

Elemental Analysis Manual

for Food and Related Products

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

Version 1.1 (July 2010)
Authors: William R. Mindak
John Cheng

GLOSSARY

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.

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 TEM 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 (≥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 µg/L. Typical standard solutions for cadmium analysis are 0.3, 0.5, 1.0, 2.0 µg/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) Wash down any material on walls and wet sample with 1 to 2 mL reagent water. Do not add more than 2 mL of water. Pipet 8.0 mL or weigh 10.0 g of high purity nitric acid into vessel liner. Use the acid to wash down any material on walls. Alternatively, one can first wash down any material on walls and wet sample with 1 to 2 mL reagent water. Do not add more than 2 mL of water. If foaming or reaction with the acid is observed (usually

foods high in sugar), let the vessels sit uncovered for 20 minutes or until reaction subsides. If a clean air hood is unavailable for this operation, place caps on vessels without pressing down fully or, if so equipped, cap vessels and loosen the pressure relief safety membrane nut 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 2 mL 30% hydrogen peroxide, 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)	10
Control Temperature (°C)	200
^a Only use with non-venting vessels.	

- (4) After vessels have cooled to less than 50 °C remove to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer and dilute digestion solution to 25 mL with reagent water. 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_o) 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 approximately 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 LOD and $<$ 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 $<$ 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 μ g/kg; LOD = 3 μ g/kg. Levels found for three different samples were 10 μ g/kg, 5 μ g/kg and 2 μ g/kg mg/kg.

10 μ g/kg is \geq LOQ; report 10 μ g/kg

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

2 μ g/kg is $<$ LOD; report 0 μ g/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.

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