

**LIMITS OF ELEMENTAL CONTAMINANTS**

The levels of elemental contaminants should be restricted as shown in *Table 1. Elemental Limits* unless otherwise stated in the individual monograph.

**BRIEFING**

**⟨2232⟩ Elemental Contaminants in Dietary Supplements.** This new dietary supplement general chapter is developed to replace general chapter *Heavy Metals* ⟨231⟩ for dietary supplements and dietary ingredients. The term elemental contaminants is adopted here as an alternative to the term “heavy metals”. The limits presented in this chapter are based on in-depth review of the toxicological literature and discussions involving several experts in metals toxicology. These limits are based on documented toxicity and recommendations from regulatory agencies and international/trade organizations and focus on the four most toxic and well-understood elements (Pb, Hg, As, and Cd). The chapter provides the default methods of analysis which include speciation methods for inorganic As (FCC Kelp monograph) and methylmercury (AOAC Official Method 990.04). This chapter also describes three separate options for determination of compliance to the limits. These options are similar to those presented in general chapter *Residual Solvents* (467).

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**Add the following:**

**▲⟨2232⟩ ELEMENTAL CONTAMINANTS IN DIETARY SUPPLEMENTS**

**INTRODUCTION**

The objective of this general chapter is to limit the amounts of elemental contaminants in dietary supplements labeled as conforming to *USP* or *NF* standards. This general chapter applies to dietary supplements only. Drug products and their ingredients are addressed in general chapter *Elemental Impurities—Limits* ⟨232⟩.

The focus of this general chapter is on the four major elements of toxicological concern: arsenic, cadmium, lead, and mercury (Class 1 elements in *Elemental Impurities—Limits* ⟨232⟩). The extent of testing can be determined using a risk-based approach considering the likelihood of contamination. Manufacturers should consider the presence of unexpected elemental contaminants when the manufacturers determine compliance.

**Table 1. Elemental Limits**

Element	Individual Component Limit <sup>a</sup> (µg/g)	PDE <sup>b</sup> (µg/day)
Arsenic (inorganic) <sup>c</sup>	1.5	15
Cadmium	0.5	5
Lead	1.0	10
Mercury (total)	1.5	15
Methylmercury (as Hg) <sup>d</sup>	0.2	2

<sup>a</sup> The limits for individual components are based on a maximum daily intake of 10 g of a dietary supplement and are intended for use only with *Options for Compliance with Limits of Elemental Contaminants* under *Individual Component Option*.

<sup>b</sup> Permitted Daily Exposure (PDE) is derived from the Provisional Tolerable Weekly Intake (PTWI) that is recommended by the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) by subtracting the daily exposure (µg/day) to each elemental contaminant from air, food, and drinking water. A body weight of 50 kg and a safety factor are used to calculate the PDE.

<sup>c</sup> Arsenic may be measured using a nonspeciation procedure under the assumption that all arsenic contained in the supplement is in the inorganic form. Where the limit is exceeded using a nonspeciation procedure, compliance with the limit for inorganic arsenic shall be demonstrated on the basis of a speciation procedure.

<sup>d</sup> Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury. Specific monographs may provide exceptions for articles that may need to be consumed in larger quantities in order to justify the claims.

**OPTIONS FOR COMPLIANCE WITH THE LIMITS OF ELEMENTAL CONTAMINANTS**

In order for a dietary supplement to comply with the limit for elemental contaminants as described in this chapter, the level of elemental contaminant in the finished dietary supplement should be NMT the PDE. The





## ANALYTICAL PROCEDURES FOR TOTAL ELEMENTAL CONTAMINANTS

Performance-based methodology for analysis of total elemental contaminants in general chapter *Elemental Impurities—Procedures* (233) is applicable for dietary supplements. The validation necessity will vary depending on the situation. In all three options described in the section *Options for Compliance with the Limits of Elemental Contaminants*, the use of *Validation of Limit Procedures* (see *Elemental Impurities—Procedures* (233)) may be appropriate. However, for the *Summation Option* acceptable levels of validation must be determined on a case-by-case basis. Validation of a procedure using the *Validation of Quantitative Procedures* (see *Elemental Impurities—Procedures* (233)) is acceptable for all options under all circumstances and is generally preferred. The determination of the level of validation necessity is at the discretion of the manufacturer and the competent regulatory authority.

## ANALYTICAL PROCEDURE FOR INORGANIC ARSENIC

Where the level of total arsenic exceeds the limit recommended in this chapter, speciation may be used to determine the amount of inorganic arsenic present. The following procedure is suggested for determination of inorganic arsenic, but any validated procedure shown to give equivalent or better results can be used.

### Apparatus

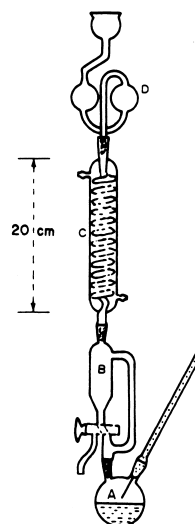


Figure 1. Special apparatus for the determination of inorganic arsenic. (A, 250-mL distillation flask; B, receiver chamber, approximately 50-mL capacity; C, reflux condenser; D, splash head.)

### Reagents

**Distillation-Reducing Solution**—72 mg/mL of ACS-grade, low-arsenic, ferrous chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ) in 6.6 N hydrochloric acid. [NOTE—Prepare fresh on the day of use.]

**Control**—6.0  $\mu\text{g}$  of As (6.0 mL of *Standard Arsenic Solution*.) [NOTE—Use this amount rather than the 3.0 mL specified for *Standard Preparation* under general chapter *Arsenic, Method I* (211).]

### Sample Solution

Take a 2.00-g sample that has previously been ground to pass through a 60-mesh screen and transfer to a distillation flask (A). Add 50 mL of *Distillation-reducing Solution*, connect the flask to the receiver chamber (B), complete the assembly of the apparatus, and begin circulating tap water through the condenser (C). Half-fill the lower two bulbs of the splash head (D) with water. Maneuver the stopcock to cause the contents of the re-





gen inlet and mercury vapor outlet, respectively. Connect the nitrogen inlet through the flowmeter and the mercury outlet to the test tube trap by means of spaghetti-type tubing. Connect the nitrogen tank to the flowmeter by means of spaghetti-type tubing and standard Swagelok fittings and unions. Connect the outlet from the LC column to the 0.01-inch (0.25-mm) ID stainless steel tube, which is connected to the inlet of the heating tube by standard 1/16-inch (1.6-mm) Swagelok fittings and zero dead volume union. Connect the outlet of the test tube trap (spaghetti tubing, item 11) to the AAS cell by the small rubber stopper inserted into the side arm of the cell.

### Operating Conditions for the HPLC/AAS Interface

**Turning the System ON**—(1) Adjust the *Mobile Phase* flow rate to 0.7 mL/min. (2) Introduce water into the condenser. (3) Adjust the nitrogen sweep to 0.1 L/min (tank pressure 15 psi (1.04 kPa) and 10.0 setting on the flowmeter). (4) Gradually adjust the temperature of the interface heater to 550° (transformer setting approximately 65). (5) After the temperature reaches 550°, check the system stability by injecting several aliquots of methylmercury standard solutions. (The retention time of methylmercury is 5–6 minutes.)

The precision between the methylmercury peak heights should be NMT 5%. Inject all standard solutions to check linearity. If these parameters cannot be achieved, check for leaks or, after long use, replace the effluent tubing. [NOTE—To conserve analytical standard solutions, another set of standards of the same concentration may be prepared by direct dilution of *Methylmercuric Chloride Stock Standard Solution* with *Sodium Thiosulfate Solution*. Use these standards only for instrument checking. To prepare solutions of 0.05, 0.100, 0.150, 0.200, and 0.250 µg Hg/100 µL, dilute 100 µg

Hg/mL *Methylmercuric Chloride Stock Standard Solution* with *Sodium Thiosulfate Solution* as follows: 1, 1, 3, 2, and 5 mL to 200, 100, 200, 100, and 200 mL, respectively.]

**Turning the System OFF**—(1) Turn off the interface heater, and let the system cool to near room temperature. (2) Shut off other components, but do not shut off the *Mobile Phase* flow while the heater is hot. If this is done, carbon may deposit and clog the effluent tube. For the same reason do not pump neat organic solvents, such as methanol, to clean the column while the heater is hot. (3) After the heater has cooled to room temperature, pump methanol to rinse the column.

### Preparation of Test Solutions

**For Supplements in Tablet Form**—Weigh and finely powder not fewer than 20 tablets. Transfer an accurately weighed portion of about 10.0 g of the powder to a 100-mL beaker. Prepare an analytical mixture by adding *Hydrochloric Acid Solution* so that the mass of the analytical portion of the powdered tablets plus the mass of the *Hydrochloric Acid Solution* totals  $25.00 \pm 0.30$  g. Blend the analytical mixture in a homogenizer (approximately 1 minute) to obtain a fine suspension. Immediately weigh 10.0 g of the fine suspension into a beaker containing 10 g of *Chromatographic Siliceous Earth*, and mix well. Quantitatively transfer the mixture to a glass chromatographic column containing a pledget of glass wool at the bottom. Compact the mixture moderately with a tamping rod to a height of approximately 8 cm, and place the pledget of glass wool on top. Elute the column by adding 20 mL followed by four 5-mL aliquots of chloroform. Collect the first 20 mL of the eluate in a tall 25-mL glass-stoppered graduated cylinder. Add 4.0 mL of *Sodium Thiosulfate Solution*, shake the mixture gently for 1 minute, and let stand 5 minutes. Transfer the upper aqueous layer con-



$$\text{Result (mg/kg)} = (R_T/R_S) \times (W_S/W_T)$$

$R_T$  = average peak height of the *Test Solution* (A)

$R_S$  = average peak height of the *Standard Solution* (A)

$W_S$  = amount of standard injected ( $\mu\text{g Hg}$ )

$W_T$  = amount of analytical portion injected (g),

and

$$W_T = (D/E) \times [F \times (0.100 \text{ mL}/4.0 \text{ mL})]$$

D = weight of the analytical portion (g)

E = weight of the analytical mixture prepared (g)

F = weight of the analytical mixture added to the  
*Chromatographic Siliceous Earth* (g)

If necessary, correct the peak height for the *Test Solution* using the response of the diluted *Reagent Blank Solution*.

The quantitation limit, defined as 10 standard deviations of the reagent blank, is 0.006  $\mu\text{g Hg}/100 \mu\text{L}$  injected. This corresponds to a quantitation limit of 0.06  $\mu\text{g Hg/g}$  for a 10-g analytical portion treated according to the procedure. The intraday variation, calculated as the standard deviation of 5 replicate injections of duplicate sample preparations, is NMT 0.12 and the relative standard deviation is NMT 20%.▲USP34