Literature

Irvine's Participation in the Industry



INDUSTRY UPDATE

ELEMENTAL IMPURITIES

USP HAS ANNOUNCED THE NEW REQUIRED METHODOLOGY TO REPLACE THE GENERAL CHAPTER FOR HEAVY METALS <231>

In an effort to improve and modernize the USP General Chapter for Heavy Metals <231>, USP has proposed the addition of two new General Chapters and one Supplemental General Chapter.

- <233> Elemental Impurities – Procedure
- <232> Elemental Impurities – Limits
- <2232> Elemental Contaminants in Dietary Supplements

The updated methodologies utilize modern technologies to provide better precision and yield higher recoveries. To comply with these changes, drug products will be required to fall within the proposed limits. General Chapters <232> and <233> will be made official. The implementation date is proposed for 2015.

WHO WILL BE REQUIRED TO COMPLY?

- Pharmaceutical
- Biopharmaceutical
- Excipient
- Medical Device
- Nutraceutical

HOW CAN IRVINE HELP YOU PREPARE?

Irvine has over 25 years of analytical experience with <231> Heavy Metals. Additionally, Irvine has over ten years experience working towards developing new methodologies. Irvine has actively participated in the industry for years on this subject matter via technical posters, papers, presentations, and through helping clients.

UTILIZING MODERN, PRECISE INSTRUMENTATION

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Irvine Pharmaceutical Services

Update on the Elemental Impurities General Chapters
<232> *Elemental Impurities—Limits* and <233> *Elemental Impurities—Procedures*

October 25, 2013

Background

On September 23, 2013, the Elemental Impurities Expert Panel met to review the Step 2 limits of the ICH Q3D Elemental Impurities Working Group, which were released in June 2013. At its meeting the Expert Panel recommended revisions to General Chapter <232> *Elemental Impurities—Limits* to partially align with the ICH Q3D limits. In addition, the Expert Panel recommended other minor editorial changes to both General Chapter <232> and General Chapter <233> *Elemental Impurities—Procedures*. On October 16-17, 2013 the General Chapters—Chemical Analysis Expert Committee met and endorsed the recommendations of the Expert Panel.

These revisions will be proposed according to the timeline below.

Revision Timeline

- **January 1, 2014:** USP pre-publishes on the USP Elemental Impurities Key Issues web page the proposed revisions to <232> and <233>. Subject to further discussion with the Council of Experts Executive Committee (which is responsible for General Notices), with input from the Elemental Impurities Implementation Advisory Group, USP also anticipates posting at this time an announcement regarding the date of implementation of <232> and <233> as specified in General Notices section 5.60.30.
- **March 1, 2014:** USP publishes the proposed revisions to <232> and <233> in *PF* 40(2) [March-April 2014] for public comment. Comments will be accepted only on the proposed revisions to the general chapters.
- **May 31, 2014:** The 90-day comment period ends (comment period is from March 1, 2014 through May 31, 2014).
- **June 2014:** The Expert Panel considers comments received on the proposed revisions and also reviews ICH Q3D Step 4 limits, which are expected in June 2014.
- **July-August 2014:** Expert Panel meets to make recommendations regarding any final revisions to the general chapters.
- **September 2014:** General Chapters—Chemical Analysis Expert Committee meets to review Elemental Impurities Expert Panel recommendations and consider final revisions to the general chapters.
- **October 2014:** The General Chapters—Chemical Analysis Expert Committee ballots on the revised General Chapters <232> and <233>, and the removal of General Chapter <231>. Note that the removal of <231> was previously proposed in *PF* 39(1). The removal of <231> will coincide with the implementation of General Chapters <232> and <233>.
- **October 2014:** USP pre-posts the approved revised General Chapters <232> and <233>, the Commentary, and a notice about the removal of General Chapter <231> on the USP Elemental Impurities Key Issues web page.
- **February 2015:** The approved revised General Chapters are published in the *USP 38-NF 33 First Supplement*, which publishes in February 2015 and becomes official August 1, 2015.

Note: USP anticipates that the implementation date for the General Chapters as elaborated in section 5.60.30 of General Notices is expected to be on or after the official date of the revised General Chapters.

Frequently Asked Questions Regarding the Implementation of USP General Chapters <232> Elemental Impurities—Limits and <233> Elemental Impurities—Procedures

Version 2: June 7, 2013

1. Are General Chapters <232> Elemental Impurities—Limits and <233> Elemental Impurities—Methods currently official? Are the new general chapters applicable to any monographs?

   Both General Chapters <232> and <233> became official February 1, 2013, but are not applicable to any monographs at this time.

2. How does USP plan to apply General Chapters <232> and <233> to monographs? Do they apply to all monographs or just to drug products?

   USP plans to apply General Chapters <232> and <233> to monographs via a proposed provision (5.60.30) in General Notices. General Chapters <232> and <233> will apply only to drug product monographs currently in the *USP-NF*.

3. USP has deferred the proposed General Notices provision 5.60.30 Elemental Impurities. What is a "deferral" and what are the possible outcomes?

   USP's standards-setting process requires that a standard be balloted by the responsible Expert Committee before it is published and becomes official. Under certain circumstances, e.g., when public comments indicate the need for a standard or part of a standard to be reconsidered, the Expert Committee may choose to defer the balloting of the text in question to allow time for additional review. Following a deferral, the standard may be balloted at a future date in its original or slightly modified form. Typically if the Expert Committee decides to significantly modify the original proposal, it would be reproposed for public comment, or canceled altogether.

4. Why has USP deferred the proposed General Notices provision 5.60.30 Elemental Impurities?

   USP believes that manufacturers need a single set of tests with limits (acceptance criteria) for drug products affected by the improved testing of elemental impurities described in <232> and <233>. USP expects the ICH Q3D EWG to conclude a Step 2 document soon, which will allow USP to align the elements and limits (acceptance criteria) in the ICH document with those in General Chapters <232> and <233>. In addition, USP believes a reasonable timeframe for implementation needs consideration based on comments received on the General Notices provision 5.60.30 as it appeared in PF 39(1).

5. How long will the General Notices provision be deferred? How will USP assess the implementation issues?

   USP has not yet established a new implementation date for General Notices 5.60.30. USP believes time is needed to adjust General Chapters <232> and <233> and for drug product manufacturers to implement the new requirements. The USP Council of Experts will consider the recommendations from an Advisory Group that will consist of scientific experts from industry and the Food and Drug Administration (FDA) who will assess the implementation issues. Based on these recommendations, the Council of Experts,

balancing manufacturer needs with public health impact, will decide the implementation timing.

6. How will the Advisory Group be formed? Who will be its members?

   In accordance with the Bylaws of the USP Convention, "The EVP-CEO may appoint advisory bodies to advance the work of the Council of Experts and the Convention and provide advice to staff on policy matters." In this case, Dr. Roger Williams has appointed an Advisory Group on the Implementation of General Chapters <232> and <233>. The members comprise:
   - Representatives of key trade organizations that submitted comments to USP on the implementation of the General Chapters
   - Three representatives of FDA
   - The rapporteurs of the ICH Q3D Expert Working Group
   - The chair of USP's Toxicology Expert Committee

   See the announcement about the Advisory Group on the Elemental Impurities Key Issues Page.

7. How will USP coordinate with the ICH Expert Working Group (EWG) Q3D on Elemental Impurities? How will the General Chapters be updated to align with ICH limits?

   USP is participating in the ICH Q3D process as an observer and looks forward to a Step 2 document in June.

8. Does USP plan to make revisions to General Chapters <232> and <233>?

   Adjustments are expected through the decision-making process of the Council of Experts. The adjustments will be proposed by the Elemental Impurities Expert Panel to the Chemical Analysis Expert Committee. USP will post further information about future revisions to <232> and <233> in the Key Issues: Elemental Impurities area of its website.

9. Will General Chapter <231> be omitted once General Chapters <232> and <233> become applicable? If so, when? Will there be a phased approach?

   USP General Chapter <231> will be omitted once General Chapters <232> and <233> become applicable, with consideration of a reasonable period of time for manufacturers to meet the new requirements.

10. Will General Chapter <231> still be applicable in monographs where cited? Will it still be applicable in monographs for veterinary articles where cited?"

    Chapter <231> still is applicable where cited in monographs. Although General Chapter <232> indicates that veterinary products are exempt from the new general chapters' requirements, many monographs for veterinary articles still require conformance to General Chapter <231>. The responsible Expert Committee for these monographs is working with interested stakeholders, including FDA, to consider future options.

11. Can manufacturers work with USP on a specific product that may not meet the limits of a particular element?

    USP hopes to work with FDA and with individual manufacturers on resolving scientific issues arising from the new Elemental Impurities requirements, again balancing manufacturer interests with public health impact.

Revision History:

Version 2: June 7, 2013

- Added FAQs
  - 'What is a "deferral" and what are the possible outcomes?'
  - 'How will the Advisory Group be formed? Who will be its members?"
- Revised FAQ
  - Will General Chapter <231> still be applicable to veterinary monographs?
- Omitted FAQ
  - "What does USP plan to do with other general chapters that address elemental impurities such as <251> Lead, <211>Arsenic and <261> Mercury, etc.?"

Version 1: May 29, 2013

- Initial version posted

Frequently Asked Questions: USP's Proposed Standards for Elemental Impurities

Q. Why is USP revising its standards for elemental impurities?

A. USP is revising its standards for elemental impurities in the interest of better protecting public health. The revisions focus on two areas of work:

- Updating the methodology used to test for elemental impurities in drugs and dietary supplements to include procedures that rely on modern analytical technology; and
- Establishing limits for acceptable levels of elemental impurities (including, but not limited to, lead, mercury, arsenic, and cadmium) in drugs and dietary supplements.

Q. Why is any level of elemental impurities considered "acceptable?" Shouldn't the level always be zero?

A.The human body requires trace elements of many substances to function properly. For example, iron is an element that would be harmful or toxic beyond certain levels, but is frequently taken as a dietary supplement to help ensure healthy blood. The human body is also well suited to eliminate a small amount of most toxins. For most toxic elemental impurities, toxicologists have indicated that daily ingestion of low part-per-million levels constitutes a very low risk even in chronic applications.

Additionally, the definition of "zero" or "absence" is very easy in a general sense (i.e. there are zero apples in a basket) but much more difficult from a measurement perspective. Requiring that "zero" molecules of an impurity may be present bases the standard on the technical ability to make the measurement rather than making it health based. Basing the standard on the best available detection technology may be prohibitively difficult for users to implement and not best serve public health.

Q. What is wrong or deficient about the current test methodology?

A. The test methodology currently described in the USP–NF, was first introduced more than 100 years ago. The test can be difficult to conduct, and can fail to detect some important elementals such as mercury at toxicologically-relevant levels.

Q. Why has USP waited until now to revise standards for elemental impurities? Was there a specific event that prompted the revision?

A. USP undergoes regular re–evaluation and revision of all its standards to update their scientific and public health relevance. There was no specific event that triggered the revision of elemental impurities standards, but our scientific experts felt that the elemental impurity standards should be updated to incorporate modern methods and health information. As we have gained a better understanding of the limitations of the current methods, it has become clear that a revision is called for.

Q. How is USP approaching the revision?

A. USP is taking a risk–based approach that focuses on the likelihood of a given impurity being found in a drug or dietary supplement and on a consensus-based evaluation of the health implications of the impurity at levels that may be found. We have included toxicologists as well as chemists in the group of experts revising the standards to obtain the best available input on both health and methodology issues.

Q. What is the timing of the new standard?

A. USP's standards–revision process involves international collaboration among USP experts, industry, regulators, and the general public. When there is no specific medical emergency, as is the case with elemental impurities, it is beneficial to allow careful deliberation and scientific dialog to reach conclusions that are supported by a maximum number of interested stakeholders. In addition, where the new standards represent a significant change from existing standards, as is also the case with elemental impurities, it is important to provide sufficient time for manufacturers to incorporate the changes in their processes necessary to implement the new standards. The new elemental impurities standards, which are intended to replace the existing methods in General Chapter <231> Heavy Metals, are expected to be finalized sometime in 2010 and become official at a later date which has not yet been determined.

Q. Some in the pharmaceutical industry believe that USP is creating unrealistic, unworkable requirements for testing, which could lead to non–compliance and shortages of key medicines. For example, the article published in USP's Pharmacopeial Forum (PF) (2008, 34(5), page 1345) includes a list of 31 substances to be tested. And the proposed limits for each individual element may be unworkable across the many quality assurance labs that would be affected.

A. USP does not intend to burden industry with unwieldy and unnecessary testing requirements. The list in the PF article was intended as a

proposal for discussion. As the revision moved forward, that list has been shortened. USP will not mandate the methodology that each lab must use. Manufacturers will have the flexibility to choose a test that best fits their processes.

Q. The proposed leeway for manufacturers to choose their own test methods is attractive because of the added flexibility. But doesn't that expose manufacturers to added risk of FDA rejection?

A. Potentially, but USP is going to great lengths to work with both FDA and industry to ensure widespread agreement on interpretation of the revised standard. And the revision will include two referee methods, which manufacturers can choose from if they want to ensure a means of demonstrating unquestioned compliance to the standard.

Q. Have imports posed an increased problem with elemental impurities? How is USP dealing with this?

A. To date, there have been no known incidents involving elemental impurities in pharmaceuticals. However, there are continuing concerns above the quality of imports. Ultimately, manufacturers are responsible for assuring conformance to FDA requirements and USP standards, no matter what the source. As more ingredients are sourced abroad, the presence of modern, scientifically sound quality standards will help protect both manufacturers and patients in the United States.

Add the following:

•⟨232⟩ ELEMENTAL IMPURITIES—LIMITS

•Official February 1, 2013• (RB 1-Feb-2013)

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⟩.

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*.

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*.

$$\textit{Daily Dose PDE} \geq \text{measured value } (\mu g/g) \times \text{maximum daily dose (g/day)}$$

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:

$$\textit{Daily Dose PDE} \geq [\Sigma^{M}{}_{1}(C_M \times W_M)] \times D_D$$

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 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	—[c]	—[c]	25	—[c]
Molybdenum	100	10	●10● (ERR 1-Oct-2012)	1.0
Nickel	500	50	1.5	5.0
Vanadium	100	10	30	1.0
Copper	1000	100	●100● (ERR 1-Feb-2013)	●10● (ERR 1-Feb-2013)

[a] PDE = Permissible daily exposure based on a 50-kg person.
[b] See *Speciation* section.
[c] Not a safety concern.

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	●10● (ERR 1-Oct-2012)	●1.0● (ERR 1-Oct-2012)	●0.15● (ERR 1-Oct-2012)
Chromium	—[a]	—[a]	2.5
Molybdenum	10	1.0	●1.0● (ERR 1-Oct-2012)
Nickel	50	5.0	0.15
Vanadium	●10● (ERR 1-Oct-2012)	●1.0● (ERR 1-Oct-2012)	●3.0● (ERR 1-Oct-2012)
Copper	100	10	●10● (ERR 1-Feb-2013)

[a] 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, •Pb,• (ERR 1-Oct-2012) and Hg in the *Target Element* evaluation.• (RB 1-Feb-2013)

Add the following:

•⟨233⟩ ELEMENTAL IMPURITIES—PROCEDURES

•Official February 1, 2013• (RB 1-Feb-2013)

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, •Pb,• (ERR 1-Oct-2012) 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⟩, *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 ⟨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 an NMI should be used. An example of an NMI in the United States is the National Institute of Standards and Technology.

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 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 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 analysis 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 Ele-*

ment 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%–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%–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.

● (RB 1-Feb-2013)

Metal Analysis by Inductively Coupled Plasma/Microwave Digestion versus Traditional Heavy Metals Limit USP <231>

Jose Perez, Jaleh Pourmansour, Hamid R. Forouhar and Joseph Bordas-Nagy
Irvine Pharmaceutical Services, Inc. | 10 Vanderbilt, Irvine, CA 92618

INTRODUCTION

The original USP method, chapter <231>, for a limit test of heavy metals is based on the sulfide precipitation of copper arsenic metals. The test demonstrates that the content of metallic impurities that are colored by sulfide ion does not exceed the specified limit. The following metals respond to this test: lead (Pb), mercury (Hg), bismuth (Bi), arsenic (As), antimony (Sb), tin (Sn), cadmium (Cd), silver (Ag), copper (Co), and molybdenum (Mo). There are significant problems associated with the reliability of this method. The most problematic is the procedure (Method II) for the analysis of samples that does not produce a clear solution. Method II involves carbonization using sulfuric acid followed by ashing in a furnace at 500-600 ºC. The remainder is taken up in a solution and treated with a sulfide reagent. The color produced is compared to the color of a standard solution to demonstrate that the heavy metals in the sample is under a specified limit. This method is not specific. It also has been known to be highly unreliable. There can be loss of analyte during the sample preparation. The color density is not stable. Comparison of the color is subjective.

New USP chapters <1232>, <232> and <233> under development will provide information on the safety profile of a range of elemental impurities; safety limits and approaches to establish their threshold concentrations in materials; and methodologies to determine concentrations of elemental impurities

Irvine Pharmaceutical Services participated in a collaboration between the Expert Committee for Heavy Metals at USP and independent laboratories to evaluate the original test for heavy metals versus new approaches to establishing limits of elemental impurities in materials used in the manufacturing of pharmaceutical products. The study described here was aimed at providing a systematic comparison of the performance of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and the USP Chapter <231> Method II limit test for the quantitation of elemental impurities corresponding to nine of the ten elements corresponding to the copper arsenic metals group.

METHODOLOGY

Sample Preparation

Microwave Digestion:
Approximately one gram of sample is accurately weighed into a microwave digestion vessel. 10 mL of aqua regia is added and the sample is digested in a microwave oven. The microwave settings is shown in Table 1.

Table 1. Microwave Oven Settings

Cycle	%Power	Pressure (bar)	Time (minute)
1	30	100	10
2	50	100	12

The sample is cooled and transferred into a 25 mL volumetric flask and diluted to volume with 1% nitric acid.

USP <231> Method II:
Approximately 2 gram of sample is accurately weighed. Sufficient amount of sulfuric acid was added to wet the sample and ignited on a hot plate until thoroughly charred. To the carbonized mass 2 mL of nitric acids and 5 drops of a sulfuric acid was added and heated until white fumes no longer evolved. Heated in furnace at 550 ºC until the carbon was completely burned off. Cooled and added 4 mL of 6N HCl. Covered and digested for 15 minutes on a steam bath followed by evaporation dryness after uncovering sample. The residue was moistened with 1 drop of HCl and added 10 mL water and digested for 10 minutes. Diluted to 50 mL with 1% nitric acid.

Spiked samples were prepared in a similar fashion as above, except that appropriate solutions containing the elements studied were added before microwave digestion.

Standard preparation:
Standards were prepared in 1% nitric acid containing the nine metals studied in the 0.5 to 20.0 ppm concentration range. Six point calibration curves were obtained.

ICP-OES Method
Samples and standards were analyzed using ICP-OES instrumentation. Table 2 shows the elements studied and the selected wavelengths for each.

Table 2. ICP-OES Wavelengths for Elements Studied

Element	Ag	Cd	Cu	Mo	Pb	Sb	Sn	Bi	As
Wavelength (nm)	328.068	228.802	327.393	202.031	220.353	206.836	189.927	223.061	188.979

RESULTS

Samples of caffeine and microcrystalline cellulose was spiked at concentrations of 2, 5 and 10 ppm with the following elements: Ag, Cd, Cu, Mo, Pb, Sb, Sn, Bi and As. The resulting samples were prepared for ICP-OES analysis by following two different procedures as described in the Methodology section of this poster. One used concentrated oxidizing acid solution (aqua regia) and microwave digestion the other followed the sample preparation procedure for USP Chapter <231> Method II. Overall, two sets of spiked samples were generated both with caffeine and microcrystalline cellulose for ICP-OES analysis. All samples were prepared and analyzed in triplicate. The values reported are the average of three determinations. The objective of this study design was to evaluate the recoveries achieved for individual elements after undergoing these sample preparation procedures.

Tables 3 and **4** show recoveries of the elements studied for spiked caffeine samples after undergoing microwave digestion and Method II sample preparation procedures, respectively. The microwave digestion process, see **Table 3**, resulted in better than 80% recoveries for all the elements and at all the spiking levels. The slope and correlation coefficient were determined for spiking level versus measured value assuming a zero intercept for the correlation. These also indicate a close to complete recovery for all the elements when using this approach over the concentration range studied. On the other hand, the results corresponding to the sample treatment process associated with Method II, see **Table 4**, reflect a very poor performance regarding the recovery of the majority of the elements studied. For Ag, Sn, Bi no recovery was achieved, and for Sb the recovery is also close to zero. For As and Mo the recoveries were consistently in the 30% range. For Pb there was actually a negative slope and correlation obtained between spiking level and measured value, that is the higher the spiking the lower the percent recoveries were. Only for Cd and Cu were any reasonable recoveries obtained.

Table 3. Results for Spiked Recoveries for Caffeine Samples Prepared Using Microwave Digestion.

Element	Spiking level (ppm)	Measured (ppm)	%Recovery	Slope	Correlation coefficient
Ag	2	1.55	77	0.979	0.9957
	5	4.30	86		
	10	9.71	97		
Cd	2	1.79	90	0.925	1.000
	5	4.58	92		
	10	9.24	92		
Cu	2	1.73	86	0.915	0.9999
	5	4.50	90		
	10	9.12	91		
Mo	2	1.75	87	0.915	0.9999
	5	4.54	91		
	10	9.12	91		
Pb	2	1.72	86	0.919	0.9999
	5	4.53	91		
	10	9.15	91		
Sb	2	1.72	86	0.917	0.9999
	5	4.55	91		
	10	9.13	91		
Sn	2	1.93	96	0.903	0.9993
	5	4.74	95		
	10	9.05	90		
Bi	2	1.78	89	0.906	1.000
	5	4.55	91		
	10	9.05	90		
As	2	1.84	92	0.913	0.9996
	5	4.72	94		
	10	9.12	91		

Table 4. Results for Spiked Recoveries for Caffeine Samples Prepared According to USP <231> Method II.

Element	Spiking level (ppm)	Measured (ppm)	%Recovery	Slope	Correlation coefficient
Ag	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
Cd	2	1.31	66	0.695	0.9997
	5	3.27	65		
	10	6.85	69		
Cu	2	1.50	75	0.829	0.9994
	5	3.78	76		
	10	8.10	81		
Mo	2	0.95	47	0.230	0.9940
	5	1.83	37		
	10	2.82	28		
Pb	2	1.15	57	-0.081	-0.7645
	5	1.42	28		
	10	0.58	6		
Sb	2	0.08	4	0.036	0.9924
	5	0.22	4		
	10	0.37	4		
Sn	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
Bi	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
As	2	0.521	26	0.306	0.9925
	5	1.72	34		
	10	3.01	30		

Tables 5 and **6** show recoveries of the elements studied for spiked microcrystalline cellulose samples after undergoing microwave digestion and Method II sample preparation procedures, respectively. The results observed for this material are closely similar to those obtained for caffeine samples. The microwave digestion process, see **Table 5**, resulted in better than 80% recoveries for all the elements and at all the spiking levels. These also indicate a close to complete recovery for all the elements when using this approach over the concentration range studied.

On the other hand, the results corresponding to the sample treatment process associated with Method II, see **Table 6**, reflect a very poor performance regarding the recovery of the majority of the elements studied. For Ag, Sn, Bi no recovery was achieved, and for Sb the recovery is also close to zero. For As and Mo the recoveries were consistently in the 30% range. For Pb there was actually a negative slope and correlation obtained between spiking level and measured value, that is the higher the spiking the lower the percent recoveries were. Only for Cd and Cu were any reasonable recoveries obtained.

Table 5. Results for Spiked Recoveries for Microcrystalline Cellulose Samples Prepared Using Microwave Digestion.

Element	Spiking level (ppm)	Measured (ppm)	%Recovery	Slope	Correlation coefficient
Ag	2	2.58	129	1.430	0.9981
	5	6.57	131		
	10	14.27	143		
Cd	2	1.68	84	0.884	0.9998
	5	4.32	86		
	10	8.81	88		
Cu	2	1.71	86	0.871	0.9999
	5	4.27	85		
	10	8.71	87		
Mo	2	1.74	87	0.879	0.9999
	5	4.33	87		
	10	8.79	88		
Pb	2	1.73	86	0.880	0.9999
	5	4.27	85		
	10	8.80	88		
Sb	2	1.71	86	0.880	0.9998
	5	4.30	86		
	10	8.81	88		
Sn	2	1.93	96	0.880	0.9998
	5	4.60	92		
	10	9.15	91		
Bi	2	1.77	89	0.880	0.9998
	5	4.34	87		
	10	8.79	88		
As	2	1.78	89	0.880	0.9998
	5	4.50	90		
	10	9.00	90		

Table 6. Results for Spiked Recoveries for Microcrystalline Cellulose Samples Prepared According to USP <231> Method II.

Element	Spiking level (ppm)	Measured (ppm)	%Recovery	Slope	Correlation coefficient
Ag	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
Cd	2	1.30	65	0.678	0.9999
	5	3.29	66		
	10	6.72	67		
Cu	2	1.49	74	0.812	0.9998
	5	3.82	76		
	10	7.97	80		
Mo	2	0.93	47	0.219	0.9902
	5	1.82	36		
	10	2.72	27		
Pb	2	1.15	58	-0.084	-0.7755
	5	1.41	28		
	10	0.56	6		
Sb	2	0.06	3	0.036	0.9965
	5	0.19	4		
	10	0.35	3		
Sn	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
Bi	2	0.00	0	NA	NA
	5	0.00	0		
	10	0.00	0		
As	2	0.53	26	0.299	0.9931
	5	1.69	34		
	10	2.96	30		

CONCLUSION

Comparison of the microwave digestion sample preparation method normally applied in ICP based elemental analysis to the sample treatment associated with current USP <231> Method II demonstrated the superior performance of ICP technique based elemental analysis approach relative to the Heavy Metal testing methodology currently in effect. The new USP chapters <1232>, <232> and <233> under development, and expected to become effective in 2010, will provide a much needed improvement in the approaches used to assess elemental impurities in materials used in the manufacturing of pharmaceutical products.

AAPS 2009 Annual Meeting , November 8-13, 2009 Poster: T2177

USP Elemental Impurities Initiatives and Current Status

Assad J. Kazeminy, Ph.D.
Irvine Pharmaceutical Services, Inc.

Discussion Overview

- Introduction
- Background
- Plasma Spectrochemistry
- Advisory Panel Discussion
- Elemental Impurities – Limits <232>
- Elemental Impurities – Procedures <233>
- Harmonization
- Timeline

Introduction

Elemental Impurities May Arise from:

- Added Intentionally
  - Drug Substance Synthetic Process (catalysts)
- Introduced Unintentionally
  - During Manufacturing / Processing Equipment, Packaging Component or Container Closure
  - Through non GMP routes (paint chip in drum leaching from pipes, etc.)
- Naturally Occurring

Introduction

Control Metal Impurities

- Controls in material attributes
  - Raw materials
  - Starting materials
  - Reagents
  - Excipients
  - Packaging materials
- Controls in manufacturing
  - Sequence of purification steps
  - Order of additional excipients or drug components
- In-process Testing

Introduction

Metal Impurities – Controls Cont.

- Control of drug substances
- Control of the final drug product
- GMP quality system
  - Control utilities, equipment, air, water, etc.

Background

USP Chapter <231> Heavy Metals

- Introduced in USP VIII (1905)
- Consists of three procedures, all involving:
  - Sulfide precipitation of metals
  - Visual comparison to lead standards
- EP and JP methods are similar to USP methods

Background

- 1995 - Pharmacopieal Forum stimuli article identified issues with <231> method II (K. Blake)
- 2000 - Second stimuli article in 2000 proposed ICP-MS as an instrumental alternative (T. Wang)
- 2004 - Lewen, et al, J. Pharm and Biomed Analysis

Background

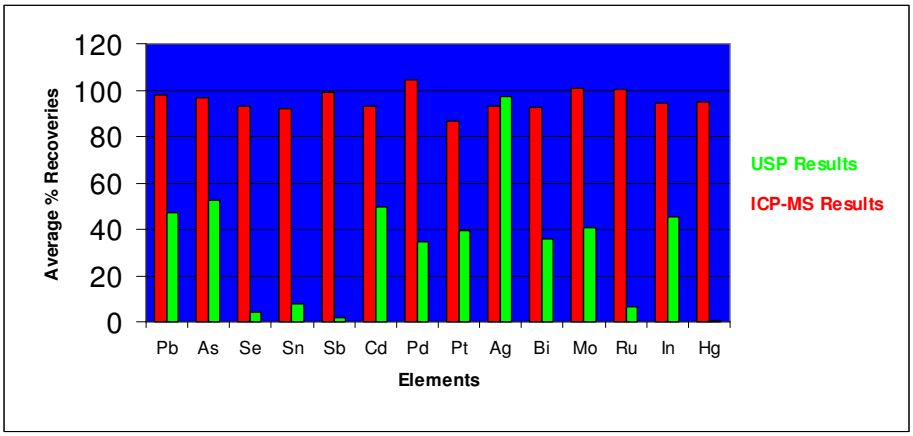
Comparisons Between Instrumentation Methods and <231>

*"Although still widely accepted and used in the pharmaceutical industry, these methods based on the intensity of the color of sulfide precipitation are **non-specific**, **insensitive**, **time-consuming**, **labor intensive**, and more often than hoped, **yield low recoveries or no recoveries** at all."*

(Wang, T. et al, J. Pharm. & Biomed. Anal., Vol. 23 (2000) 867-890)

Background

Comparisons Between Instrumental Methods and <231>
(Lewen, N. et al J. Pharm. & Biomed. Anal. 35 (2004) 739-752)



Plasma Spectrochemistry

An Alternative Method to Heavy Metals

ICP/ICP-MS

- Element Specific
- Quantitative
- Cost issues
- Dilute and shoot (70-80% of API)

Plasma Spectrochemistry

- USP published a new chapter on inductively-coupled plasma in Pharmacopeial Forum
  -Volume 28(6) {Nov. – Dec. 2002}
- USP/NF 2005 1st Supplement
  Official: April 1, 2005

Advisory Panel Discussions

Heavy Metals <231> Advisory Panel

- 2007-2008
  - Panelists recruited from industry and GC-EC
  - Objective to investigate instrument-based methods to replace Heavy Metals <231>

Advisory Panel Discussions

Detection Techniques

- Atomic absorption (AA)
  - Flame, graphite furnace, cold vapor
- Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)
- Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
- X-ray Fluorescence (XRF)
- Laser-Induced Breakdown Spectroscopy (LIBS)
- Ion chromatography (IC)
- Flame emission spectroscopy (FES)

Advisory Panel Discussions

Methods for Sample Preparation

- Microwave digestion
  - Open vessel
  - Closed vessel
- Hot plate
- Parr bomb
- Pre-digestion

Advisory Panel Discussion

Advisory Panel Recommendations

Heavy Metals <231> Advisory Panel

- 2007-2008
  - Resulted in Stimuli Article published in Pharmacopeial Forum 34 (5) [Sept-Oct., 2008] proposed new instrument-based chapter to replace <231>

Advisory Panel Discussions

Heavy Metals <231> Advisory Panel

- 2008 – present
  - Panelists recruited from agencies, industry, USP, and includes three professional toxicologists
  - Objective to develop USP standards to replace Heavy Metals <231>

Advisory Panel Discussions

Recommendations <232>:

The USP Metal Impurities Advisory Panel has made the following recommendations in development of the USP Metal Impurities standard:

- API and excipients will be tested for arsenic, cadmium, lead, mercury plus...
  - EMEA Metal Catalysts, including their scope as outlined in the EMEA Guideline (12 Catalysts; EMEA list with EMEA limits, less iron and zinc). Additional metals listed in table under consideration.
- Establish multiple options for limit calculation following the USP <467> Residual Solvents model.

Elemental Impurities – Limits <232>

Methods for Establishing Exposure Limits

- European Medicines Agency (EMEA) guidance, "Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents" (2008)
- 10g/day dose for drug products for calculation of ppm limits
- 50 kg person for extrapolation from animal data on body weight-basis
- 70-year lifetime
- 10% bioavailability for extrapolation from the oral permissible daily exposure (PDE) to the parenteral PDE

Elemental Impurities <233>

Advisory Panel Recommendations <233>

- Dilute-and-shoot (direct dilution) and closed-vessel microwave digestion provided the best results and options for sample preparation procedures.
  - Flow chart provided to assist analysts with sample preparation procedures.
  - Analysts are free to go directly to closed vessel microwave digestion, if desired.
- ICP-OES and ICP-MS are options for analytical determinations.

Elemental Impurities <232>

Advisory Panel Accomplishments to Date

- USP Stimuli Article in PF 34(5) for public comment (November, 2008)
- USP published draft revisions for comment in PF 36(1), January, 2010
  - Elements and limits chapter <232>
  - Methodology chapter <233>
  - Dietary supplement chapter <2232>
  - Stimuli Article with rationales for limits
  - Stimuli Article with responses to comments on PF 34(5) Stimuli Article
- Latest revisions appear in PF 37(3), May-June, 2011

Elemental Impurities <232>

- Applies to drug products
  - Drug Substances
  - Excipients
- Does not apply to dietary supplements
- Does not apply to Veterinary products
- Does not apply to conventional vaccines
- Speciation is not addressed in this Chapter
- Procedures are specified in *Elemental Impurities*
  - Procedures <232>

Elemental Impurities - Limits <232> for Drug Products

Element	Oral Daily Dose PDE[a] (µg/day)	Parental Daily Dose PDE[a] (µg/day)	Inhalational Daily Dose PDE[a] (µg/day)	LVP Component Limit[b] (µg/day)
Cadmium	25	2.5	1.5	0.25
Lead	5	5	5	0.5
Inorganic arsenic	1.5	1.5	1.5	0.15
Inorganic mercury	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	Not a safety concern	Not a safety concern	25	Not a safety concern
Molybdenum	100	10	250	1.0
Nickel	500	50	1.5	5.0
Vanadium	100	10	30	1.0
Copper	1000	100	100	10

[a] PDE = Permissible daily exposure based on a 50-kg person.

[B] When the daily dose of an injection is greater than 100mL (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. Each component used in an LVP must be less than the values listed.

Elemental Impurities - Limits <232> for Drug Substances

Element	Concentration limits (µg/day) for Oral Drug Product with a Maximum Daily Dose of ≤ 10g/day	Concentration Limits (µg/day) for Parenteral Drug product with a Maximum Daily Dose of ≤ 10g/day	Concentration Limits (µg/day) for Inhalational Drug Product with a Maximum Daily Dose Of ≤ 10g/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	1.5
Palladium	10	1.0	1.5
Platinum	10	1.0	1.5
Rhodium	10	1.0	1.5
Ruthenium	10	1.0	1.5
Chromium	Not a safety concern	Not a safety concern	25
Molybdenum	10	1.0	1.0
Vanadium	10	1.0	3.0
Copper	100	10	10

ICH Q3D Step 2 Permitted Daily Exposures for Elemental Impurities

Element	Oral PDE (µg/day)	Parental PDE (µg/day)	Inhalational PDE (µg/day)
As	15	15	1.9
Cd	5.0	6.0	13
Li	780	390	25
Ni	600	60	6.0
Sb	1200	600	22
Sn	180	180	7.6
Se	6400	640	64

ICH Q3D Step 2 Permitted Daily Exposures for Elemental Impurities

Element	Oral PDE (µg/day)	Parental PDE (µg/day)	Inhalational PDE (µg/day)
Cr	11000	1100	2.9
Cu	1300	130	3.4
Hg	40	4.0	1.2
Pb	5.0	5.0	5.0
Co	50	5.0	2.9
Mo	180	180	7.6
Se	170	85	140
V	120	12	1.2
Au	130	130	1.3
Ir	1000	10	1.4
Os	1000	10	1.4
Pd	100	10	1.0
Pt	1000	10	1.4
Rh	1000	10	1.4
Ru	1000	10	1.4
Tl	8.0	8.0	69
Ba	13000	1300	340

Elemental Impurities <232>

Permitted Daily Exposures for Elemental Impurities

Element	Concentration Limits (µg/g) for Oral Drug Products with a Max Daily Dose of ≤ 10g/day	Concentration Limits (µg/g) for Parenteral Drug Products with a Max Daily Dose of ≤ 10g/day	Concentration Limits (µg/g) for Inhalational Drug Products with a Max Daily Dose of ≤ 10g/day
Inorganic arsenic	15	15	1.9
Cadmium	5.0	6.0	3.4
Inorganic mercury	15	1.5	1.2
Lead	5.0	5.0	5.0
Iridium	100	10	1.5
Osmium	100	10	1.5
Palladium	100	10	1.0
Platinum	100	10	1.5
Rhodium	10	1.0	1.5
Ruthenium	100	10	1.5
Chromium	Not a safety concern	Not a safety concern	2.9
Molybdenum	180	10	7.6
Nickel	600	60	6.0
Vanadium	120	12	1.2
Copper	1300	1000	13

Elemental Impurities – Procedures <233>

Options to Determine Content

- Drug Product Analysis Option
  - Sample and measure dosage form
  - Scale results to daily dose
- Summation Option
  - Sample and measure all components
  - Sum each metal and scale to daily dose
- Individual Component Approach for LVP
  - Validation process will add no additional impurities

Elemental Impurities – Procedures <233>

- Elemental Impurities – Procedures <233>
  - Compendial Procedures
    - Procedure 1: ICP-OES
    - Procedure 2: ICP-MS
- Validation
    - Limit Procedures
    - Quantitative Procedures
  - Calculations and Reporting
  - Method Verification per <1226>

Elemental Impurities – Procedures <233>

- Validation Requirements
- Limit Procedure
  - Detect ability
  - Precision
  - Specificity

Elemental Impurities – Procedures <233>

Validation Requirements

- Quantitative Procedure
  - Accuracy
  - Detectability
  - Precision
    - Repeatability
    - Ruggedness
  - Specificity
  - LOQ
  - Range
  - Linearity

Implementation Timeline

- December 2, 2012
  - General Chapters <232> and <233> are made official
- December, 2015
  - General Chapter <231> Heavy Metals will be removed from all compendia monographs
  - General Chapters <232> and <233> will be implemented through the new General Notices provision

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Questions? Comments?

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- Roland Frotschl

Chuck Barton

- Richard Ko

FDA Liaison(s)

- John Kauffman
- Jill C. Merrill
- Mamata De

Yin Guo

USP Liaison

- Kahkashan Zaiadi

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Commentary

Metal Impurities in Food and Drugs

Darrell R. Abernethy,[1] Anthony J. DeStefano,[1,3] Todd L. Cecil,[1] Kahkashan Zaidi,[1] Roger L. Williams,[2] and the USP Metal Impurities Advisory Panel

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Abstract. The major metals of potential health concern found in food, drugs (medicines), and dietary supplements are lead, cadmium, mercury, and arsenic. Other metals, such as chromium, copper, manganese, molybdenum, vanadium, nickel, osmium, rhodium, ruthenium, iridium, palladium, and platinum, may be used or introduced during manufacturing and may be controlled in the final article as impurities. Screening for metals in medicines and dietary supplements rarely indicates the presence of toxic metal impurities at levels of concern. The setting of heavy metal limits is appropriate for medicines and is appropriate for supplements when heavy metals are likely or certain to contaminate a given product. Setting reasonable health-based limits for some of these metals is challenging because of their ubiquity in the environment, limitations of current analytical procedures, and other factors. Taken together, compendial tests for metals in food and drugs present an array of issues that challenge compendial scientists.

KEY WORDS: analysis; impurities; limits; metals; standards; US Pharmacopeia.

INTRODUCTION

The US Pharmacopeial (USP) Convention's Council of Experts has worked for several years to improve approaches in the *United States Pharmacopeia* (*USP*) for metals testing and control. As inorganic impurities, metals are one of three types of impurities (organic, inorganic, and residual solvents) that must be controlled in medicines and their ingredients (1) and, by extension, in certain foods and dietary supplements. The current *USP* test for metals is nonspecific and is insufficiently sensitive to control highly toxic metals at levels that present health concerns. A proposed *USP* compendial revision provides health-based (Permissible Daily Exposure, PDE) criteria for testing metals and establishing health-based limits.

In the current cycle (2005–2010), a Metal Impurities Advisory Panel* to the General Chapters Expert Committee in the Council of Experts working with USP staff has devoted considerable attention to these issues. The Advisory Panel began by evaluating modern instrumental techniques to detect metals of interest and then considered, on the basis of health concerns, which metals should be controlled and the associated control limits. This commentary focuses primarily on the establishment of PDE for lead, cadmium, mercury and arsenic, the broader process of selecting metals for update, and the establishment of health-based limits, along with a brief discussion of instrumental techniques that are capable of detecting or quantifying the metals at the required levels. The evolving standards (*USP* General Chapters describing limits and testing requirements for the selected metals in compendial articles) that will arise from recommendations of the Advisory

USP Metal Impurities Advisory Panel Members: Nancy Lewen (Chair), Timothy L. Shelbourn (Vice Chair), Charles Barton, Courtney M. Callis, Steven J. Dentali, Anna M Fan, Roland Frotschl, Assad Kazeminy, Richard Ko, Gregory C. Turk, Robert Wiens; Government Liaisons: Renee Blosser, FDA, Mamata De, FDA, Bruce A. Fowler, CDC, John F. Kauffman, FDA

**The views expressed in this paper are those of the authors and do not represent official positions of the US Food and Drug Administration, the Centers for Disease Control and Prevention/ Agency for Toxic Substances and Disease Registry (CDC/ATSDR), or other US government bodies.

[1] Documentary Standards Division, US Pharmacopeia, Rockville, Maryland 20852-1790, USA.
[2] Chief Executive Officer, US Pharmacopeia, Rockville, Maryland 20852-1790, USA.
[3] To whom correspondence should be addressed. (e-mail: ajd@usp.org)

ABBREVIATIONS: ATSDR, Agency for Toxic Substances and Disease Registry; CDC, Centers for Disease Control and Prevention; cGMP, Current Good Manufacturing Practices; EPA, Environmental Protection Agency; FDA, Food and Drug Administration; GFAAS, Graphite Furnace Atomic Absorption Spectroscopy; IARC, International Agency for Research on Cancer; ICP-OES, Inductively Coupled Plasma–Optical Emission Spectroscopy; ICP-MS, Inductively Coupled Plasma–Mass Spectroscopy; IPCS, International Program on Chemical Safety; IRIS, Integrated Risk Information System; JECFA, Joint Expert Committee on Food Additives; LOAEL, Lowest Observed Adverse Effect Level; MRL, Minimal Risk Level; NA, Not Applicable; ND, Not Determined; NOAEL, No Observed Adverse Effect Level; OEHHA, Office of Environmental Health Hazard Assessment; PDE, Permissible Daily Exposure; RfD, Reference Dose; USP, US Pharmacopeial Convention; WHO, World Health Organization.

Panel to the General Chapters Expert Committee may be reported at a later date. Deliberations of the Advisory Panel regarding metals of concern, limits for these metals, and methodology for their analysis were aided by an Institute of Medicine meeting (2).

THERAPEUTIC VALUE

Metals have been used as medicines through the ages. In 1820, the first *Pharmacopoeia of the United States of America* provided a listing of "simple medicines kept in the shop of the apothecary, but not necessarily prepared by him," including arsenious acid (white arsenic), antimony, bismuth, copper (various salts including copper sulfate or "blue vitriol"), iron, mercury, lead (various salts including lead subcarbonate or "white lead"), tin, silver, gold, and zinc (3).

In 1941 *The Pharmacological Basis of Therapeutics, 1st Edition,* included a series of arsenicals, antimony, mercurials, bismuth, zinc, copper, silver, gold, chromium, iron, magnesium, and selenium, all with designated therapeutic indications for various diseases (4). This textbook described in some detail the toxicities of these metal-containing drugs and included several instances in which the margin between therapeutic effect and toxicity is deemed insufficient to justify the use of certain metals.

At the present time, metals that are approved for therapeutic use in the US include aluminum, arsenic, bismuth, copper, iron, lithium, manganese, magnesium, and selenium (5).

METAL TOXICITY

A number of sources provide information about the toxicity of metals based on animal and human data and may be considered for use by regulatory and public health authorities. In addition to the published scientific literature, they include the following:

- Agency for Toxic Substances and Disease Registry (ATSDR) of the US Department of Health and Human Services (http://www.atsdr.cdc.gov/, accessed June 16, 2009)
- Integrated Risk Information System (IRIS) of the US Environmental Protection Agency (http://cfpub.epa.gov/ncea/iris/index.cfm, accessed June 16, 2009)
- World Health Organization (WHO) International Program on Chemical Safety (IPCS) (http://www.who.int/ipcs/en/, accessed June 16, 2009)
- Joint Expert Committee on Food Additives (JECFA) of the WHO and the Food and Agriculture Organization (http://www.who.int/ipcs/food/jecfa/en/, accessed June 16, 2009).
- State of California Office of Environmental Health Hazard Assessment (OEHHA) (http://oehha.ca.gov/, accessed June 16, 2009)—reproductive/developmental toxicity and carcinogenicity information for articles marketed in California (relative to Proposition 65)

Chemical-specific assessments that address the most current issues are also published by federal and state agencies.

The types of toxicity considered include acute, subchronic, and chronic, and the major concerns are related to neurotoxicity, nephrotoxicity, hepatic toxicity, cardiovascular effects, reproductive/developmental toxicity, neurodevelopmental toxicity, immunotoxicity, and carcinogenicity. In general, exposure limits for environmental media or dietary items are established for chronic or long-term exposure because of the anticipated long-term exposures or intakes. Such limits also will be protective for short-term exposures using standard risk-assessment methodology. Special situations may require limits for shorter-term exposures. Speciation of a metal can be important for toxicity characterization.

In the Institute of Medicine Meeting (2), there was a clear consensus that the most toxic and environmentally ubiquitous metals to focus on with respect to control in pharmaceutical ingredients were mercury, lead, cadmium, and arsenic. To this list were added the metal catalysts considered to be the most important by the European Medicines Agency (EMEA), less iron and zinc, which are essential minerals (6). An extensive review of the toxicity of these catalysts is presented in the EMEA Guideline. A brief discussion of the toxicity of these four most toxic metals follows.

Neurotoxicity

Chronic lead exposure, even at very low levels, has been associated with decreased intelligence quotient in children (7). Methyl mercury poisoning from eating contaminated fish in Japan and contaminated bread in Iraq resulted in parathesis, loss of gait coordination, slurred speech, sensory deficits, mental disturbances, and neurodevelopmental effects (8,9). More recent studies in fish-eating populations conducted in the Seychelles Islands, the Faroe Islands, and New Zealand showed that in utero exposure was associated with neuropsychological effects in the offspring (Environmental Protection Agency (EPA), 2001, http://www.epa.gov/ncea/iris/subst/0073.htm, accessed December 02, 2009). Combined exposure to methyl mercury and lead could certainly occur, but it is unknown if the toxicity is additive, synergistic, or targeted to unique cellular targets and unrelated. Methyl mercury is not an issue for medicines, where the typical form of mercury is mercuric but is present in some dietary supplements, such as fish oil.

Nephrotoxicity

Lead, cadmium, and mercury are nephrotoxic (10–12). Again, it is unclear how toxicity to combined exposures would manifest. When an individual is exposed to more than one metal that has the same or similar organ toxicity, present risk assessment models assume the toxins are additive in their effects, although this is based on limited data (13). In the case of chronic co-exposure to arsenic and cadmium, at least additive nephrotoxicity has been reported (14). At present, data are insufficient to support establishing science-based limits for specific articles based on combined multiple metal exposures with similar toxicities. Thus, at present, the metal limits will be treated individually.

Populations at Increased Risk

Metals as developmental neurotoxins are of particular concern during brain and nervous system development. Exposure of the prepartum mother and of the child during the neonatal and early childhood periods to lead as a prototype neurotoxic metal presents increased risk by comparison to exposure at later ages (7). For nephrotoxic metals,

individuals with pre-existing renal dysfunction are more susceptible than those with normal renal function (13,15). Similarly, individuals with diabetes may be especially sensitive to the renal toxicity of cadmium (16,17). The limits described in Table I are set for healthy adults with a 50-kg body weight. For medicines or dietary supplements that are likely to be used in vulnerable patient groups, acceptable limits may be lower.

Selection of Metals for Update and Development of Health-Based Limits

An assessment of acceptable exposure for metals in food and drugs requires careful evaluation of the following:

1. Human (preferred if good-quality data are available) and animal toxicity data associated with exposure to the metal
2. Likelihood of presence of the metal in the article to be tested
3. Level and pattern of use or consumption of the article or product
4. Level of exposure to the metal
5. Other sources of exposure to the metal
6. Other factors that may affect toxicity (e.g., co-exposure to other metals)
7. Data quality and individual variability
8. Special populations at increased risk for toxicity.

These considerations and other factors form the basis for a risk-based approach for the selection of metals that should be controlled and their control limits. For example, if a metal catalyst was used during drug substance synthesis, some amount of the metal may be present in the drug ingredient, but concerns may be mitigated if the catalyst was not used during manufacture. Equipment used in the manufacture of the ingredients or the final product is another source of metal contamination. For pharmacopeial purposes, this source of contamination is considered a cGMP issue that is controlled by process validation. Some metals, such as lead, mercury, cadmium, and arsenic, are ubiquitous in the environment in appreciable quantities. These may add to the total exposure when consumers use drugs or consume dietary supplements that may contain the same metals and other metals of concern (18–21). Still, some dietary supplements and many drugs have been evaluated repeatedly over time, and no significant levels of metals of interest have been found (22,23).

At times, risk evaluation is complicated by the necessity to identify the species of the metal that is likely to be present. In the case of arsenic, mercury, and chromium, the metal species determines its toxicity (24). The International Union of Pure and Applied Chemistry definition of chemical species is "a specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure." Inorganic arsenicals (As^{+3}, As^{+5}) are highly toxic, methyl arsenates are of limited toxicity, and

Table I. Limits of Metals for Pharmaceuticals

Metal	Oral Daily Dose PDE (μg/day)	Oral Component Limit (μg/g)[a]	Parenteral Component Limit (μg/g)[a]	Detection Limit, ICP–OES, (μg/g)[b,c]	Detection Limit, GFAAS, (μg/g)[b,c]	Detection Limit, ICP-MS, (μg/g)[c,d]
High Toxicity						
Arsenic (inorganic)	15	1.5	0.15	3.5	0.1	0.01
Cadmium	25	2.5	0.25	0.06	0.0008	0.002
Lead	10	1	0.1	2	0.04	0.003
Mercury (Hg^{+2})	15	1.5	0.15	3	0.6	0.001
Intermediate Toxicity						
Chromium III	250	25	2.5	0.3	0.05	0.02
Molybdenum	250	25	2.5	0.12	0.006	0.002
Nickel	250	25	2.5	0.6	0.05	0.02
Palladium	100	10	1.0	4	0.05	ND
Platinum	100	10	1.0	2	0.02	0.003
Osmium[e]	100 (Combination not to exceed)	10 (Combination not to exceed)	1.0 (Combination not to exceed)	2	NA	0.001[f]
Rhodium[e]				2	0.01	ND
Ruthenium[e]				5	1	ND
Iridium[e]				2	0.05	ND
Vanadium	250	25	2.5	0.78	0.1	0.004
Low Toxicity						
Copper	2500	250	25	0.2	0.001	0.01
Manganese	2500	250	25	0.05	0.005	0.02

[a] Assumes 10-g oral or parenteral dose
[b] Dean JA, ed. Lange's Handbook of Chemistry, 15th ed. New York: McGraw-Hill; 1999:7.29–7.33.
[c] All limits are Limits of Detection (3σ) corrected for a 1 g/100 mL dilution.
[d] Fernandez-Turiel JL, *et al.* Strategy for water analysis using ICP-MS. J Anal Chem. 2000;368:601–06.
[e] The sum of these four metals should not exceed the limits specified in this row.
[f] Tyutyunnik OA, Koshcheeva IYa, Orlova VA, Shumskaya TV, Gorbacheva SA. Determination of osmium traces in natural samples. J Anal Chem. 2004;59(9):885–88.

organic arsenicals such as arsenobetaine are nontoxic (25). In contrast, methyl mercury is highly toxic, Hg^{+2} is less toxic, and Hg^{+1} and metallic Hg^{0} have very limited toxicity (24). Chromium toxicity similarly depends on species: Cr^{+6} is highly toxic and carcinogenic, but Cr^{+3} is an essential trace element (26). Unless preparatory separations for these species are undertaken, the analytical method will simply detect total metal content, which may be unrelated to potential toxicity (27).

Plant-derived (botanical) dietary supplements may accumulate metals from the soil where they are grown or from other environmental sources, such as air or water. Similarly, animal- or mineral-based dietary supplements may contain metals associated with their local environments (Table II). Taking into account metals likely to be used as catalysts in manufacturing (6) and adding highly toxic metals that are ubiquitous in the environment (lead, mercury, cadmium, and arsenic) and other similarly distributed metals (35,36) allows categorization of metals based on health concern (Table I).

Development of Health-based Limits for Pharmaceuticals

The sources of toxicity noted above were used to develop a consensus oral permissible daily exposure (oral PDE) for each metal of interest in pharmaceutical products. In particular, the PDEs for the 12 medium- and low-toxicity metals in Table I are adopted from those presented in the recent EMEA guideline on the presence of residual metal catalysts in pharmaceuticals (6).

For arsenic, both the International Agency for Research on Cancer (IARC) and EPA classify inorganic arsenic as carcinogenic to humans (37,38). EPA Reference Dose (RfD) for chronic oral exposures, 0.3 μg/kg/day, is based on a no-observed-adverse-effect level (NOAEL) of 0.8 μg/kg/day and a lowest-observed-adverse-effect level (LOAEL) of 14 μg/kg/day for hyperpigmentation, keratosis, and possible vascular complications in a human population in Taiwan consuming arsenic-contaminated drinking water. Using the oral RfD of 0.3 μg/kg/day, an oral PDE of 15 μg/day based on a 50-kg person is derived.

For cadmium, the major effect is kidney damage producing tubular proteinuria. A concentration of 200 μg Cd/g wet human renal cortex is the highest renal level not associated with significant proteinuria (39). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) that results in 200 μg Cd/g wet human renal cortex (39). The toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 5 and 10 μg/kg/day from water and food, respectively. Thus, based on an estimated NOAEL of 5 μg/kg/day for Cd in drinking water and an uncertainty factor of 10, an RfD of 0.5 μg/kg/day (water) was calculated. An equivalent RfD for Cd in food is 1 μg/kg/day. Both values reflect incorporation of an uncertainty factor of 10. ATSDR determined that the adverse effect levels for renal effects were similar to those observed for skeletal effects, but the renal effects database was stronger and, therefore, was used for derivation of a chronic-duration oral Minimal Risk Level (MRL). Data were derived from select environmental studies worldwide that examined the relationship of urinary cadmium and the prevalence of elevated levels of biomarkers of renal function. The 95% lower confidence limit of urinary cadmium dose corresponding to the probability of exceeding 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. Applying a safety factor of 3 for human variability, ATSDR has set the MRL at 0.1 μg/kg/day. Using the ATSDR MRL as the oral PDE yields a PDE of 5 μg/day.

The 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. Instead, the EPA has developed the Integrated Exposure Uptake Biokinetic Model. In 1994, the FDA adopted an allowable level for lead at 5 ppb as a bottled water quality standard regulation (59 FR 26933). Assuming an average consumption of 2 L/day of the bottled water, the oral PDE is 10 μg/day for a 50-kg person.

With regard to mercury, as discussed above, the presence of methyl mercury in pharmaceutical products is extremely unlikely. Therefore, the EPA recommended RfD for mercuric chloride—0.3 μg/kg/day or 15 μg/day for a 50-kg person—is used as the oral PDE. The RfD was based on formation of mercuric-mercury–induced autoimmune glomerulonephritis in rats (EPA, last revised 1995, searched 2009, http://www.epa.gov/ncea/iris/subst/0692.htm). Because for oral products a 10-gram daily dose is assumed, the

Table II. Toxic Metal Impurities in Dietary Supplements

Mineral	Contaminating Metal Impurity	Levels of Metal Impurity	Reference
Calcium (bone meal, dolomite, fossil oyster shells)	Lead	0.6–190 ppm	(28)
Zinc	Lead	More than 1 μg/daily dose	(29)
Women's and children's vitamins	Lead	Median exposure 0.576 μg/daily dose	(30)
Tums chewable tablets	Lead	2.67 μg/daily dose	(29)
Vitamin Shoppe multivitamins Especially for Women	Lead	15.3 μg/daily dose	(31)
Botanical/other natural ingredient	**Contaminating metal impurity**	**Levels of metal impurity**	**Reference**
Panax pseudoginseng	Lead	48.6 ppm	(32)
Licorice extract	Arsenic	0.5 ppm	(33)
Ginkgo	Lead	12.5 μg/daily dose	(34)
Ginseng	Lead	9.2 μg/daily dose	(34)
St. John's Wort	Lead	5.8 μg/daily dose	(34)
Shark cartilage	Lead	1.4 ppm	(32)

maximum permissible metal concentration on a per-gram basis (μg/g) is one-tenth of the oral PDE. To account for the limited bioavailability of many metals after oral ingestion, for pharmaceuticals that are administered parenterally, a safety factor of 10 (the oral-route concentration is divided by 10) is applied to the metal PDE and is reflected as a factor of 10-fold decrease in concentration relative to the corresponding oral concentration in Table I.

The table, including elements and PDE, is subject to change as usage patterns change or as new toxicity data become available. For pharmaceuticals, high-toxicity metals that are ubiquitous in the environment must be verifiably absent above the limits noted in Table I. This could be established by determination of levels in ingredients that make up the product or by determination of levels in the product after manufacture. For metal catalysts, a specific catalyst must be verifiably absent above the limits noted when the catalyst was used in the manufacturing process. Metals of low toxicity not listed in Table I should be controlled in the context of current Good Manufacturing Practices (cGMP). A separate table will be reported in future communications to accommodate the elements and exposures associated with dietary supplements.

TESTING FOR METALS

Current Approaches

The first appearance of a pharmacopeial test for metals occurred in *USP VIII* (1905) and was titled "Time-Limit Test for Heavy Metals" (40). This was a nonspecific sulfide-precipitation method and was put forward as a screen for antimony, arsenic, cadmium, copper, iron, lead, and zinc. This test was modified in *USP XII* (1942) with the addition of a lead standard comparison solution (41). With various modifications, the test procedure remains official in *USP*'s General Chapter *Heavy Metals* <231> (42). It also can be required in dietary supplement food articles that indicate conformance to a *USP* monograph. Variants of this test are also the current standard in the *European Pharmacopoeia 6.0* Chapter 2.4.8 "Heavy Metals" (43), the *Japanese Pharmacopoeia XV* Chapter 1.07 "Heavy Metals Limit Test" (44), and the *International Pharmacopoeia* 4th Edition Chapter 2.2.3 "Limit Test for Heavy Metals" (45).

The nonspecific metals limit test in <231> has been criticized for 1) the large sample size required for analysis, 2) the lack of element-specific information, 3) the use of a visual comparison to the black precipitate of lead sulfide reference material, 4) the low recovery of essentially all the elements and lead standard during sample preparation if the sample is insoluble and requires heating or digestion, and 5) the safety and other issues associated with the generation of hydrogen sulfide in a laboratory setting. In the last decade and more, USP has issued calls to revise the metals test procedure described in <231> (46–48).

Modern Instrumental Methods

Many procedures have been developed for selective detection and quantification of metal species. Some procedures use excitation and emission phenomena to detect metals in intact material, such as X-ray fluorescence and neutron activation analysis. Other procedures separate the metals from the organic matrix. These procedures require an initial atomization and ionization process. This process is accomplished using flame, furnace, plasma, laser, or spark techniques. Once ionized, the metals are quantified using optical emission, chromatographic techniques, or mass spectrometry. These procedures are all options for the research laboratory, but in the manufacturing environment operating under cGMP, the list of possibilities is more limited. Because of the constraints with methods of sufficient sensitivity and selectivity for toxicologically based metal limits, analysts may find that electrothermal atomic absorption spectrometry, inductively coupled plasma–optical emission spectrometry (ICP-OES), and inductively coupled plasma–mass spectrometry (ICP-MS) are the most suitable procedures (47,48). The choice of analytical procedure depends on the solubility of the drug ingredient or dietary supplement and other components of the material (matrix). To provide guidance about the range of sensitivities of ICP-OES, ICP-MS, and graphite furnace atomic absorption spectrometry (GFAAS), Table I lists the approximate limits of detection for each element by each method.

CONCLUSION

The PDE and approaches described in this paper represent a substantial revision of the current pharmacopeial approaches to metals testing. Modern instrumental procedures offer the possibility of detecting all metals at levels below those corresponding to the listed PDE. Evolving standards for levels of metals in compendial drug products therefore must be clear about the choice of metals and specified PDE to avoid unnecessary testing. The risk-based approach presented in this communication provides a way forward. Evolution of the considerations of the Advisory Panel into compendial standards for *USP–NF* is in progress (see www.usp.org/hottopics/metals.html for periodic reports). The standards will be applied to drugs in *USP* and excipients in *NF,* as well as dietary supplements labeled to indicate conformance to *USP* standards.

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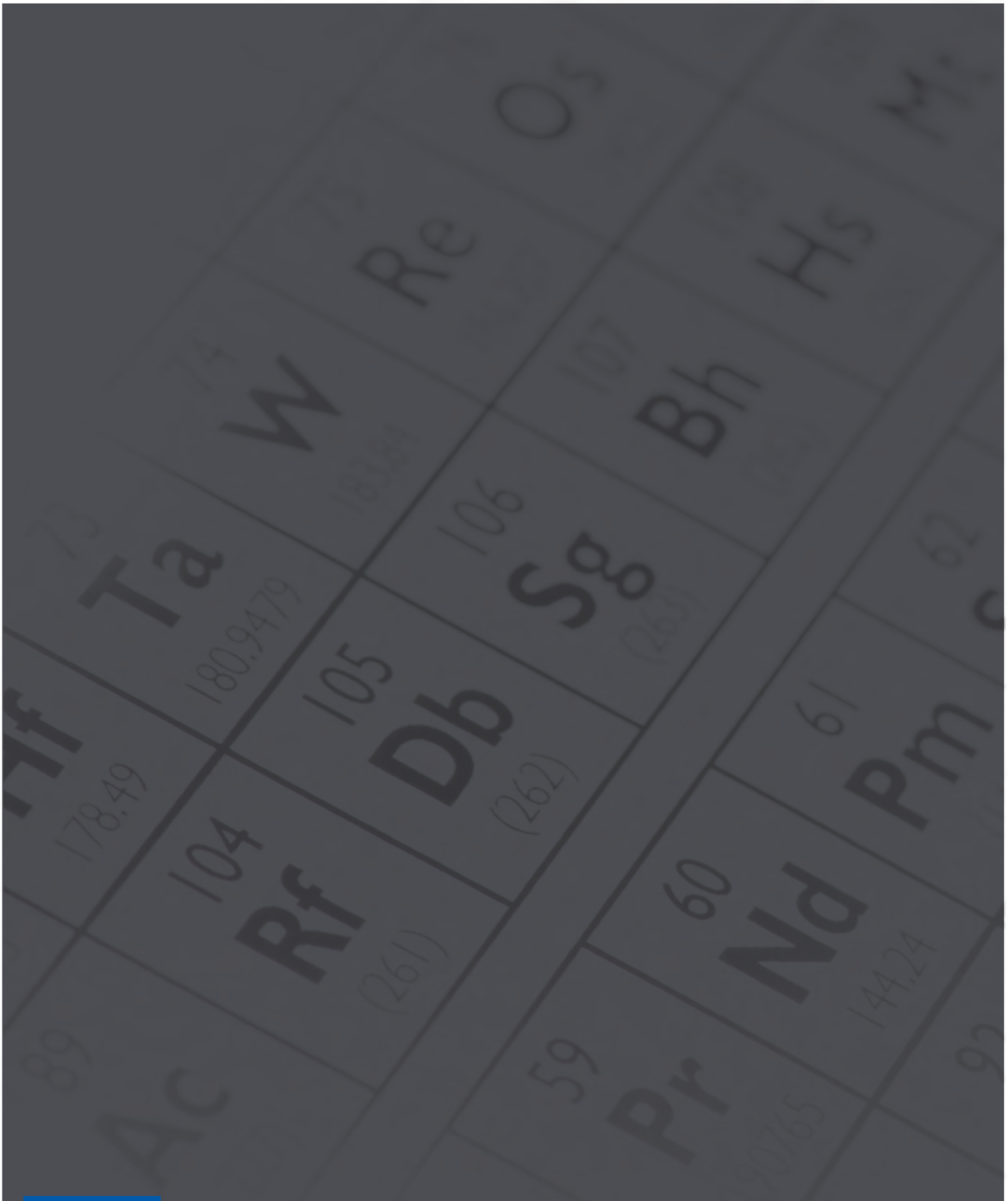

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10 VANDERBILT, IRVINE CA 92618
TEL: 949.951.4425
TOLL FREE: 877.445.6554
FAX: 949.951.4909
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