9.1.09

AOAC Official Method 999.11 Determination of Lead, Cadmium, Copper, Iron, and Zinc in Foods

Atomic Absorption Spectrophotometry after Dry Ashing

First Action 1999

NMLK–AOAC Method

[Applicable to the determination of lead, cadmium, zinc, copper, and iron in food by dry ashing and flame atomic absorption spectrometry (GFAAS), flame and graphite furnace procedures. *See* Table **999.11A** for the results of the interlaboratory study supporting the acceptance of the method.]

Caution: Always gently add acid to water.

Avoid environmental contamination by Pb. Store quartz crucibles in 20% HNO_3 and rinse with deionized water before use. When necessary, crucibles may be boiled with 20% HNO_3 before use. Heat platinum crucibles until red hot and boil with 50% (v/v) HCl prior to use.

Ash products with a high fat content (≥40%), e.g., margarine or lard, with great care to avoid self-ignition. Pre-ash such products according to **D**(**c**)(*2*), even if a programmable furnace is used.

A. Principle

Test portions are dried and then ashed at 450°C under a gradual increase (≤50°C/h) in temperature. 6*M* HCl (1 + 1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1M HNO_3, and the analytes are determined by flame and graphite procedures.

B. Apparatus

(**a**) *Atomic absorption spectrophotometer*.—With an air–acetylene burner or nitrous oxide–acetylene burner for flame and a graphite furnace for electrothermal determinations, with appropriate background (nonatomic) correction (*see* Table **999.11B**).

See Table **999.11C** for example of instrumental parameters for graphite furnace AAS. (The parameters listed are for a Perkin Elmer HGA-500 instrument. For other instruments, the parameters may have to be changed. Suitable parameters are usually given in the manual provided with the instrument.)

Table 999.11A Interlaboratory study results

Metal	Sample	Analyte range	Mean, mg/kg	*n*[a]	s_r[b]	s_R[c]	RSD_r[d]	RSD_R[e]	r[f]	R[g]
Pb-HGAAS	Liver paste/milk powder	≥0.04	0.040	11	0.019	0.019	46	46	0.052	0.052
	Apple sauce		0.27	10		0.10		38		0.29
	Minced fish		0.53	10		0.11		20		0.31
	Wheat bran		0.111	12		0.056		50		0.16
	Simulated diets D/E		0.246	10[1]	0.034	0.048	14	20	0.096	0.14
Cd-HGAAS	Liver paste	≥0.05	0.0491	11		0.0058		12		0.016
	Minced fish/wheat bran		0.175	8[1]	0.021	0.032	12	18	0.058	0.089
	Simulated diets D/E		0.51	8	0.10	0.13	19	26	0.27	0.37
Zn-FAAS	Liver paste/minced fish	≥0.7	6.63	13	0.35	0.50	5.3	7.5	0.98	1.4
	Apple sauce		0.699	11[3]		0.047		6.8		0.13
	Wheat bran		71.5	12[2]		4.9		6.8		14
	Milk powder		35.0	12[2]		2.8		8.0		7.9
	Simulated diets D/E		37.82	11[2]	0.68	1.31	1.8	3.5	1.9	3.7
Cu-FAAS	Apple sauce/minced fish	≥0.2	0.240	12[1]	0.076	0.076	32	32	0.21	0.21
	Milk powder		0.51	14[2]		0.23		45		0.65
	Liver paste		5.34	13[2]		0.37		6.9		1.0
	Wheat bran		9.52	12		0.98		10		2.7
	Simulated diets D/E		45.4	12[1]	1.6	3.1	3.6	6.9	4.6	8.8
Fe-FAAS	Liver paste	≥4	24.3	14		2.8		11		7.8
	Minced fish/milk powder		3.99	11[3]	0.44	0.54	11	14	1.2	1.5
	Wheat bran		124	13[1]		14		11		39
	Simulated diets D/E		216	13	18	23	8.2	11	49	64

[a] *n* = Number of laboratories remaining after elimination of outliers [in brackets].

[b] s_r = Repeatability standard deviation.

[c] s_R = Reproducibility standard deviation.

[d] RSD_r = Relative repeatability standard deviation.

[e] RSD_R = Relative reproducibility standard deviation.

[f] r = 2.8 × s_r.

[g] R = 2.8 × s_R.

Table 999.11B Instrumental parameters for flame determination

Element	Flame	Wavelength, nm
Fe	Nitrous oxide–acetylene, oxidizing	248.3
Cu	Air–acetylene, oxidizing	324.7
Zn	Air–acetylene, oxidizing	213.9

(**b**) *Hollow cathode, or electrodeless discharge lamps for all elements determined*.

(**c**) *Furnace*.—Programmable, or muffle furnace with thermostat maintaining 450 ± 25°C. If muffle furnace is used, a separate pre-ashing device is required. *See* (**d**)–(**h**).

(**d**) *Hot plate*.—With heating control, to heat up to about 300°C.

(**e**) *Lamp*.—IR 250 W, fixed to a retort stand in a way that allows adjustment of the distance to the plate.

(**f**) *Ceramic plate*.—e.g., Desiccator plate on a low stand, with a diameter that suits the hot plate.

(**g**) *Glass cover*.—e.g., Crystallizing dish, 185 mm diameter, 100 mm height, to fit on (**f**) or equivalent.

(**h**) *Wash-bottle*.—"Scrubber," containing H_2SO_4 for purification of air.

See Figure **999.11** for assembly of items (**d**)–(**h**).

(**i**) *Quartz or platinum crucibles*.—50–75 mL.

(**j**) *Polystyrene bottles*.—With leak-proof closures, 100 mL.

Carefully clean and rinse all glassware and plasticware with HNO_3 or HCl to avoid metal contamination. *Cleaning procedure for glass and plasticware*.—Acid solution: 500 mL concentrated HNO_3, **C**(**c**) +4500 mL deionized water, **C**(**a**). Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4–5 times with deionized water.

C. Reagents

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

(**a**) *Water*.—Redistilled or deionized, resistivity ≥18 MΩ·cm.

(**b**) *Hydrochloric acid*.—6*M*. Dilute 500 mL HCl (37% w/w) with water to 1 L.

(**c**) *Nitric acid*.—65% (w/w).

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

(**e**) *Lead standard solution*.—1 mg/mL. Dissolve 1.000 g Pb in 7 mL HNO_3, (**c**), 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.]

(**f**) *Cadmium standard solution*.—1 mg/mL. Dissolve 1.000 g Cd in 14 mL water + 7 mL HNO_3, (**c**), in 1 L volumetric flask. Dilute to volume with water.

(**g**) *Zinc standard solution*.—1 mg/mL. Dissolve 1.000 g Zn in 14 mL water + 7 mL HNO_3, (**c**), in 1 L volumetric flask. Dilute to volume with water.

(**h**) *Copper standard solution*.—1 mg/mL. Dissolve 1.000 g Cu in 7 mL HNO_3, (**c**), in 1 L volumetric flask. Dilute to volume with water.

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

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

D. Procedures

(**a**) *Pre-treatment*.—Homogenize product if necessary, using noncontaminating equipment. Check for leaching metals if the apparatus consists of metal parts.

(**b**) *Drying*.—In a crucible, weigh 10–20 g test portion to nearest 0.01 g. Dry in a drying oven, on a water-bath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace.

(**c**) *Ashing*.—(*1*) *Ashing in a programmable furnace*.—Place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C/h to 450°C. Let dish stand for at least 8 h or overnight. Continue according to (**e**). (*2*) *Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in **B(d)**–(**h**).—See* Figure **999.11**. Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp down at the cover. Pre-ash product sample by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200–250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool. Wet ash with 1–3 mL water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than

Table 999.11C Example of instrumental parameters for graphite furnace AAS

Metal	Wavelength, nm	Temperature program				Test solution volume, µL	Graphite tube
		Step 1	Step 2	Step 3	Step 4		
Pb	283.3	Temp., 130°C	650	1900	2500	20	L'vov
		Ramp, 10 s	5	0	2		
		Hold, 30 s	10	2	2		
Cd	228.8	Temp., 130°C	350	1200	2500	10	Uncoated
		Ramp, 1 s	5	0	2		
		Hold, 19 s	10	2	2		

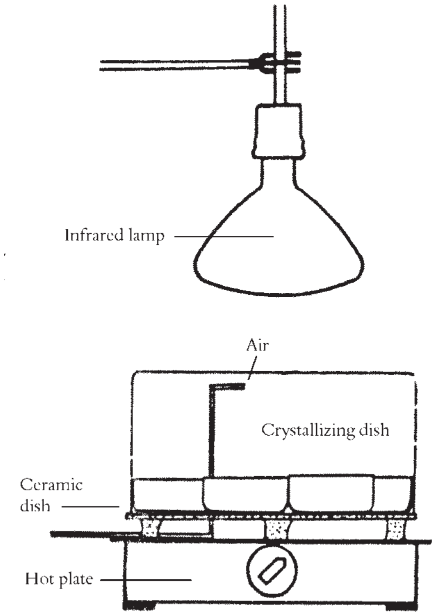

Figure 999.11—Apparatus for pre-ashing of samples.

200°C and raise temperature (50–100°C/h) to 450°C. Proceed with ashing at 450°C for 1–2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product. Add 5 mL 6*M* HCl, **C**(**b**), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0–30.0 mL, to the nearest 0.1 mL, of 0.1M HNO_3, **C**(**d**). Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1–2 h. Then stir solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blanks with each analytical batch.

(**d**) *Atomic absorption spectrophotometry*.—Pb and Cd in foods generally require graphite furnace AAS for determination. Zn, Cu, and Fe can, in most foods, be determined by flame AAS.

Wavelength, gas mixture/temperature program, and other instrumental parameters that are most appropriate for each metal are found in the manual provided with the instrument.

Background correction must always be used in flameless AAS and for flame applications at low concentrations.

When results are outside of the linear range, the test solutions should be diluted with 0.1M HNO_3, **C**(**d**).

Flame technique.—Prepare calibration curves from a minimum of 3 standards.

Graphite furnace (flameless) technique.—The method of addition should always be used. Measurements must be made in the linear range when method of addition is used. Measurements are preferably made with peak area rather than peak height.

E. Calculations and Evaluation of Results

Detection limit.—Calculate the detection limit, DL, for each metal as:

$$\begin{gathered}\text{DL} = 3 \times \text{standard deviation of the mean} \\ \text{of the blank determinations} \\ (n = \geq 20)\end{gathered}$$

Calculate the concentration, c, of metal in the test sample according to the formula:

$$\frac{\qquad \times}{\qquad\qquad}$$

where c = concentration in the test sample (mg/kg); a = concentration in the test solutions (mg/L); b = mean concentration in the blank solutions (mg/L); V = volume of the test solution (mL); m = weight of the test portion (g).

If (a – b) is lower than the DL, then (a – b) is substituted with DL for calculation of the limit of detection in the test portion.

If test solution has been diluted, dilution factor has to be taken into account. When running replicates, the average of the results should be given with 2 significant figures.

Reference: *J. AOAC Int*. **83**, 1204(2000).

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