RESIDUES AND TRACE ELEMENTS

Determination of Metals in Foods by Atomic Absorption Spectrometry after Dry Ashing: NMKL[1] Collaborative Study

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A method for determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrometry (AAS) after dry ashing at 450°C was collaboratively studied in 16 laboratories. The study was preceded by a practice round of familiarization samples and another round in which solutions were distributed and the metals were determined directly by AAS. The study included 5 different foods (liver paste, apple sauce, minced fish, wheat bran, and milk powder) and 2 simulated diets. A single analysis was carried out with each sample. Suitable sample combinations were used as split-level combinations for determination of the repeatability standard deviation. The reproducibility relative standard deviation for each of the elements ranged from 20 to 50% for lead concentrations of 0.040–0.25 mg/kg, from 12 to 352% for cadmium concentrations of 0.001–0.51 mg/kg, from 4 to 8% for zinc concentrations of 0.7–38 mg/kg, from 7 to 45% for copper concentrations of 0.51–45 mg /kg, and from 11 to 14% for iron concentrations of 4–216 mg/kg.

Most of the collaboratively studied and approved methods available today for trace element determinations are very specific and apply only to one or 2 elements, usually in a very specific matrix. Only a few methods exist that are approved for simultaneous determination of more than one element in more general types of food matrixes (1). For many elements commonly determined, there are no approved methods at all.

Most types of samples require a procedure to get the sample into solution before analysis by atomic absorption spectrometry (AAS). The 2 most commonly used techniques to accomplish this are dry ashing at a defined temperature and wet digestion with mineral acid. Over the years, several investigators have pointed out the possible loss of analyte during dry ashing. Gorsuch (2, 3) showed that certain metals could be lost through volatilization or retention on silica crucible walls when metallic standard solutions were added to the samples, or when metallic standard solutions were ashed with certain chlorides. Losses of Cd in specific sample tissues were reported by Feinberg and Ducauze (4) and by Slabyj et al. (5). In the first case, however, the samples were ashed at 750°C, with H_2SO_4 as an ashing aid. In the second case, the indications that dry ashing contributed to the poor results were not substantiated. Koirtyohann and Hopkins (6) showed that no losses of Cd, Zn, or Fe through volatilization occurred when tissues were ashed at temperatures of <600°C. Loss by retention on crucible walls at an ashing temperature of 500°C was observed for Zn in porcelain crucibles. In platinum or silica crucibles, only insignificant retention was observed at an ashing temperature of 500°C. Using radioactive isotopes in biological materials, van Raaphorst et al. (7) demonstrated that no losses of Cd occurred by volatilization or retention at an ashing temperature of 450°C. The papers cited above present strong indications that the method for dry ashing at a maximum temperature of 450°C presented here yields results free from losses by volatilization or retention. This method has been used for many years; moreover, numerous recovery studies and frequent use of certified reference materials (CRMs)

have given no indication of systematic losses. Ashing aids and modification of the sample matrix during ashing have often been used to eliminate potential losses and/or to speed up the ashing procedure. This always increases the risk of contamination, however, and results in poorer detection limits.

Dry ashing is generally rather time consuming; it usually takes a day or more before a result can be obtained, although very little attention from the analyst is necessary. Contamination can sometimes be a problem, however, because the samples are exposed to ambient air for long periods of time. One advantage of dry ashing is that the resulting ash can be dissolved in a small amount of diluent. This provides much better detection limits than wet digestion, especially when dry ashing is used with flame AAS (FAAS).

Wet digestion methods are generally rapid; an analysis may be finished within several hours, and the methods are not as sensitive to contamination, especially when closed decomposition vessels are used. The disadvantages of wet digestion are that only fairly small samples can be used and also that the solutions normally have to be strongly diluted before the analysis. This results in rather poor detection limits, especially when FAAS is used.

The 2 decomposition techniques should, therefore, be considered complementary because both have advantages as well as drawbacks. The selection of the technique should be based on individual laboratory requirements.

AAS is now probably the most widely used technique for determination of metals in biological materials. AAS determinations are usually made by FAAS when the concentrations are high enough, or by graphite furnace AAS (GFAAS) when the concentrations are low. It is probably not meaningful to try to define exactly when to use FAAS or GFAAS. Both techniques should basically give the same result as long as the flame results are above the detection limit. In practice, FAAS should be selected instead of GFAAS whenever possible, because it is less time consuming and also less sensitive to interference (e.g., background absorption).

The metals that were considered to be of greatest interest for this collaborative trial were the toxic metals Pb and Cd, for which many countries have established legal limits and for which low detection limits are of interest. Also of interest were the essential metals Zn, Cu, and Fe, for which there are recommendations regarding a safe and adequate daily intake.

In 1986, 3 methods suggested by a working group within the NMKL were subjected to a pretrial in which the participants were free to choose which method to use. The methods were (*1*) wet digestion with HNO_3 in an open vessel; (*2*) wet digestion with H_2SO_4/HNO_3, followed by extraction with ammonium-pyrrolidinedithiocarbamate/methyl isobutyl ketone (APDC/MIBK); and (*3*) dry ashing at 450°C, according to the method described here. Of the responding laboratories, 15 used method *3*, 5 used method *2*, and 2 used method *1*. The results by all 3 methods were similar, and the reproducibility between the laboratories was encouraging for further work. But because the interest was focused on the dry ashing method, that method was selected to be collaboratively tried, and work on the 2 wet digestion methods was terminated.

In an attempt to elucidate the contribution by the determination step to the total variance in the results, a number of prepared solutions were sent to the participants and analyzed directly by AAS before the start of the collaborative trial. The results of this pretrial are discussed in the **Discussion** section.

The dry ashing method described here was based on the method of Dalton and Malanoski (8), used with a modified pre-ashing apparatus from the method of Thiers (9).

This trial was finished in 1989, and the method was approved by the NMKL in 1990 (10).

999.11 Determination of Lead, Cadmium, Copper, Iron, and Zinc in Foods—Atomic Absorption Spectrophotometry after Dry Ashing

First Action 1999

[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. 6M 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.)

(**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**).

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] $r = 2.8 \times s_r$.

[g] $R = 2.8 \times s_R$.

(**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.

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

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.

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		

(**c**) *Nitric acid.*—65% (w/w).

(**d**) *Nitric acid.*—0.1M. 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.
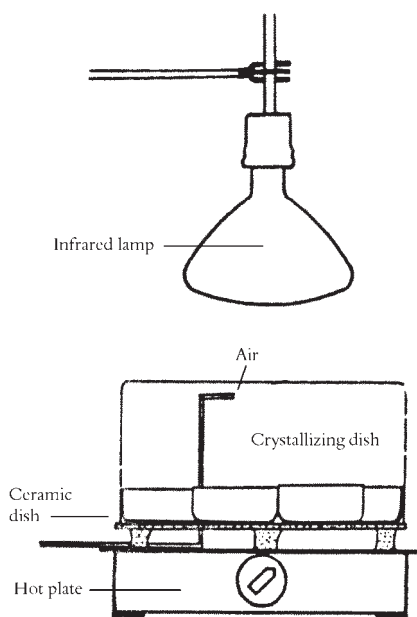

Figure 999.11   Apparatus for pre-ashing of samples.

(**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 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 6M 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:

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

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

$$c = \frac{(a-b) \times V}{m}$$

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.

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Collaborative Study

Test Materials

Test materials *1–5* were produced in Denmark under the guidance of the official adviser who was previously responsible. The test materials were (*1*) liver paste, packed in 100 mL Al cans; (*2*) apple sauce, packed in 100 mL Al cans and fortified with Pb at 0.2 mg/kg; (*3*) minced fish, packed in 100 mL Al cans and fortified with Pb at 0.5 mg/kg and Cd at 0.2 mg/kg; (*4*) wheat bran, packed in 250 mL plastic bottles;

(*5*) milk powder, packed in 100 mL plastic bottles; and (*6*) and (*7*) simulated diets D and E, packed in 50 mL plastic bottles.

The levels of Pb in apple sauce and of Pb and Cd in minced fish were fortified as shown above in order to extend the ranges of these elements. The concentrations of the different elements ranged between 0.025 and 0.5 mg/kg for Pb, between 0.001 and 0.6 mg/kg for Cd, between 0.7 and 55 mg/kg for Zn, between 0.2 and 45 mg/kg for Cu, and between 2 and 235 mg/kg for Fe. These ranges cover the natural levels found in most foods.

Test materials *6* and *7*, simulated diets, consisted of different proportions of a number of foods, e.g., meat, liver, potatoes, milk, and flour. These 2 diets were originally produced as reference samples for another project (11, 12) and are now established as CRMs (13).

To deduce the contribution of the AAS determination to the total analytical error, before the study the participating laboratories were given 4 samples of aqueous solutions to determine the metals directly by AAS: 2 mixed standard solutions containing Pb, Cd, Cu, and Fe at 2 different levels and 2 solutions of dry-ashed pork and pig liver.

Homogeneity of the Test Materials

The within- and between-container variation was determined by 2-way analysis of variance (ANOVA) of duplicate determinations of 10 randomly selected containers from each type of sample. The results are presented in Table 1. 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 will be 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 is used to compute P-values (P = probability).

Normally, P-values of >0.05 are interpreted as if no inhomogeneity is indicated, whereas P-values of <0.05 are normally interpreted as if inhomogeneity is present. However, in this latter case, there is a risk equal to the P-value of drawing the wrong conclusion because the P-value gives only the probability that random effects alone are the cause of the results. This means that the risk for a randomly caused statistical significance increases if many tests are performed at a P-level of 0.05. Thirty-five tests were performed at this level (Table 1) and consequently 2–3 random significant inhomogeneities could be expected. Inhomogeneity can still be present if it is evenly distributed between and within containers, which would result in a P-value of >0.05. To some extent, this can be identified by high relative standard deviation (RSD) values. "Normal" or low RSDs for which the P-value is <0.05 indicate that the inhomogeneity is probably insignificant, although the contrary is indicated by the P-value. The Fe concentration in sample 2 was judged as too inhomogeneous to be determined in the collaborative trial.

Table 1. Results (mg/kg dw) of the homogeneity study: means, *P*-values, and relative standard deviation (RSD) values

Metal	Results	Sample No.						
		1 Liver paste	2 Apple sauce	3 Minced fish	4 Wheat bran	5 Milk powder	6 Diet 1	7 Diet 2
Pb	Mean	0.0375	0.278	0.444	0.0930	0.0298	0.204	0.253
	P	<0.01	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05
	RSD	26	12	6.8	12	37	12	11
Cd	Mean	0.0494	0.0017	0.210	0.170	0.0005	0.492	0.566
	P	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01	>0.05
	RSD	18	45	8.1	11	50	6.6	4.0
Zn	Mean	8.29	0.639	4.22	55.1	31.3	35	39
	P	<0.01	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05
	RSD	2.3	5.3	3.7	5.3	3.8	2.9	4.1
Cu	Mean	4.78	0.184	0.184	7.53	0.320	39	45
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01
	RSD	3.0	9.2	11	2.9	11	4.5	8.0
Fe	Mean	25.7	23.2	6.96	127	1.80	212	235
	P	<0.05	<0.01	>0.05	>0.05	>0.05	<0.05	<0.01
	RSD	6.6	13	7.7	4.4	15	7.1	8.5

In general, the higher the RSD, the lower the concentration of the metal. Consequently, the highest concentrations generally had the lowest RSDs, in accordance with expectations when the levels are close to the detection limits.

Collaborators' Comments

Very few comments on, or deviations from, the method were noted on the reply forms.

Laboratory 5 took 5–10 g sample and, after ashing, diluted it to 100 mL. The large volume resulted in a strongly reduced detection limit.

Laboratory 17 added 0.3 mL concentrated HNO_3 to the samples before the last round of ashing. According to the author's experience, this has no effect on the results.

Laboratory 20 cleaned the utensils with 3N HCl-ethylendiaminetetraacetic acid.

Laboratory 25 claimed that sample 7 was not in the package.

The participants were instructed to report the results to 3 significant digits (i.e., 0.0111, 1.11, or 111). There were, however, a considerable number of deviations from this instruction in the replies.

Elimination of Outliers

Outliers were eliminated accordance with the AOAC INTERNATIONAL guidelines (14).

Results

Results were received from 16 of the original 17 participating laboratories. Not all of the laboratories determined all of the metals. All determinations were made as single determinations.

Samples with nearby or split levels were used to calculate the repeatability (within-laboratory variation) and reproducibility (within- and between-laboratory variation) standard deviations. In those instances in which samples had a unique level, only reproducibility was calculated.

The contamination level was controlled by blank determinations. The participants were instructed to carry out at least 5 blanks/metal. The mean value of the blanks were deducted from the readings before the results were calculated.

The overall mean of the blank determinations was decided for each metal on the basis of results only from laboratories that reported results acceptable according to the statistical evaluation. Limits of detection were calculated as 3 times the standard deviation of the mean of these blanks.

Lead

Fourteen of the participating laboratories performed Pb determinations. All of the determinations were made with background correction. Most of the analyses were carried out by GFAAS. FAAS was used by Laboratory 6 for sample 3; by Laboratory 7 for all samples; by Laboratory 10 for samples 2, 3, 6, and 7; and by Laboratory 11 for sample 2. These results were not included in the statistical evaluation. Matrix modification was used by Laboratories 2, 3, 6, 14, and 16. Laboratory 1 reported the level in sample 5 to be undetectable. Laboratory 11 reported problems with the background correction during the measurements. Laboratory 16 claimed that it had probably missed a dilution factor when calculating the result for sample 2. These results, therefore, were not included in the statistical evaluation. The 2 results reported as below a laboratory's detection limit were taken at face value in the calculations.

The mean of the blank determinations was 0.0019 mg/L, and the range was 0.0004–0.0055 mg/L. The limit of detection was calculated to be 0.0021 mg/L sample solution, with a range of 0.0003–0.0047 mg/L. This corresponds to an average limit of detection in the actual sample of 0.0063 mg/kg, assuming a sample weight of 10 g and dilution to 30 mL.

Cadmium

Fifteen of the participating laboratories performed Cd determinations, all with background correction. Most of the determinations were by GFAAS. FAAS was used by Laboratory 6 for samples 3, 6, and 7; by Laboratory 8 for all samples; by Laboratory 10 for all samples; by Laboratory 11 for samples 1, 3, 4, 6, and 7; and by Laboratory 12 for samples 3, 4, 6, and 7. These results were treated separately and were included for information. Matrix modification was used by laboratories 3, 6 (sample 5), 14, and 16.

The result reported as below the laboratory's detection limit was taken at face value in the calculations.

The mean of the blank determinations was 0.0004 mg/L, and the range was 0.00001–0.0022 mg/L. The limit of detection was calculated to be 0.0013 mg/L sample solution with a range of 0.00003–0.0088 mg/L. This corresponds to an average limit of detection in the actual sample of 0.0039 mg/kg, assuming a sample weight of 10 g and dilution to 30 mL.

Zinc

Fifteen of the participating laboratories performed Zn determinations. Laboratories 1, 13, and 14 did not use background correction. All analyses were made by FAAS.

The mean of the blank determinations was 0.017 mg/L, and the range was 0.0032–0.030 mg/L. The limit of detection was calculated to be 0.019 mg/L sample solution, with a range of 0.000–0.039 mg/L. This corresponds to an average limit of detection in the actual sample of 0.057 mg/kg, assuming a sample weight of 10 g and dilution to 30 mL.

Copper

Fifteen of the participating laboratories performed Cu determinations. Laboratories 6, 11, 13, and 14 did not use background correction. Laboratory 1 did not submit the information. All laboratories used FAAS.

The mean of the blank determinations was 0.015 mg/L, and the range was 0.000–0.060 mg/L. The limit of detection was calculated to be 0.036 mg/L sample solution, with a range of 0.000–0.072 mg/L. This corresponds to an average detection limit in the actual sample of 0.108 mg/kg, assuming a sample weight of 10 g and dilution to 30 mL.

Iron

Fourteen of the participating laboratories performed Fe determinations. Background correction was not used by Laboratories 13 and 14. All laboratories used FAAS.

The mean blank level was 0.108 mg/L, and the range was 0.010–0.590 mg/L. The limit of detection was calculated to be 0.267 mg/L sample solution, with a range of 0.000–2.00 mg/L. This corresponds to an average detection limit in the actual sample of 0.800 mg/kg, assuming a sample weight of 10 g and dilution to 30 mL.

Discussion

Pretrial

The results of the pretrial with ready-made solutions gave several indications:

(*1*) At similar concentration levels in the standard solutions and the sample solutions, there was no significant difference in the variance for Cd, Cu, and Fe. At the lowest concentration (0.0106 ± 0.0012 mg/L in the standard solution and 0.008 ± 0.008 in the sample solution), the variance for Pb was significantly higher (variance ratio, 48) in the sample solution, which indicates that the higher mineral content in the sample solution causes greater variance in the result. This is due to several factors, among which is the correction process for the background absorption.

(*2*) When the concentration of the sample solutions of pork and liver were multiplied by a factor, 2.5, to give the approximate concentration in the actual samples and then compared with similar concentrations in samples from the trial, the variance of Cu and, to a varying degree, those of Cd and Fe were significantly higher for the trial samples than for the pretrial. It may then be assumed that the ashing procedure makes a contribution to the total variance for these metals. Pb, which is notoriously difficult to determine, apparently receives the major part of the variance, at least at very low concentrations, from the AAS determination. It must, however, be emphasized that this pretrial was of a limited nature, and that the conclusions therefore are only indicative.

Collaborative Trial

At higher concentrations of Zn, Cu, and Fe, results can be obtained without background correction that are not systematically too high. This must, of course, be verified in each individual case.

With the exception of Pb, the difference between the S_r and the S_R for the split-level sample combinations increased with increasing concentrations. For Pb, Cd, Zn, Cu, and Fe, the results for samples 6 and 7 showed very good agreement with the certified reference values. It can therefore be assumed that no loss of these metals occurred during the dry ashing.

The RSD_R values for all the metals determined agreed reasonably well with what Horwitz et al. (15) showed to be generally expected at different concentrations. When the ratios of RSD_R found/RSD_R predicted (HORRAT; 16) are calculated, ratios between 0.5 and 2 indicate acceptable precision of the method according to the International Union of Pure and Applied Chemistry (IUPAC; 17).

Conclusions

The results of the collaborative trial, at the concentrations tested, correspond to the requirements for reproducibility that according to Horwitz et al. can be expected of a method.

The agreement of the trial results for the CRMs, samples 6 and 7, with their reference values (13), was generally very good.

The method has low detection limits, making the method suitable for quantitative analysis at low concentrations.

Based on these conclusions, the method must be considered to give acceptable results for the elements determined.

Recommendation

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

Acknowledgments

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