AOAC Official Method 986.15
Arsenic, Cadmium, Lead, Selenium, and Zinc in Human and Pet Foods

Multielement Method

First Action 1986
Final Action 1988

Codex-Adopted–AOAC Method*

A. Principle

Material is digested with HNO_3 in closed system. Cd and Pb are determined by anodic stripping voltammetry (ASV). As, Se, and Zn are determined by atomic absorption spectrophotometry (AAS) after generation of metal hydrides (for As and Se).

B. Apparatus

(**a**) *Polarograph.*—With anodic stripping accessories. Typical operating parameters for Princeton Applied Research Model 174 with hanging drop Hg electrode are: scan rate, 5 mV/s; scan direction, + ; scan range, 1.5 V; initial potential, –0.7 V; modulation amplitude, 25 mV; operation mode, differential pulse; display direction, "–"; drop time, 0.5 s; low pass filter, off; selector, off; pushbutton, initial; output offset, off; and current range, 5–10 μA, or as needed.

Other instruments and electrodes such as wax impregnated graphite may be used according to manufacturer's directions.

(**b**) *Atomic absorption spectrophotometer.*—With Zn, As, and Se hollow cathode lamps or As and Se electrodeless discharge lamps, 3 slot, 10 cm Bolling burner head, air–C_2H_2 and H_2–N_2–entrained air flames, and deuterium arc background corrector.

(**c**) *Decomposition vessel.*—70 mL. *See* **974.14A** (*see* 9.2.24).

(**d**) *Hydride generator.*—*See* Figure **986.15A**. Constructed from following: (*1*) *Flat bottom flask.*—Borosilicate glass, 50 mL (Corning No. 5160, or equivalent). (*2*) *Stopper fittings.*—Two-hole (1 through center) No. 9 rubber stopper, fitted with gas outlet tube of 100 mm × ⅛ in (3 mm) id polyethylene tubing through center hole. Place bottom of gas outlet tube through cut off bottom 1 in (25 mm) segment of ⅝ in (16 mm) polyethylene test tube with hole in bottom so that 3 mm of tube protrudes through test tube. Insert through second hole 75 mm × ⅛ in (3 mm) id polyethylene tubing as N_2 inlet tube. Seal bottom end of tube with burner and then punch several holes at sealed end with 21 gage needle. Alternatively, prepare similarly 500 mm × 1/16 in (1.5 mm) id polyethylene tubing and hold in place in stopper with hole-through septum. Connect other end of tubing to AA spectrophotometer with 500 mm Tygon tubing by cutting auxilliary line at ca 75 mm from mixing chamber and attaching tubing. (*3*) *Generator mount.*—(Optional.) 64 mm × 0.5 in (13 mm) id pipe secured to laboratory ring stand by means of clamp holder. Insert extension clamp into pipe and attach another clamp to back of clamp to hold clamp in place and to serve as handle; clamp is now free to rotate ca 180°. Attach rubber stopper of hydride generator to extension clamp with stiff wire and position just at level of clamp jaws. In operation, place flask of generator between jaws of extension clamp, insert stopper firmly into neck of flask, then tighten clamp jaws around neck of flask. Unit can be rapidly and uniformly inverted by rotating handle on extension clamp, thus allowing sample and sodium borohydride to mix rapidly and reproducibly.

(**e**) *Pipets.*—50 and 100 μL Eppendorf micropipets, or equivalent.

C. Reagents

(Use double distilled H_2O. Rinse all glassware with HNO_3 [1 + 1] followed by thorough H_2O rinse. Decontaminate digestion vessels by digesting with reagents to be used in digestion. Rinse thoroughly with H_2O. Decontamination is necessary to reduce blanks, especially for Pb, to acceptable level.)

(**a**) *Acids.*—(*1*) *Nitric acid.*—Redistilled. (*2*) *Perchloric acid.*—70%, double vacuum distilled. (*3*) *Hydrochloric acid.*—8M. Dilute 66 mL HCl to 100 mL with H_2O.

(**b**) *Nitrate solution.—Equimolar solution of KNO_3 and $NaNO_3$.*—Dissolve 54.3 g KNO_3 and 45.7 g $NaNO_3$ (available as Suprapur®, Nos. 5065 and 6546, respectively, EM Science) in H_2O in 200 mL volumetric flask, dilute to volume, and mix. To further purify, add 1–2 drops NH_4OH to 25 mL aliquot and extract with 2 mL 10 μg dithizone/mL CCl_4 until lower solvent layer is colorless.

(**c**) *Magnesium solutions.*—(*1*) *Magnesium chloride solution.*—37.5 mg/mL. Dissolve total of 3.75 g MgO, USP, by adding small amounts at time to 100 mL 8M HCl. (*2*) *Magnesium nitrate solution.*—75 mg/mL. Mix 3.75 g MgO, USP, with ca 30 mL H_2O, slowly add HNO_3 to dissolve (ca 10 mL), cool, and dilute to 50 mL with H_2O.

(**d**) *Sodium borohydride solution.*—4.0 g $NaBH_4$/100 mL 4% NaOH.

(**e**) *Potassium iodide solution.*—Dissolve 20 g KI in H_2O and dilute to 100 mL. Prepare just before use.

(**f**) *Metal powders.*—Purity: 99.99 + % Cd, Pb, Zn; 99.99% Se.

(**g**) *Cadmium standard solutions.*—(*1*) *Stock solution.*—1 mg/mL. Dissolve 1.000 g Cd powder in 20 mL HNO_3 (1 + 1) in 1 L volumetric flask, and dilute to volume with H_2O. (*2*) *Working solution.*—2 μg/mL. Pipet 10 mL stock solution into

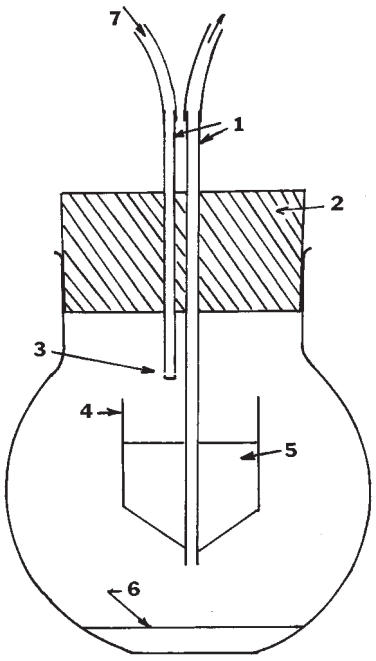

Figure 986.15A—Hydride generator: 1, polyethylene tubing; 2, rubber stopper; 3, flame sealed polyethylene tubing with holes punched at one end; 4, reagent cup; 5, sodium borohydride solution; 6, test solution; 7, nitrogen inlet from "auxilliary" line of AAS.

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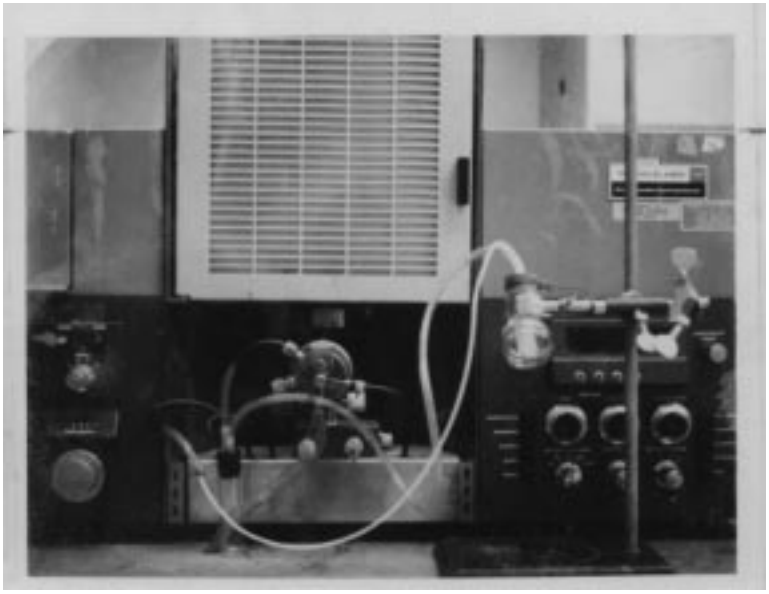

Figure 986.15B—Hybrid generator and mount connected to auxilliary line of spectrophotometer. Test tube acid trap connected between generator and instrument is not included in method.

100 mL volumetric flask, and dilute to volume with H_2O. Pipet 2 mL diluted solution into 100 mL volumetric flask and dilute to volume with H_2O.

(**h**) *Lead standard solutions.*—(*1*) *Stock solution.*—1 mg/mL. Dissolve 1.000 g Pb powder in 20 mL HNO_3 (1 + 1) in 1 L volumetric flask, and dilute to volume with H_2O. (*2*) *Working solution.*—5 µg/mL. Pipet 1 mL stock solution into 200 mL volumetric flask and dilute to volume with H_2O.

(**i**) *Zinc standard solutions.*—(*1*) *Stock solution.*—1 mg/mL. Dissolve 1.000 g Zn powder in 20 mL HCl (1 + 1) in 1 L volumetric flask, and dilute to volume with H_2O. (*2*) *Working solutions.*—0.2, 0.5, 1.0, and 1.5 µg/mL. Pipet 1 mL stock solution into 100 mL volumetric flask and dilute to volume with H_2O. Pipet 2, 5, 10, and 15 mL diluted solution into separate 100 mL volumetric flasks, each containing 1 mL $HClO_4$, and dilute to volume with H_2O.

(**j**) *Arsenic standard solutions.*—(*1*) *Stock solution.*—Dissolve 1.320 g As_2O_3 in minimum volume 20% NaOH in 1 L volumetric flask, acidify with HCl (1 + 1), and dilute to volume with H_2O. (*2*) *Working solutions.*—1, 2, 3, 4, and 5 µg/mL. Pipet 10 mL stock solution into 100 mL volumetric flask, and dilute to volume with H_2O. Pipet 1, 2, 3, 4, and 5 mL diluted solution into separate 100 mL volumetric flasks, and dilute to volume with H_2O.

(**k**) *Selenium standard solutions.*—(*1*) *Stock solution.*—1 mg/mL. Dissolve 1.000 g Se powder in minimum volume HNO_3 in 200 mL beaker and evaporate to dryness. Add 2 mL H_2O and evaporate to dryness. Repeat addition of H_2O and evaporation to dryness twice. Dissolve in minimum volume HCl (1 + 9) in 1 L volumetric flask, and dilute to volume with HCl (1 + 9). (*2*) *Working solutions.*—1, 2, 3, 4, and 5 µg/mL. Pipet 10 mL stock solution into 100 mL volumetric flask and dilute to volume with H_2O. Pipet 1, 2, 3, 4, and 5 mL diluted solution into separate 100 mL volumetric flasks and dilute to volume with H_2O.

Table 986.15 Flow rates and pressures for arsenic and selenium determinations

Gas	Tank psi	AA control box, psi	Perkin-Elmer Model 403 flowmeter, divisions
H_2	20	10	20 (4 L/min)
N_2	40	30	25 (10 L/min)

D. Closed System Digestion

(Do not exceed manufacturer's specifications of 0.3 g solids with 70 mL vessel. Proceed cautiously with new or untried uses. Let such test portions stand with HNO_3 overnight or heat on hot plate cautiously until any vigorous reaction subsides. Then proceed with closed vessel digestion. Open vessel in hood since nitrogen oxides are released.)

Weigh 0.3 g test portion (dry basis) into decontaminated decomposition vessel, add 5 mL HNO_3, close vessel with lid, and heat in 150°C oven 2 h. Cool in hood, remove vessel from jacket, and transfer contents to 10 mL volumetric flask. Add 4 mL H_2O to vessel, cover with lid, and while holding lid tightly against rim, invert several times, and add rinse to flask. Dilute to volume with H_2O and mix.

E. Anodic Stripping Voltammetry

(For Cd and Pb.)

Pipet aliquot of digested test solution into decontaminated 50 mL Vycor crucible and add 2 mL nitrate solution, **C**(**b**). Conduct reagent blank simultaneously. Heat on hot plate at low heat to dryness; then increase heat to maximum (ca 375°C). Nitrate salts will melt and digest organic matter in 15–20 min. Place crucibles in 450°C furnace to oxidize any remaining carbonaceous matter (10–20 min). Digestion is complete when melt is clear. Let cool, add 1 mL HNO_3 (1 + 1) to solidified melt, and heat on hot plate to dryness to expel carbonates and nitrites and to control acidity. Dissolve in 5.0 mL HNO_3 (0.5 mL/L), warming on hot plate to speed solution. Transfer to polarographic cell with 5.0 mL H_2O. Bubble O_2-free N_2 through solution 5 min; then direct N_2 over solution.

Set dial for Hg drops at 4 µm divisions. Stir solution with magnetic stirrer at constant and reproducible rate so Hg drop is not disturbed. Slide selector switch to "Ext. Cell" and measure time for

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120 s with stopwatch. Turn off stirrer and let stand 30 s. Press "Scan" button to obtain peaks corresponding to Cd and Pb at ca –0.57 and –0.43 V, respectively, against saturated calomel electrode.

Add known volumes of each standard to test solution in cell from Eppendorf pipet. Amounts added should be ca 1×, 2×, etc. of amount metal present initially in cell, and each addition should not change original volume significantly. After each addition, bubble N_2 through solution briefly and perform deposition and stripping operations exactly as for original solution. Plot µg metal added on *x*-axis against peak height on *y*-axis. Extrapolate linear line to *x*-axis to obtain µg metal in cell.

$$\text{Metal/µg metal/g test portion, } = \frac{M - M'}{\text{g test portion}} \times \frac{10}{\text{mL aliquot taken}}$$

where M and M' = µg metal from standard curve for test portion and blank, respectively.

F. Atomic Absorption Spectrophotometry

(For As, Se, and Zn.)

(**a**) *Arsenic*.—Pipet aliquot digested test solution into decontaminated 50 mL round, flat-bottom borosilicate flask, and add 1 mL $Mg(NO_3)_2$ solution, (**c**)(*2*). Heat on hot plate at low heat to dryness; then increase heat to maximum (ca 375°C). Place flask in 450°C furnace to oxidize any carbonaceous matter and to decompose excess $Mg(NO_3)_2$ (≥30 min). Cool, dissolve residue in 2.0 mL 8M HCl, add 0.1 mL 20% KI to reduce As^{5+} to $As,^{3+}$ and let stand ≥2 min. Conduct reagent blank with sample.

Prepare standards as follows: To six 50 mL flasks (same type as used for sample) add 2.0 mL $MgCl_2$ solution, (**c**)(*1*), and to 5 flasks add 50 µL aliquots of respective working standard solutions so that series will contain 0, 0.05, 0.1, 0.15, 0.20, and 0.25 µg As. (Other amounts may be used depending on sensitivity of system.) Add 0.1 mL 20% KI to each flask, mix, and let stand ≥2 min.

Connect generator to instrument as shown in Figure **986.15B** and adjust pressures and flows for H_2 - H_2-entrained air flame as in Table **986.15**. Operate instrument according to manufacturer's instructions, with As lamp in place and recorder set for 20 mm/min.

Add 2.0 mL 4% $NaBH_4$ solution to reagent dispenser of generator, and insert rubber stopper tightly into neck of flask containing sample or standard. With single rapid, smooth motion, invert flask, letting solution mix with sample or standard. (This operation must be performed reproducibly.) Sharp, narrow *A* peak will appear immediately. When recorder pen returns to baseline, remove stopper from flask, and rinse reagent dispenser with H_2O from squeeze bottle; then suck out H_2O. Proceed with next sample or standard. When series is complete, rinse glassware thoroughly.

Plot calibration curve of µg As against *A*, and obtain µg As in sample aliquot from this curve. Correct for reagent blank.

(**b**) *Selenium*.—Proceed as in (**a**), using Se lamp and standards, but omit addition of KI solution. KI will reduce Se to elemental state and cause loss of signal. Instead, cover flask with small watch glass and place on steam bath 10 min, and cool to room temperature.

(**c**) *Zinc*.—Pipet 1 mL aliquot digested test solution into decontaminated 25 mL Erlenmeyer, and add 0.1 mL $HClO_4$. Heat on hot plate to white fumes of $HClO_4$. Sample should be completely digested as indicated by clear, practically colorless solution. If sample chars, add 0.5 mL portions HNO_3 and again heat to white fumes. Finally, heat just to dryness but do not bake. Cool, and dissolve residue in 3.0 mL $HClO_4$ (1 + 99).

Operate instrument in accordance with manufacturer's instructions, using air–C_2H_2 flame, and measure *A* of sample and standards, **C**(**i**)(*2*). Dilute test solution with $HClO_4$ (1 + 99), if solution is too concentrated. Plot calibration curve of µg Zn against *A*, and obtain µg Zn in test solution aliquot from this curve. Correct for reagent blank.

Reference: *JAOAC* **63**, 485(1980).

CAS-7440-38-2 (arsenic)
CAS-7440-43-9 (cadmium)
CAS-7439-92-1 (lead)
CAS-7782-49-2 (selenium)
CAS-7440-66-6 (zinc)

Revised: March 1996

* Adopted as a Codex Alternative Approved Method (Type III) for atomic absorption spectrophotometry of arsenic in all foods.
Adopted as a Codex Alternative Approved Method (Type III) for anodic stripping voltammetry of cadmium, lead, and zinc in all foods.
Adopted as an Alternative Approved Method (Type III) for atomic absorption spectrophotometry of cadmium and selenium in natural mineral waters.
Adopted as a Codex Reference Method (Type II) for atomic absorption spectrophotometry of arsenic in natural mineral waters.
Paragraph E: Adopted as a Codex Reference Method (Type II) for colorimetry, dithizone of lead in chocolate. Also adopted as a Codex Method for anodic stripping voltammetry of lead in cocoa powders (cocoa) and dry cocoa–sugar mixtures.
Paragraph F: Adopted as a Codex Alternative Approved Method (Type III) for atomic absorption spectrophotometry of arsenic in fruit juices.

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