

Elemental Analysis Manual

for Food and Related Products

Archive Notes

This method has been placed in archive status only because it is no longer used at FDA laboratories. It remains the most current version and is still considered a valid analysis option.

4.3 Graphite Furnace Atomic Absorption Spectrometric Determination of Cadmium and Lead in Food Using Microwave Assisted Digestion

Version 1.2 (August 2010)
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GLOSSARY

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4.3.1 SCOPE AND APPLICATION

This method describes procedures for using graphite furnace atomic absorption spectrometry (GFAAS) for determination of total element concentration (mass fraction) in a variety of food products such as fruits, vegetables, cheese, grains, meats, and nuts. Other matrices may be analyzed by these procedures if performance is demonstrated for an applicable analyte in the matrix of interest, at the concentration levels of interest. This method is applicable to the analytes listed in 4.3 Table 1.

4.3 Table 1. Analytical Limits

Element	Symbol	ASDL ^a (µg/L)	LOD ^b (µg/kg)	LOQ ^b (µg/kg)
Cadmium	Cd	0.014	0.35	2.7
Lead	Pb	0.35	8.8	68
^a Based on fortified method blanks.				
^b Based on 1 g analytical portion.				

The limits listed above are intended as a guide and actual limits are dependent on the sample matrix, instrumentation and selected operating conditions.

This method should be used by analysts experienced in the use of graphite furnace atomic absorption spectrometry, including the interpretation of spectral and matrix interferences, and procedures for their correction; and should be used only by personnel thoroughly trained in the handling and analysis of samples for determination of trace elements in food products.

4.3.2 SUMMARY OF METHOD

An analytical portion (0.4 to 5 g depending on food composition) is digested with nitric acid and hydrogen peroxide in a high-pressure Teflon[®] lined digestion vessel using microwave heating and a feedback program to control temperature and pressure. A 25 mL analytical solution is prepared from the digest. Cadmium and lead are determined in the analytical solution by GFAAS with platform atomization, matrix modification with magnesium and phosphate, and Zeeman effect background correction. Fortified analytical solutions are used to check for matrix interferences.

Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the U.S. Food and Drug Administration. Equivalent performance may be achievable using apparatus and materials other than those cited heror matrix interferences.

4.3.3 EQUIPMENT AND SUPPLIES

Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the U.S. Food and Drug Administration. Equivalent performance may be achievable using apparatus and materials other than those cited here.

- (1) Graphite furnace atomic absorption spectrometer—Capable of displaying and recording fast, transient signals, measuring peak area, and having a minimum sensitivity (m_o based on peak area) of 30 pg lead at 283.2 nm wavelength and 1.3 pg cadmium at 228.8 nm wavelength. Equipped with light sources (hollow cathode or electrodeless discharge lamps) specific for lead and cadmium, Zeeman effect background correction, autosampler, and electrothermal atomizer (graphite furnace) with pyrolytically coated graphite tubes and platforms.

Safety Notes:

The graphite furnace emits UV radiation during the atomization and clean-out steps. Avoid looking at the furnace during these steps.

Zeeman effect background correction systems use a magnet that creates strong magnetic fields. Stay at least 3 feet away from the magnet when it is on.

- (2) Microwave digestion system—Requires temperature control to 200 °C, pressure control to at least 600 psi, power range of 0-100% in 1% increments, minimum 1000 watts for 12 position carousel, feedback control of temperature and pressure and multi-step programming with ramp to temperature capability. Digestion vessels must be quartz or Teflon[®] lined. System must be able to reach at least 200 °C and at least 600 psi. Vessels designed to vent and reseal can be used provided they vent at pressures >300 psi. Directions on use of microwave digestion equipment are specific to CEM Corporation brand equipment and assume familiarity. Use of the method with other brands of equipment may require procedural modifications and performance verification.

Safety Note: Microwave digestion systems can be potentially dangerous. Vessels contain concentrated nitric acid at high temperatures and pressures. Analyst must be familiar with manufacturer's recommended safety precautions including connection of the system to an appropriate exhaust system.

4.3.4 REAGENTS AND STANDARDS

Reagents may contain elemental impurities that can affect the quality of analytical results. Reagents should be sought that minimize analyte contamination (ideally, analyte level is below the IDL). Use of high purity or trace element “metals” grade reagents is usually required.

Safety Note: Reagents should be regarded as potential health hazards and exposure to these compounds should be limited. Material safety data sheets for these chemicals are to be available to the user.

- (1) Reagent water—Water that meets specifications for ASTM Type I water¹.
- (2) High purity nitric acid—Concentrated (sp gr 1.41), trace element (*i.e.*, metals) grade or double distilled.
- (3) Nitric acid—Concentrated (sp gr 1.41), ACS reagent grade.
- (4) Nitric acid 1% (v/v)—Dilute 10 mL high purity nitric acid to 1000 mL with reagent water.
- (5) Hydrogen peroxide—30% H₂O₂ solution. High purity or trace metals grade.
- (6) Ammonium phosphate solution (NH₄H₂PO₄) 10% (m/v)—Dissolve 10 g NH₄H₂PO₄ in reagent water. Dilute to 100 mL. Use matrix modifier grade. Solution may be purchased commercially.
- (7) Magnesium stock standard solution 10,000 mg/L—Use commercially available solution made specifically for use as a matrix modifier.
- (8) Matrix modifier—Dilute 1 mL 10,000 mg/L Mg and 10 mL 10% NH₄H₂PO₄ to 100 mL with 1% nitric acid. Solution will be 1% NH₄H₂PO₄ (m/v) and 100 mg/L Mg. Analyze matrix modifier for cadmium and lead contamination before use. Alternate matrix modifiers may be useful depending on the instrument model, volume of sample used, and the configuration of the platform. The acceptability of alternate modifiers must be verified.

- (9) Cadmium and lead stock standard solutions—Commercially prepared single element 1000 or 10,000 mg/L solutions in a nitric acid matrix prepared specifically for spectrometric analysis. Do not use solutions containing hydrochloric or sulfuric acid. Alternatively, prepare in the laboratory from high purity ($\geq 99.99\%$) metals or salts.
- (10) Intermediate standard solutions—Dilute cadmium and lead stock standards with 1% nitric acid into acid rinsed volumetric flask. Store in plastic bottles (Teflon[®] FEP or HDPE bottles recommended; check for contamination before use). Both elements can be combined in the same solution.
- (11) Standard solutions—Dilute cadmium and lead intermediate standards with 1% nitric acid in a Class A volumetric flask or prepare by gravimetrically diluting intermediate standards. Store in plastic bottles (Teflon[®] FEP, LDPE or HDPE bottles recommended; check for contamination before use). Typical standard solutions for lead analysis are 3, 5, 10 and 20 $\mu\text{g/L}$. Typical standard solutions for cadmium analysis are 0.3, 0.5, 1.0, 2.0 $\mu\text{g/L}$. Concentrations can be adjusted depending on instrument sensitivity but must be within linear response range. Do not use standard solutions that are more than 30 days old since element concentrations can change with age. The autosampler may be used to inject varying amounts of a standard solution as an alternative to making a series of standard solutions. The auto--sampler must be programmed to inject varying amounts of standard and standard blank such that the total injection volume remains constant.
- (12) Standard blank—1% nitric acid.
- (13) Independent check solution (ICS)—Dilute an appropriate volume of cadmium and lead stock solutions (obtained from a different source than used to prepare intermediate standard solutions) volumetrically (or gravimetrically) with 1% nitric acid so analyte concentration will be approximately the midpoint of the standard curve. Do not use prepared ICS that is more than 30 days old since element concentrations can change with age. Commercial solutions may be substituted for prepared solutions and used to expiration date.
- (14) Check solution—Use mid-concentration standard solution for the check solution.
- (15) Gas supply for furnace—High purity (99.9%) argon. A 95% argon-5% hydrogen gas mixture can also be used during the dry and char steps of the furnace program to reduce interference from high levels of chloride present in high-salt samples. This gas mixture can also be used for all steps.

4.3.5 DIGESTION PROCEDURE

The following operations should be performed in a clean environment to reduce contamination. An exhausting hood must be used when working with nitric acid. See §2.3.1 for additional information on performing microwave digestions.

- (1) Weigh analytical portion into clean vessel liner and determine mass of analytical portion. Generally, for samples of unknown composition, weight the equivalent of about 0.5 dry material to an accuracy of 0.001 g. If maximum pressure attained for this unknown is less than the vessel limit then a greater mass may be analyzed. Less than the maximum mass should be used for samples high in salt content. A maximum analytical portion of 5 g should not be exceeded even if calculations based on the food's energy indicate that a larger portion could be taken. Use 1 g reagent water for method blanks (MBKs). For dry samples and dry CRM materials adding 1 g of reagent water can help control exothermic reactions during the digestion.
- (2) Pipette 8.0 mL or weigh 11.3 g of high purity nitric acid (sp gr 1.41 g/mL) into vessel

liner, washing down any material on walls. Weighing acid using a top loading balance and Teflon[®] FEP wash bottle is suggested. Use double distilled grade for lowest method blank values. The trade name for double distilled grade will vary by manufacturer. Acid should be added drop wise for the first few mL until it can be established that the sample will not react violently. Some foods, especially those high in sugar, will react with nitric acid within several minutes. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a class 100 clean hood for 20 minutes or until reaction subsides. If a clean hood is unavailable, place caps on vessels without pressing down fully or, if so equipped, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid.

- (3) Add 1 mL high purity 30% H₂O₂. Seal vessels, apply correct torque to cap (tighten pressure relief nuts if equipped) and run the digestion program in 4.3 Table 2.

4.3 Table 2. Microwave Digestion Program

<i>Digestion Programs for CEM MARS 5 with 12-Position Carousel with Ramp to Temperature Feature</i>	
Power is applied for the Ramp Time minutes or until Control Pressure or Control Temperature is met. If Control Pressure or Control Temperature are met before end of Ramp Time then program proceeds to Hold Time	
	Digestion
Maximum Power (Watts)	1200
Control Pressure (psi) ^a	800
Ramp Time (min)	25
Hold Time (min)	15
Control Temperature (°C)	200
^a Only use with non-venting vessels.	

- (4) After vessels have cooled to less than 50° C remove them to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer and dilute digestion solution with reagent water to 25 mL. This analytical solution should be transferred to a plastic bottle or a capped polypropylene centrifuge tube for storage.

Note: Dilution volumes <25 mL can be used but the analyst should be aware of potential problems. The higher acid concentration might reduce tube life and will require careful determination of the drying step parameters to ensure proper drying of analytical portion. The reduced volume will also result in a higher concentration of potentially interfering matrix components. Diluting to >25 mL might be advantageous for high-salt foods.

4.3.6 DETERMINATION PROCEDURE

The determination procedure was developed using a PerkinElmer 5100PC spectrometer equipped with a 5100 ZL furnace module (transverse heated graphite furnace), end-capped graphite tubes and AS71 autosampler. 4.3 Table 3 is an example of a furnace program used with this

instrument. The optimum furnace program and amount and type of matrix modifier must be determined for the equipment used. Quantification may be performed by either standard curve or standard additions. However, complex matrices may require additional dilution or the determination to be made by standard additions.

4.3 Table 3. Typical GFAAS Instrument Conditions

Conditions for PerkinElmer 5100C AAS with 5100 ZL furnace using end-capped tubes							
Cadmium				Lead			
Step	Temp (°C)	Ramp (sec)	Hold (sec)	Step	Temp (°C)	Ramp (sec)	Hold (sec)
1	110	5	25	1	110	5	25
2	130	15	25	2	130	15	25
3	200	5	5	3	200	5	5
4	600	10	20	4	820	10	20
5	1600	0	4	5	1700	0	3
6	2400	1	4	6	2400	1	4
Injection temperature: 100 °C Wavelength: 228.8 nm Slit width: 0.7 nm Sample Volume: 20 µL Matrix Modifier: 5 µL 1% NH ₄ H ₂ PO ₄ in 100 µg/mL Mg				Injection temperature: 100 °C Wavelength: 283.3 nm Slit width: 0.7 nm Sample Volume: 20 µL Matrix Modifier: 4 µL 1% NH ₄ H ₂ PO ₄ in 100 µg/mL Mg			

Instrument Setup

- (1) Setup graphite furnace atomic absorption spectrometer according to the manufacturer's recommendations and with the following attributes:
 - Program the system for 2 replicate measurements of all solutions from the same auto-sampler cup and to use the mean of these measurements for calculations. Only 1 measurement from the same autosampler cup is required if the determination is by method of standard additions.
 - If argon-hydrogen mixture used, then configure gas flow to switch from argon to the argon-hydrogen mixture during the dry and char steps. Alternatively, the argon-hydrogen mixture can be used for all steps.
 - Use peak area (integrated absorbance) mode for concentration calculations.
 - Program instrument to use a linear, least squares calculated intercept, curve fit algorithm for converting absorbance values to µg/L concentration units. Do not subtract standard blank response from standard solution response.
 - Program instrument to display and print peak height absorbance, peak area absorbance, concentration result, dilution factor applied to analytical solution and absorbance verses time graphics plot.
- (2) Optimize furnace program and the amount of modifier for analyte.
 - Follow manufacturer's recommendations for optimizing each step of the furnace program to obtain near ideal peak profile (shape).

- The dry step may need to be extended from what is normally used because of high acid concentrations of analytical solutions (approximately 15–20% nitric acid).
 - A long slow multi-step drying stage was found to be necessary to prevent spattering of some food analytical solutions.
 - Use a MBK to determine drying parameters and then confirm with a food analytical solution.
 - A slightly higher than normal atomization temperature (by 50–100 °C) was found helpful for food analytical solutions.
- (3) Check instrument performance
- Verify characteristic mass (m_0) is within 20% of expected value.
 - Verify short term precision is less than 5% relative standard deviation with a mid-range standard (n=5).

Determination of Analyte Concentration Using Standard Curve

- (1) Standardize the instrument using the standard blank and at least 4 standard solutions (or 4 concentration levels of autosampler “made” standards).
- (2) Check standardization performance
- Correlation coefficient (r) of linear regression (integrated absorbance verses pg added) is ≥ 0.998 .
 - ICS recovery within $100 \pm 5\%$ (initial calibration verification).
 - Standard blank <ASDL.
- (3) Analyze analytical solutions and quality control solutions. Interpolate analyte concentration from standard curve. A typical sequence for an analytical run is listed in 4.3 Table 4.
- (4) Check instrument measurement performance
- RPD of the measurements of 2 replicate injections is 7% or less for all solutions when instrument response ≥ 0.012 A-sec.
 - Check solution analyzed at a frequency of 10% and at the end of the analytical run has a recovery of $100 \pm 10\%$ (continuing calibration verification).
 - Background absorbance for reported measurements is ≤ 1.0 A-sec. Dilute analytical solution if necessary to comply with criteria. If software does not permit background to be reported in A-sec then use 1.0 A as criteria.
 - Measurements are below highest standard solution. Dilute analytical solution with standard blank if necessary to comply with criteria.
 - FAS recovery is $100 \pm 10\%$. Dilute analytical solution with standard blank if necessary to comply with criteria.
 - Peak profile of analytical solution is comparable to standard solution.

4.3 Table 4. Typical Analytical Sequence^a

Auto-Sampler Cup #	Solution	QC Criteria	Auto-Sampler Cup #	Solution	QC Criteria
	m_o check	$m_o \pm 20\%$ of expected	13	sample 3 FAS	90–110% recovery
	precision check	$n=5$, < 5% RSD	14	check solution	90–110%
	standardization	$r \geq 0.998$	15	sample 4	A-sec < high std.
			16	sample 4 FAS	90–110% recovery
1	standard blank	< ASDL	17	sample 5	A-sec < high std.
2	ICS	95–105%	18	sample 5 FAS	90–110% recovery
3	MBK 1	$\leq MBK_C$	19	sample 6	A-sec < high std.
4	MBK 2	$\leq MBK_C$	20	sample 6 FAS	90–110% recovery
5	MBK 3	$\leq MBK_C$	21	sample 7	A-sec < high std.
6	RM	80–120% recovery ^b	22	sample 7 FAS	90–110% recovery
7	sample 1	A-sec < high std.	23	sample 8	A-sec < high std.
8	sample 1 FAS	90–110% recovery	24	sample 8 FAS	90–110% recovery
9	sample 1 FAP	80–120% recovery	25	check solution	90–110%
10	sample 2	A-sec < high std.	26	sample 9	A-sec < high std.
11	sample 2 FAS	90–110% recovery	27	sample 9 FAS	90–110% recovery
12	sample 3	A-sec < high std.	28	check solution	90–110%

^a All solutions analyzed in duplicate. Precision between the required 2 injections must be $\leq 10\%$ RSD analytical solutions with ≥ 0.012 A-sec.

^b Or within the uncertainty on the certificate.

Determination of Analyte Concentration Using Standard Additions

- (1) Analyze analytical solutions and quality control solutions using minimum of 3 additional portions of solution with added amounts of analyte deposited on platform at ~2 and 5 times, respectively, of the amount of analyte in solution but not less than ASQL. Measurements are made where the relationship between absorbance and concentration is linear. Extrapolate analyte concentration from x-intercept of linear regression curve.
- (2) Check Performance of Standard Additions
 - Check solution analyzed at a frequency of 10% and at the end of the analytical run has a recovery of $100 \pm 10\%$ (continuing calibration verification).
 - Background absorbance for reported measurements is ≤ 1.0 A-sec. Dilute analytical solution if necessary to comply with criteria.
 - Correlation coefficient (r) of linear regression (integrated absorbance verses pg added) is ≥ 0.995 .
 - Slope of standard addition curve for analytical solution is $\pm 50\%$ of the slope of standard addition curve for a standard blank (or a standard solution without any matrix effect such as the ICS).
 - Peak profile of analytical solution is comparable to standard solution.

Note: If analysis fails to meet control limits then sample probably has a large matrix effect that is not fully corrected by standard additions. For this situation, dilute sample by a factor of 2 and re-analyze using additions based on the level in analytical solution and the dilution factor.

4.3.7 CALCULATIONS

Calculate the concentration (mass fraction) of the analyte in the analytical portion according to the formula

$$\text{Concentration } (\mu\text{g/kg}) = \left[(S \times \text{DF}) - \text{MBK}_L \right] \times \frac{V}{m \times \text{MCF}}$$

where

S = concentration (mean of 2 or more determinations) of analyte in analytical solution (or diluted analytical solution) ($\mu\text{g/L}$)

MBK_L = laboratory MBK ($\mu\text{g/L}$)

V = volume (L) of analytical solution (usually 0.025 L)

m = mass of analytical portion (kg)

DF = dilution factor (1 if analytical solution not diluted)

MCF = mass correction factor (1 if no water or other solvent was added to aid homogenization)

Round calculated concentration to at most 3 significant figures. Concentration may be converted to other convenient units (*e.g.*, mg/kg, ng/kg).

4.3.8 METHOD VERIFICATION

The following is the minimum number of quality control samples to be analyzed with each batch of samples: 1 reference material (RM), 1 fortified analytical portion (FAP), 3 method blanks (MBKs) and 1 replicate. Replicate analytical portions should be analyzed for each sample whenever analyte nonhomogeneity may be an issue.

Reference Material

Control limits for RM Recovery are $100 \pm 20\%$ or within concentration uncertainty (converted to percent relative uncertainty) supplied on certificate, whichever is greater. The z-score procedure, which allows for greater deviation and is discussed in §3.5.3, may also be used, although it requires additional calculations. If three or more RMs are analyzed then only two-thirds of an element's RM recovery results must meet the control limit.

FAP Recovery

Control limit for FAP recovery is $100 \pm 20\%$.

Method Blanks (MBK)

Minimum of 3 MBKs analyzed. At least two-thirds of MBKs are $\leq \text{MBK}_C$.

Relative Percent Difference (RPD) of Two Replicate Analytical Portions

Control limit for RPD is 20%.

4.3.9 REPORT

Report results only when quality control criteria for a batch have been satisfactorily met. Report results that are $\geq \text{LOQ}$ as the mass fraction determined followed by the units of measurement. Report results that are $\geq \text{LOD}$ and $< \text{LOQ}$ as the mass fraction determined followed by the units of measurement and the qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification (TR). Report results that are $< \text{LOD}$ as 0 followed by the units of measurement and the qualifier that indicates analyte is below the level of reliable detection or is

not detected (ND).

Example: LOQ = 6 µg/kg; LOD = 3 µg/kg. Levels found for three different samples were 10 µg/kg, 5 µg/kg and 2 µg/kg mg/kg.

10 µg/kg is \geq LOQ; report 10 µg/kg

5 µg/kg is \geq LOD but also $<$ LOQ; report 5 µg/kg (TR)

2 µg/kg is $<$ LOD; report 0 µg/kg (ND)

4.3.10 METHOD VALIDATION

Closed-vessel microwave digestion procedures are commonly applied to trace element analysis of food samples because of superior contamination control, speed and ease of use²⁻³. Combining microwave digestion and GFAAS for food analysis has been demonstrated⁴⁻⁵ and includes a collaborative study⁶ resulting in a validated method⁷.

Single Lab Validation. Results of an FDA in-house validation of the method are presented in Appendix A. Recovery results of fortified analytical portions of selected foods averaged 96% for cadmium and 93% for lead. Recovery results for RMs ranged from 88% to 108% for cadmium and 92% to 109% for lead.

Uncertainty. A result above LOQ has an estimated combined uncertainty of 10%. Use of a coverage factor of 2 to give an expanded uncertainty at about 95% confidence corresponds with the RM Recovery control limit of $\pm 20\%$. A result above LOD but below LOQ is considered qualitative and is not reported with an uncertainty.

A detailed discussion of method uncertainty is presented in §3.3. This method conforms to the information contained in that discussion. Derivation of an estimated uncertainty specific to an analysis is discussed §3.3.2.

Interlaboratory Trial. Results of an FDA interlaboratory trial are presented in Appendix B. Mean recovery results of fortified analytical portions of selected foods averaged 99% for cadmium and 97% for lead. Mean recovery results for RMs ranged from 87% to 102% for cadmium. The lead levels in the RMs were either too low to be quantified or an interference was present.

REFERENCES

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Appendix A – Supplemental Information on In-house Method Validation

Version 1 (June 2008)
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GLOSSARY

In-house validation of EAM Method 4.3 (draft E, January 2002) was performed using a CEM Corporation model MDS 2000 microwave digestion system with UDV vessels and a PerkinElmer 5100PC atomic absorption spectrometer equipped with a 5100 ZL furnace module (transverse heated graphite furnace), end-capped graphite tubes and AS71 autosampler.

4.3A.1 ANALYTICAL LIMITS

Analytical limits were determined by the analysis of fortified method blanks (FMBs). Method blanks were fortified at a level estimated to be approximately 3-5 times the detection limit. Results are summarized in 4.3A Table 1. The characteristic mass, m_0 , for cadmium was 1.1 pg and for lead was 25 pg.

4.3A Table 1. Estimate of Analytical Limits

	Cadmium	Lead
Number of FMBs	10	10
Fortification level (µg/L)	0.05	1
Standard deviation (µg/L)	0.00363	0.0911
ASDL (µg/L)	0.014	0.35
ASQL (µg/L)	0.11	2.7
LOD (µg/kg) based on 1 g anal. portion	0.35	8.8
LOD (µg/kg) based on 4 g anal. portion	0.087	2.2
LOQ (µg/kg) based on 1 g anal. portion	2.7	69
LOQ (µg/kg) based on 4 g anal. portion	0.68	17

4.3A.2 REFERENCE MATERIAL RESULTS

Three replicates of several reference materials (RMs) were prepared for the method validation of accuracy. Analyte concentrations were determined using both standard curve and standard additions procedures. When using the standard curve procedure, the required dilution of the analytical solution was based on recovery of the fortified analytical solution (FAS). Matrix interference was assumed if the FAS recovery was <90%, which necessitated increasing the dilution factor. Standard addition results compared favorably with standard curve results. A moisture correction factor was determined for each RM and applied to the results, which are summarized in 4.3A Table 2.

4.3A Table 2. Reference Material Results

Reference Material ^a	Cadmium			Lead		
	Ref. Value (mg/kg)	Mean ^b Result (mg/kg)	RM Rec. (%)	Ref. Value (mg/kg)	Mean ^b Result (mg/kg)	RM Rec. (%)
Dogfish Muscle (NRCC DORM-1)	0.086	0.093	108	0.4	0.43	107
Spinach (NIST 1570)	-	-	-	1.2	1.13	94
Mussel (NIES-6)	-	-	-	0.91	0.84	92
Bovine Liver (NIST 1577)	0.27	0.29	107	0.34	0.37	109
Oyster Tissue (NIST 1566)	3.5	3.09	88	0.48	0.5	104
Cocoa Powder (FDA CP)	0.39	0.371	95	-	-	-
Wheat Flour (NIST 1567)	0.032	0.032	100	-	-	-
Rice Flour (NIST 1568a)	0.022	0.021	95	-	-	-

^aNRCC=National Research Council of Canada, NIST=National Institute of Standards and Technology, NIES=National Institute for Environmental Studies of Japan, FDA=Food and Drug Administration.
^bn=3; corrected for moisture.

Cadmium

Six RMs were analyzed to evaluate method performance accuracy for cadmium (4.3A Table 2). These RMs provided a variety in matrix (vegetative, organ meat and animal protein) and cadmium concentration (0.022–3.5 mg/kg). The dilution factor required for the analytical solution varied from 1 (Dogfish Muscle, Wheat and Rice Flours) to 50 (Oyster). The dilution factors were based on cadmium concentration and not interference. FAS recoveries were acceptable for the dilutions used. Results were within the reference value uncertainty except for Oyster, which was slightly below the uncertainty window, but was recovered at 88%.

Lead

Five RMs were analyzed to evaluate method performance accuracy for lead (4.3A Table 2). These RMs provided a variety in matrix (vegetative, organ meat and animal protein) and lead concentration (0.34–1.2 mg/kg). The dilution factor (based on FAS recovery) required for the analytical solution varied from 1 (Oyster and Bovine Liver) to 4 (Spinach). The Spinach exhibited unacceptably low FAS recoveries and low recovery based on reference values at dilution factors less than 4. The Spinach reference value recoveries for dilution factors of 2 and 3 were 42% and 75%, respectively. All results were within the reference value uncertainty except for Mussel, which was slightly below the uncertainty window, but was recovered at 93%.

4.3A.3 FOOD RESULTS

Fourteen foods were selected from the FDA Total Diet Study market basket 1999-1 and analyzed to evaluate method performance accuracy and precision (see 4.3A Table 3). Foods were chosen based on analytical challenge (either sample preparation or analysis) or analyte level. Two replicate portions of both unfortified and cadmium and lead fortified portions were prepared and analyzed. Analytical portions were limited by the energy content of the food but with an upper limit of 5 g to limit dilution of the nitric acid and ensure a complete digestion. Portions varied from approximately 0.5 g for high-fat foods like nuts to 5 g for high-water content foods like pickles. 4.3A Table 3 lists the average mass used for each food. TDS results were obtained using a dry ashing mineralization and measurement of lead and cadmium by graphite furnace atomic absorption spectrometry¹.

4.3A Table 3. Foods and Analytical Portion Mass for In-House Validation

Food	Portion (g)	Food	Portion (g)
American cheese	0.87	Iceberg lettuce	3
Beef liver	1.4	Canned spaghetti	2.5
Peanut butter	0.47	Dill pickles	5
White bread	1.1	Catsup	2.8
Raisin bran cereal	0.85	Mixed nuts	0.51
Canned fruit cocktail	3.8	Canned peaches	4.5
Spinach	4.4	Yellow mustard	3.3

Cadmium

The results for cadmium are listed in 4.3A Table 4. Cadmium results ranged from 1 µg/kg in canned fruit cocktail to 170 µg/kg in spinach. All results were >LOQ.

Agreement between duplicates was generally good with a few exceptions. American cheese, iceberg lettuce and mixed nuts had relatively high relative percent differences (RPD) but only American cheese was greater than the control limit of 20%. American cheese was challenging because the cadmium level was near the LOQ and the cheese has a very high mineral content. Even though there was an acceptable FAS recovery for American cheese, a matrix effect (peak broadening) was noted which probably contributed to the high RPD.

The required dilution factors (DFs) based on FAS recovery are listed in 4.3A Table 4. Foods highest in salt had the greatest matrix effect indicated by the low FAS recovery (<90%) when analyzed without further dilution. 4.3A Table 5 lists results for three high-salt foods at various dilutions and illustrates the utility of using the FAS recovery to detect interference and determine the necessary dilution to lower matrix interference to an acceptable level. Without the necessary dilution factor the results would be biased low. The high dilution factor for spinach was the result of the high cadmium concentration and not matrix interference.

Results were in good agreement with TDS results. The largest difference was for raisin bran cereal, which was probably the result of nonhomogeneity.

4.3A Table 4. Cadmium in Food Results

Food	Found ^a (µg/kg)	LOQ (µg/kg)	DF	RPD (%)	Fortification (µg/kg)	Recovery ^a (%)	TDS Result ^b (µg/kg)
American cheese	3	3.0	1	26.9	14	100.2	<10
Beef liver	37	1.9	1	4.2	34	90.2	36
Peanut butter	87	5.8	1	0.5	106	99.9	79
White bread	20	2.5	1	7.8	23	95.6	14
Raisin bran cereal	43	3.2	1	1.4	59	96.1	33
Canned fruit cocktail	1	0.7	1	9.2	7	94.8	<5
Spinach	170	6.2	10	9.4	234	97.9	176
Iceberg lettuce	18	0.91	1	12.2	17	94.1	15
Canned spaghetti	12	1.1	1	2.3	20	96.7	10
Dill pickles	5	2.2	4	6.8	5	91.1	<7 (6)
Catsup	24	2.9	3	7.5	18	98.6	15
Mixed nuts	20	5.3	1	11.6	48	84.2	20
Canned peaches	1	0.61	1	6.5	11	95.0	<5
Yellow mustard	34	3.3	4	5.6	40	109.2	28
Mean:				8.0		96.0	

^a Results are the mean of two replicate analytical portions. Results displayed in parentheses are trace levels <LOQ.
^b Foods are from FDA Total Diet Study (TDS) market basket 1999-1. Results displayed with "<" are less than the LOQ; the LOQ is provided and a trace level is provided in parentheses if analyte was detected. LOQ = 10xσ.

4.3A Table 5. Cadmium Results for High-Salt Foods at Various Dilutions

Food	DF =1		DF =2		DF =4	
	FAS Rec. (%)	Found (µg/kg)	FAS Rec. (%)	Found (µg/kg)	FAS Rec. (%)	Found (µg/kg)
Dill pickles	13	1	58	3	>90	5
Catsup	57	0	51	22	>90	24 ^a
Yellow mustard	0	23	52	31	>90	34
^a DF=3						

Lead

The results for lead are listed in 4.3A Table 6. Result for canned peaches was 15 µg/kg. All other results were trace levels <LOQ.

Agreement between duplicates was generally good with a few exceptions. American cheese was challenging because of very low lead level and a very high mineral content, which resulted in some matrix interference. A dilution factor of 4 was required for lead in mustard and pickles, which resulted in a very low lead level in the analytical solution. The 41% difference between raisin bran cereal duplicates for lead was most likely due to nonhomogeneity (inconsistent ratio of raisins to cereal in the analytical portions even though the sample was processed through a food mill) and the low levels measured (<LOQ).

The required DFs based on FAS recovery are listed in 4.3A Table 6. Foods highest in salt had the greatest matrix effect and thus low FAS recovery (<90%) when analyzed without further dilution. 4.3A Table 7 lists results for three high-salt foods at various dilutions and illustrates the utility of using the FAS recovery to detect interference and determine the necessary dilution to lower matrix interference to an acceptable level. Without the necessary dilution factor, the results

would be biased low.

The levels of lead were quantifiable in 10 of the foods but TDS results were all below LOQ. Results were generally higher than TDS results when considering TDS LOQ and trace results. For example, the level of lead found in dill pickles, catsup, mixed nuts, and yellow mustard are above the TDS LOQ but the TDS results were nonquantifiable. The disagreements with TDS results could be due to issues related to homogeneity or interferences compensation based on FAS recovery.

4.3A Table 6. Lead in Food Results

Food	Found ^a (µg/kg)	LOQ (µg/kg)	DF	RPD (%)	Fortification (µg/kg)	Recovery ^a (%)	TDS Result ^b (µg/kg)
American cheese	(76)	237	3	16	283	95.5	<50
Beef liver	(44)	49	1	6.5	171	92.8	<50 (18)
Peanut butter	(55)	145	1	8.1	531	89.4	<50
White bread	(38)	124	2	10	233	88.7	<30
Raisin bran cereal	(53)	160	2	41	295	96.6	<30 (15)
Canned fruit cocktail	(13)	18	1	5.8	66	94.8	<20 (13)
Spinach	(23)	31	2	0.6	58	96.7	<20 (13)
Iceberg lettuce	(9)	24	1	2.5	84	97.3	<20
Canned spaghetti	(17)	55	2	4.9	100	96.6	<20 (9)
Dill pickles	(54)	55	4	27	49	95.2	<30 (29)
Catsup	(43)	98	4	4.2	92	93.3	<30
Mixed nuts	(64)	130	1	11	478	90.9	<50 (16)
Canned peaches	15	15	1	2.5	55	91.9	<20 (13)
Yellow mustard	(42)	83	4	28	80	84.6	<20
Mean:				12.1		93.2	
^a Results are the mean of two replicate analytical portions. Results displayed in parentheses are trace levels <LOQ.							
^b Foods are from FDA Total Diet Study (TDS) market basket 1999-1. Results displayed with "<" are less than the LOQ; the LOQ is provided and a trace level is provided in parentheses if analyte was detected. LOQ = 10xσ.							

4.3A Table 7. Lead Results for High-Salt Foods at Various Dilutions

Food	DF =1		DF =2		DF =4	
	FAS Rec. (%)	Found (µg/kg)	FAS Rec. (%)	Found (µg/kg)	FAS Rec. (%)	Found (µg/kg)
Dill pickles	43	9	50	18	>90	54
Catsup	33	7	60	22	>90	43
Yellow mustard	28	4	38	15	>90	42

4.3A.4 CONCLUSION

The method is applicable to the analysis of food for cadmium and lead. A single microwave digestion program can be utilized by varying the analytical portion based on energy content. Foods of different nutritional composition (fat, protein, carbohydrate) can be digested in a single microwave carousel. Analyte recovery in the fortified analytical solution aids in determining the dilution necessary to minimize matrix interference. This interference might otherwise go unnoticed. Method performance was validated by the analysis of food type RMs and recovery of analyte from consumer foods. Recovery was acceptable in both the reference materials (mean 99% for cadmium and 101% for lead) and foods (mean 96% for cadmium and 93% for lead). By

carefully adhering to the QC requirements and analyzing analytical solutions at dilutions dictated by the FAS recovery, accurate results can be obtained. Quality control analyses are necessary to ensure data quality.

REFERENCES

- (1) U.S. Food and Drug Administration (2005) (INTERNET) [FDA Total Diet Study](#) Home Page [cited 26 February 2010].

Appendix B – Supplemental Information on Interlaboratory Trial

Version 1 (June 2008)
Authors: William R. Mindak

GLOSSARY

An FDA interlaboratory method trial was undertaken to evaluate proposed EAM Method 4.3 (draft E, January 2002). The method describes procedures for determination of cadmium and lead in food by graphite furnace atomic absorption spectrometry (GF-AAS) after microwave assisted nitric acid decomposition and includes specific analytical quality controls. An important quality control measure is the recovery of the analyte from a fortified analytical solution (FAS) to determine if matrix interference is present. If matrix interference is present then additional dilution of the analytical solution or standardization by method of standard additions was required.

Four FDA laboratories participated in the study administered by CFSAN/Elemental Research Branch: Northeast Regional Laboratory, Southeast Regional Laboratory, Atlanta Center for Nutrient Analysis and the Center for Food Safety and Applied Nutrition (CFSAN). All participating laboratories used the same brand of instrument (Perkin Elmer) equipped with a transverse heated graphite furnace. Each laboratory was given 3 reference materials and 3 foods for analysis by EAM Method 4.3 (draft E) for cadmium and lead. In addition, each laboratory was also given high-purity nitric acid, cadmium and lead stock solution, independent check solution, a copy of the method, and reporting forms. CFSAN also determined cadmium and lead in its GF-AAS analytical solutions by inductively coupled plasma-mass spectrometry (ICP-MS) using an Agilent 7500C instrument.

4.3B.1 ANALYTICAL LIMITS

4.3B Tables 1 and 2 list GF-AAS instrument conditions and standardization information for cadmium and lead, respectively, used by the participating laboratories. The limit of quantification (LOQ) for each element is calculated using the analytical solution quantification limit (ASQL), analytical portion and dilution of the analytical portion. Only results above the laboratory's LOQ are used for assessing accuracy and precision except results of reference materials, which are assessed only if the certified value is above the LOQ. The characteristic mass (m_o), ASDL, and ASQL reported by each laboratory are listed in 4.3B Table 3.

Note: When the interlaboratory trial was conducted, the protocol at the time required ASQL and LOQ be calculated based on 10 times the standard deviation of the blank. The values reported in this appendix thus reflect " $10 \times \sigma$ " values rather than the current protocol of " $30 \times \sigma$ ".

Instrument sensitivity for cadmium was similar among the laboratories as indicated by the small variation in m_o values. Except for Laboratory 1, ASDL and ASQL values for cadmium varied by a factor of 5, which is not an unreasonable variation between laboratories. Laboratory 1 reported a value of 0 for cadmium's ASDL and ASQL that is not a real representation of these analytical

limits. As directed by the method, a value of 0 is avoided by obtaining an appropriate number of significant digits for the data used to calculate ASDL and ASQL.

Instrument sensitivity for lead was similar for Laboratories 2, 3 and 4 as indicated by the small variation in m_0 values. The difference in m_0 between Laboratory 1 and the other laboratories is probably due to the use of end-capped tubes by Laboratory 1 versus regular tubes by the other laboratories. ASDL and ASQL values only varied by about a factor of 2 (excluding Laboratory 1's values).

4.3B Table 1. GF-AAS Instrument Conditions for Cadmium

Laboratory:	1	2	3	4
GF-AAS Manufacturer	PE	PE	PE	PE
Instrument Model	PE 4100ZL	PE 4100ZL	PE 5100ZL	PE 5100ZL
Lamp Type (Cd)	EDL	EDL	EDL	EDL
Wavelength (nm)	228.8	228.8	228.8	228.8
Char Temp. °C	600	600	600	650
Atomization Temp. °C	1600	1600	1650	1700
<i>Standardization</i>				
Standard 1 (µg/L)	0.3	0.5	1	0.5
Standard 2 (µg/L)	0.5	1	2	1
Standard 3 (µg/L)	1	2	3	2
Standard 4 (µg/L)	2	3	4	4
Standard 5 (µg/L)	3	4	-	-
Algorithm	Linear	Linear	Linear	Linear

4.3B Table 2. GF-AAS Instrument Conditions for Lead

Laboratory:	1	2	3	4
GF-AAS Manufacturer	PE	PE	PE	PE
Instrument Model	PE 4100ZL	PE 4100ZL	PE 5100ZL	PE 5100ZL
Lamp Type (Pb)	HCL	HCL	EDL	HCL
Wavelength (nm)	283.3	283.3	283.8	283.3
Char Temp. °C	750	750	820	800
Atomization Temp. °C	1700	1700	1700	1600
<i>Standardization</i>				
Standard 1 (µg/L)	3	5	5	2.5
Standard 2 (µg/L)	5	10	10	5
Standard 3 (µg/L)	10	20	15	10
Standard 4 (µg/L)	20	30	20	20
Standard 5 (µg/L)	30	40	-	-
Algorithm	Linear	Linear	Linear	Linear

4.3B Table 3. Analytical Sensitivity and Limits

Laboratory:	1	2	3	4
<i>Cadmium</i>				
m_o (pg)	1	1.5	1.6	1
ASDL (µg/L)	0	0.027	0.047	0.010
ASQL (µg/L)	0	0.064	0.11	0.030
<i>Lead</i>				
m_o (pg)	23	36	31	33
ASDL (µg/L)	0.65	0.90	0.87	0.39
ASQL (µg/L)	1.5	2.1	2.2	0.93

4.3B.2 REFERENCE MATERIAL RESULTS

Three reference materials were included in the study: Cocoa Powder (CFSAN in-house reference material), Bovine Liver (NIST SRM 1577b) and Trace Elements in Spinach (NIST SRM 1570). These materials were chosen because the reference value lead levels are near the estimated limit of quantification (LOQ) or because of the analytical challenge presented by the matrix. Reference materials were analyzed, in duplicate, without drying. Reference material results were corrected for moisture as determined at CFSAN by freeze-drying portions of the reference materials. The moisture values used for conversion are listed in 4.3B Table 4. 4.3B Tables 5 to 10 list the reference material results. Additional dilution of the analytical solution was necessary in some cases to overcome matrix interference. The HORRAT value¹ was calculated and used to assess results. The HORRAT value is the ratio of the reproducibility relative standard deviation, expressed as a percent (RSD_R , %) to the predicted reproducibility relative standard deviation, expressed as a percent ($PRSD_R$, %) Results with HORRAT values greater than 2 were investigated for outliers.

4.3B Table 4. Moisture in RMs

Reference Material	Moisture (%)
Cocoa Powder (CPFDA)	4.90
Bovine Liver (NIST SRM 1577b)	4.40
Spinach (NIST SRM 1570)	5.70

Cadmium

Cadmium results were generally good (4.3B Tables 5 to 7) and all levels were well above LOQ in each reference material. Mean RM recoveries for the 3 reference materials were excellent (87% to 102%). Results from Laboratory 1 were consistently lower than the other laboratories but no outliers were detected using the Dixon outlier statistical test. Results from Laboratory 4 were consistently higher than the reference value, although by an acceptable amount. Unfortunately, Laboratory 1 reported an ASDL and ASQL of 0 so no estimate of LOQ was possible. Assuming Laboratory 1's LOQ was similar to those of the other laboratories then all cadmium values were well above LOQ. All analytical solutions required additional dilution to obtain a cadmium concentration within the calibration curve or to diminish matrix interference.

The dilution factor (DF) required for a given sample varied approximately by a factor of 2. The HORRAT value was ≤ 1.5 for each reference material indicating between lab variability was in the expected range. ICP-MS results were in agreement with GF-AAS results and reference values.

4.3B Table 5. Cadmium in Cocoa Powder (CPFDA)

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Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5043	0.4871	0.5071	0.5626
Rep 2 Anal Portion (g)	0.5022	0.4961	0.4908	0.5512
ASQL ($\mu\text{g/L}$)	-	0.064	0.11	0.030
Rep 1 Result ($\mu\text{g/L}$)	5.25	7.22	7.60	8.96
Rep 2 Result ($\mu\text{g/L}$)	5.15	6.92	7.22	8.84
DF	5	4.3	2	4
LOQ (mg/kg)	-	0.02	0.02	0.006
Rep 1 Result (mg/kg)	0.273	0.384	0.394	0.419
Rep 2 Result (mg/kg)	0.268	0.370	0.387	0.422
Mean Result (mg/kg)	0.271	0.377	0.391	0.421
Interlaboratory Mean Result \pm SD = 0.365 ± 0.0653 mg/kg RSD _R = 17.9%; PRSD _R = 18.6%; HORRAT = 1.0 Reference Value \pm SD = 0.388 ± 0.059 mg/kg				
RM Recovery (%)	70	97	101	109
Interlaboratory Mean RM Recovery = 94%				
ICP-MS Result (mg/kg)	0.387			

4.3B Table 6. Cadmium in Bovine Liver (NIST SRM 1577b)

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5224	0.4998	0.5089	0.5386
Rep 2 Anal Portion (g)	0.5145	0.5017	0.5148	0.5284
ASQL ($\mu\text{g/L}$)	-	0.064	0.11	0.030
Rep 1 Result ($\mu\text{g/L}$)	8.65	9.54	11.4	11.3
Rep 2 Result ($\mu\text{g/L}$)	8.50	9.30	11.1	11.2
DF	5	6	4	10
LOQ (mg/kg)	-	0.02	0.03	0.02
Rep 1 Result (mg/kg)	0.433	0.498	0.586	0.548
Rep 2 Result (mg/kg)	0.432	0.484	0.563	0.552
Mean Result (mg/kg)	0.433	0.491	0.574	0.550
Interlaboratory Mean Result \pm SD = 0.512 ± 0.0634 mg/kg RSD _R = 12.4%; PRSD _R = 17.7%; HORRAT = 0.7 Reference Value \pm SD = 0.50 ± 0.03 mg/kg				
RM Recovery (%)	87	98	115	110
Interlaboratory Mean RM Recovery = 102%				
ICP-MS Result (mg/kg)	0.507			

4.3B Table 7. Cadmium in Spinach (NIST SRM 1570)

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5295	0.4917	0.4892	0.5270
Rep 2 Anal Portion (g)	0.5285	0.4922	0.4880	0.5553
ASQL (µg/L)	-	0.064	0.11	0.030
Rep 1 Result (µg/L)	22.9	25.1	21.9	30.9
Rep 2 Result (µg/L)	22.4	25.6	23.5	33.3
DF	10	16	10	10
LOQ (mg/kg)	-	0.06	0.06	0.02
Rep 1 Result (mg/kg)	1.15	1.36	1.25	1.56
Rep 2 Result (mg/kg)	1.12	1.38	1.27	1.59
Mean Result (mg/kg)	1.13	1.37	1.26	1.57
Interlaboratory Mean Result \pm SD = 1.33 \pm 0.186 mg/kg RSD _R = 13.9%; PRSD _R = 15.3%; HORRAT = 0.9 Reference Value (non-certified) = 1.5 mg/kg				
RM Recovery (%)	76	91	84	105
Interlaboratory Mean RM Recovery = 87%				
ICP-MS Result (mg/kg)				1.48

Lead

Lead results (4.3B Tables 8 to 10) did not agree as well as the cadmium results. This can be attributed, in part, to the low levels of lead (near or below LOQ) in reference materials Cocoa Powder and Bovine Liver. However, results for the reference material Spinach was quite variable even though lead was present at approximately 5 times LOQ. ICP-MS results were in agreement with reference values.

The reference value of lead in Cocoa Powder was near or below the LOQ for Laboratories 2-4 and the obtained results are consistent with the LOQ (4.3B Table 8). Laboratory 1's LOQ indicated the ability to measure the reference value but the RM recovery was very high (255%) and matrix interference was not observed. The reason for this excessively high result is unclear but one possible explanation is that LODs and LOQs based on measurements of a pristine matrix (1% nitric acid or method blanks) do not accurately estimate these limits for a sample matrix. The trace level found by Laboratory 4 was very close to the reference value (109%). This laboratory also determined that there was matrix interference (using FAS recovery) and diluted the analytical solution by a factor of 3. The trace levels of Laboratories 2 and 3 were 136% and 48%, respectively, of the reference value. Neither of these laboratories experienced matrix interference and thus did not dilute the analytical solution. Cocoa Powder was a challenging sample because of the low level of lead and the possibility of matrix interference. FAS recovery did not detect possible matrix interference for Laboratories 1-3. Inaccurate assessment of or inability to detect matrix interference could have contributed to the poor results for Laboratories 1-3 but the low lead concentration was probably a factor as well. The high result from Laboratory 1 (255%) cannot be explained from matrix interference because this type of interference usually causes signal suppression and thus low results.

The reference value of lead in Bovine Liver was near or below the LOQ for Laboratories 2-4 and the obtained results are consistent with the LOQ (4.3B Table 9). Laboratory 1's LOQ indicated the ability to measure the reference value but the RM recovery was very high (232%) and matrix interference was not observed. Laboratories 3 and 4 had acceptable recoveries of 111% and 99%, respectively even though Laboratory 4's value is a trace level. Laboratory 1's lead result of 0.299 mg/kg (232% recovery) failed the Dixon outlier test (at 5% possible false

rejection) and was labeled as an outlier. Although no reason for this outlier is clear, this laboratory's LOQ may be an under estimated value. As with Cocoa Powder, only Laboratory 4 found it necessary to dilute the analytical solution due to matrix interference.

The reference value of lead in Spinach was above the LOQ for all laboratories (4.3B Table 10). Recovery of lead in Spinach ranged from 12% to 101% of the reference value with a mean of 65%. This reference material was meant to provide a challenging matrix with a lead concentration well above LOQ. The relatively high lead content was supposed to allow quantification even after extensive dilution of the analytical solution due to matrix interference. Laboratory 4 found extensive matrix interference that required a DF of 4 in order to achieve an accurate result (101% recovery). In contrast, Laboratory 2 was able to achieve a good result (99% recovery) without the need for further dilution. The reason behind this difference in interference assessment needs further investigation. Laboratories 1 and 3 had poor recoveries of 12% and 49%, respectively. A possible explanation for these low recoveries is an improper assessment of interference. A DF of 3 or more should have been required.

4.3B Table 8. Lead in Cocoa Powder (CPFDA)^a

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5043	0.4871	0.5071	0.4811
Rep 2 Anal Portion (g)	0.5022	0.4961	0.4908	0.5512
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Result (µg/L)	6.6	2.34	0.86	2.16
Rep 2 Result (µg/L)	5.9	3.32	1.12	2.56
DF	1	1	1	3
LOQ (mg/kg)	0.08	0.2	0.1	0.2
Rep 1 Result (mg/kg)	0.299	0.124	0.042	0.118
Rep 2 Result (mg/kg)	0.264	0.176	0.063	0.122
Mean Result (mg/kg)	0.281	0.150 TR	0.053 TR	0.120 TR
Reference Value ± SD = 0.110 ± 0.022 mg/kg				
RM Value Recovery (%)	255	136	48	109
ICP-MS Result (mg/kg)				0.117
^a Interlaboratory statistics were not calculated since two or more laboratories reported values less than LOQ.				

4.3B Table 9. Lead in Bovine Liver (NIST SRM 1577b)^a

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5224	0.4998	0.5089	0.5386
Rep 2 Anal Portion (g)	0.5145	0.5017	0.5148	0.5284
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Result (µg/L)	6.70	2.05	3.80	2.36
Rep 2 Result (µg/L)	6.90	1.74	2.40	2.82
DF	1	1	1	3
LOQ (mg/kg)	0.07	0.1	0.1	0.2
Rep 1 Result (mg/kg)	0.292	0.108	0.163	0.115
Rep 2 Result (mg/kg)	0.306	0.091	0.122	0.140
Mean Result (mg/kg)	0.299	0.099 TR	0.143	0.128 TR
Reference Value \pm SD = 0.129 \pm 0.004 mg/kg				
RM Value Recovery (%)	232	77	111	99
ICP-MS Result (mg/kg)				0.114
Dixon Outlier Test Value: 0.783 Laboratory 1 Dixon Tabulated Value (5%): 0.765 Laboratory 1 Outlier				
^a Interlaboratory statistics were not calculated since two or more laboratories reported values less than LOQ.				

4.3B Table 10. Lead in Spinach (NIST SRM 1570)

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	0.5295	0.4917	0.4892	0.5270
Rep 2 Anal Portion (g)	0.5285	0.4922	0.4880	0.5553
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Result (µg/L)	3.3	20.9	11.5	23.7
Rep 2 Result (µg/L)	3.8	23.0	10.1	25.6
DF	1	1	1	4
LOQ (mg/kg)	0.07	0.2	0.1	0.2
Rep 1 Result (mg/kg)	0.137	1.12	0.625	1.19
Rep 2 Result (mg/kg)	0.147	1.24	0.546	1.22
Mean Result (mg/kg)	0.142	1.18	0.585	1.21
Interlaboratory Mean Result \pm SD = 0.779 \pm 0.5130 mg/kg RSD _R = 65.8%; PRSD _R = 16.6%; HORRAT = 4.0 Reference Value \pm SD = 1.2 \pm 0.2 mg/kg				
RM Value Recovery (%)	12	99	49	101
Interlaboratory Mean RM Recovery = 65%				
ICP-MS Result (mg/kg)				1.14

4.3B.3 FOOD RESULTS

Three food materials were included in the study: ketchup, sweet potato (puréed baby food) and yellow mustard. These foods were chosen because of the expected low levels of lead or cadmium or because of the analytical challenge presented by the matrix. Two replicate analytical portions of each food were analyzed. A third fortified analytical portion (FAP) was prepared and analyzed by the laboratory to estimate analyte recovery in the food matrix. 4.3B Tables 11 to 16 list the food results. Additional dilution of the analytical solution was necessary in some cases to overcome matrix interference.

Cadmium

Cadmium results between laboratories were generally in good agreement (4.3B Tables 11 to 13) and RSD_R ranged from 6.6% to 13.4%. The only outlier was the result for ketchup from Laboratory 1. The level of cadmium in the foods was well above LOQs. Unfortunately, Laboratory 1 reported an ASQL of 0 so no estimate of LOQ was possible. Assuming Laboratory 1's LOQ was similar to those of the other laboratories then all cadmium values were well above LOQ. Two replicate analytical portions analyzed by each laboratory agreed well with each other. FAP recovery was good with a range of 90% to 105% and a mean of 99%. Results from Laboratory 4 were consistently higher than the others while results from Laboratory 1 were generally lower. The HORRAT value was ≤ 1.5 for each food and indicates between lab variability was in the expected range. Some analytical solutions required additional dilution to obtain a cadmium concentration within the calibration curve or to diminish matrix interference. The DF required for a given sample varied approximately by a factor of 2. However, the analytical portion mass varied between laboratories for a given sample and a smaller mass would lessen the potential for interference. Laboratory 4's ICP-MS results were in good agreement with GF-AAS results with a maximum relative percent difference (RPD) between the two techniques of 15% for yellow mustard.

4.3B Table 11. Cadmium in Sweet Potato Baby Food

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.5145	2.4204	8.5526	3.1087
Rep 2 Anal Portion (g)	2.5422	2.4226	8.6700	3.0389
ASQL ($\mu\text{g/L}$)	-	0.064	0.110	0.030
Rep 1 Result ($\mu\text{g/L}$)	0.436	0.393	1.34	0.620
Rep 2 Result ($\mu\text{g/L}$)	0.43	0.428	1.45	0.609
DF	1	1	1	1
LOQ ($\mu\text{g/kg}$)	0	0.7	0.3	0.3
Rep 1 Result ($\mu\text{g/kg}$)	4.2	4.1	4.0	5.0
Rep 2 Result ($\mu\text{g/kg}$)	4.1	4.4	4.0	5.0
Mean Result ($\mu\text{g/kg}$)	4.2	4.3	4.0	5.0
Interlaboratory Mean Result \pm SD = $4.4 \pm 0.45 \mu\text{g/kg}$ $RSD_R = 10.2\%$; $PRSD_R = 36.2\%$; HORRAT = 0.3				
Fortification ($\mu\text{g/kg}$)	12.4	20.0	24.3	322
FAP Recovery (%)	93	102	98	105
Interlaboratory Mean FAP Recovery = 99%				
ICP-MS Result ($\mu\text{g/kg}$)				3.8

4.3B Table 12. Cadmium in Ketchup

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.7017	1.0180	2.0212	2.2000
Rep 2 Anal Portion (g)	2.7268	1.0387	2.0208	2.0337
ASQL (µg/L)	-	0.064	0.11	0.030
Rep 1 Result (µg/L)	0.954	0.951	1.80	2.24
Rep 2 Result (µg/L)	1.09	0.992	1.82	2.10
DF	1	1	1	4
LOQ (µg/kg)	-	2	1	2
Rep 1 Result (µg/kg)	9.0	23.4	22.0	25.4
Rep 2 Result (µg/kg)	10.0	23.9	23.0	25.8
Mean Result (µg/kg)	9.5	24	23	26
Interlaboratory Mean Result \pm SD = 20 ± 7.3 µg/kg RSD _R = 36%; PRSD _R = 28.8%; HORRAT = 1.2				
Fortification (µg/kg)	16	50	96	459
FAP Recovery (%)	96	104	90	102
Interlaboratory Mean FAP Recovery = 98%				
ICP-MS Result (µg/kg)				21
Dixon Outlier Test Value: 0.808 Laboratory 1 Dixon Tabulated Value (5%): 0.765 Laboratory 1 Outlier				
<i>Results Excluding Laboratory 1</i>				
Interlaboratory Mean Result \pm SD = 24 ± 1.6 µg/kg Interlaboratory Mean FAP Recovery = 99% RSD _R = 6.6%; PRSD _R = 28%; HORRAT = 0.2				

4.3B Table 13. Cadmium in Yellow Mustard

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.5461	1.0135	2.6180	2.1585
Rep 2 Anal Portion (g)	2.5503	0.9894	2.5710	2.3195
ASQL (µg/L)	-	0.064	0.11	0.030
Rep 1 Result (µg/L)	2.77	1.42	3.36	3.23
Rep 2 Result (µg/L)	2.76	1.35	3.56	3.49
DF	2	1	4	4
LOQ (µg/kg)	0	2	4	2
Rep 1 Result (µg/kg)	27.0	35.0	32.0	37.4
Rep 2 Result (µg/kg)	26.9	34.0	35.0	37.6
Mean Result (µg/kg)	27	35	34	38
Interlaboratory Mean Result \pm SD = 33 ± 4.4 µg/kg RSD _R = 13.4%; PRSD _R = 26.7%; HORRAT = 0.5				
Fortification (µg/kg)	40	50	79	496
FAP Recovery (%)	96	97	100	105
Interlaboratory Mean FAP Recovery = 100%				
ICP-MS Result (µg/kg)				32

Lead

Unfortunately, the lead levels in the foods were near or below the LOQs (4.3B Tables 14 to 16). However, the laboratories correctly obtained results that were consistent with LOQs and the concentration obtained by Laboratory 4 using ICP-MS. Two replicate analytical portions

analyzed by each laboratory agreed well with each other considering the low levels. FAP recovery was good and ranged from 89% to 121% with a mean of 98%. Statistics were not calculated because the measured levels in the foods were not above LOQ in 2 or more laboratories. Some analytical solutions required additional dilution because of matrix interference. The DF required for a given sample varied approximately by a factor of 3. The analytical portion variation between laboratories must be taken into consideration when comparing results. The ICP-MS results appear to be considerably lower than the GF-AAS results indicating that a positive bias in the GF-AAS results may be present. This discrepancy supports the qualification of results below LOQ. Analytical limits were estimated using a pristine matrix (method blanks) and probably do not accurately estimate these limits for a sample matrix especially a high-salt containing matrix (e.g., yellow mustard) which is known to interfere with measurements by GF-AAS.

Ketchup (4.3B Table 14) was a challenging sample because of low lead concentration and high salt content. Laboratory 1 found lead to be below LOD. The other laboratories determined lead to be at trace levels. There was inconsistency in the dilution required for interference free analysis (as determined by the FAS recovery). Laboratory 1 (2.7 g analytical portion) found that no additional dilution was necessary whereas Laboratory 4 (2.1 g analytical portion) determined that a DF of 3 was necessary. FAP recovery was very good but for one slightly high result (121%). The results from Laboratories 2–4 were similar even though they were at trace levels. However, the ICP-MS lead result of <0.006 mg/kg indicates a positive bias may be present by GF-AAS.

4.3B Table 14. Lead in Ketchup^a

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.7017	1.0180	2.0212	2.2000
Rep 2 Anal Portion (g)	2.7268	1.0387	2.0208	2.0337
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Anal Result (µg/L)	0	1.30	0.84	2.92
Rep 2 Anal Result (µg/L)	0	1.57	1.74	1.97
DF	1	1	2	3
LOQ (µg/kg)	70	50	50	40
Rep 1 Result (µg/kg)	0	32	20	33
Rep 2 Result (µg/kg)	0	38	43	24
Mean Result (µg/kg)	0 (ND)	35 (TR)	32 (TR)	29 (TR)
Fortification (µg/kg)	32	500	96	459
FAP Recovery (%)	101	96	121	93
Interlaboratory Mean FAP Recovery = 103%				
ICP-MS Result (µg/kg)	< 6			
^a Interlaboratory statistics were not calculated since two or more laboratories reported values less than LOQ.				

Sweet potatoes (4.3B Table 15) were challenging because of the very low lead concentration. Three laboratories determined lead to be below LOQ. Results had a wide variation (41% RPD) as would be expected at such low concentrations. Laboratory 4 (3 g analytical portion) found it necessary to dilute the analytical solution because of matrix interference in contrast to Laboratory 3 that found no interference even with an 8.5 g analytical portion. FAP recovery was excellent. The very low levels by GF-AAS, considering some are trace levels, are in close agreement with the level found by ICP-MS. As with ketchup, the LOQ is probably underestimated by using method blanks.

4.3B Table 15. Lead in Sweet Potato Baby Food^a

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.5145	2.4204	8.5526	3.1087
Rep 2 Anal Portion (g)	2.5422	2.4226	8.6700	3.0389
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Result (µg/L)	1.8	1.55	2.05	1.58
Rep 2 Result (µg/L)	1.9	1.25	2.33	1
DF	1	1	1	3
LOQ (µg/kg)	20	20	6	30
Rep 1 Result (µg/kg)	17.9	16	6	12.7
Rep 2 Result (µg/kg)	18.7	13	7	8.2
Mean Result (µg/kg)	18 (TR)	15 (TR)	6.5	11 (TR)
Fortification (µg/kg)	115	200	24	322
FAP Recovery (%)	107	97	96	97
Interlaboratory Mean FAP Recovery = 99%				
ICP-MS Result (µg/kg)				8.5
^a Interlaboratory statistics were not calculated since two or more laboratories reported values less than LOQ.				

Yellow mustard (4.3B Table 16) presented a similar challenge as ketchup having a low lead and high salt concentration. Laboratory 1 reported that the level of lead was below LOD and the other laboratories reported trace levels. Laboratory 4 found interference severe enough to require a 3-fold dilution. In contrast, Laboratory 3 did not find dilution necessary even though they used the largest analytical portion. Laboratory 2 also did not find it necessary to dilute the analytical solution but they used the smallest (1 g) analytical portion that may have minimized any matrix interference. FAP recovery was very good. The ICP-MS lead result of 5.7 µg/kg indicates a positive bias may be present by GF-AAS.

4.3B Table 16. Lead in Yellow Mustard^a

Laboratory:	1	2	3	4
Rep 1 Anal Portion (g)	2.5461	1.0135	2.6180	2.1585
Rep 2 Anal Portion (g)	2.5503	0.9894	2.5710	2.3195
ASQL (µg/L)	1.6	2.1	2.2	0.93
Rep 1 Result (µg/L)	0	1.53	2.10	2.59
Rep 2 Result (µg/L)	0	1.16	2.14	2.91
DF	2	1	1	3
LOQ (µg/kg)	70	50	30	40
Rep 1 Result (µg/kg)	0	38	20	30
Rep 2 Result (µg/kg)	0	29	21	31
Mean Result (µg/kg)	0 (ND)	34 (TR)	21 (TR)	31 (TR)
Fortification (µg/kg)	55	500	79	496
FAP Recovery (%)	94	93	89	92
Interlaboratory Mean FAP Recovery = 92%				
ICP-MS Result (µg/kg)				5.7
^a Interlaboratory statistics were not calculated since two or more laboratories reported values less than LOQ.				

4.3B.4 OTHER SUPPORTING RESULTS

Other supporting information includes results from the use of the method by FDA laboratories other than the trial participants. One laboratory (Laboratory A) reported lead results for reference materials analyzed in support of regulatory analyses that are summarized below.

Corn Bran, NIST Reference Material 8433

Lead Reference Value = 0.140 mg/kg

Mean RM Recovery = 92.8% (n=12)

Minimum 86.4%, Maximum 102.0%

Oyster Tissue, NIST Standard Reference Material 1566

Lead Reference Value = 0.480 mg/kg

Mean RM Recovery = 92.5% (n=7)

Minimum 80.4%, Maximum 106.3%

Cadmium Reference Value = 3.5 mg/kg

Mean RM Recovery = 92.9% (n=5)

Minimum 85.1%, Maximum 101.1%

The laboratory reporting the above results and another laboratory (Laboratory B) also reported FAP recovery in support of regulatory analyses using the method. These results are summarized below.

Lead FAP Recovery

Laboratory A

Mean = 99.6% (n = 33)

Minimum 82.0%, Maximum 127.0%

Laboratory B

Mean = 99.9% (n=55)

Minimum 80.0%, Maximum 122.0%

Cadmium FAP Recovery

Laboratory B

Mean = 98.2% (n=56)

Minimum 82.0%, Maximum 116.0%

4.3B.5 CONCLUSION

The results of this interlaboratory method trial show that accurate results can be obtained for cadmium and lead in food using GF-AAS. FAS recovery provided a means to assess the presence of matrix interference and, therefore, a need for further dilution of the analytical solution. However, the need for and degree of additional dilution varied between laboratories. Therefore, analysts should carefully evaluate the possibility of interference not only by the FAS recovery but also by observing the peak profile for any irregularities. In addition, when

interference is suspected, the analyst should compare results obtained at different dilutions if analyte level is sufficient (*i.e.*, if dilutions provide measurements above LOQ).

Good estimates of analytical limits are necessary for qualifying results that are below LOQ. Detection and quantification limits determined in a pristine matrix are probably very optimistic for “real” samples especially samples with a challenging matrix. Actual analytical limits in foods are almost certainly greater than analytical limits estimated using method blanks. Although analytical limits probably vary among foods, it is impractical to determine limits for each food matrix. Therefore rules for estimating analytical limits include very conservative rounding and the analyst should consider raising the estimate of the analytical limit when interference is suspected. In some cases, for example, an estimated LOQ of 37 µg/kg should be rounded up to 50 µg/kg.

The results of the interlaboratory trial have presented three issues that should be monitored with use of the method: analytical limits, utility of FAS recovery, and measurement of lead above the ASQL.

- The trial assumed that the procedure for determining analytical limits was followed and did not require submission of the data used to determine analytical limits. Therefore, critical aspects of determining analytical limits such as using sufficient significant figures and adding analyte to method blanks to obtain a signal near ASDL were not documented. The procedures given for analytical limits should be followed and if they do not provide good estimates then alternative procedures should be investigated.
- The FAS recovery did not consistently indicate the presence of interference. The trial did not require submission of FAS recovery data but assumed the analyst would perform this task and correctly interpret the results. The utility of the FAS to correctly indicate interferences should be monitored and if it fails to function properly then alternative procedures should be investigated.
- Unfortunately, the level of lead in the foods and 2 of the reference materials was below the LOQ. Even though FAP recoveries are very good for lead, trial data on native levels of lead above LOQ are needed to demonstrate interlaboratory reproducibility. A small follow-up to the trial should be undertaken to provide this information.

REFERENCES

- (1) Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis. [AOAC website](#).