BRIEFING

〈2232〉 Elemental Contaminants in Dietary Supplements, page 258 of *PF* 36(1) [Jan.–Feb. 2010]. This previously proposed general information chapter was scheduled to become official in *USP 34*. However, due to the postponement of related general test chapters *Elemental Impurities—Limits* 〈232〉 and *Elemental Impurities—Procedures* 〈233〉 by the General Chapters Expert Committee, the official publication was suspended. This chapter has been modified to reflect changes made in chapters 〈232〉 and 〈233〉, as well as comments received. This new version includes clarifying language to indicate that the chapter is intended only for dietary supplement dosage forms. The chapter describes three separate options to determine compliance with the limits. Examples of calculations have been removed.

(GCCA: C. Okunji.) Correspondence Number—C91568

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.~~

~~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.~~

~~Table 1. Elemental Limits~~

~~Element~~	~~Individual Component Limit[a] (µg/g)~~	~~PDE[b] (µg/day)~~
~~Arsenic (inorganic)[c]~~	~~1.5~~	~~15~~

Cadmium	0.5	5
Lead	1.0	10
Mercury (total)	1.5	15
Methylmercury (as Hg)[d]	0.2	2

[a] 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*.

[b] 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.

[c] 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.

[d] 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 following three options are available when manufacturers determine compliance with the limits for elemental contaminants in dietary supplements.

Dietary Supplement Analysis Option

This option is generally applicable. In this option the results obtained from the analysis of a typical serving size, scaled to a maximum daily intake, are compared to the PDE, as stated in *Table 1*.

Calculate the measured value (in µg of contaminant)/daily intake as:

$$\text{Result} = MVSS \times N$$

MVSS = measured value (in µg of contaminant)/serving size
N = maximum number of serving sizes recommended/day.

Acceptance Criteria: The measured value/daily intake is NMT the PDE value given in *Table 1*.

Individual Component Option

For dietary supplements with a maximum daily intake of NMT 10 g, the product meets the requirements when each component meets the limits given for the *Individual Component Limit* in *Table 1*. If all components in a formulation meet the limits given for the *Individual Component Limit*, these components can be used in any proportion. No further calculation is necessary.

Summation Option

This option can be used for products that are consumed in quantities greater than 10 g/day or where any component of the dietary supplement exceeds the applicable *Individual Component Limit*. The PDE, as stated in *Table 1*, can be used to calculate the concentration of elemental contaminants allowed in a dietary supplement. Apply this option by separately adding the

amounts of each elemental contaminant (in µg/day) present in each of the components of the dietary supplement product using the following equation:

$$\text{Result} = \Sigma_{n}^{1}(C_i \times W_i)$$

n = each component used to manufacture the dietary supplement product

C_i = element concentration in that component (µg/g)

W_i = weight of each component in the dietary supplement (weight in g of the maximum number of serving sizes/day).

Examples

Consider an example of the application of the different options to the lead concentration in a dietary supplement. The PDE is 10 µg per day and the *Individual Component Limit* is 1.0 µg/g (ppm). The maximum administered daily weight of a dietary supplement product is 5.0 g, and the dietary supplement contains two additional components. The composition of the dietary supplement and the calculated maximum content of lead are given in *Table 2*.

Table 2

Component	Amount of Component/ Daily Intake (g)	Lead Content (µg/g)	Daily Exposure (µg/day)
Dietary Ingredient	0.3	3.0	0.9
Additional Component 1	0.9	1.0	0.9
Additional Component 2	3.8	1.5	5.7
Dietary Supplement	5.0	—	7.5

Additional Component 1 meets the *Individual Component Limit*, but the *Dietary Ingredient* and the *Additional Component 2* do not. Thus, the *Individual Component Option* cannot be used. Nevertheless, with the *Summation Option* the dietary supplement product meets the PDE limit of 10 µg/day and thus conforms to the acceptance criteria in this general chapter.

Consider another example using cadmium as the elemental contaminant. The certificates of analysis from the suppliers of each component indicate that the dietary ingredient contains NMT 0.4 µg/g and the additional components contain NMT 0.5 µg/g each. The serving size is two capsules, the weight of each capsule is 2.5 g, and the maximum number of servings/day is three. The daily intake/weight of the dietary supplement is therefore 2.5 × 2 × 3 = 15.0 g. The composition of the dietary supplement and the calculated maximum content of cadmium based on the certificates of analysis from the suppliers are given in *Table 3*.

Table 3

Component	Amount of Component/ Daily Intake (g)	Cadmium Content (µg/g)	Daily Exposure (µg/day)
Dietary Ingredient	1.0	0.4	0.4
Additional Component 1	2.6	0.5	1.3
Additional Component 2	11.4	0.5	5.7
Dietary Supplement	15.0	—	7.4

In this example the *Individual Component Option* is not applicable because the maximum daily intake is more than 10g. The dietary supplement exceeds the limit for cadmium in *Table 1* using certificates of analysis from suppliers and the *Summation Option*. Although the cadmium content is within the *Individual Component Limit* (µg/g), the dietary supplement product fails for cadmium PDE using the *Dietary Supplement Analysis Option* considering the amount of daily intake.

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

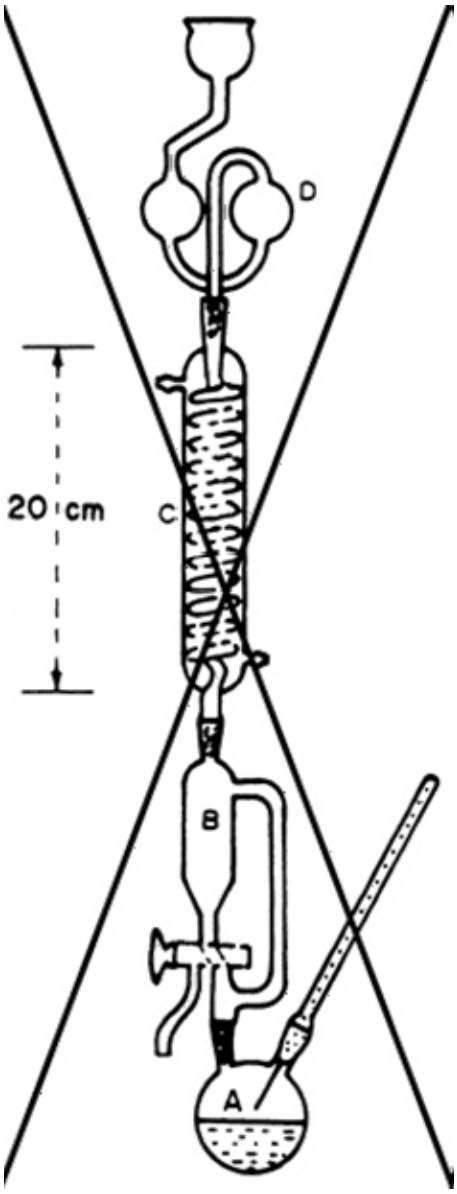

Figure 1. Special apparatus for the determination of inorganic arsenic. (*A*, 250-mL distillation flask; *B*, receiver chamber, approximately 50-mL capacity; *C*, reflux condenser; *D*, splash head.)

Reagents

Distillation-Reducing Solution—72 mg/mL of ACS-grade, low-arsenic, ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) in 6.6 N hydrochloric acid. [NOTE—Prepare fresh on the day of use.]

Control—6.0 µg 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 receiver chamber to drain into the distillation flask, heat the flask until the temperature above the solution reaches 106° to 108°, and continue refluxing at this temperature for 45 min. Close the stopcock, continue heating at 108° to 110°, and collect 30 to 33 mL of distillate in the receiver chamber. Remove the heating source and allow the temperature to drop to about 80°.

Drain the distillate from the receiver chamber into a 250-mL beaker that is contained in an ice-water bath. Close the stopcock, and add a second 50-mL portion of the *Distillation-reducing Solution* through the thermometer opening to the distillation flask. Replace the thermometer, increase the temperature to 108° to 110°, and collect a second 30- to 33-mL portion of distillate in the receiver chamber.

Drain the second distillate into the beaker containing the first portion, and continue cooling in the ice-water bath until the combined distillate cools to room temperature. Remove the splash head, and wash its contents into the beaker. Also, wash down the inside of the condenser and receiver chamber with water, collecting the washings in the beaker. Filter the beaker contents through a Whatman No. 40, or equivalent, filter paper, collecting the filtrate in a 300-mL Erlenmeyer flask having a 24/40 standard-taper joint, to be used later as an arsine generator flask. Wash the filter three times with water so that the final volume of filtrate measures 200 mL.

Analysis

Add 2 mL of potassium iodide TS and 0.5 mL of stronger acid stannous chloride TS to the *Sample Solution* contained in the Erlenmeyer flask, and proceed as directed in the *Procedure* under general chapter *Arsenic, Method I* ⟨ 211 ⟩ beginning with "Allow to stand at room temperature for 30 minutes."

ANALYTICAL PROCEDURE FOR METHYLMERCURY

Where methylmercury determination is required, the following procedure is suggested. However, any validated procedure shown to give equivalent or better results can be used.

Apparatus

[NOTE—Wash all glassware with a laboratory detergent, and rinse thoroughly with hot tap water followed by purified water. Rinse with acetone, and let dry.]

The system consists of an HPLC connected through an interface to an atomic absorption detector for mercury determination at 253.7 nm with a mercury hollow-cathode lamp, deuterium background corrector, and gas flow-through cell with open ends or quartz closed ends (10–25-mm ID × 100–115 mm).

Interface Assembly (see **Figure 2**)—Consists of the following components:

1. *Heater*—With 1-inch thick magnesia and alumina insulation
2. *Flowmeter*
3. *Temperature-indicating device*—Ranging from 0° to 1370°
4. *Short condenser*—175 mm jacket length, standard taper 24/40
5. *Rubber stopper*—No. 5, solid neoprene. [NOTE—A suitable rubber stopper is available as No. 14–141F from Fisher Scientific Co.]
6. *Stainless steel tubing*—1/16 inch (1.6 mm) OD, 0.04 inch (1 mm) ID
7. *Trap*—Test tube, 125×15 mm
8. *Boiling flask*—2 neck, 500 mL [NOTE—A suitable boiling flask is available as Kontes No. 605000.]

9. *Stainless steel tubing*—Two 6-inch (15 cm) lengths
10. *Plastic tubing*—Spaghetti type, 0.057–0.067 inch (1.45–1.7 mm) ID
11. *Plastic tubing*—Spaghetti type, as connector to AAS system
12. *Electrical connection*—Standard 120-V plug to variable-voltage transformer

Atomic Absorption Spectrophotometer (AAS)—Follow the manufacturer's operating instructions for mercury determination at 253.7 nm with deuterium background correction. Typical response for an injection of 0.100 µg Hg/100 µL standard is approximately 0.20 A using a cell of 25-mm ID × 115-mm. Use a recording device set to obtain approximately 30%–50% full scale for an injection of 0.100 µg Hg/100 µL standard. The working range is between approximately 0.01 and 0.25 µg Hg/100 µL injected.

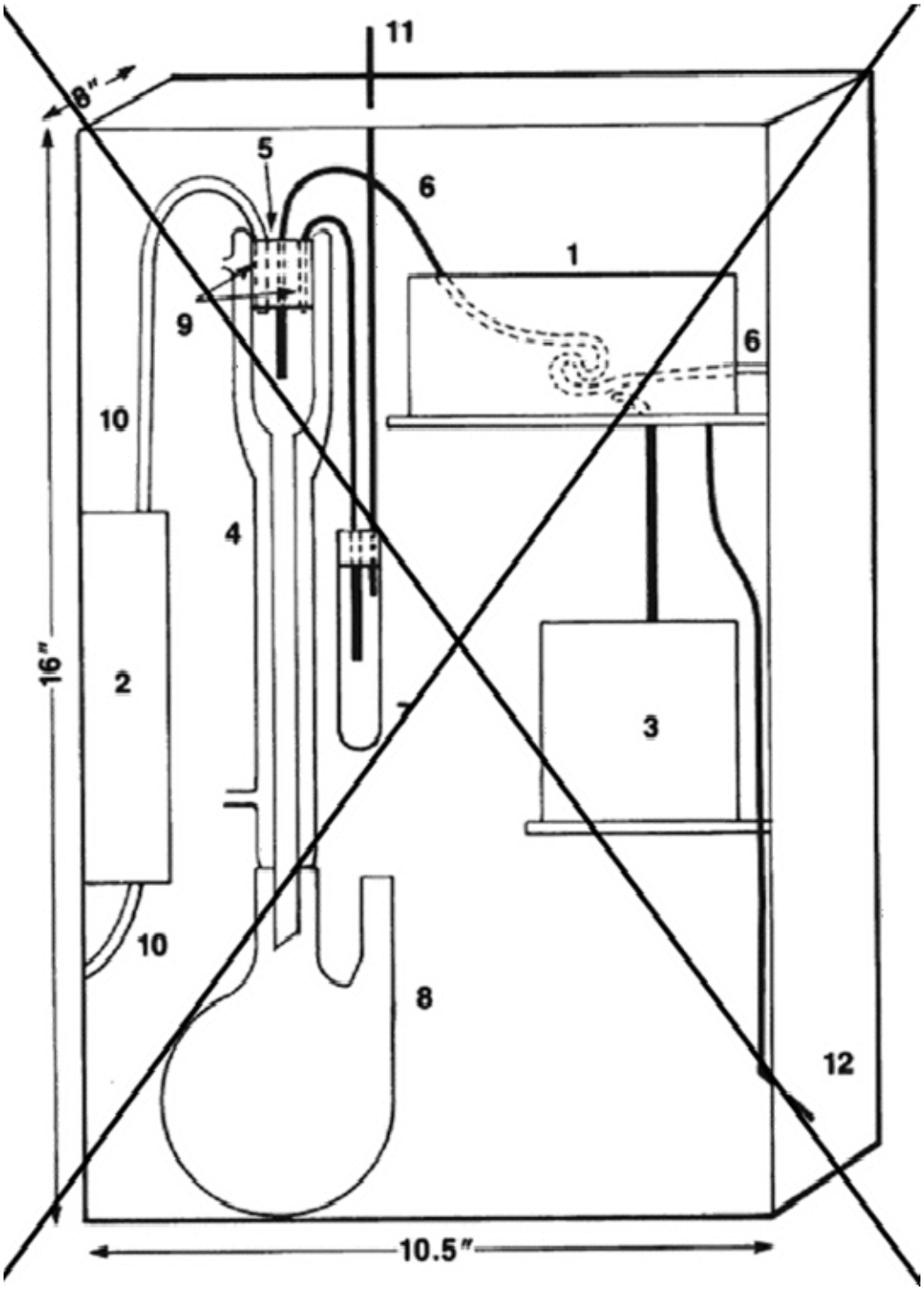

Figure 2. A diagram of the HPLC/AAS interface.

Reagents

[NOTE—Use water double-distilled in glass.]

Sodium Thiosulfate Solution—Use a 0.01M solution.

Hydrochloric Acid Solution—Use a 1.8M solution.

Chromatographic Siliceous Earth—Acid-washed [NOTE—A suitable grade is available as acid-washed Celite 545.]

Methylmercuric Chloride Stock Standard Solution—Use a solution equivalent to 100 µg/mL of mercury from methylmercuric chloride prepared by dissolving 125 mg of methylmercuric chloride in 20 mL of methanol and diluting with water to 1 L.

Ammonium Acetate Solution— Use a 0.05*M* solution.

Mobile Phase— Methanol and ammonium acetate solution (3:2) adjusted with glacial acetic acid to a pH of 5.7±0.2. Add 0.1 mL of 2-mercaptoethanol/L immediately before use.

Instrument Set-up

Figure 2 is a diagram of the HPLC/AAS interface. Components are placed inside a shop-made box of the dimensions shown. The box has a Plexiglas door at the front, and the back and top are removable. Items 1–3 are bolted to the sides of the box. Set up the remaining items as follows: Bend a 30-inch (76 cm) stainless steel tubing (item *6*) as shown to provide additional heating surface. Place the bent portion, together with the thermocouple element, between 2 disks of the heater held tightly together by a screw at the center of the upper disk. Enclose the heater assembly in 1-inch (25-mm) thick magnesia–alumina insulation, and secure to the aluminum plate support by means of the aluminum cover and screws. Push the stainless steel tubing from the heater outlet through the center of the rubber stopper (item *5*) so that the end of the tubing is near the constructed portion of the condenser when the stopper is inserted tightly into the top of the condenser. Push two additional 6-inch (15-cm) lengths of the stainless steel tubing through the rubber stopper to serve as the nitrogen 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 ± 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 containing the methylmercury–thiosulfate complex together with any emulsion into a 25-mL Erlenmeyer flask. Blow a moderately strong stream of nitrogen into the flask for 1–2 minutes to break up any emulsion, and expel droplets of chloroform. [NOTE—To aid in breaking the emulsion, hold and rotate the flask at a 45-degree angle with one hand, and direct the nitrogen stream at the thin layer of emulsion that adheres to the bottom of the flask as it rotates.]

[NOTE—Some supplements may produce cloudy extracts. If this occurs, the extract can be passed through a membrane filter.]

For Supplements in Capsule Form—Weigh accurately not fewer than 20 capsules and determine the average weight. Place a number of capsules equivalent to about 10.0 g in a 100-mL beaker, and add the *Hydrochloric Acid Solution* so that the mass of the analytical portion of capsules taken plus the mass of the *Hydrochloric Acid Solution* totals 25.00 ± 0.30 g. Proceed as directed in *For Supplements in Tablet Form* beginning with "Blend the analytical mixture..."

For Supplements in Liquid Form—Weigh accurately 10.0 g of the liquid in a 100-mL beaker, and prepare an analytical mixture by adding *Hydrochloric Acid Solution* so that the mass of the analytical portion of the dietary supplement liquid taken plus the mass of the *Hydrochloric Acid Solution* totals 25.00 ± 0.30 g. Proceed as directed in *For Supplements in Tablet Form* beginning with "Blend the analytical mixture..."

Preparation of the Reagent Blank Solution

Prepare the reagent blank analytical solution by weighing 25.00 g of *Hydrochloric Acid Solution* into a 100-mL beaker. Proceed as directed in *Preparation of Test Solutions—For Supplements in Tablet Form*, beginning with "Immediately weigh 10.0 g..."

Preparation of Standard Solutions

Prepare 0.050, 0.100, 0.150, 0.200, and 0.250 µg Hg/100 µL of *Standard Solutions* by adding, respectively, 20-, 40-, 60-, 80-, and 100-µL aliquots of *Methylmercuric Chloride Stock Standard Solution* to 20 mL of chloroform in separate 25-mL glass-stoppered graduated cylinders. Proceed as directed in *Preparation of Test Solutions—For Supplements in Tablet Form*, beginning with "Add 4.0 mL of *Sodium Thiosulfate Solution*..."

Chromatographic System

The liquid chromatograph is equipped with a 4.6-mm × 25-cm L1 column and a 2.1-mm × 7-

~~cm L2 guard column. Inject a 100-µL aliquot of *Test Solution* (0.100 g injected for 10.0 g analytical portion) into the HPLC/AAS system. After the methylmercury peak appears, inject a 100-µL aliquot of *Standard Solution* that produces a peak height equal to or slightly higher than the *Test Solution* peak height. Repeat by injecting the *Test Solution* again followed by the selected *Standard Solution*. If the *Test Solution* peak height is higher than the peak height for the highest standard, dilute quantitatively an appropriate aliquot of *Test Solution* with *Sodium Thiosulfate Solution*. Account for the dilution in the final calculation.~~

~~Calculations~~

~~Additional dilutions must be accounted for in the final calculation. *Do not vary the injection volume.*~~

~~Measure peak heights above the base line, and calculate the methyl-bound mercury concentration in the test portion, in µg Hg/g, by comparing the average peak heights of the *Test Solution* to the average peak heights of the *Standard Solution* as follows:~~

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

~~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 (µg 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 µg Hg/100 µL injected. This corresponds to a quantitation limit of 0.06 µg 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%.~~

The objective of this general chapter is to limit the amounts of elemental contaminants in finished dietary supplement dosage forms labeled as conforming to *USP* or *NF* standards. This general chapter is not intended to set limits for dietary ingredients. Those limits are set in the corresponding individual monographs.
The focus of this general chapter is on the four major elements of toxicological concern: arsenic, cadmium, lead, and mercury. The extent of testing can be determined using a risk-based approach that takes into account the likelihood of contamination. Manufacturers should consider the presence of unexpected elemental contaminants to determine compliance.

LIMITS OF ELEMENTAL CONTAMINANTS

The levels of elemental contaminants should be restricted as shown in *Table 1* unless otherwise stated in the individual monograph. Specific monographs may provide different limits for articles that need to be consumed in large quantities.

Table 1

Element	PDE (µg/day)[a]
Arsenic (inorganic)	15
Cadmium	5
Lead	10
Mercury (total)	15
Methylmercury (as Hg)	2

[a] 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. Other regulations (i.e.: Proposition 65 in California) may require different limits; manufacturers are responsible for compliance with applicable local requirements differing from these PDE values.

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. Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.

OPTIONS FOR COMPLIANCE WITH THE LIMITS OF ELEMENTAL CONTAMINANTS

In order for a dietary supplement finished dosage form to comply with the limits for elemental contaminants as described in this chapter, the level of elemental contaminant in the finished dietary supplement should be NMT the PDE. The following three options are available for determining compliance with the limits for elemental contaminants in dietary supplements.

Dietary Supplement Analysis Option

This option is generally applicable. In this option the finished dietary supplement dosage form is analyzed according to the procedures in the general chapter *Elemental Impurities—Procedures* ⟨ 233 ⟩ or the speciation procedures given in this chapter. The results obtained from the analysis of a typical serving size, scaled to a maximum daily intake, are compared to the PDE, as stated in *Table 1*.

Analysis: Proceed as directed below in this chapter.

Calculate the measured amount of each elemental contaminant, in µg/daily intake, as:

$$\text{Result} = MVSS \times N$$

MVSS = measured amount of each elemental contaminant (µg /serving size)
N = maximum daily intake of the supplement recommended in the labeling (servings/day).

Acceptance criteria: The measured amount/daily intake is NMT the PDE value given in *Table 1*.

Individual Component Option

This option is applicable to finished dietary supplement dosage forms with a maximum daily intake of NMT 10 g of the dietary supplement finished product.

Analysis: Unless otherwise specified in the individual monograph, proceed with the individual ingredient as directed below in this chapter.

Acceptance criteria: The product meets the requirements when each component used to prepare the finished dietary supplement meets the limits given in *Table 2*.

Table 2

Element	Individual Component Limits (µg/g)[a]
Arsenic (inorganic)[b]	1.5
Cadmium	0.5
Lead	1.0
Mercury (total)	1.5
Methylmercury (as Hg)[c]	0.2

[a] 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*, *Individual Component Option*.

[b] 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.

[c] Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.

[NOTE—If all components in a formulation meet the limits given for the *Individual Component Limits*, these components can be used in any proportion. No further calculation is necessary.]

Summation Option

This option can be used for finished dietary supplement dosage forms that are consumed in quantities greater than 10 g/day or where the acceptance limit for any contaminant in any component of the dietary supplement exceeds the applicable *Individual Component Limit*.

Analysis: Unless otherwise specified in the individual monograph, proceed with the individual ingredient as directed below in this chapter.

Calculate the amount of each elemental contaminant, in µg/daily intake, present in the finished dietary supplement dosage form:

$$\text{Result} = \Sigma(C_i \times W_i) \times N$$

C_i = elemental contaminant concentration in the individual component (µg/g)
W_i = weight of each individual component per serving of the dietary supplement (g/serving)
N = maximum daily intake of the supplement recommended in the labeling (servings/day)

Acceptance criteria: The calculated amount of each elemental contaminant/daily intake is NMT the PDE value given in *Table 1*.

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

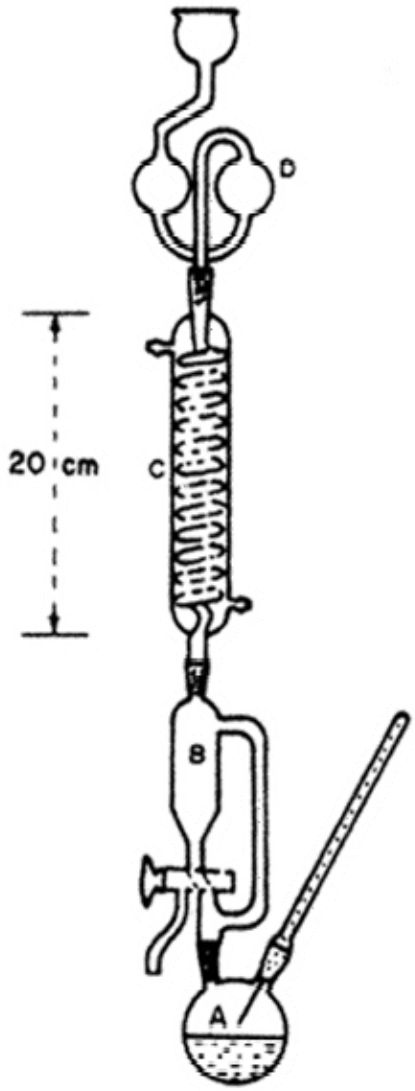

Figure 1. Special apparatus for the determination of inorganic arsenic (A, 250-mL distillation

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 ($FeCl_2 \cdot 4H_2O$) in 6.6 N hydrochloric acid. [NOTE—Prepare fresh on the day of use.]

Control: 6.0 µg of As (6.0 mL of *Standard Arsenic Solution*). [NOTE—Use this amount rather than the 3.0 mL specified for *Standard Preparation* in the general chapter *Arsenic* ⟨211⟩, *Method I*.]

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). To the flask 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 receiver chamber to drain into the distillation flask, heat the flask until the temperature above the solution reaches 106°–108°, and continue refluxing at this temperature for 45 min. Close the stopcock, continue heating at 108°–110°, and collect 30–33 mL of distillate in the receiver chamber. Remove the heating source, and allow the temperature to drop to about 80°.

Drain the distillate from the receiver chamber into a 250-mL beaker that is contained in an ice-water bath. Close the stopcock, and add a second 50-mL portion of the *Distillation-reducing solution* through the thermometer opening to the distillation flask. Replace the thermometer, increase the temperature to 108°–110°, and collect a second 30–33-mL portion of distillate in the receiver chamber.

Drain the second distillate into the beaker containing the first portion, and continue cooling in the ice-water bath until the combined distillate cools to room temperature. Remove the splash head, and wash its contents into the beaker. Also, wash down the inside of the condenser and receiver chamber with water, collecting the washings in the beaker. Pass the beaker contents through a Whatman No. 40, or equivalent, filter paper, collecting the filtrate in a 300-mL Erlenmeyer flask having a 24/40 standard-taper joint, to be used later as an arsine generator flask. Wash the filter three times with water so that the final volume of filtrate measures 200 mL.

Analysis: Add 2 mL of potassium iodide TS and 0.5 mL of stronger acid stannous chloride TS to the *Sample solution* contained in the Erlenmeyer flask, and proceed as directed in the *Procedure* in *Arsenic* ⟨211⟩, *Method I*, *Procedure*, beginning with “Allow to stand at room temperature for 30 min.”

Analytical Procedure for Methylmercury

Where methylmercury determination is required, the following procedure is suggested. However, any validated procedure shown to give equivalent or better results can be used.

Apparatus

NOTE—Wash all glassware with a laboratory detergent, and rinse thoroughly with hot tap water followed by purified water. Rinse with acetone, and let dry.

The system consists of an HPLC connected through an interface to an atomic absorption detector for mercury determination at 253.7 nm with a mercury hollow-cathode lamp, deuterium background corrector, and gas flow-through cell with open ends or quartz closed

ends (10–25-mm ID × 100–115 mm).

Interface assembly (see *Figure 2*)**:** Consists of the following components:

1. *Heater*—With 1-inch thick magnesia and alumina insulation
2. *Flowmeter*
3. *Temperature-indicating device*—Ranging from 0°–1370°
4. *Short condenser*—175-mm jacket length, standard taper 24/40
5. *Rubber stopper*—No. 5, solid neoprene. [NOTE—A suitable rubber stopper is available as No. 14–141F from Fisher Scientific Co.]
6. *Stainless steel tubing*—1/16 inch (1.6 mm) OD, 0.04 inch (1 mm) ID
7. *Trap*—Test tube, 125 × 15 mm
8. *Boiling flask*—2 neck, 500 mL [NOTE—A suitable boiling flask is available as Kontes No. 605000.]
9. *Stainless steel tubing*—Two 6-inch (15-cm) lengths
10. *Plastic tubing*—Spaghetti type, 0.057–0.067 inch (1.45–1.7 mm) ID
11. *Plastic tubing*—Spaghetti type, as connector to AAS system
12. *Electrical connection*—Standard 120-V plug to variable voltage transformer

Atomic absorption spectrophotometer (AAS): Follow the manufacturer's operating instructions for mercury determination at 253.7 nm with deuterium background correction. Typical response for an injection of 0.100 µg Hg/100 µL standard is approximately 0.20 A using a cell of 25-mm ID × 115-mm. Use a recording device set to obtain approximately 30%–50% full scale for an injection of 0.100 µg Hg/100 µL standard. The working range is approximately 0.01–0.25 µg Hg/100 µL injected.

Figure 2

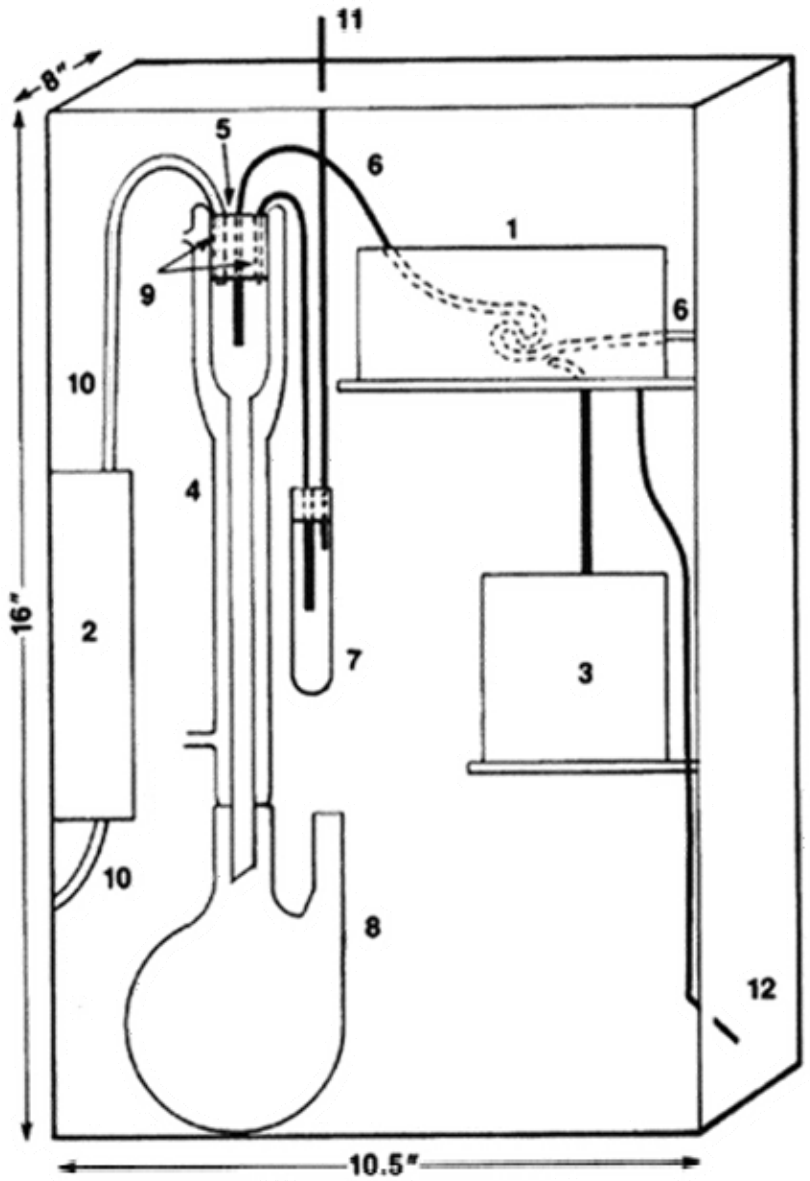

Figure 2. A diagram of the HPLC/AAS interface.

Reagents [NOTE—Use water double-distilled in glass.]

Sodium thiosulfate solution: Use a 0.01 M solution.

Hydrochloric acid solution: Use a 1.8 M solution.

Chromatographic siliceous earth: Acid-washed. [NOTE—A suitable grade is available as acid-washed Celite 545.]

Methylmercuric chloride stock standard solution: Use a solution equivalent to 100 µg/mL of mercury from methylmercuric chloride prepared by dissolving 125 mg of methylmercuric chloride in 20 mL of methanol and diluting with water to 1 L.

Ammonium acetate solution: Use a 0.05 M solution.

Mobile phase: Methanol and ammonium acetate solution (3:2) adjusted with glacial acetic acid to a pH of 5.7 ± 0.2. Add 0.1 mL of 2-mercaptoethanol per L immediately before use.

Instrument Set-Up

Figure 2 is a diagram of the HPLC/AAS interface. Components are placed inside a shop-made box of the dimensions shown. The box has a Plexiglas door at the front, and the back and top are removable. Items 1–3 are bolted to the sides of the box. Set up the remaining items as follows: Bend a 30-inch (76 cm) stainless steel tubing (item 6) as shown to provide additional heating surface. Place the bent portion, together with the thermocouple element, between 2 disks of the heater held tightly together by a screw at the center of the upper disk. Enclose the heater assembly in 1-inch (25-mm) thick magnesia–alumina insulation, and secure to the aluminum plate support by means of the aluminum cover and screws. Push the stainless steel tubing from the heater outlet through the center of the rubber stopper (item 5) so that the end of the tubing is near the constructed portion of the condenser when the stopper is inserted tightly into the top of the condenser. Push two additional 6-inch (15-cm) lengths of the stainless steel tubing through the rubber stopper, one to serve as the nitrogen inlet and the other as the mercury vapor outlet. 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 min.)

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 Sample Solutions

For supplements in tablet form: Weigh and finely powder NLT 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 ± 0.30 g. Blend the analytical mixture in a homogenizer (approximately 1 min) 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 min, and allow to stand for 5 min. Transfer the upper aqueous layer containing the methylmercury–thiosulfate complex together with any emulsion into a 25-mL Erlenmeyer flask. Blow a moderately strong stream of nitrogen into the flask for 1–2 min to break up any emulsion, and expel droplets of chloroform.
NOTE—To aid in breaking the emulsion, hold and rotate the flask at a 45-degree angle with one hand, and direct the nitrogen stream at the thin layer of emulsion that adheres to the bottom of the flask as it rotates.
NOTE—Some supplements may produce cloudy extracts. If this occurs, the extract can be passed through a membrane filter.

For supplements in capsule form: Weigh accurately NLT 20 capsules and determine the average weight. Place a number of capsules equivalent to about 10.0 g in a 100-mL beaker, and add the *Hydrochloric acid solution* so that the mass of the analytical portion of capsules taken plus the mass of the *Hydrochloric acid solution* totals 25.00 ± 0.30 g. Proceed as directed in *For Supplements in Tablet Form* beginning with “Blend the analytical mixture...”

For supplements in liquid form—Weigh accurately 10.0 g of the liquid in a 100-mL beaker, and prepare an analytical mixture by adding *Hydrochloric acid solution* so that the mass of the analytical portion of the dietary supplement liquid taken plus the mass of the *Hydrochloric acid solution* totals 25.00 ± 0.30 g. Proceed as directed in *For Supplements in Tablet Form* beginning with “Blend the analytical mixture...”

Preparation of the Reagent Blank Solution

Prepare the reagent blank analytical solution by weighing 25.00 g of *Hydrochloric acid solution* into a 100-mL beaker. Proceed as directed in *Preparation of Sample Solutions, For Supplements in Tablet Form*, beginning with “Immediately weigh 10.0 g...”

Preparation of Standard Solutions

Prepare 0.050, 0.100, 0.150, 0.200, and 0.250 µg Hg/100 µL of *Standard Solutions* by adding, respectively, 20-, 40-, 60-, 80-, and 100-µL aliquots of *Methylmercuric chloride stock standard solution* to 20 mL of chloroform in separate 25-mL glass-stoppered graduated cylinders. Proceed as directed in *Preparation of Sample Solutions, For Supplements in Tablet Form*, beginning with “Add 4.0 mL of *Sodium thiosulfate solution*...”

Chromatographic System

(See *Chromatography* 〈621〉.)

Mode: LC

Detector: Cold vapor atomic absorption at 253.7 nm

Guard column: 2.1-mm × 7-cm; packing L2

Analytical column: 4.6-mm × 25-cm; packing L1

Injection volume: 100 µL

Analysis

Inject the *Sample solution* into the HPLC/AAS system. After the methylmercury peak appears, inject a 100-µL aliquot of *Standard solution* that produces a peak height equal to or slightly higher than the *Sample solution* peak height. Repeat by injecting the *Sample solution* again, followed by the selected *Standard Solution*. If the *Sample solution* peak height is higher than the peak height for the highest standard, dilute quantitatively an appropriate aliquot of *Sample solution* with *Sodium thiosulfate solution*. Account for the dilution in the final calculation.

Calculations

Additional dilutions must be accounted for in the final calculation. *Do not vary the injection volume*.

Measure peak heights above the base line, and calculate the methyl-bound mercury concentration in the test portion, in µg Hg/g, by comparing the average peak heights of the *Sample solution* to the average peak heights of the *Standard Solution* as follows:

$$\text{Result } (\mu g/g) = (r_T/r_S) \times (W_S/W_T)$$

r_T = average peak height of the *Sample solution* (A)
r_S = average peak height of the *Standard solution* (A)
W_S = amount of standard injected (µg Hg)
W_T = amount of analytical portion injected (g)

where

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

D = weight of the analytical portion (g)
E = weight of the analytical mixture prepared (g)
F = weight of the anaytical mixture added to the *Chromatographic siliceous earth* (g)

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

The quantitation limit, defined as 10 times the standard deviation of the reagent blank, is 0.006 µg Hg/100 µL injected. This corresponds to a quantitation limit of 0.06 µg Hg/g for a 10-g analytical portion treated according to the procedure. The intraday variation, calculated as the standard deviation of five replicate injections of duplicate sample preparations, is NMT 0.12 and the relative standard deviation is NMT 20%.

▪1S (*USP36*)