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⟨251⟩ LEAD

Change to read:

▲INTRODUCTION▲ (Official 1-Jun-2023)

The imposition of stringent limits on the amounts of lead (Pb) that may be present in pharmaceutical products has resulted in the use of ▲three▲ (Official 1-Jun-2023) methods, of which ▲*Procedure 1*,▲ (Official 1-Jun-2023) set forth in this chapter, depends upon extraction of lead by solutions of dithizone.

▲Use *Procedure 1*, *Procedure 2*, or *Procedure 3* as indicated in the individual monograph. *Procedure 2* or *Procedure 3* can be used in all circumstances, provided that suitability is demonstrated by meeting the requirements in *Requirements for Procedure Validation*.▲ (Official 1-Jun-2023)

Change to read:

▲PROCEDURES

• **Procedure 1: Chemical Method▲** (Official 1-Jun-2023)

Select all reagents for this test to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Thoroughly rinse all glassware with warm dilute nitric acid (1 in 2), followed by water.

Ammonium cyanide solution: Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

Dithizone extraction solution: Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of alcohol. Store the solution in a refrigerator. Before use, shake a suitable volume of the *Dithizone extraction solution* with about half its volume of dilute nitric acid (1 in 100), discarding the nitric acid.

Ammonium citrate solution: Dissolve 40 g of citric acid in 90 mL of water. Add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a reddish color. Remove any lead that may be present by extracting the solution with 20-mL portions of *Dithizone extraction solution*, until the dithizone solution retains its orange–green color.

Diluted standard lead solution: Dilute an accurately measured volume of standard lead solution TS (containing 10 µg of lead per milliliter), with 9 volumes of dilute nitric acid (1 in 100) to obtain a solution that contains 1 µg of lead per milliliter.

Hydroxylamine hydrochloride solution: Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make approximately 65 mL. Transfer to a separator, add 5 drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 min. Extract this solution with successive 10- to 15-mL portions of chloroform until a 5-mL portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 3 N hydrochloric acid until the solution is pink (if necessary, add 1 or 2 drops more of thymol blue TS), then dilute with water to 100 mL.

Potassium cyanide solution: Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from this solution by extraction with successive portions of *Dithizone extraction solution*, as described in *Ammonium citrate solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

Standard dithizone solution: Dissolve 10 mg of dithizone in 1000 mL of chloroform. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light, and store in a refrigerator.

Test preparation: [Note—If, in the following preparation, the substance under test reacts too rapidly and begins charring with 5 mL of sulfuric acid before heating, instead use 10 mL of cooled dilute sulfuric acid (1 in 2), and add a few drops of hydrogen peroxide before heating.] Where the monograph does not specify preparation of a solution, prepare a *Test preparation* as follows.

[**Caution—**Exercise safety precautions in this procedure, because some substances may react with explosive violence when digested with hydrogen peroxide.]

Transfer 1.0 g of the substance under test to a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest on a hot plate in a hood until charring begins. Other suitable means of heating may be substituted. (Add additional sulfuric acid, if necessary, to wet the substance completely, but do not add more than a total of 10 mL.) Add, dropwise and with caution, 30 percent hydrogen peroxide, allowing the reaction to subside and heat between drops. Add the first few drops very slowly, mix carefully to prevent a rapid reaction,

and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls of the flask. [Note—Add peroxide whenever the mixture turns brown or darkens.]

Continue the digestion until the substance is completely destroyed, copious fumes of sulfur trioxide are evolved, and the solution is colorless. Cool, and cautiously add 10 mL of water. Evaporate until sulfur trioxide again is evolved, and cool. Repeat this procedure with another 10 mL of water to remove any traces of hydrogen peroxide. Cautiously dilute with 10 mL of water, and cool.

Analysis: Transfer the *Test preparation*, rinsing with 10 mL of water, or the volume of the prepared sample specified in the monograph to a separator, and, unless otherwise directed in the monograph, add 6 mL of *Ammonium citrate solution* and 2 mL of *Hydroxylamine hydrochloride solution*. (For the determination of lead in iron salts, use 10 mL of *Ammonium citrate solution*.) Add 2 drops of phenol red TS, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution if necessary, and add 2 mL of *Potassium cyanide solution*. Immediately extract the solution with 5-mL portions of *Dithizone extraction solution*, draining off each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 mL of dilute nitric acid (1 in 100), and discard the chloroform layer. Add to the acid solution 5.0 mL of *Standard dithizone solution* and 4 mL of ▲*Ammonium*▲ *(ERR 1-Jun-2023)* *cyanide solution*, and shake for 30 s.

Acceptance criteria: The color of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *Diluted standard lead solution* equivalent to the amount of lead permitted in the sample under examination and made with the same quantities of the same reagents and in the same manner as in the test with the sample.

- **▲Procedure 2 and Procedure 3**

Both *Procedure 2* and *Procedure 3* are ICP-based procedures and can be used for the determination of lead. *Procedure 2* can be used for the determination of lead by inductively coupled plasma atomic (or optical) emission spectroscopy (ICP–AES or ICP–OES). *Procedure 3* can be used for the determination of lead 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 *Requirements for Procedure Validation*.

Where a monograph specifies a limit for lead concentration, the value listed in the monograph should be used as the *J* value for the purposes of this test.

System standardization and suitability evaluation using applicable reference materials should be performed on the day of analysis.

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 appropriately validated preparation procedure. In cases where spiking of a material under test is necessary to provide an acceptable signal intensity, the blank should be spiked with lead using, where possible, the same spiking solution. [Note—All liquid samples should be weighed.]

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 as follows. Dehydrate and predigest 0.5 g of the primary sample in 5 mL of freshly prepared concentrated acid. Allow to sit loosely covered for 30 min in a fume hood. Add an additional 10 mL of concentrated acid, and digest using a closed vessel technique until the digestion or extraction is complete. Repeat, if necessary, by adding an additional 5 mL of concentrated acid. [Note—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 2: ICP–OES

Standardization solution 1: 1.5*J* of lead in a matched matrix

Standardization solution 2: 0.5*J* of lead in a matched matrix

Sample stock solution: Prepare as directed in *Sample preparation*.

Sample solution: Dilute the *Sample stock solution* with an appropriate solvent to obtain a final lead concentration of not more than 1.5*J*.

Blank: Matched matrix

Elemental spectrometric system

(See ⟨730⟩.)

Rinse: Use diluent.

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 solution*.

Suitability criteria: Not more than 20% for lead. [Note—If samples are high in mineral content, rinse the system well 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 3: ICP–MS

Follow *Procedure 2* except for *Detector* and *Analysis*.

[NOTE—An instrument with a cooled spray chamber is recommended. (A collision cell or reaction cell may also be beneficial.)]

Detector: Mass spectrometer

Analysis: Analyze according to the manufacturer's suggestions for program and mass-to-charge ratio. Calculate and report results based on the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences.]▲ (Official 1-Jun-2023)

Add the following:

▲REQUIREMENTS FOR PROCEDURE VALIDATION

The following section defines the validation parameters and the acceptance criteria for performance-based procedures. Meeting these requirements must be demonstrated experimentally using an appropriate system suitability procedure and reference materials. Any alternative procedure (e.g., an atomic-absorption-based procedure) that has been validated and meets the acceptance criteria that follow is considered to be suitable for use.

Meeting these validation acceptance criteria is sufficient to demonstrate that the procedure will produce comparable results to those obtained using the procedure prescribed in the monograph.

• **ACCURACY**

Standard solutions: Prepare solutions containing lead at concentrations ranging from 50% to 150% of *J* using appropriate reference materials.

Test samples: Spike the material under test with the appropriate reference materials before any sample preparation steps (digestion or solubilization). Prepare three replicate samples at concentrations ranging from 50% to 150% of *J* for lead.

Acceptance criteria

Spike recovery: 70%–150% for the mean of three replicate preparations at each concentration

• **PRECISION**▲▲ (ERR 1-JUN-2023)

Repeatability

Test samples: Six independent samples of material under test (taken from the same lot) spiked with appropriate reference materials for lead at the indicated concentration

Acceptance criteria

Relative standard deviation: Not more than 20% (*N* = 6) for lead

Intermediate precision (Ruggedness)

Analysis: Perform the *Repeatability* analysis again on a different day, with different instrumentation, with a different analyst, or a combination thereof. Combine the results of this analysis with the *Repeatability* analysis so the total number of analyses is 12.

Acceptance criteria

Relative standard deviation: Not more than 25% (*N* = 12) for lead

• **SPECIFICITY:** The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) lead in the presence of components that may be expected to be present, including matrix components.

• **LIMIT OF QUANTITATION, RANGE, AND LINEARITY:** Demonstrated by meeting the *Accuracy* requirement.▲ (Official 1-Jun-2023)

Add the following:

▲GLOSSARY

Concentrated acid: Concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acid 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.

Matched matrix: Solutions having the same solvent composition as the *Sample solution*. In the case of an aqueous solution, a matched matrix would indicate that the same acids and acid concentrations are used in both preparations.

Target limit or target concentration: The acceptance value for the elemental impurity being evaluated, in this case lead. Where a monograph specifies a threshold limit, this shall become the target limit or target concentration of lead for the material. Exceeding the target limit indicates that a material under test exceeds the acceptable value. The determination of compliance is addressed in other chapters.

***J*:** The concentration (w/w) of the element of interest, in this case lead, 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 (CRMs) 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 (NIST).▲ (Official 1-Jun-2023)

Topic/Question	Contact	Expert Committee
<251> LEAD	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

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