RESIDUES AND TRACE ELEMENTS

Determination of Lead, Cadmium, Zinc, Copper, and Iron in Foods by Atomic Absorption Spectrometry after Microwave Digestion: NMKL[1] Collaborative Study

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A method for determination of lead, cadmium, zinc, copper, and iron by atomic absorption spectrometry (AAS) after microwave digestion was subjected to a collaborative study in which 16 laboratories participated [including users of inductively coupled plasma (ICP) and ICP–mass spectrometry (MS)]. The types of samples included in the study were minced fish, wheat bran, milk powder, bovine and pig liver, mushroom, 2 simulated diets, and bovine muscle; the last 4 were certified reference materials. These were analyzed as single (4 samples), double blind (1 sample), or split level (2 samples) samples. Before the collaborative study, a pretrial was conducted in which 4 ready-made solutions and one fish tissue sample were analyzed for Pb and Cu. The reproducibility relative standard deviation (RSD_R) values, for results above the detection limit, ranged from 59% at 0.155 mg/kg to 16% at 1.62 mg/kg for Pb, from 28% at 0.0124 mg/kg to 11% at 0.482 mg/kg for Cd, from 9.3% at 35.3 mg/kg to 1.7% at 147 mg/kg for Zn, from 39% at 0.241 mg/kg to 3.0% at 63.4 mg/kg for Cu, and from 17% at 7.4 mg/kg to 5.9% at 303 mg/kg for Fe. The RSD_R values agreed well with the norms described by the International Union of Pure and Applied Chemistry. As a complement to the AAS determinations, a number of laboratories analyzed the samples either by ICP or by ICP–MS. The results of these analyses agreed well with the AAS results. On the basis of the results of the collaborative study, the method was adopted Official First Action by AOAC INTERNATIONAL.

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The recommendation was approved by the Methods Committee on Residues and Related Topics, and was adopted by the Official Methods Board of AOAC INTERNATIONAL. *See* "Official Methods Board Actions," (1999) *Inside Laboratory Management*, November/December issue.

[1] Nordic Committee on Food Analysis (Secretariat General c/o National Veterinary Institute, Department of Food and Feed Hygiene, PO Box 8156, Dep. N-0033 Oslo, Norway).

Most samples need to be brought into solution by some means in order to have their element content determined. The 2 most widely used techniques are based on dry ashing or wet digestion. Both techniques have advantages as well as limitations. The choice of technique should be based on the needs of the specific user. Dry ashing provides good detection limits and needs little attendance, but it is time consuming and sensitive to contamination. Wet digestion is very rapid and normally not as sensitive to contamination, but it is labor intensive and usually results in rather dilute solutions.

The first use of microwave energy as a heat source in wet digestion was demonstrated in 1975 (1). Numerous applications have since been described, both for open-vessel and closed-vessel digestions. Closed-vessel digestion has several advantages over open-vessel digestion: smaller quantities of reagent (no evaporation), less contamination, and a higher reaction rate. Many examples of practical and theoretical concepts are given by Kingston and Jassie (2). The most commonly used acid in digestion of biological materials is nitric acid, but a variety of different acid mixtures have been used. Hydrogen peroxide in combination with nitric acid gives a higher oxidizing power and has been shown by Matusiewicz et al. (3) to be an efficient and safe oxidizing mixture.

After the successful completion of the collaborative study of a method for determination of metals in foods by atomic absorption spectrometry (AAS) after dry ashing (4) in 1989, it soon became evident that a collaborative study of a method based on wet digestion was needed as well. A survey of a large number of laboratories in the Nordic countries in 1992 showed that wet digestion using microwaves as the source of energy was the technique most rapidly increasing in use. We, therefore, set out to develop a method for wet digestion under pressure, using microwaves.

Because the number of metals that can be determined in a collaborative trial is limited for practical reasons, it was decided that the method should be restricted to the toxic metals Pb and Cd and the essential metals Zn, Cu, and Fe. A larger number of metals would have put a heavy burden on the participants.

Fourteen laboratories participated in a pretrial in which Pb (chronically difficult to determine) and Cu (which is deter-

mined at the highest wavelength and could thus reveal problems with D2 background correction systems) were determined. The samples were 2 ready-made standard solutions, 2 ready-made sample solutions, and one sample of fish tissue to be digested by the participating laboratory. The pretrial showed that the participants had both the digestion and determination steps well under control, and the results were encouraging for the subsequent collaborative trial.

Because of the urge to improve, or at least include some of our own ideas, it is often difficult to impress upon the participants in collaborative trials the importance of following the method exactly. Therefore, before the start of the collaborative trial, we organized meetings with the participants in the different countries. During these meetings, the participants were instructed about the importance of adhering to the method during the analytical work, how to report the results, and where other difficulties could be reported. The participants considered these meetings very beneficial in explaining the purpose of the study and the importance of following the protocol in detail.

Five participants analyzed the samples by inductively coupled plasma–atomic emission spectrometry (ICP–AES) or ICP–mass spectrometry (ICP–MS), which provided an excellent complement to the AAS determinations. The determinations were made with the same sample solution in the 3 laboratories that used both AAS and ICP techniques.

Collaborative Study

The collaborative trial followed the AOAC INTERNATIONAL (5) and NMKL (6) guidelines. A total of 16 laboratories from Finland, Norway, and Sweden participated.

Test Materials

The following sample types (replication types) were used in the collaborative study: (*1*) minced fish, fresh, containing Pb added at 0.5 mg/kg and Cd added at 0.2 mg/kg, and packed in Al cans (single); (*2*) wheat bran (double blind); (*3*) milk powder, freeze-dried (single); (*4*) and (*5*) bovine liver and pig liver, freeze-dried (split); (*6*) mushroom, air-dried, National Food Administration (NFA) certified reference material (CRM; single); (*7*) and (*8*) simulated diets E and F, freeze-dried, NFA CRM (split); (*9*) bovine muscle, National Institute of Standards and Technology (NIST) standard reference material (SRM) 8414 (single); and (*10*) reagent blanks (*see* method description; double).

The minced fish, wheat bran, and milk powder were also used in a previous collaborative trial (4) and therefore had estimated assigned values. Two of the laboratories in this trial took part in the earlier trial in 1989 and might thus have had prior information on the nature of these samples. However, all test materials with the exception of the minced fish were repackaged in 12.5 mL plastic containers and sent with code markings to the participants. The possibility that the 2 laboratories participating in 1989 would recognize the minced fish sample was considered to be insignificant. This material, in contrast to the dry powders, was intended to be analyzed fresh. The bovine and pig livers were in-house reference materials with known metal levels. Both the mushroom reference material, consisting of dried and pulverized *Cantharellus tubaeformis*, and the 2 simulated diets (E and F), produced from a number of foods mixed in different proportions, were CRMs produced at the NFA (7, 8). The Bovine Muscle Powder (NIST SRM 8414) was purchased from NIST, Gaithersburg, MD. The blanks were used as double blind samples in accordance with AOAC INTERNATIONAL guidelines (5).

Homogeneity of Test Materials

The homogeneities of the minced fish, wheat bran, and milk powder used in a previous collaborative trial were described in an earlier report (4). The mushroom and the simulated diets E and F were CRMs, and their homogeneities were described in separate reports (7, 8). The bovine liver and pig liver were analyzed for within- and between-container variation by one-way analysis of variance (ANOVA) of duplicate determinations of 10 randomly selected containers of each type of sample. The results of the homogeneity study of these samples are shown in Table 1. Inhomogeneity was indicated for Cd and Fe in the bovine liver, but was considered insignificant for the reasons described below. The homogeneity of the Bovine Muscle Powder (SRM 8414) was defined by NIST.

The statistical test of homogeneity was based on a comparison between (*1*), the variation between determinations made within the containers pooled over all containers analyzed (error of method), and (*2*), the variation between containers (error of method + inhomogeneity). These 2 variations are equal if no inhomogeneity is present. Random variations, however, are generated that will sometimes cause the ratio (*2*) divided by (*1*) to deviate from 1, even if no inhomogeneity is present. Therefore, only large values for this ratio can indicate inhomogeneity. The *F*-distribution was used to compute *P*-values.

P-values of >0.05 are normally interpreted as if no inhomogeneity is indicated, whereas *P*-values of <0.05 are normally interpreted as if inhomogeneity is present. However, in the latter case, there is a risk equal to the *P*-value of drawing the wrong conclusion because the *P*-value gives only the probability of random effects alone being the cause of the results. This means that the risk of a randomly caused statistically significant result increases if many tests are performed at a *P* level of 0.05. Inhomogeneity may still be present if it is evenly distributed between and within containers (but will be undetected if the sample mass is small), which would result in a *P*-value of >0.05. To some extent, this situation can be identified by high relative standard devation (RSD) values. "Normal" or low RSDs when the *P*-value is <0.05 indicate that the inhomogeneity is probably insignificant although the *P*-value indicates the contrary.

Range of Metal Concentrations

The Pb levels in the samples ranged from <0.055 to 1.6 mg/kg, which covers the levels likely to be found in most foods. Cd ranged from <0.002 to 0.76 mg/kg. Only a few foods may be expected to fall outside this range, e.g., kidneys and certain wild-growing fungi. Zn ranged from 4 to 182 mg/kg, Cu was between 0.24 and 108 mg/kg, and Fe

Table 1. Homogeneity data[a] for metals in bovine liver and pig liver test materials

Parameter	Pb	Cd	Zn	Cu	Fe
			Bovine liver		
Mean, mg/kg	0.149	0.233	132	162	221
SD, mg/kg[b]	0.056	0.009	1.4	3.7	2
RSD, %[c]	37	4	1	2	1
P[d]	>0.05	0.047	>0.05	>0.05	0.006
S_s, mg/kg[e]	0	0.0068	0.74	1.0	2.0
S_a, mg/kg[f]	0.058	0.0067	1.2	3.6	1.3
RSD_s, %[g]	0.0	2.9	0.56	0.62	0.89
			Pig liver		
Mean, mg/kg	0.135	0.083	237	57.7	716
SD, mg/kg	0.021	0.004	2.5	1.6	18
RSD, %	16	5	1	3	3
P	>0.05	>0.05	>0.05	>0.05	>0.05
S_s, mg/kg	0	0.0011	1.18	0.95	6.0
S_a, mg/kg	0.0246	0.0042	2.18	1.25	17.3
RSD_s, %	0.0	1.4	0.50	1.6	0.83

[a] Results are in mg/kg dry wt.
[b] SD = standard deviation.
[c] RSD = relative standard deviation of all replicates.
[d] *P* = probability value of the one-way ANOVA (*P*-values of <0.05 indicate inhomogeneity with a 95% probability).
[e] S_s= sampling standard deviation.
[f] S_a = analytical standard deviation.
[g] RSD_s = sampling relative standard deviation.

ranged from 7 to nearly 500 mg/kg. These ranges include the levels found in most foods.

999.10 Lead, Cadmium, Zinc, Copper, and Iron in Foods—Atomic Absorption Spectrophotometry after Microwave Digestion

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[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 (0.1), Cd (0.01), Zn (4), Cu (0.2), 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 the 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. For disposal of 4% acetic acid solutions, follow local regulations.

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, Mattews, NC

Table 999.10A Interlaboratory study results

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

[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.

28106-2000, USA. Microwave oven should be regularly checked 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:

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

(**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.

All glassware and plasticware should be carefully cleaned and rinsed, e.g., with HNO_3 or HCl , in order to avoid metal contamination.

C. Reagents

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

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

Table 999.10C Instrumental parameters for GFAAS

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

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

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

(**f**) *Zinc standard solution*.—1 mg/. 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**) *Pb standard solution*.—1 mg/mLDissolve 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**) *Pre-treatment*.—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:

$$H_2O = \frac{W_f - W_d}{W_f} \times 100$$

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 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.

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

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:

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

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:

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

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 ($n = \geq 20$). 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.

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Results and Discussion

Results of the Pretrial Study

Pb and Cu were determined in 5 samples consisting of 2 ready-made standard solutions, 2 ready-made sample solutions (made from solutions of digested wheat flour and bran and pig kidney) and one sample of canned (minced) fish. The results are shown in Table 2. They indicate that most of the deviation is due to the analytical steps before the AAS determinations. The RSD values for the different solutions, which were all very small, indicate that the participants had their AAS instruments in good working order.

Table 2. Results[a] for Pb (GFAAS) and Cu (GFAAS and FAAS) from the pretrial study

Parameter	Sample				
	Std soln 1	Std soln 2	Sample soln 1	Sample soln 2	Fish tissue
			Pb		
n[b]	11	13	11	12	12
No. of outliers	1	0	0	1	1
Mean	0.00143	0.0171	0.00149	0.0168	0.483
S_r[c]					0.011
S_R[d]	0.00035	0.0016	0.00082	0.0013	0.130
RSD_r, %[e]					2.3
RSD_R, %[f]	24	9.4	55	7.9	27
r[g]					0.031
R[h]	0.00098	0.0045	0.0023	0.0036	0.363
			Cu		
n	14	14	13	14	13
No. of outliers	0	0	1	0	1
Mean	0.049	4.79	0.268	1.94	0.310
S_r					0.045
S_R	0.008	0.18	0.012	0.06	0.086
RSD_r, %					14
RSD_R, %	16	3.7	4.6	3.3	28
r					0.125
R	0.022	0.49	0.35	0.18	0.240

[a] Results in mg/L (solutions) and mg/kg fresh weight (fish tissue).
[b] n = number of laboratories after elimination of outliers.
[c] S_r = repeatability standard deviation.
[d] S_R = reproducibility standard deviation.
[e] RSD_r = repeatability relative standard deviation.
[f] RSD_R = reproducibility relative standard deviation.
[g] $r = 2.8 \times S_r$.
[h] $R = 2.8 \times S_R$.

The canned fish was to be freeze-dried by the participants before digestion. The fish tissue sample was selected for analysis for 2 reasons: to see how the participants managed all steps of the analysis and to enable a comparison with the analysis of the fish in the collaborative study, in which the fish sample was analyzed in the fresh state. The RSD values for the digested fish tissue were fully satisfactory. The results for the fortified minced fish agreed well with the fortified level of 0.5 mg Pb/kg.

Collaborators' Comments

One participant emphasized the importance of a sentence in the method description that explicitly permitted the use of matrix modifiers. Although the use of matrix modifiers is very widespread, this is an area in which most analysts use their own approach. Optimization of the method for the best modifier for each combination of metal and food matrix was regarded to be outside the scope of this project. It is, however, up to each analyst to decide to use the matrix modifier of his/her choice, and to demonstrate its effects.

Another participant questioned whether brand names should be mentioned in the method. When brand names or other trademarks are used, it is only to clarify the quality of an item that is required.

The need to transfer the sample solution from the 25 mL volumetric flask to a larger plastic vessel was questioned by one participant. The reasons for the transfer are that plastic beakers are easier to handle and that volumetric flasks have a rather narrow neck, sometimes making pipetting difficult.

Results of the Collaborative Study

A total of 16 laboratories participated. Fourteen laboratories used AAS. Three used both AAS and ICP and/or ICP–MS (with the same sample solutions). Two laboratories used only ICP and/or ICP–MS. However, not all the participants determined all the metals.

Table 3. Collaborative results (mg/kg) for the GFAAS determination of Pb in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10:1[b]	10:2[b]
1	0.486	0.0923	0.109	0.0107	0.155	0.0835	1.56	0.277	0.461	0.381	0.000092	0.000125
2	0.5059	0.1047	0.1107	–0.0199	0.149	0.05102	1.78	0.245	0.444	0.375	–0.00005	0.00105
3	0.529	0.097	0.093	0.010	0.117	0.028	1.41	0.262	0.471	0.431	0.00	0.00
4	0.533	0.105	0.144	0.0148	0.217	0.0964	1.73	0.238	0.457	0.508	0.0003	0.0005
5	0.459	0.0897	0.144	–0.00724	0.0885	0.0546	1.49	0.203	0.399	0.319	0.00095	0.00085
6	0.256	0.112	0.357	0.00646	0.875[c]	0.105[c]	1.84	0.385	0.759	5.73[d]	0.001276	0.001322
7	0.558	0.135	0.46	0.0239	0.159	0.255	1.63	0.273	0.305	0.337	0.0001	0.0005
10	—	—	—	—	—	—	—	—	—	—	0.0009	0.0021
11	0.238	0.127	0.169	0.0114	0.153	0.0768	1.23	0.358	0.658	0.489	0.0006	0.0004
13	0.393	0.094	0.178	–0.0303	0.208	0.169	1.34	0.252	0.398	0.344	–0.00033	0.00032
14	0.475	0.102	0.117	–0.0882[d]	0.125	0.14	1.41	0.362	0.538	0.868[d]	0.0002	–0.0002
15	0.683	0.213	0.285	0.286[d]	0.224	0.063	2.137	0.297	0.631	0.523	0.0006	0.0008
16	0.591	0.132	0.149	0.0295	0.16	0.0968	1.86	0.286	0.487	0.272	0.00009	–0.00001

[a] Double blind.
[b] Double blank.
[c] Cochran's outlier.
[d] Grubbs outlier.

Table 4. Collaborative results (mg/kg) for the GFAAS determination of Cd in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10.1[b]	10.2[b]
1	0.184	0.166	0.167	0.00049	0.224	0.0863	0.479	0.55	0.919	0.0122	3.7E-06	4.1E-06
2	0.246	0.189	0.199	–0.00448	0.251	0.0979	0.568	0.588	1.084	0.00752	0.00002	0.00004
3	0.186	0.165	0.17	0.001	0.219	0.104	0.513	0.603	0.861	0.01	0.00	0.00
4	0.179	0.156	0.157	–0.00045	0.213	0.0763	0.424	0.535	0.933	0.0116	0.00002	0.00004
5	0.2	0.161	0.165	0	0.205	0.0802	0.484	0.55	0.981	0.0084	—	—
6	0.197	0.197	0.222	0.0241[c]	0.281	0.0807	0.762[c]	0.73	1.14	0.013	0.00024[c]	0.00014[c]
7	0.202	0.234[d]	0.357[d]	0.00438	0.312	0.102	0.498	0.758[d]	1.41[d]	0.0204	0.00013[d]	0.00000[d]
10	—	0.141	0.143	0.001	0.181	0.075	0.411	0.449	0.775	0.012	0	0
11	0.242	0.225[c]	0.31[c]	0.00569	0.327	0.129	0.676[c]	0.728	1.02	0.0167	0.00006	0.00004
13	0.195	0.188	0.207	–0.001	0.244	0.0966	0.56	0.655	1.06	0.0134	0.00009	0.00005
14	0.173	0.145	0.145	–0.00207	0.189	0.0736	0.411	0.473	0.75	0.0116	0	–0.00004
15	0.231	0.16	0.161	–0.00498	0.221	0.076	0.48	0.565	0.974	0.012	0.00009	0.00007
16	0.293	0.171	0.185	0.0236[c]	0.221	0.107	0.474	0.531	0.883	0.0273[c]	0.000004	0.000001

[a] Double blind.
[b] Double blank.
[c] Grubbs outlier.
[d] Cochran's outlier.

Table 5. Collaborative results (mg/kg) for the FAAS determination of Zn in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10:1[b]	10:2[b]
1	4.16	74.3	74.8	36.5	131	227	57.7	39	56.9	146	–0.0034	0.0012
2	4.11	74.2	75.5	35.9	133	228	56.4	39.8	55.9	145	0.005	0.002
3	4.06	65.4[c]	31.2[c]	28.1	128	218	53.1	34.2	53.0	134[d]	0	0
4	4.91	71.7	72.7	36.4	135	229	58.2	39.9	55.8	150	0	0
5	4.21	71.4	71.6	33.2	137	231	57.2	38.9	55.1	144	0.004	0.005
6	4.46	70	78.3	38.4	149	238	61.4	43.8	55.9	147	0.001	0.002
7	4.7	73.2	73.6	35.8	127	229	55	37	56.1	136[d]	0	0
9	5.22	75.1	73.1	40.2	142	244	62.0	42.8[c]	77[c]	171[d]	0.0044	0.0002
10	—	69.3	68.2	33.1	116	209	52.5	42.1	52.9	146	0.003	0.004
11	4.19	72.6	78.7	32.4	138[c]	211[c]	58.4	40.6	58.8	150	0	0
13	4.18	76.1	75.8	36.5	136	231	56.8	40.8	56.2	146	—	—
14	—	79.2	76.9	37.3	143	235	58.5	43.5	59.8	152	—	—
15	4.72	63.6	69.8	31.4	133.0[c]	243.8[c]	51.9	34.3	54.9	148.7	0.0148[d]	0.008[d]
16	5.07	76.5	73.8	38.7	138	231	57.7	40.5	57.3	146	—	—

[a] Double blind.
[b] Double blank.
[c] Cochran's outlier.
[d] Grubbs outlier.

Table 6. Collaborative results (mg/kg) for the FAAS determination of Cu in foods

	Sample										
Lab	1	2:1[a]	2:2[a]	4	5	6	7	8	9	10:1[b]	10:2[b]
1	0.174	9.86	10	158	55.4	38.3	50.7	77.7	2.44	—	—
2	—	10.78	11.02	157	54.6	38.3	49.8	76.8	—	—	—
3	0.176	10.3	13.1	165	53	35.9	49.7	74.8	2.81	0	0
4	—	—	—	168	53.3	36.6	48.8	75.5	—	—	—
5	—	—	—	155	53.8	36.7	48.3	75.8	—	0.00153	0.00118
6	—	9.7	10.4	159	56.1	39.8	50.6	77.6	—	0.002	0.003
7	—	9.99	11.2	158	55.4	40.5	51.9	80.6	—	—	—
9	0.375	10.2	9.87	163	53.9	40.3	48.8	75	2.51	0.0509	0.0664
10	—	10.2	9.68	155	54.7	36.6	45.3	75.3	2.75	0.005	0.010
11	—	—	—	164	57.6	35.9	55.7[c]	83.8[c]	—	—	—
13	—	11	10.9	165	54.5	38.8	50.9	76.9	—	—	—
14	—	11.2	10.8	165	55.8	39.04	52.4	79.3	2.78	—	—
15	0.24	8.4	9.01	151.5	45.6	32.5	41.8[c]	68.1[c]	2.49	0.0166	0.0184
16	—	9.82	8.87	167	55	38.7	50.4	79.2	—	—	—

[a] Double blind.
[b] Double blank.
[c] Grubbs outlier.

Table 7. Collaborative results (mg/kg) for the GFAAS determination of Cu in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10:1[b]	10:2[b]
1	—	—	—	0.422	—	—	—	—	—	—	0.00015	0.00023
2	0.2108	—	—	0.539	—	—	—	—	—	3.26	0.00034	0.00121
3	—	—	—	—	—	—	—	—	—	—	—	—
4	0.243	—	—	0.475	—	—	—	—	—	2.74	0.0006	0.0002
5	0.232	—	—	0.903	—	—	—	—	—	2.52	—	—
6	0.319	—	—	0.658	—	—	—	—	—	2.98	—	—
7	0.343	—	—	0.589	—	—	—	—	—	3.54	0.0043	0.0047
9	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—
11	0.203	—	—	0.398	—	—	—	—	—	3.04	0.0010	0.0008
13	0.214	—	—	0.437	—	—	—	—	—	2.82	0.0002	0.0002
14	0.206	—	—	—	—	—	—	—	—	—	0.00698	0.00459
15	—	—	—	—	—	—	—	—	—	—	—	—
16	0.365	—	—	0.577	—	—	—	—	—	3.83	0.0008	0.0009

[a] Double blind.
[b] Double blank.

Table 8. Collaborative results (mg/kg) for the FAAS determination of Fe in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10:1[b]	10:2[b]
1	9.95	133	132	—	218	840	111	245	414	81.9	—	—
2	5.84	134	135	—	208.3	779	102.7	239	380.7	74.1	—	—
3	6.21	129	134	—	224	767	106	234	383	72.2	0	0
4	6.76	124	124	5.45	214	783	101	233	379	71.8	0	0
5	7.82	120	109	1.6	201	728	108	238	377	70.4	0.035	0.021
6	—	123	129	—	202	808	111	242	376	74.8	–0.038	–0.002
7	6.47	123	115	—	185	707	103	215	336	68.1	—	—
9	7.64	127	126	6.41	232	755	120	229[c]	430[c]	95.2	0.0497	0.0607
10	—	102	98.4	3.15	177	723	90.2	191	365	68.3	—	—
11	—	120	128	—	197	747	—	233	367	65	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—
15	8.27	119.1	113.8	–13.4	213	750.3	109.5	237.7	368	80.1	0.270	0.263
16	7.86	130	127	—	201	827	97.8	226[c]	283[c]	77.8	—	—

[a] Double blind.
[b] Double blank.
[c] Cochran's outlier.

Table 9. Collaborative results (mg/kg) for the GFAAS determination of Fe in foods

	Sample											
Lab	1	2:1[a]	2:2[a]	3	4	5	6	7	8	9	10:1[b]	10:2[b]
1	—	—	—	1.70	—	—	—	—	—	—	0.00143	0.00146
2	—	—	—	1.39	—	—	—	—	—	—	0.029	0.032
3	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—
6	7.59	—	—	4.06	—	—	—	—	—	—	—	—
7	—	—	—	2.91	—	—	—	—	—	—	0.0056	0.0051
9	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—
11	6.20	—	—	3.98	—	—	90.3	—	—	—	0.013	0.013
13	10.1	—	—	1.95	—	—	98.7	—	—	—	0.002	0.002
15	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	3.80	—	—	—	—	—	—	0.0041	0.0022

[a] Double blind.
[b] Double blank.

Results for the AAS determinations were received from 14 collaborating laboratories (Tables 3–9). These results were divided into subgroups, depending on whether results were derived from FAAS or GFAAS. The statistical evaluation was performed with results remaining after elimination of outliers according to the guidelines of AOAC INTERNATIONAL (5). Detection limits for the 5 metals were calculated from the blanks by using the S_r.

Pb.—All results were derived from GFAAS analyses. Laboratory 10 experienced problems with the low and intermediate levels, and was therefore eliminated before the calculations. Four results were eliminated by the Grubbs test and 2 by Cochran's test. The detection limits were calculated to be 0.055 mg/kg for a 0.5 g sample and 0.014 mg/kg for a 2 g sample. The result for analysis of the fortified fish sample agreed well with the fortified level as well as with the result from the pretrial.

Cadmium.—All results were derived from GFAAS. Nine results were eliminated by the Grubbs test and 6 by Cochran's test. The detection limits were calculated to be 0.002 mg/kg for a 0.5 g sample and 0.0006 mg/kg for a 2 g sample. The result for analysis of the fortified fish sample agreed well with the fortified level of 0.2 mg/kg.

Zinc.—All results were derived from FAAS. Five results were eliminated by the Grubbs test and 8 by Cochran's test. The detection limits were calculated to be 0.24 mg/kg for a 0.5 g sample and 0.060 mg/kg for a 2 g sample.

Copper.—The results were derived from both FAAS and GFAAS. Four FAAS results were eliminated by the Grubbs test. The detection limits for FAAS were calculated to be 0.71 mg/kg for a 0.5 g sample and 0.18 mg/kg for a 2 g sample. For GFAAS, the detection limits were calculated to be 0.098 mg/kg for a 0.5 g sample and 0.024 mg/kg for a 2 g sample.

Table 10. HORRAT values calculated from the collaborative results

Element determined	Method of analysis	Sample							
		1	2	3	4 & 5	6	7 & 8	9	10
Pb	GFAAS	1.5	2.8	—	1.9	1.1	1.4	1.2	—
Cd	GFAAS	0.8	0.6	—	1.0	0.6	0.8	0.9	—
Zn	FAAS	0.7	0.6	1.0	0.7	0.6	0.6	0.2	—
Cu	FAAS	2.0	0.7	—	0.5	0.6	0.4	0.5	—
Fe	FAAS	1.4	1.0	74	1.0	0.9	0.9	1.3	—
Cu	GFAAS	1.3	—	1.6	—	—	—	1.0	—
Fe	GFAAS	2.1	—	3.0	—	0.8	—	—	—

Iron.—The results were derived from both FAAS and GFAAS. Four FAAS results were eliminated by Cochran's test. The detection limits for FAAS were calculated to be 1.6 mg/kg for a 0.5 g sample and 0.41 mg/kg for a 2 g sample. For GFAAS, the detection limits were calculated to be 0.15 mg/kg for a 0.5 g sample and 0.038 mg/kg for a 2 g sample.

Calculation of HORRAT values (RSD acceptance intervals).—Table 10 shows the HORRAT values for the various concentrations from the collaborative study. The HORRAT is calculated as follows:

$$\text{HORRAT} = \text{RSD}_R/2^{(1 - 0.5 \log c)}$$

where c is the concentration expressed as a decimal fraction. With any method, HORRAT values of 0.5–2 indicate acceptable performance with respect to precision. The procedure is described as the RSD acceptance interval by the International Union of Pure and Applied Chemistry (IUPAC; 9) and referred to by NMKL (6).

Results for the reference materials.—The use of reference materials supplied by standards organizations is, on certain conditions, encouraged by AOAC INTERNATIONAL for use in collaborative studies. However, no guidelines are given on how to interpret the results for these reference materials. The NIST *Handbook for SRM Users* (10) gives a procedure, based on confidence intervals for both the results found and the certified means, of how results can be evaluated. Although the relevance of such a comparison can be argued, it has over the years developed into a de facto norm, in which (*1*) the found mean is compared with the certified interval (usually given as a

Table 11. Comparison between the levels found in the collaborative study and the means ± 95% confidence intervals for the CRMs[a]

Parameter	Mushroom, NFA	Simulated diet E, NFA	Simulated diet F, NFA	Bovine Muscle, NIST SRM 8414
		Pb, GFAAS		
Study mean ± S_R[b]	1.62 ± 0.26	0.287 ± 0.055	0.501 ± 0.127	0.398 ± 0.086
Cert. mean ± 95% CI[c]	1.43 ± 0.10	0.273 ± 0.017	0.439 ± 0.026	0.38 ± 0.24
Mean within range	N[d]	Y[e]	N	Y
Range overlap	Y	Y	Y	Y
		Cd, GFAAS		
Study mean ± S_R	0.482 ± 0.053	0.580 ± 0.088	0.948 ± 0.119	0.0124 ± 0.0034
Cert. mean ± 95% CI	0.437 ± 0.031	0.536 ± 0.031	0.877 ± 0.045	0.013 ± 0.011
Mean within range	N	N	N	Y
Range overlap	Y	Y	Y	Y
		Zn, FAAS		
Study mean ± S_R	56.9 ± 3.0	39.6 ± 3.0	56.0 ± 1.9	147 ± 2
Cert. mean ± 95% CI	55.0 ± 2.0	39.5 ± 3.1	55.8 ± 3.9	142 ± 14
Mean within range	Y	Y	Y	Y
Range overlap	Y	Y	Y	Y
		Cu, FAAS		
Study mean ± S_R	37.7 ± 2.2	49.8 ± 1.9	77.0 ± 1.9	2.63 ± 0.17
Cert. mean ± 95% CI	34.4 ± 3.5	46.5 ± 1.4	72.7 ± 1.0	2.84 ± 0.45
Mean within range	Y	N	N	Y
Range overlap	Y	Y	N	Y
		Fe, FAAS		
Study mean ± S_R	105 ± 8	231 ± 16	375 ± 19	75.0 ± 8.1
Cert. mean ± 95% CI	101 ± 11	216 ± 17	334 ± 50	71.2 ± 9.2
Mean within range	Y	Y	Y	Y
Range overlap	Y	Y	Y	Y

[a] Results in mg/kg dry wt.
[b] S_R = reproducibility standard deviation.
[c] CI = confidence interval.
[d] N = no.
[e] Y = yes.

Table 12. Comparison of results from the AAS determinations with those from the ICP/ICP–MS determinations after microwave digestion[a]

Parameter	Minced fish	Wheat bran	Milk powder	Livers	Mushroom, NFA	Simulated diets E and F, NFA	Bovine Muscle, NIST 8414
				Pb			
Mean GFAAS ± S_R[b]	0.476 ± 0.129	0.150 ± 0.091	0.0049 ± 0.0188[c]	0.130 ± 0.055	1.62 ± 0.26	0.394 ± 0.098	0.398 ± 0.086
Mean ICP–MS ± S_R	0.431 ± 0.048	0.091 ± 0.012	0.0071 ± 0.0016	0.122 ± 0.042	1.28 ± 0.13	0.315 ± 0.030	0.415 ± 0.068
Agreement[d]	Y[e]	N[f]	Y	Y	N	Y	Y
				Cd			
Mean GFAAS ± S_R	0.211 ± 0.035	0.171 ± 0.022	–0.000038 ± 0.0032[c]	0.164 ± 0.034	0.482 ± 0.053	0.764 ± 0.105	0.0124 ± 0.0034
Mean ICP–MS ± S_R	0.183 ± 0.013	0.175 ± 0.018	0.0041 ± 0.0029	0.167 ± 0.021	0.489 ± 0.052	0.742 ± 0.059	0.0179 ± 0.0086
Agreement	Y	Y	N	Y	Y	Y	Y
				Zn			
Mean FAAS ± S_R	4.50 ± 0.41	73.5 ± 3.5	35.3 ± 3.3	182 ± 9	56.9 ± 3.0	47.8 ± 2.5	147 ± 3
Mean ICP[g] ± S_R	4.25 ± 0.59	79.1 ± 2.2	35.4 ± 3.5	189 ± 5	58.1 ± 6.8	50.4 ± 9.4	150 ± 20
Agreement	Y	N	Y	Y	Y	Y	Y
				Cu			
Mean FAAS ± S_R	0.241 ± 0.094	10.1 ± 0.8		108 ± 4	37.7 ± 2.2	63.4 ± 1.9	2.63 ± 0.17
Mean ICP[g] ± S_R	0.263 ± 0.032	10.8 ± 0.8		107 ± 9	38.1 ± 1.5	64.0 ± 4.3	2.99 ± 0.35
Agreement	Y	Y		Y	Y	Y	Y
				Fe			
Mean FAAS ± S_R	7.42 ± 1.27	123 ± 10	0.6 ± 8.1	487 ± 31	105 ± 8	303 ± 18	75.0 ± 8.1
Mean ICP[g] ± S_R	7.36 ± 1.28	130 ± 5	6.17 ± 9.20	479 ± 21	105 ± 8	305 ± 13	74.4 ± 7.4
Agreement	Y	Y	Y	Y	Y	Y	Y

[a] Results in mg/kg dry wt (minced fish, mg/kg fresh wt).
[b] S_R = reproducibility standard deviation.
[c] Result below the detection limit.
[d] Agreement is based on 2-tailed *t*-test, $P = 0.05$.
[e] Y = yes.
[f] N = no.
[g] Both ICP–AES and ICP–MS.

Table 13. Comparison of results for the same samples analyzed by AAS in this collaborative study and by the dry ashing method in the earlier collaborative study

Parameter	Minced fish, mg/kg fresh wt	Wheat bran, mg/kg dry wt	Milk powder, mg/kg dry wt
	Pb, GFAAS		
Mean ± S_R[a], wet[b]	0.476 ± 0.129	0.150 ± 0.091	0.0049 ± 0.0188[c]
Mean ± S_R, dry[d]	0.518 ± 0.104	0.114 ± 0.054	0.0249 ± 0.0185
Agreement[e]	Y[f]	Y	N[g]
	Cd, GFAAS		
Mean ± S_R, wet	0.211 ± 0.035	0.171 ± 0.022	–0.000038 ± 0.0032[c]
Mean ± S_R, dry	0.209 ± 0.040	0.177 ± 0.020	0.0020 ± 0.0016
Agreement	Y	Y	Y
	Zn, FAAS		
Mean ± S_R, wet	4.50 ± 0.41	73.5 ± 3.5	35.3 ± 3.3
Mean ± S_R, dry	4.40 ± 0.55	71.5 ± 4.9	35.0 ± 2.8
Agreement	Y	Y	Y
	Cu, FAAS		
Mean ± S_R, wet	0.241 ± 0.094	10.1 ± 0.8	
Mean ± S_R, dry	0.222 ± 0.077	8.75 ± 2.00	
Agreement	Y	N	
	Fe, AAS		
Mean ± S_R, wet	7.42 ± 1.27	123 ± 10	0.6 ± 8.1
Mean ± S_R, dry	6.28 ± 0.44	122 ± 13	1.74 ± 0.61
Agreement	N	Y	

[a] S_R = reproducibility standard deviation.
[b] Wet digestion (present study).
[c] Result below the detection limit.
[d] Dry ashing (previous study; ref. 4).
[e] Agreement is based on 2-tailed *t*-test, $P = 0.05$.
[f] Y = yes.
[g] N = no.

95% confidence interval), and (*2*) the found interval (usually given as a standard deviation) is compared with the certified interval. If the found mean falls within the certified interval, the result is considered excellent. If the 2 intervals overlap, the result is considered acceptable. If there is no overlap, the result is considered biased. In Table 11, the results for the CRMs, mushroom (sample 6), simulated diets E and F (samples 7 and 8), and Bovine Muscle (sample 9), are compared according to this model. An alternative method for evaluation of reference materials is described in an earlier paper (7).

Comparison with ICP/ICP–MS.—Five laboratories analyzed the samples by ICP–AES or ICP–MS (for Pb and Cd, only ICP–MS) after microwave digestion. The results are too few for evaluation of the ICP–AES and ICP–MS techniques, but comparison of the results (using a 2-tailed *t*-test, $P = 0.05$) with the AAS results will strengthen the validation of the microwave AAS method (Table 12).

Comparison with results of the dry ashing method.—Three samples (1, minced fish; 2, wheat bran; and 3, milk powder) were used in an earlier collaborative study of a dry ashing method (4) in 1989. The comparison of the results from these 2 trials (using a 2-tailed *t*-test, $P = 0.05$) indicate that they are not statistically different (*see* Table 13). It should be noted that the results for Pb and cadmium by the dry ashing method are derived from both FAAS and GFAAS.

Comparison with results of the pretrial.—The results of the analysis of the minced fish in the fresh state in the collaborative study agreed very well with the results of the pretrial in which the minced fish was analyzed after freeze-drying.

Conclusions

The HORRAT values are satisfactory. There is good agreement between the levels found and the certified means and ranges for the CRMs. There is good agreement between the microwave AAS method and the dry ashing AAS method. There is good agreement between the results from AAS and ICP/ICP–MS after microwave digestion.

Recommendation

The Associate Referee recommends that this method be adopted First Action by AOAC INTERNATIONAL.

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