9.1.08

AOAC Official Method 999.10
Lead, Cadmium, Zinc, Copper, and Iron in Foods

Atomic Absorption Spectrophotometry
after Microwave Digestion
First Action 1999
Final Action 2005

NMKL–AOAC Method

[Applicable to determination of Zn, Cu, and Fe in a variety of foods by microwave digestion and flame atomic absorption spectrophotometry (FAAS), and Cd and Pb by microwave digestion and graphite furnace atomic absorption spectroscopy (GFAAS). Method is capable of determining these elements at concentrations above approximately Pb (0.4), Cd (0.01), Zn (4), Cu (3), and Fe (7) mg/kg. Method is not applicable to foods with a fat content 40%. Not applicable to milk powder.]

See Table **999.10A** for the results of the interlaboratory study supporting acceptance of the method.

Caution: Digestion vessels must cool for an appropriate time before opening in order to avoid burns from hot and corrosive vapors. Always gently add acid to water. Maintain safe distance from furnaces equipped with Zeeman background correction when the magnet is on. Consult manufacturer's instructions to determine safe distance, which varies for different instruments. *See* Appendix B, *Laboratory Safety*, for safe use of compressed gases, inorganic acids, and atomic absorption spectrometer.

Table 999.10A. Interlaboratory study results for lead, cadmium, zinc, copper, and iron in foods

Metal	Sample	Analyte range, mg/kg	Mean, mg/kg	n^a	Outliers	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat
Pb (GFAAS)	Liver	0.1	0.130	11	1	0.049	0.055	37	42	0.14	0.15	1.95
	Wheat bran		0.155	12	0	0.088	0.091	57	59	0.25	0.26	2.81
	Diets[b]		0.394	12	0	0.063	0.098	16	25	0.18	0.27	1.37
	Bovine muscle		0.398	10	2		0.086		22		0.24	1.21
	Fish		0.48	12	0		0.13		27		0.36	1.52
	Mushroom		1.62	12	0		0.26		16		0.73	1.08
Cd (GFAAS)	Bovine muscle	0.01	0.0124	12	1		0.0034		28		0.0097	0.91
	Liver		0.164	13	0	0.025	0.034	15	20	0.070	0.094	0.96
	Wheat bran		0.171	11	2	0.0078	0.022	4.6	13	0.022	0.063	0.63
	Fish		0.211	12	0		0.035		17		0.099	0.85
	Mushroom		0.482	11	2		0.053		11		0.149	0.62
	Diets[b]		0.764	12	1	0.050	0.105	6.5	14	0.14	0.294	0.85
Zn (FAAS)	Fish	4	4.50	12	0		0.41		9.1		1.1	0.51
	Milk powder		35.3	14	0		3.3		9.3		9.1	1.00
	Diets[b]		47.8	13	1	1.9	2.5	4.0	5.3	5.4	7.1	0.60
	Mushroom		56.9	14	0		3.0		5.3		8.4	0.61
	Wheat bran		73.5	13	1	2.5	3.5	3.4	4.8	7.1	9.9	0.58
	Bovine muscle		147.3	11	3		2.5		1.7		7.0	0.23
	Liver		181.9	12	2	2.8	8.8	1.6	4.8	7.9	25	0.66
Cu (FAAS)	Fish	0.2	0.241	4	0		0.094		39		0.26	1.98
	Bovine muscle		2.63	6	0		0.17		6.4		0.47	0.47
	Wheat bran		10.14	10	1	0.44	0.81	4.3	7.9	1.2	2.3	0.70
	Mushroom		37.7	14	0		2.2		5.7		6.0	0.62
	Diets[b]		63.42	12	2	0.95	1.9	1.5	3.0	2.7	5.3	0.35
	Liver		107.5	14	0	3.3	4.1	3.1	3.8	9.3	12	0.48
Fe (FAAS)	Fish	7	7.4	9	0		1.3		17		3.5	1.44
	Bovine muscle		75.0	12	0		8.1		11		23	1.32
	Mushroom		105.5	11	0		7.9		7.5		22	0.95
	Wheat bran		123.1	12	0	3.9	9.9	3.2	8.1	11	28	1.05
	Diets[b]		303	10	2	12	18	4.0	5.9	33	50	0.88
	Liver		487	12	0	27	31	5.4	6.4	74	88	1.02

[a] n = Number of laboratories after outlier elimination. Values for s_r, RSD_r, and r are only available for duplicate or split level determinations.

[b] Simulated diets E and F.

Table 999.10B. Instrumental parameters for FAAS

Metal	Flame type	Wavelength, nm
Zn	Air–acetylene, oxidizing	213.9
Cu	Air–acetylene, oxidizing	324.7
Fe	Air–acetylene, oxidizing	248.3
Fe	N_2O–acetylene, oxidizing	248.3

A. Principle

Products are digested with HNO_3 and H_2O_2 under pressure in a closed vessel heated by microwaves. Solution is diluted with H_2O. Pb and Cd are determined by GFAAS. Zn, Cu, and Fe are determined by FAAS.

B. Apparatus

(**a**) *Atomic absorption spectrophotometer.*—With air–acetylene burner or nitrous oxide–acetylene burner for flame (FAAS; *see* Table **999.10B**) and a graphite furnace for electrothermal (GFAAS; *see* Table **999.10C**) determinations, with appropriate background (nonatomic) correction.

(**b**) *Hollow cathode or electrodeless discharge lamps*.—For Pb, Cd, Zn, Cu, and Fe.

(**c**) *Microwave oven.*—Designed for laboratory use, e.g., MDS-2000 (CEM Corp., PO Box 200, Matthews, NC 28106-2000, USA). Check microwave oven regularly for delivered power. If the measured effect does not agree with the specification, adjust the program: Fill a plastic beaker (polypropylene or Teflon) with 1.000 kg water (room temperature) and measure temperature (T_b). Place beaker in microwave oven and heat water at full power for 2 min. Take beaker out of oven, stir water, and measure temperature (T_a). The delivered power in watts:

$$P = 35 \quad (T_a - T_b)$$

(**d**) *Teflon digestion vessels*.—100 mL, withstanding a pressure of at least 1.4 MPa.

(**e**) *Volumetric flasks.*—25 and 1000 mL.

(**f**) *Funnels*.—Glass or plastic.

(**g**) *Plastic bottles*.—e.g., Polystyrene bottles with tightly fitting lids, 50–100 mL.

(**h**) *Drying oven*.—Or equipment for freeze-drying.

Carefully clean all glassware and plasticware and rinse, e.g., with HNO_3 or HCl, to avoid metal contamination.

C. Reagents

Reagents should be of at least analytical reagent grade (p.a.), preferably ultrapure (suprapur), or equivalent.

(**a**) *Water.*—Redistilled or deionized, 18 M cm.

(**b**) *Nitric acid*.—65% (w/w).

(**c**) *Nitric acid*.—0.1M. Dilute 7 mL concentrated HNO_3, (**b**), with water to 1 L.

(**d**) *Nitric acid.*—3M. Dilute 200 mL concentrated HNO_3, (**b**), with water to 1 L.

Table 999.10C. Instrumental parameters for GFAAS

		Temperature (C)/ramp-hold (s)		Cleaning out
Metal	Wavelength, nm	Ashing step	Atomization step	step (C)
Pb	283.3	650/15-10	1900/0-4	2500
Cd	228.8	350/15-10	1200/0-4	2500

(**e**) *Hydrogen peroxide*.—30% (w/w).

(**f**) *Zinc standard solution*.—1 mg/mL. Dissolve 1.000 g Zn in 14 mL water + 7 mL nitric acid, (**b**), in 1 L volumetric flask. Dilute to volume with water. [*Note*: Commercially available standard solutions for AAS (e.g., BDH Chemicals Ltd., Poole, UK) may be used for all metal standard solutions.]

(**g**) *Copper standard solution*.—1 mg/mL. Dissolve 1.000 g Cu in 7 mL nitric acid, (**b**), in 1 L volumetric flask. Dilute to volume with water.

(**h**) *Iron standard solution*.—1 mg/mL. Dissolve 1.000 g Fe in 14 mL water + 7 mL nitric acid, (**b**), in 1 L volumetric flask. Dilute to volume with water.

(**i**) *Lead standard solution*.—1 mg/mL. Dissolve 1.000 g Pb in 7 mL HNO_3, (**b**), in 1 L volumetric flask and dilute to volume with water.

(**j**) *Cadmium standard solution*.—1 mg/mL. Dissolve 1.000 mg Cd in 14 mL water + 7 mL HNO_3, (**b**), in 1 L volumetric flask and dilute to volume with water.

(**k**) *Working standard solutions.*—(*1*) *For flame analysis*.—Dilute standard, (**f**)–(**j**), with 0.1M HNO_3, (**c**), to a range of standards that covers the concentration of the element to be determined. (*2*) *For graphite furnace analysis*.—Dilute standard solutions, (**f**)–(**j**), with 0.1M HNO_3, (**c**), to a range of standards that covers the linear range of the element to be determined.

D. Procedures

(**a**) *Cleaning procedure.*—(*1*) *For glass and plasticware*.—Acid solution: 500 mL concentrated HNO_3, **C**(**b**), + 4500 mL deionized water, **C**(**a**). Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with acid solution. Finally rinse 4–5 times with deionized water. (*2*) *For Teflon digestion vessels*.—Rinse with acetone, wash with deionized water, keep vessels covered with 0.1M HNO_3, **C**(**c**), for at least 30 min, rinse with deionized water, and let vessels dry.

Use separate vessels for different applications, depending on the concentration of metals. If, however, the same digestion vessels are used for heavily contaminated products, e.g., sludge, it may be necessary to use a more severe cleaning procedure, e.g., heating vessels together with concentrated HNO_3, **C**(**b**). The instrument manual usually provides detailed instructions for such cleaning procedures.

(**b**) *Pretreatment*.—If product is to be analyzed fresh, proceed to (**d**), *Homogenization.* Otherwise, continue at (**c**), *Drying*.

(**c**) *Drying*.—Dry to constant weight in drying oven at 105 C, or freeze-dry. Freeze-drying is usually preferable because it renders the product less compact and easier to homogenize. If final result is based on fresh weight, weigh test portion before and after drying to obtain water content:

$$H_2O = \frac{W_f \quad W_d}{W_f} \quad 100$$

where H_2O, % = water content of the test portion (%); W_f = weight of the test portion (g); W_d = weight after drying (g).

(**d**) *Homogenization.*—Homogenize products using noncontaminating equipment. Check for leached metals if the apparatus consists of metal parts.

(**e**) *Digestion.*—Weigh 0.2–0.5 g dry material into digestion vessel. If water-containing materials are used, maximum weight is restricted to 2 g, but dry matter content must never exceed 0.5 g. For

Table 999.10D. Parameters for microwave oven program

Step	Power, watts	Duration, min
1	250	3
2	630	5
3	500	22
4	0	15

example, if product has a water content of 50%, take a maximum of 1 g (= 0.5 g dry matter). If a product has a water content of 95%, take 2 g (<0.5 g dry matter). When unknown products are digested, too much solids may cause the safety membrane in the digestion vessel to rupture.

Add 5 mL HNO_3, **C**(**b**), and 2 mL 30% H_2O_2, **C**(**e**). Close vessels, place vessels in holder, place vessel holder in microwave oven, and close door. Set oven program according to the parameters given in Table **999.10D** and start program.

The program is valid only when 12 vessels are being digested simultaneously. If fewer are being digested, the remaining vessels must be filled with reagent blank. When a microwave oven other than the one given as an example is used, it may be necessary to use a slightly different time/power program.

Remove digestion vessels from microwave oven and let cool thoroughly before opening them. Open vessel and rinse down lid and walls into container. Transfer solution to 25 mL volumetric flask and dilute to mark with deionized water. Then, transfer solution to plastic container. Treat blanks in the same way as tests. One blank should be included in every set.

(**f**) *Dilution*.—If test solution needs to be further diluted (due to high metal concentrations), dilute with 3M HNO_3, **C**(**d**), in order to maintain same acid concentration prior to metal determination, (**g**).

High acid concentration is environmentally undesirable and may depress the analytical signal. Reduce acid strength by diluting the test solution ½ with 0.1M nitric acid and standard solutions ½ with 3M nitric acid. The tests and standards are thereby brought to the same acid concentration. Matching of acid concentrations is important when a calibration curve is used.

(**g**) *Atomic absorption spectrophotometry*.—Use of flame or graphite furnace technique is determined by the concentration of the metal to be determined. Flame technique should be used as far as possible, since this technique is less sensitive to interference than the GFAAS. The most appropriate wavelength, gas mixture/temperature program, and other instrumental parameters for each metal are found in the manual provided with the instrument. Always use background correction.

Measurements must be within the linear range when the method of standard addition is used. A standard addition curve consists of at least 3 points, of which at least 2 are standards. The concentration of the highest standard should be 3–5 times the concentration in the test solution. The lower standard should have a concentration approximately half of the highest standard. A simplified version of the method of standard addition is to use a matrix-matched standard curve, which is applicable to products with the same matrix: The test and standard solutions are mixed and used to make a standard addition curve. This curve is then parallel transferred to origin and is used as the standard curve for the tests that followed and that have been diluted in the same proportions. The matrix-matched standard curve and the test solutions will thus have the same matrix concentration. On most modern instruments, this function is included in the software.

(*1*) *Flame technique*.—The concentration of Zn, Cu, and Fe are usually at levels suitable for determination by FAAS. When calibration curve is to be used, standards and test solutions must have the same acid concentration.

Since Fe may be strongly affected by interferences from the matrix, use either the method of standard addition or matrix-matched standards. When experiencing severe interferences, an oxidizing nitrous oxide acetylene flame may be an alternative.

(*2*) *Graphite furnace technique*.—This technique is generally required for determination of Pb and Cd in foods. Use pyrolytically coated tubes with platforms. Since the method results in a fairly large dilution of the analyte, it may frequently be needed also for the determination of, e.g., Cu. The method of standard addition or matrix-matched standards should always be used unless shown to be unnecessary (i.e., no significant difference between the slopes of calibration curves of pure working standard and standard addition curves of the test product). Measurements must be made in the linear range when the method of addition is used.

Program the autosampler to deliver a volume that gives as large an absorbance as possible within the linear range and producing a background absorbance not larger than approximately 0.5 absorbance units. Multiple injection may enhance the absorbance at very low concentrations. Evaluate each new matrix by means of ash- and atomization-curves in order to optimize the graphite furnace parameters.

E. Calculations and Evaluation of Results

Calculate the concentration (C) of metal in the test sample according to the formula:

$$C = \frac{(a \quad b)df \quad 25}{m}$$

where C = concentration in the test sample (mg/kg); a = concentration in the test solutions (mg/L); df = dilution factor; b = mean concentration in the blank solutions (mg/L); m = weight of the test portion (g).

If (a – b) is lower than the detection limit, DL, then (a – b) is replaced by DL for calculation of the limit of detection in the test sample.

If the test solution has been diluted, the dilution factor (df) has to be taken into account. If the test portion was dried and the result should be based on fresh weight, correct according to the following:

$$C_{FW} = C \quad \frac{100 \quad H_2O\%}{100}$$

where C_{FW} = concentration in the test portion corrected to fresh weight (mg/kg); $H_2O\%$ = the water content of the test portion (%).

When running replicates, the average of the results should be given with 3 significant figures.

Detection limit.—The DL for each metal is calculated as DL = 3 standard deviation of the mean of the blank determinations (n = 20). A large number of blanks must be analyzed before DL can be established. A DL is not static and will need to be re-evaluated from time to time in accordance with changes in the blank levels.

Reference: J. AOAC Int. 83, 1189(2000).