
BRIEFING

⟨233⟩ Elemental Impurities—Procedures. This proposed new general test chapter is the second of two being developed to replace the general test chapter *Heavy Metals* (231); the first chapter is *Elemental Impurities—Limits* (232). The procedures described in Chapter (231) are inadequate to provide the basis for control of the elements in Chapter (232) at their proposed limits.

This chapter describes the validation of two types of procedures, limit and quantitative, for the measurement of elemental impurities and provides criteria for the approval of alternative procedures. The chapter also describes two referee procedures, inductively coupled plasma–atomic (optical) emission spectroscopy (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS), both using closed vessel microwave digestion.

The choice of procedure, including the sample preparation and the instrument parameters, is the responsibility of the user.

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Add the following:

▲⟨233⟩ ELEMENTAL IMPURITIES— PROCEDURES

INTRODUCTION

This chapter describes analytical procedures for the evaluation of elemental impurities in *USP* for drug substances and drug products (including natural-source

and rDNA biologics); in *NF* for excipients; and in the *USP Dietary Supplements Compendium* for dietary supplements and dietary ingredients (all drug articles). Two referee procedures are described. Criteria for the approval of alternative procedures are also described. An alternative procedure will require complete validation for each element of interest. In addition, a system suitability evaluation using a USP Reference Standard or its equivalent should be demonstrated on the day of analysis. Alternative procedures that meet the validation requirements described herein are considered to be equivalent to Procedures 1 and 2. A decision-tree that can be used to guide a user to an appropriate alternative procedure is presented in *Figure 1*. The test requirement is specified in *General Notices* or the individual monograph.

Speciation

When elements are present in certain complexes, oxidation states, or organic combinations, they may show more significant toxicity than in other forms and may require further testing and control. The determination of the oxidation state or organic complex or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter.

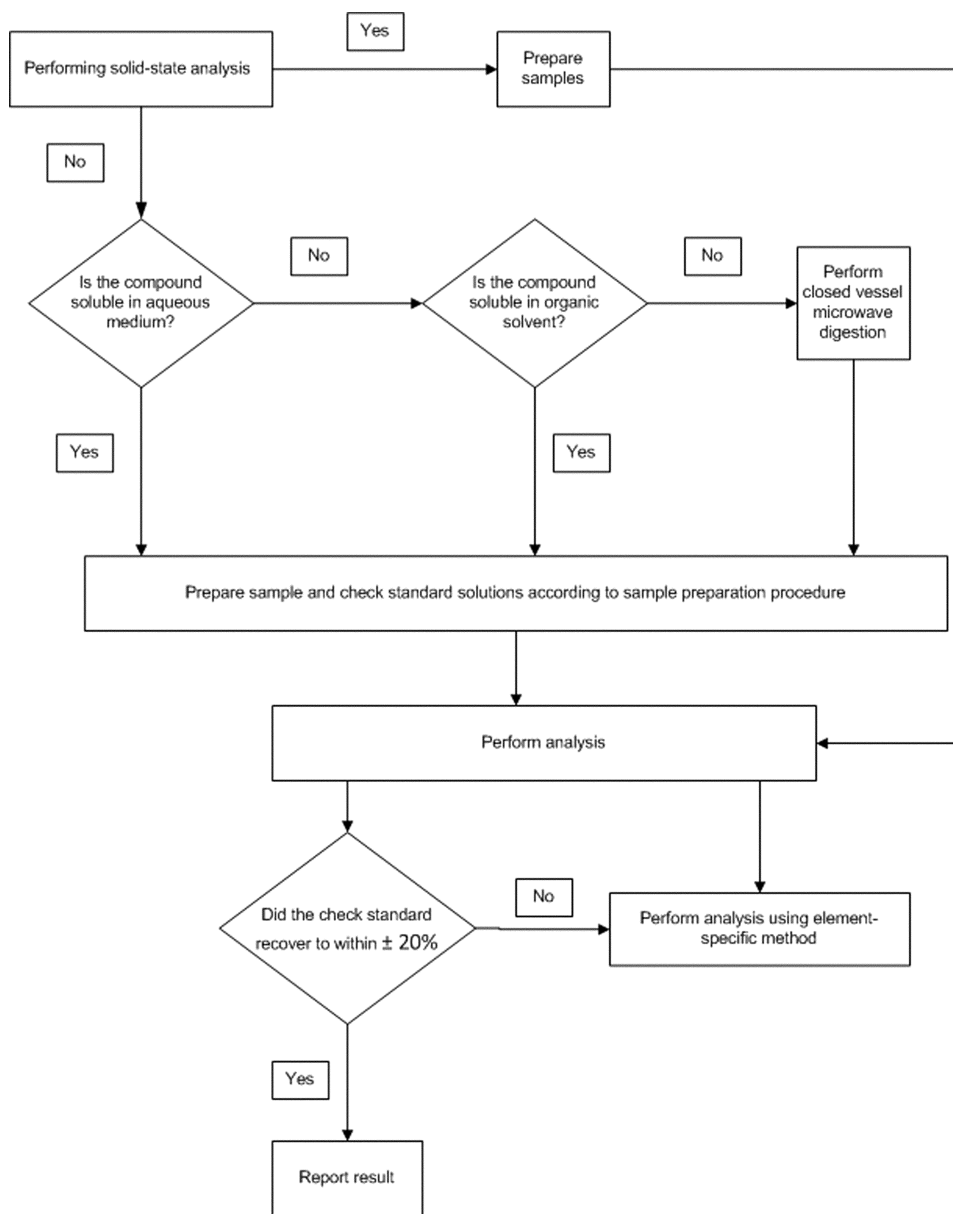


Figure 1. Elemental impurities decision tree.

ALTERNATIVE PROCEDURE VALIDATION REQUIREMENTS

The level of validation necessary to ensure that a procedure is appropriate for its intended purpose—that is, that it is acceptable—will differ, depending on whether a limit test or a quantitative determination is necessary. The requirements for validation of an elemental impurities procedure for either type of determination are described below.

VALIDATION OF LIMIT PROCEDURES

For elemental impurities, validation of a limit procedure should include accuracy, precision, and specificity. Following are acceptable validation parameters that allow a procedure to be deemed appropriate as a limit procedure:

Accuracy

Control Sample—A preparation of certified reference materials for the element of interest at the indicated level

Test Sample—A sample of material under test, spiked with certified reference materials for the element of interest at the indicated level, prepared in triplicate

Acceptance Criteria—Each *Test sample* provides a signal of intensity or value equivalent to or greater than that of the *Control sample*. [NOTE—The signal obtained must show a change from the value obtained compared to a blank determination.] The accuracy of the method must be determined by conducting studies with test materials supplemented with known concentrations of each element at the appropriate acceptance limit concentration. The test materials must be spiked before any sample preparation steps are taken. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked before the digestion procedure.

Precision for Instrumental Methods (Repeatability)

[NOTE—Noninstrumental precision is demonstrated by meeting the *Accuracy* requirement above.]

Test Samples: Six independent samples of the material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria: Relative standard deviation, NMT 20%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained for that element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

VALIDATION OF QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of a quantitative procedure. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference material.

Accuracy

Control Sample 1: $0.5J$, of the certified reference materials for the element of interest, where J is the indicated limit

Control Sample 2: J , of the certified reference materials for the element of interest, where J is the indicated limit

Control Sample 3: $1.5J$, of the certified reference materials for the element of interest, where J is the indicated limit

Test Sample 1: Sample of material under test, spiked with certified reference materials for the element of interest at $0.5J$, where J is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 2: Sample of material under test, spiked with certified reference materials for the element of interest at J , where J is the indicated limit [NOTE—Prepare in triplicate.]

Test Sample 3: Sample of material under test, spiked with certified reference materials for the element of interest at $1.5J$, where J is the indicated limit [NOTE—Prepare in triplicate.]

Acceptance Criteria: Spike recovery: 80%–150% for the mean of three replicate preparations at each concentration. The test materials must be supplemented before any sample preparation steps. For example, if a test material is to be digested with a closed vessel microwave digestion apparatus, the material must be spiked at the beginning of the digestion procedure.

Precision

REPEATABILITY

Test Samples: Six independent samples of material under test, spiked with certified reference materials for the element of interest at the indicated level

Acceptance Criteria: Relative standard deviation, NMT 20%

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the method must be established. Acceptable experiments for establishing intermediate precision include performing the *Repeatability* analysis

1. On different days,
2. With different instrumentation, or
3. With different analysts.

Note that executing only one of the three experiments listed is required in order to demonstrate intermediate precision.

Acceptance Criteria: Relative standard deviation, NMT 25%

Specificity

Specificity (false-negative) for an element in the material under test will be deemed acceptable if acceptance criteria for accuracy and precision are obtained

for that element in the presence of other elements that may interfere with the evaluation, at their indicated limits.

Specificity (false-positive) must also show an absence of signal for an element in the presence of other elements that, at their indicated limits, may interfere with the evaluation.

Limit of Quantitation (Sensitivity)—Demonstrated by meeting the *Accuracy* requirement.

REFEREE PROCEDURES 1 AND 2

Procedure and Detection Technique

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP-OES). Procedure 2 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP-MS).

Verification

Before the initial use of a referee procedure, the analyst should ensure that the procedure is appropriate for the instrument and sample used. This is accomplished by procedure verification, as described in *Verification of Compendial Procedures* (1226).

Sample Preparation

Sample preparation is critical to the successful completion of the evaluation. Use the flow chart in *Figure 1* to determine the means of sample preparation. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the specified limit stated in the corresponding monograph or chapter. [NOTE—All liquid samples should be weighed.]

Closed Vessel Microwave Digestion—This sample preparation procedure is designed for samples that must be digested. The procedure also applies to samples that are not soluble in nitric acid. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the microwave digestion apparatus used, if proportions remain constant.]

Sample Preparation—Dehydrate and predigest 0.5 g of sample in 5 mL of freshly prepared aqua regia.¹ Sulfuric acid may also be used as a last resort.² Allow the sample to sit loosely covered for 30 min in a fume hood. Add 10 mL more of aqua regia, and digest, using a closed vessel microwave technique. Microwave until digestion or extraction is complete. Repeat if necessary by adding 5 mL more of aqua regia. [NOTE—Where closed vessel microwave digestion is necessary, follow the manufacturer's recommended procedures to ensure safe usage.][NOTE—In closed vessel microwave digestion, the use of concentrated hydrofluoric acid (HF) is not recommended. However, when its use is necessary, practice the utmost caution in the preparation of test articles, and review or establish local procedures for safe handling, safe disposal, and HF-tolerant instrumental configurations.]

Reagents—All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* (730). Reagents should be commercial elemental stock standards that are National Institute of Standards and Technology (NIST)—traceable, at a recommended concentration of 100 µg/mL or greater; or appropriate USP Reference Standards, as either single element or multielement.

¹ Ultra pure nitric acid/hydrochloric acid (1 : 3) prepared as needed. (A 1%–5% solution of aqua regia is used as a rinsing solution between analyses and as calibration blanks.)

² Sulfuric acid should be used only when absolutely needed, for the following reasons: Upon addition of sulfuric acid, elements may be lost as a result of extreme exothermic reaction. The viscosity of sulfuric acid is higher than that of other acids, which affects the overall flow of solution.

Procedure 1: ICP-OES

Sample Solution: Proceed as directed in *Sample preparation* above. When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool (add an appropriate stabilizer, such as gold at about 0.1 ppm, for mercury measurement), and dilute with *Purified Water* to 50.0 mL.

Calibration Solution 1: $2J$ of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where J is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2: $0.1J$ of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where J is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Check Standard Solution: 1 ppm of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*) [NOTE—Multiple elements of interest may be included in this solution at 1 ppm each. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank: Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* (730))

Mode: ICP

Detector: Optical emission spectroscopy

Rinse: 5% aqua regia

Calibration: Two-point, using *Calibration solution 1*, *Calibration solution 2*, and *Blank*

System Suitability

Sample: *Check Standard Solution*

Suitability requirements—

Drift: differs from actual concentration by NMT 20%.

[NOTE—If samples are high in mineral content, to minimize sample carryover, rinse system well (60 sec) before introducing *Check Standard Solution*.]

Analysis: Analyze according to manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size.

Procedure 2: ICP-MS

Sample Solution: Proceed as directed in *Sample preparation* above, and add appropriate internal standards at appropriate concentrations.

When closed vessel microwave digestion is used, proceed as directed above, allow the digestion vessel to cool, add appropriate internal standards at appropriate concentrations (gold should be one of the internal standards for mercury measurement), and dilute with *Purified water* to 50.0 mL.

Calibration Solution 1: $2J$ of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where J is the limit for the specific elemental impurity. [NOTE—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Calibration Solution 2: $0.1J$ of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample solution*), where J is the limit for the specific elemental impurity. [NOTE—Multiple elements

of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

Blank: Matched matrix (acid concentrations similar to that of the *Sample solution*)

Elemental Spectrometric System (see *Plasma Spectrochemistry* (730))

Mode: ICP [NOTE—An instrument with a cooled spray chamber is recommended.]

Detector: Mass spectrometer

Rinse: 5% aqua regia

Calibration: *Calibration solution 1*, *Calibration solution 2*, and *Blank*

System Suitability

Sample: *Calibration solution 1*

Suitability requirements—

Drift: differs from actual concentration by NMT 20%.

[NOTE—If samples are high in mineral content, rinse system well (60 sec) before introducing *Check Standard Solution* to minimize sample carryover.]

Analysis: Analyze per manufacturer's suggestions for program and m/z . Calculate and report results based on the original sample size. [NOTE: Arsenic is subject to interference from argon chloride. Appropriate measures, including a sample preparation without aqua regia, must be taken to correct for the interference, depending on instrumental capabilities.]

CALCULATIONS AND REPORTING

Upon completion of the analysis, calculate the final concentration of a given element in the test article ($\mu\text{g/g}$) from the solution element concentration ($\mu\text{g/mL}$) as follows:

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

where

C = concentration of analyte ($\mu\text{g/g}$)

A = instrument reading ($\mu\text{g/mL}$)

V_1 = volume of initial test article preparation (mL)

W = weight of test article preparation (g)

V_2 = total volume of any dilution performed (mL)

V_3 = aliquot of initial test article preparation used in any dilution performed (mL)

Similarly, calculate the final concentration of a given element in the test article ($\mu\text{g/g}$) from the solution element concentration (ng/mL) as follows:

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

C = concentration of analyte ($\mu\text{g/g}$)

A = instrument reading (ng/mL)

V_1 = volume of initial test article preparation (mL)

W = weight of test article preparation (g)

V_2 = total volume of any dilution performed (mL)

V_3 = aliquot of initial test article preparation used in any dilution performed (mL)^{▲USP34}