<233> ELEMENTAL IMPURITIES—PROCEDURES

INTRODUCTION

This chapter describes analytical procedures for the evaluation of elemental impurities that are suitable for the limits described in *Elemental Impurities – Limits* <232> and *Elemental Contaminants in Dietary Supplements* <2232>. Two procedures and criteria for the acceptability of alternative procedures are described. Alternative procedures that meet the validation requirements described herein are considered equivalent to Procedures 1 and 2 for the purposes of this test. In addition, system standardization and suitability evaluation using appropriate reference materials should be performed on the day of analysis. The test requirement is specified in *General Notices* or the individual monograph.

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

Strong acid : concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or Aqua Regia.

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

Target Elements: Elements with the potential to be present in the material under test. *Target Elements* must include lead, mercury, arsenic, and cadmium, and should include any of the remaining elemental impurities presented in General Chapter *Elemental Impurities – Limits <232>* used in the production of the material under test or the components therein. *Target Elements* should also include any other elements that may be added through material processing or storage or whose presence may interfere with the operation of the analytical procedures [Note: exclusion of elements from the list does not exempt the user from compliance with the requirement described in <232> or this chapter]

Target Limit or Target Concentration: The uppermost acceptance value for the elemental impurity being evaluated. Exceeding the Target limit would indicate that a material under test exceeds the acceptable value. The determination of compliance is addressed in other chapters. [Note: *Target Limits* can be approximated by dividing the *Modified Daily Dose PDEs* by the maximum daily dose for the Drug Product analysis option in <232> or the *Daily Serving PDE* divided by the maximum daily serving size in <2232>] (see *Elemental Impurities – Limits* <232> or *Elemental Contaminants in Dietary Supplements* <2232>).

J: The concentration (w/w) of the element(s) of interest at the *Target limit*, appropriately diluted to the working range of the instrument

Appropriate reference materials: Where "appropriate reference materials" are specified in the chapter, certified reference materials (CRM) from a national metrology institute (NMI) (e.g. NIST) or a reference materials that are traceable to the CRM of the NMI should be used.

COMPENDIAL PROCEDURES 1 AND 2

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

Verification
Before initial use, the analyst should ensure that the procedure is appropriate for the instrument and sample used by meeting the *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 is dependent on the material under test and is the responsibility of the analyst. When a sample preparation is not indicated in the monograph, an analyst may use any of the following appropriately verified preparation procedures. Samples and Blanks may be spiked with *Target Elements* where an analyte has limited solubility to the solvent system of choice. Standard solutions may contain multiple *Target Elements*. [NOTE—All liquid samples should be weighed.]

Neat: Used for liquids or alternative procedures that allow the examination of unsolvated samples.
Direct aqueous solution: Used when the sample is soluble in an aqueous solvent.
Direct organic solution: Used where the sample is soluble in an organic solvent.
Indirect solution: Used when a material is not directly soluble in aqueous or organic solvents. Digest the sample using a closed-vessel digestion procedure, similar to the procedure provided below. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the limit specified in the corresponding monograph or chapter.

Closed Vessel Digestion—This sample preparation procedure is designed for samples that must be digested in a strong acid using a closed vessel digestion apparatus. Closed vessel digestion minimizes the loss of volatile impurities. The choice of a strong acid is dependent upon the sample matrix. The use of any of these strong acids may be appropriate, but each introduces inherent safety risks. Therefore, appropriate safety precautions should be employed at all times. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the digestion apparatus used.]
An example procedure that has been shown to have broad applicability follows.

Dehydrate and predigest 0.5 g of primary sample in 5 mL of freshly prepared *Strong Acid*. Allow to sit loosely covered for 30 min in a fume hood. Add 10 mL more of *Strong Acid*, and digest, using a closed vessel technique, until digestion or extraction is complete. Repeat if necessary by adding 5 mL more of *Strong Acid*. [NOTE—Where closed vessel digestion is necessary, follow the manufacturer's recommended procedures to ensure safe usage.]

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: Dilute acid
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 sec) before introducing *Sample* to minimize carryover.]

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

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 to *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target Elements* at NMT 2*J*.
Blank: *Matched matrix*
Elemental Spectrometric System (see *Plasma Spectrochemistry* <730>)

Mode: ICP [NOTE—An instrument with a cooled spray chamber is recommended.]
Detector: Mass spectrometer
Rinse: Dilute *acid*
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 sec) before introducing *Sample* to minimize carryover.]

Analysis: Analyze per 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.]

PROCEDURE VALIDATION REQUIREMENTS

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 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 a procedure is appropriate for its intended purpose—that is, that it is an acceptable alternative procedure—will depend 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.

VALIDATION OF LIMIT PROCEDURES

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

The suitability of the method must be determined by conducting studies with test materials supplemented with known concentrations of each *Target Element* of interest at the appropriate acceptance limit concentration. The test materials must be spiked before any sample preparation steps are taken.

Limit of Detection

Standard solutions—A preparation of reference materials for the *Target Element(s)* at the *Target Concentration*.

Spiked sample solutions 1—A sample of material under test, spiked with appropriate reference materials for the *Target Elements* at the *Target Concentration*, prepared in a minimum of 3 preparations.
Spiked sample solutions 2 —A sample of material under test, spiked with appropriate reference materials at 80% of the *Target Concentration* for the *Target Elements*, prepared in a minimum of 3 preparations.

Acceptance Criteria—Each *Spiked sample solutions 1* provides a signal of intensity or value equivalent to or greater than that of the *Standard solutions*. *Spiked sample solutions 2* must provide a signal intensity or value less than that of the *Standard solution*. [NOTE—The signal from each sample obtained must show a change from the value obtained compared to a blank determination.]

Precision for Instrumental Methods (Repeatability)
[NOTE—Non-instrumental precision is demonstrated by meeting the *Limit of Detection* requirement above.]
Sample Solutions: Six independent samples of the material under test, spiked with appropriate reference materials for the *Target Elements* at the indicated levels

Acceptance Criteria: Relative standard deviation, NMT 20% for each *Target Element*.

Specificity - the procedure must be able to unequivocally assess each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements* and matrix components

VALIDATION OF QUANTITATIVE PROCEDURES

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

Accuracy
Standard solutions: Prepare solutions containing the *Target Elements* at concentrations ranging from 50% to 150% of their indicated limit values, using appropriate reference materials.
Test Samples: Prepare samples of the material under test spiked with appropriate reference materials prior to any sample preparations steps (digestion or solubilization) at concentrations ranging from 50% to 150% of the indicated limit value 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, 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*

Intermediate Precision
Perform the *Repeatability* analysis

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

Executing only one of the three experiments listed is required to demonstrate intermediate precision.

Acceptance Criteria: Relative standard deviation, NMT 25% for each *Target Element*

Specificity - the procedure must be able to unequivocally assess each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements* and matrix components

Limit of Quantitation, Range, and Linearity—Demonstrated by meeting the *Accuracy* requirement.

Reagents Section of the USP-NF

Aqua Regia, [*8007-56-5*]—A mixture of ultra pure nitric acid and hydrochloric acid (1:3) prepare freshly as needed.
Nitric Acid, Ultra pure, HNO_3—**63.01** [7697-37-2] Nitric Acid with sub-ppm levels of elemental impurities and other metals