



U.S. Department of Health & Human Services



U.S. Food and Drug Administration

Elemental Analysis Manual

for Food and Related Products

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4.4 Inductively Coupled Plasma-Atomic Emission Spectrometric Determination of Elements in Food Using Microwave Assisted Digestion

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4.4.1 SCOPE AND APPLICATION

This method describes procedures for using inductively coupled plasma-atomic emission spectrometry (ICP-AES) for determination of total element concentration (mass fraction) in food. The method was validated with the following foods: milk, cheese, bacon, tuna, eggs, peanut butter, corn, bread, pancakes, cereal, prune juice, lemonade, broccoli, sweet potato, spaghetti & meatballs, mayonnaise, beer, beef baby food, haddock and pears. 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 using pneumatic nebulization is applicable to the analytes listed in 4.4 Table 1. It should be noted that aluminum results could be biased low in some samples because of insoluble aluminum compounds especially if silica is present. Thallium is listed conditionally because although fortification recoveries were acceptable during method validation, no reference materials were available.

4.4 Table 1. Analytical Limits

Element	Symbol	ASDL ^a (mg/L)	LOD ^b (mg/kg)	LOQ ^b (mg/kg)
Aluminum	Al	0.054	0.8	2
Arsenic	As	0.086	2	4
Barium	Ba	0.0033	0.05	0.2
Boron	B	0.021	0.3	0.8
Cadmium	Cd	0.023	0.3	0.9
Calcium	Ca	0.63	8	30
Chromium	Cr	0.13	2	5
Cobalt	Co	0.021	0.3	0.8
Copper	Cu	0.0079	0.1	0.3
Iron	Fe	0.0083	0.2	0.3
Lead	Pb	0.17	3	6
Magnesium	Mg	0.16	2	6
Manganese	Mn	0.0099	0.2	0.4
Molybdenum	Mo	0.027	0.4	1
Nickel	Ni	0.066	0.9	3
Phosphorus	P	0.16	2	6
Potassium	K	1.1	20	40
Sodium	Na	0.12	2	5
Strontium	Sr	0.0019	0.03	0.07
Thallium	Tl	0.16	2	6
Vanadium	V	0.014	0.2	0.5
Zinc	Zn	0.023	0.3	0.8

^aBased on 3×σ of method blanks using pneumatic nebulization.
^bBased on 10×σ of method blanks, 4 g analytical portion, and 50 mL analytical solution.

The limits listed in 4.4 Table 1 are intended as a guide and actual limits are dependent on the sample matrix, instrumentation and selected operating conditions.

Note: When the method was developed, 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 4.4 Table 1 thus reflect “10×σ” values rather than the current protocol of “30×σ”.

Aluminum concentrations using the method do not account for aluminum bound to silicates. The method, especially using pneumatic nebulization, may not achieve quantitative measurement of typical concentrations in some foods for some elements. Using ultrasonic nebulization will improve analytical limits for most elements. The following elements appear prone to laboratory environmental contamination and therefore require extensive assessment of contamination control: aluminum, chromium, and lead. Successful application of the method must match the purpose of the food analysis with the laboratory's analytical limits. A subset of the elements may be selected for analysis.

This method should only be used by analysts experienced in the use of inductively coupled plasma atomic emission 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.

4.4.2 SUMMARY OF METHOD

An analytical portion (0.4 to 5 g dependent on food composition) is decomposed 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 50 mL analytical solution is prepared from the digest. Analytical solutions are nebulized and aerosol is transported to a plasma where desolvation and excitation occur. Either pneumatic or ultrasonic nebulization sample introduction is used. Characteristic atomic emission spectra are produced by radio frequency inductively coupled plasma. Spectra are dispersed by a grating spectrometer, and line intensities are measured with a light sensitive detector such as a photomultiplier tube or charge transfer device. Photocurrents are processed by a computer system. A background correction technique is required to compensate for variable background emission contribution to analyte signal and should be applied except in cases of line broadening. Extensive quality control procedures are incorporated for monitoring laboratory contamination and food matrix interference to ensure data quality. The application of microwave assisted decomposition sample preparation to ICP-AES determination of elements is well documented in the literature¹⁻¹⁰.

4.4.3 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) Inductively coupled plasma atomic emission spectrometer (ICP-AES)—Simultaneous or sequential ICP-AES with associated glassware, which uses a mass flow controller to regulate argon nebulizer flow rate supplied by a Dewar of liquid argon or tank of gaseous argon. A variable speed peristaltic pump to deliver all solutions to nebulizer. Pneumatic nebulizer which can aspirate high dissolved solids (*e.g.*, V-groove, cross flow, etc.) or an ultrasonic nebulizer.

Safety Note: Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions.

- (2) Microwave decomposition 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.

Safety Note: Microwave digestion systems are dangerous. Vessels contain concentrated nitric acid at high temperatures and pressures. Analyst must be familiar with manufacturer's recommended safety precautions.

4.4.4 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 (i.e., 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 water¹¹.
- (2) High purity nitric acid—Concentrated (sp gr 1.41), trace element 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) Nitric acid 10% (v/v)—Dilute 100 mL high purity nitric acid to 1000 mL with reagent water.
- (6) Hydrogen peroxide—30% H₂O₂ solution. High purity or trace metals grade.
- (7) Stock standard solutions—Commercially prepared single element solutions prepared specifically for spectrometric analysis (usually 1000 or 10,000 mg/L). Stock standard solutions may also be prepared in the laboratory from high purity (≥99.99%) metals or salts. Alternatively, commercial multi-element solutions prepared specifically for spectrometric analysis can be used. These multi-element solutions will be much lower in concentration (typically 10-500 mg/L) than single element solutions to avoid compatibility problems.
- (8) Intermediate standard solution(s)—Prepared to contain appropriate concentration(s) of analytes for preparation of standard solutions. Pipet an appropriate volume of stock standard solution(s) into an acid rinsed volumetric flask and dilute to volume with 10% nitric acid. Alternatively, intermediate standard solutions may be prepared gravimetrically by measuring stock standard solution and 10% nitric acid masses multiplied by solution density in a 125 or 250 mL plastic bottle. The density of 10% (v/v) nitric acid is 1.04 g/mL and stock standard solution densities are provided by their

commercial sources. Store prepared intermediate standard solutions in plastic bottles. Alternatively, commercial multi-element solutions prepared specifically for spectrometric analysis can be used.

- (9) Standard solutions—Prepare at least 3 standard solutions by combining appropriate volumes of stock standard solutions or intermediate standard solutions in volumetric flasks. Analyte concentration range should cover the LDR or a portion thereof. Lowest standard should be near the ASQL. Dilute to volume with 10% nitric acid. Many of the elements (cadmium, cobalt, molybdenum, etc.) have LDRs that far exceed the values expected in food analytical solutions. In addition, line-rich elements like iron may cause spectral interference on other emission lines if high concentrations are used to standardize the instrument. Therefore, the analyst may choose to work within part of the LDR. A recommended maximum concentration of an element in a standard solution is 10 mg/L. Exceptions would be elements usually present at high concentrations for example, calcium, sodium, potassium, magnesium and phosphorus. For convenience, each standard solution should contain all the analytes to be determined. Chemical compatibility (i.e., of analytes, acids, etc.) must be considered to avoid the formation of analyte precipitates when mixing single element stock solutions to prepare standard solutions. High quality custom-made multi-element solutions are commercially available and are recommended. Transfer prepared standard solutions to acid cleaned plastic bottles (Teflon[®] FEP is preferred) for storage. Standard solutions may also be prepared by gravimetric dilution. Gravimetric dilution can be performed by measuring mass of stock or intermediate standard solution and 10% nitric acid masses in a 125 or 250 mL plastic bottle. Volumes are calculated from solution densities. At typical laboratory temperatures, the density of 10% (v/v) nitric acid is 1.04 g/mL and stock standard densities are provided by their commercial sources. Do not use standard solutions that are more than 30 days old since element concentrations can change with age.
- (10) Standard blank—10% nitric acid. Prepare sufficient amount for use in standardization, determination of IDLs, and for nebulizer rinse between each measurement.
- (11) Independent check solution (ICS)—Dilute appropriate volumes of analyte stock solutions or intermediate standard solutions obtained from a different source than used to prepare standard solutions with 10% nitric acid so analyte concentration will be several times the ASQL or in the range of 0.5 to 10 mg/L for most elements. Do not use ICS that is more than 30 days old since element concentrations can change with age.
- (12) Check solution—Use mid-concentration multi-analyte standard solution for the check solution.
- (13) Fortification solution—Prepared such that, when 1 mL is diluted to analytical solution volume (initial analytical solution volume usually 50 mL), analyte concentration is approximately at the middle of the LDR or appropriate for the expected sample analyte concentration. A fortification solution should not be prepared that would result in an analyte concentration in the analytical solution that is less than 10 times the ASQL. In addition, the fortification solution should not increase any analyte's concentration by more than 40 mg/L relative to the analytical solution because of potential problems caused by high analyte levels (nebulizer transport effects and spectral interference, etc.) and the challenge of minimizing the fortification solution volume. Pipet an appropriate volume of stock standard solution(s) or intermediate standard solution(s) into an acid rinsed volumetric flask and dilute to volume with 10% nitric acid. Alternatively, fortification solution may be prepared gravimetrically.

Note: The concentration of analytes in the fortification solution should be adjusted based on experience and knowledge on the native analyte levels for the types of samples analyzed. Ideally, the fortification level should be 1 to 3 times the sample's native analyte concentration. However, determining ideal fortification levels is encumbered by the number of analytes, wide range of analyte concentrations found in foods, and unknown analyte concentration in the sample. For microwave digestion, it is important to control the amount of solution added to the decomposition vessel to minimize dilution of the nitric acid for a more consistent decomposition. Therefore, the total volume of fortification solution(s) added to the analytical portion must be no more than 1 mL.

Note: A fortification solution may also be used to prepare the ICS. However, in this case the stock and intermediate standard solutions used to prepare the fortification solution would have to be from a source independent from those used to prepare standard solutions. When the ICS is prepared from the fortification solution then ICS also serves to check the fortification level.

4.4.5 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 4.4 Table 2.

4.4 Table 2. Microwave Digestion Programs

<i>Digestion Program with Ramp to Temperature Feature and Pressure Control</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.	

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.

4.4.6 DETERMINATION PROCEDURE

The determination procedure was developed using an Applied Research Laboratories Model 3580 inductively coupled plasma atomic emission spectrometer. 4.4 Table 3a lists conditions used with this instrument. The optimum conditions must be determined for the equipment used. Quantification is performed by standard curve. However, complex matrices may require additional dilution or the determination to be made by standard additions.

Instrument Setup

- (1) Setup inductively coupled plasma atomic emission spectrometer according to the manufacturer's recommendations and with the following attributes:
 - Set rinse time to at least 60 sec.
 - Program instrument method for the analytes of interest. Include the following elements even if they are not analytes of interest to allow for interference correction: Al, Ca, Fe, Cr, Cu, Mn, Ti, and V.
 - Suggested emission line wavelengths are listed in 4.4 Table 3b. Other wavelengths may be used but they may not achieve the same sensitivities.
 - Use background correction.
 - Configure instrument for 3 integrations of emission. Use integration time appropriate for the particular instrument and emission line. Allow at least 10 sec after the solution reaches the plasma before starting integration. Report each emission reading and the mean and RSD.
 - Program instrument to use a linear, least squares calculated intercept, curve fit algorithm for converting emission values to mg/L concentration units. Do not subtract standard blank response from standard solution response. Use the mean of the emission integrations to calculate concentration of analyte.

(2) Optimize instrument

- Follow manufacturer's recommendations for optimizing the emission spectrometer.
- After instrument warm-up, perform optical profiling. Optical profiling is performed either with a built-in mercury lamp, a 2 mg/L Mn solution, or procedure recommended by instrument manufacturer.

(3) Check instrument performance

- Verify emission counts are within 80-100% of expected value with a mid-range standard.
- Verify short term precision is less than 5% relative standard deviation with a mid-range standard (n=5).
- Verify IDL is within a factor of 3 of expected value.

4.4 Table 3a. Typical ICP-AES Instrument Conditions

Conditions for Applied Research Laboratories (ARL) Model 3580	
<i>Plasma</i>	Incident RF power: 1200 watts Reflected RF power: <10 watts Viewing height above work coil: 15 mm Argon pressure: >90 psi Injector tube orifice internal diameter: 1 mm Coolant argon flow rate: 12 L/min Auxiliary (plasma) argon flow rate: 1 L/min Aerosol carrier Ar flow rate: 0.85 L/min
<i>Pneumatic Nebulizer</i>	ARL Maximum Dissolved Solids Nebulizer (V-groove): Sample uptake rate controlled to 2.5 mL/min
<i>Ultrasonic Nebulizer</i>	CETAC U-5000AT: Heating Temperature: 140 °C Sample uptake rate controlled to 1 mL/min Cooling Temperature: 0.5 °C
<i>Data Acquisition Parameters</i>	Integration Time: 10 sec Number of Integrations: 3

4.4 Table 3b. Typical ICP-AES Instrument Conditions: Wavelengths

Conditions for Applied Research Laboratories (ARL) Model 3580			
Element	Wavelength (nm) x Order ^a	Element	Wavelength (nm) x Order ^a
Aluminum	308.22x2	Magnesium	383.83x1 ^b
Arsenic	189.04x3	Manganese	257.61x3
Barium	493.41x1	Molybdenum	202.03x3
Boron	249.68x3	Nickel	231.60x3
Cadmium	226.50x3	Phosphorus	178.29x3 ^b
Calcium	317.93x2 ^b	Potassium	766.49x1 ^b
Chromium	267.72x3	Sodium	589.59x1 ^b
Cobalt	228.62x3	Strontium	407.77x1 ^b
Copper	324.75x2	Thallium	190.86x3
Iron	259.94x2	Vanadium	292.40x2
Lead	220.35x3	Zinc	213.86x2

^aBackground corrections performed at ± 0.070 nm except as noted
^bNo background correction performed.

Determination of Analyte Concentration Using Standard Curve

- (1) Standardize the instrument using the standard blank and at least 3 standard solution concentration levels. Allow at least 10 sec after the standard solution reaches the plasma before starting integration. Flush system with standard blank for at least 60 sec between each standard solution.
- (2) Check Standardization Performance
 - Correlation coefficient (r) of linear regression (emission intensity verses concentration) 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 4.4 Table 4. Rinse sample introduction system by aspirating standard blank for a minimum of 60 sec between all analyses (or longer if necessary). Rinse time is appropriate if results of a standard blank are $<ASDL$ when analyzed immediately after a high standard.
- (4) Check Instrument Measurement Performance
 - RSD of replicate integrations $\leq 7\%$ for all solutions when instrument response $\geq ASQL$.
 - 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).
 - Standard blank analyzed at a frequency of 10% and at the end of the analytical run $<ASDL$ (continuing calibration blank).
 - Measurements are below highest standard solution. Dilute analytical solution with standard blank if necessary to comply with criteria.

- Wavelength scan indicates absence of spectral interference that is not corrected for by background correction or inter-element correction factors.

(5) Inter-element Correction Factors

- If analytical solution has or is expected to have Al, Ca, Fe, Cr, Cu, Mn, Ti or V at concentrations >20 mg/L then inter-element correction factors must be determined as outlined in manufacturer's Instructions. Program instrument to use these factors.
- Analyze the solution(s) used to determine the inter-element correction factors as a sample to demonstrate proper correction for interference.

Note: Each analytical solution must be checked for spectral interference by performing a wavelength scan. An intensity (emission counts) versus wavelength scan must be recorded for each element for each analytical solution. Depending on ICP-AES instrument software, these scans can be incorporated into the ICP-AES analytical run or performed in a separate "scan" run. An appropriate standard solution must be scanned and the result overlaid with the scan of the analytical solution. A standard solution close in element concentration to the analytical solution should be chosen. A broad or double peak indicates an unresolved peak that may result in a positive bias. Interfering peaks could be from elements not being quantified. Peaks in the area of the background correction point(s) may result in a negative bias. Background correction points should be chosen in an area(s) free from other peaks.

4.4 Table 4. Typical Analytical Sequence

Auto-Sampler Tube #	Solution	Quality Control Criteria
	sensitivity check	Emission counts 80-100% of historical value
	precision check	n=5, RSD ≤5%
	standard curve check	r ≥ 0.998
	analyze std blank 5 times	IDL ≤ 3 × historical IDL
1	ICS	Recovery 95-105%, RSD <7%
2	standard blank	<ASDL
3	MBK 1	≤MBK _C
4	MBK 2	≤MBK _C
5	MBK 3	≤MBK _C
6	RM	Conc < high std, recovery 80-120%, RSD <7%
7	sample 1	Conc < high std, RSD <7%
8	sample 1 FAP	Conc < high std, recovery 80-120%, RSD <7%
9	sample 2	Conc < high std, RSD <7%
10	check solution	Recovery 90-110%, RSD <7%
11	standard blank	<ASDL
12	sample 3	Conc < high std, RSD <7%
13	sample 4	Conc < high std, RSD <7%
14	sample 5 #1	Conc < high std, RSD <7%
15	sample 5 #2	Conc < high std, RSD <7%
16	sample 6	Conc < high std, RSD <7%
17	sample 7	Conc < high std, RSD <7%
18	sample 8	Conc < high std, RSD <7%
19	sample 9	Conc < high std, RSD <7%
20	sample 10	Conc < high std, RSD <7%
21	sample 11	Conc < high std, RSD <7%
22	check solution	Recovery 90-110%, RSD <7%
23	standard blank	<ASDL
24	sample 12	Conc < high std, RSD <7%
25	sample 13	Conc < high std, RSD <7%
26	sample 14	Conc < high std, RSD <7%
27	check solution	Recovery 90-110%, RSD <7%
28	standard blank	<ASDL

2/3 of MBKs ≤ MBK_C

RPD < 20%

4.4.7 CALCULATIONS

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

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

where

S = concentration of analyte in analytical solution (or diluted analytical solution) (mg/L)

MBK_L = laboratory MBK (mg/L)

V = volume (L) of analytical solution (usually 0.050 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.*, µg/kg, ng/kg).

4.4.8 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 MBKs, and 1 replicate. Analysis of replicate analytical portions is encouraged for all samples but replicates should be analyzed 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 2 MBKs analyzed and concentration of both MBKs are $\leq \text{MBK}_C$. If 3 or more MBKs are analyzed then 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%.

4.4.9 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 <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 mg/kg; LOD = 3 mg/kg. Levels found for three different samples were 10 mg/kg, 5 mg/kg and 2 mg/kg.

10 mg/kg is \geq LOQ; report 10 mg/kg

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

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

4.4.10 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 use¹²⁻¹³. Combining microwave digestion and ICP-AES for food analysis has been demonstrated¹⁻¹⁰.

In-house validation. Results of an FDA in-house validation of the method are presented in Appendix A. In addition, these in-house validation results using ultrasonic nebulization were published¹⁰. In general, the recovery results for RMs using pneumatic nebulization are good except for aluminum and no assessment can be made for chromium, cobalt, molybdenum, nickel and thallium due to lack of reference value data. As expected, aluminum recovery results for RMs were low especially in materials containing appreciable levels of silicon. Low recovery of aluminum appears to be associated with incomplete dissolution of samples containing silica and requires the use of hydrofluoric acid to obtain complete recovery.

The results of replicate FAP analyses of 20 foods using ultrasonic nebulization were used to assess analyte recovery and matrix induced interference. FAPs were analyzed for all elements except calcium, magnesium, phosphorus, potassium and sodium. All foods had been prepared and analyzed for 14 elements under FDA's Total Diet Study program¹⁴ enabling comparison of analytical results for some elements. FAP recoveries for all foods were acceptable (80-120%) for arsenic, barium, cadmium, chromium, copper, iron, manganese, molybdenum, nickel, strontium thallium, and vanadium. FAP recoveries for most foods were acceptable for all other elements (aluminum, boron, lead, selenium, sodium and zinc). The precision of replicate analysis of unfortified portions was 15% or less for all element concentration measurements above LOQ except for cadmium in spaghetti (19%), calcium in haddock (30%), copper in cheddar cheese (16%), iron in cheddar cheese (19%) and mayonnaise (17%), nickel in prune juice (16%), potassium in mayonnaise (17%), and selenium in haddock (20%). FDA Total Diet Study results were available for comparison for all elements except barium, boron, chromium, molybdenum, strontium, and thallium. A majority of the results were in agreement with Total Diet Study results.

The in-house validation results for analysis of foods using microwave decomposition and element detection using ICP-AES indicate the following elements can be reliably measured at concentrations above LOQ: arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, sodium, strontium, vanadium and zinc. Thallium appears to be reliably measured but accuracy assessment is hindered by the lack of appropriate reference materials. Aluminum can be measured but the analyst must realize that the results might be biased low if some of the aluminum is bound to silica.

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. [Under development]

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4.4 Appendix A - Supplemental Information on In-house Method Validation

Version 1 (June 2008)
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GLOSSARY

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Validation experiments of EAM 4.4 were performed using a CEM Corporation model MDS 2000 microwave digestion system with TFM Teflon lined Heavy Duty Vessels and an Applied Research Laboratories (ARL) Model 3580 inductively coupled plasma atomic emission spectrometer. Both a pneumatic nebulizer (ARL Maximum Dissolved Solids Nebulizer) and an ultrasonic nebulizer (CETAC U-5000 AT) were used. In-house validation results have been published¹.

4.4A.1 ANALYTICAL LIMITS

Analytical limits were estimated by the analysis of method blanks and the results are summarized in 4.4A Table 1.

Note: When the method validation 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×σ” values rather than the current protocol of “30×σ”.

4.4A Table 1. Estimated Analytical Limits

Element	Symbol	Pneumatic Nebulizer			Ultrasonic Nebulizer		
		ASDL (mg/L)	LOD ^a (mg/kg)	LOQ ^a (mg/kg)	ASDL (mg/L)	LOD ^a (mg/kg)	LOQ ^a (mg/kg)
Aluminum	Al	0.054	0.8	2	0.0074	0.1	0.3
Arsenic	As	0.086	2	4	0.0022	0.03	0.08
Barium	Ba	0.0033	0.05	0.2	0.011	0.2	0.4
Boron	B	0.021	0.3	0.8	0.0040	0.05	0.2
Cadmium	Cd	0.023	0.3	0.9	0.00039	0.005	0.02
Calcium	Ca	0.63	8	30	1.1	20	40
Chromium	Cr	0.13	2	5	0.0035	0.05	0.2
Cobalt	Co	0.021	0.3	0.8	0.00037	0.005	0.02
Copper	Cu	0.0079	0.1	0.3	0.00071	0.009	0.03
Iron	Fe	0.0083	0.2	0.3	0.0038	0.05	0.2
Lead	Pb	0.17	3	6	0.0032	0.04	0.2
Magnesium	Mg	0.16	2	6	0.52	7	20
Manganese	Mn	0.0099	0.2	0.4	0.000079	0.001	0.003
Molybdenum	Mo	0.027	0.4	1	0.00081	0.02	0.03
Nickel	Ni	0.066	0.9	3	0.0015	0.02	0.06
Phosphorus	P	0.16	2	6	0.0048	0.06	0.2
Potassium	K	1.1	20	40	0.053	0.7	2
Selenium	Se	0.24	3	9	0.0086	0.2	0.4
Sodium	Na	0.12	2	5	0.87	20	40
Strontium	Sr	0.0019	0.03	0.07	0.027	0.4	1
Thallium	Tl	0.16	2	6	0.0031	0.04	0.2
Vanadium	V	0.014	0.2	0.5	0.00043	0.006	0.02
Zinc	Zn	0.023	0.3	0.8	0.0013	0.02	0.05

^aBased on 10×σ of method blanks, 4 g analytical portion, and 50 mL analytical solution.

4.4A.2 REFERENCE MATERIAL RESULTS

Reference Material Results

The results of replicate analyses of RMs (0.5 g to 1 g analytical portions) were used to assess accuracy and precision. Pneumatic and ultrasonic nebulization were assessed using independently prepared sets of RMs. RM recoveries were calculated based on available certified, informational, or consensus values for analytical results above LOQ.

Pneumatic nebulization

Results for 9 RMs analyzed using pneumatic nebulization are listed in 4.4A Table 2. At least one RM recovery was attainable for each element except chromium, cobalt, molybdenum, nickel, selenium, and thallium. RM recoveries were acceptable (80-120%) for arsenic, boron, cadmium, calcium, copper, lead, magnesium, manganese, phosphorus, potassium, vanadium, and zinc. As expected, aluminum RM recoveries were low especially materials containing appreciable levels of silicon. Low recovery of aluminum appears to be associated with incomplete dissolution of samples containing silica and requires the use of hydrofluoric acid to obtain complete recovery especially for plant materials²⁻³. Aluminum RM recoveries for 3 of 4 RMs were low (about 68%) and two of these were plant materials and the other, oyster tissue, has about 0.11% silicon⁴. Aluminum recovery from the fourth RM (dogfish liver; DOLT-2) was acceptable. Barium RM recoveries for 2 of 4 RMs were low. The barium recovery from oyster tissue (79%) is based on a

consensus value ($n = 4$) with a relative standard deviation of about 14% but this variability does not fully account for the somewhat low recovery. The barium recovery from non-fat milk powder (42%) is also based on a consensus value ($n=2$) without an estimate of variability. Iron RM recovery for 1 (non-fat milk powder) of 9 RMs was high (121%). The iron concentration was near the LOQ (2 mg/kg) and, as expected had a high RSD (12%). Sodium RM recovery for 1 (orchard leaves) of 7 RMs was low (70%). The sodium level of this RM (58 mg/kg) was much lower than the other RMs and was somewhat near the LOQ (20 mg/kg) but the RSD of measurements was good (2%). This low recovery may indicate a MBK correction problem. Strontium RM recovery for 1 (non-fat milk powder) of 5 RMs was high (121%). This strontium recovery is based on a consensus value ($n=2$) without an estimate of variability. Molybdenum could not be measured above the LOQ in any RM but was measurable at "trace" level in one RM (rice flour). RSD for this level was good (8%) and the mean level is in close agreement with the reference value. Nickel also could not be measured above LOQ in any RM but was measurable at "trace" levels in two RMs (spinach and lobster). The RSD for nickel measured in spinach RM was good (4%) and the mean level is in close agreement with the reference value. However, the RSD measured in lobster RM was poor (41%) as expected for this "trace" level near the LOD and the mean level was not in agreement with the reference value. Arsenic could only be measured above the LOQ in one RM. However, arsenic was found in 5 other RMs at "trace" levels and all these concentrations were in fairly good agreement to the reference values which may indicate a high estimate of the arsenic LOQ. Precision of replicate analysis (measured as RSD) was 15% or less for all element concentration measurements above LOQ. The somewhat poor precision for aluminum in dogfish muscle is probably due to non-homogeneity. In general, the RM recoveries using microwave digestion and pneumatic nebulization are good except for aluminum and no assessment can be made for chromium, cobalt, molybdenum, nickel, selenium, and thallium due to lack of data.

4.4A Table 2. Reference Material Results Using Pneumatic Nebulization^a (1 of 3)

Element	Oyster Tissue (NIST 1566)				Rice Flour (NIST 1568)				Trace Elements in Spinach (NIST 1570)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	255 ^b	172	4	67	2.8 ^b	< 3	-	-	870	593	0.3	68
Arsenic	13.4	(13.5)	13	-	0.41	< 5	-	-	0.15	< 5	-	-
Barium	4.9 ^b	3.9	3	79	0.3 ^b	(0.19)	3	-	14.6 ^b	14.7	3	101
Boron	8.0 ^b	7.4	4	93	0.64 ^b	< 1	-	-	28.0 ^b	26	1	93
Cadmium	3.5	(3.79)	4	-	0.029	< 2	-	-	1.39 ^b	< 2	-	-
Calcium	1500	1340	3	89	140	148	4	90	13500	12500	2	93
Chromium	0.69	< 7	-	-	0.06 ^b	< 7	-	-	4.6	< 7	-	-
Cobalt	0.35 ^b	< 1	-	-	0.02	< 1	-	-	1.49 ^b	< 1	-	-
Copper	63	64.7	2	103	2.2	2.16	4	98	12	12.2	1	102
Iron	195	194	3	99	8.7	9.08	3	104	550	513	1	93
Lead	0.48	< 9	-	-	0.045	< 9	-	-	1.2	< 9	-	-
Magnesium	1280	1230	3	96	474 ^b	479	2	101	8500 ^b	7980	1	94
Manganese	17.5	16.5	4	94	20.1	20.1	0.3	100	165	160	1	97
Molybdenum	0.20 ^b	< 2	-	-	1.62 ^b	(1.72)	8	-	0.31 ^b	< 2	-	-
Nickel	1.03	< 4	-	-	0.14 ^b	< 4	-	-	5.6 ^b	(6.2)	4	-
Phosphorus	7600 ^b	7300	6	96	1620 ^b	1580	3	98	5500	4970	1	90
Potassium	9690	9600	1	99	1120	1140	3	102	35600	33000	1	93
Selenium	2.1	< 20	-	-	0.4	< 20	-	-	0.043 ^b	< 20	-	-
Sodium	5100	5400	1	106	6	(19.1)	24	-	13900 ^b	14500	1	104
Strontium	10.36	10.1	2	98	0.19 ^b	(0.22)	11	-	87	86.3	2	99
Thallium	≤ 0.005 ^c	< 8	-	-	< 0.002 ^b	< 8	-	-	0.03 ^c	< 8	-	-
Vanadium	2.6 ^b	2.33	3	90	0.006 ^b	< 0.7	-	-	1.2 ^b	(1.3)	5	-
Zinc	852	764	7	90	19.4	19.5	5	100	50	45	3	90

4.4A Table 2. Reference Material Results Using Pneumatic Nebulization^a (2 of 3)

Element	Orchard Leaves (NIST 1571)				Non-Fat Milk Powder (NIST 1549)				Dogfish Muscle (NRCC DORM-1)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	330 ^b	224	4	68	2 ^c	<3	-	-	nv	14.9	16	-
Arsenic	10	(12.3)	5	-	0.00193 ^b	<5	-	-	17.7	(17.8)	9	-
Barium	43 ^b	44.4	1	103	2.2 ^b	0.93	1	42	nv	(0.27)	36	-
Boron	33	30	1	91	1.98 ^b	(2.1)	6	-	nv	<1	-	-
Cadmium	0.11	<2	-	-	0.0005	2	-	-	0.086	<2	-	-
Calcium	20900	17100	4	82	13000	12300	0.1	95	nv	1300	5	-
Chromium	2.6	<7	-	-	0.0026	<7	-	-	3.6	<7	-	-
Cobalt	0.16 ^b	<1	-	-	0.0041 ^c	<1	-	-	0.049	<1	-	-
Copper	12	12.8	3	107	0.7	(0.86)	2	-	5.22	5.23	5	100
Iron	300	257	2	86	1.78	2.15	12	121	63.6	66.3	3	104
Lead	45	40.8	6	91	0.019	<9	-	-	0.4	<9	-	-
Magnesium	6200	5260	3	85	1200	1200	0.2	100	1210	1190	4	98
Manganese	91	79.9	2	88	0.26	<0.5	-	-	1.32	(1.52)	8	-
Molybdenum	0.3	<2	-	-	0.34 ^c	<2	-	-	nv	<2	-	-
Nickel	1.3	<4	-	-	0.10 ^b	<4	-	-	1.2	<4	-	-
Phosphorus	2100	1790	3	85	10600	11000	0.3	104	nv	10100	3	-
Potassium	14700	13800	2	94	16900	17000	0.4	101	15900	15200	5	96
Selenium	0.08	<20	-	-	0.11	<20	-	-	1.62	<20	-	-
Sodium	82	58	2	70	4970	5540	0.5	111	8000	8140	5	102
Strontium	36 ^b	34.9	1	97	3.1 ^b	3.7	0.2	121	nv	8.5	8	-
Thallium	0.041 ^b	<8	-	-	0.00052 ^b	<8	-	-	nv	<8	-	-
Vanadium	0.51 ^b	<0.7	-	-	0.005 ^b	<0.7	-	-	nv	<0.7	-	-
Zinc	25	24	2	96	46.1	44.8	1	97	21.3	18.1	4	85

4.4A Table 2. Reference Material Results Using Pneumatic Nebulization^a (3 of 3)

Element	Dogfish Liver (NRCC DOLT-1)				Dogfish Liver (NRCC DOLT-2)				Lobster Hepatopancreas (NRCC TORT-1)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	nv	(5.0)	16	-	25.2	25.4	6	101	nv	35.5	8	-
Arsenic	10.1	(11.5)	8	-	16.6	(14.1)	7	-	24.6	28.2	3	114
Barium	nv	(0.19)	14	-	nv	0.6	10	-	nv	3.98	3	-
Boron	nv	<2	-	-	nv	<2	-	-	nv	5.45	11	-
Cadmium	4.18	4.66	2	111	20.8	19.4	1	93	26.3	25.4	3	96
Calcium	nv	483	4	-	nv	521	2	-	8950	7930	5	89
Chromium	0.4	<8	-	-	0.37	<8	-	-	2.4	<8	-	-
Cobalt	0.157	<2	-	-	0.24	<2	-	-	0.42	<2	-	-
Copper	20.8	20.1	1	97	25.8	27.7	1	107	439	429	1	98
Iron	712	693	1	97	1103	1010	0.4	91	186	191	4	103
Lead	1.36	<10	-	-	0.22	<10	-	-	10.4	(9.3)	20	-
Magnesium	1100	1020	2	93	nv	784	1	-	2250	2380	3	106
Manganese	8.72	8.45	1	97	6.88	5.91	0.2	86	23.4	22.2	3	95
Molybdenum	nv	<2	-	-	nv	<2	-	-	1.5	<2	-	-
Nickel	0.26	<4	-	-	0.2	<4	-	-	2.3	(4.7)	41	-
Phosphorus	nv	9820	3	-	nv	8330	0.3	-	8790	8820	5	100
Potassium	10100	9200	1	91	nv	8380	0.3	-	10410	10700	3	103
Selenium	7.34	<20	-	-	6.06	<20	-	-	6.88	<20	-	-
Sodium	7260	7240	0.3	100	nv	7630	0.3	-	36700	36300	2	99
Strontium	nv	4.7	4	-	nv	4.53	2	-	113	113	2	100
Thallium	nv	<9	-	-	nv	<9	-	-	nv	<10	-	-
Vanadium	nv	<0.8	-	-	nv	<0.8	-	-	1.4	(1.42)	9	-
Zinc	92.5	82.5	4	89	85.8	77.6	0.3	90	177	154	5	87

^aMean result based on 3 analyses except 5 analyses for Oyster Tissue, Dogfish Muscle (DORM-1), and Lobster Hepatopancreas. NIST=National Institute of Standards and Technology, NRCC=National Research Council of Canada, nv=no value available. Mean results in () are "trace" levels and mean results below the LOD are listed as < LOD.

^bReference value not certified; consensus value⁷.

^cReference value not certified; informational value provided by certifying organization.

Ultrasonic nebulization

Results for 9 RMs analyzed using ultrasonic nebulization are listed in 4.4A Table 3 (only oyster tissue and dogfish muscle were not the same analytical solutions analyzed using pneumatic nebulization). At least one RM recovery is attainable for each element except thallium. RM recoveries were acceptable (80-120%) for arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, strontium, vanadium and zinc. Aluminum RM recoveries for 2 of the 8 RMs were poor. The aluminum recovery from oyster tissue was low (65%) probably for the reasons discussed above. The aluminum recovery from bovine liver was very high (344%) and cannot be readily explained but could be due to the reference value (1.7 mg/kg) and the level found (5.8 mg/kg) being near the LOQ (2 mg/kg) or a contamination problem. A low (68%) nickel RM recovery for 1 (dogfish muscle) of 4 RMs cannot be readily explained. Selenium RM recoveries of all 4 RMs were high (mean 156%). All these RMs have an LOQ of 2 mg/kg and have reference values between 2 and 3 mg/kg, which may indicate that the estimated LOQ is too low or there is not sufficient correction of spectral background emission. Replicate analysis precision (measured as RSD) was 15% or less for all element concentration measurements above LOQ except for chromium in mussel (85%) and soy powdered infant formula (52%), and selenium in whole egg powder (22%). Extremely high variability was observed for chromium and many divergent results were excluded from performance assessment. The cause of this variability was not thoroughly investigated but environmental contamination or spectral interference is suspected. Chromium reference values for all RMs except dogfish muscle were below LOQ. The somewhat high selenium variability for whole egg powder is probably due a low estimated LOQ. Boron was not detected (<0.3 mg/kg) in soy powdered infant formula having a reference value of 0.60 mg/kg. Although the reference value is below the LOQ (1 mg/kg) trace levels should have been detected which may indicate that the estimated LOD may be too low. The trace vanadium result for total diet RM is a factor of 10 lower than a consensus value (n=1) without an estimate of variability indicating the low creditability of the value. A more recent publication by one of the same authors whose work provided the consensus value reported a vanadium concentration for the Total Diet RM of 0.037 mg/kg (mean of 2 analyses)⁵ which more closely agrees with the trace result. Cobalt was not detected (<0.03 mg/kg) in dogfish muscle having a reference value of 0.049 mg/kg. Although the reference value is below the LOQ (0.07 mg/kg) trace levels should have been detected which may indicate that the estimated LOD may be too low.

4.4A Table 3. Reference Material Results Using Ultrasonic Nebulization^a (1 of 3)

Element	Oyster Tissue (NIST 1566)				Bovine Liver (NIST 1577 ^a)				Rice Flour (NIST 1568 ^a)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	255 ^b	166	5	65	1.7 ^b	5.8	15	344	4.4	5.3	6	120
Arsenic	13.4	14.0	2	105	0.047	<0.2	-	-	0.29	(0.36)	21	-
Barium	4.9 ^b	3.9	1	80	0.08 ^b	<0.9	-	-	nv	<0.7	-	-
Boron	8.0 ^b	7.20	6	90	0.84 ^b	<0.4	-	-	0.54 ^c	(0.44)	11	-
Cadmium	3.5	3.65	3	104	0.44	0.454	6	103	0.022	(0.044)	44	-
Calcium	1500	1430	2	95	120	(153)	5	-	118	(151)	14	-
Chromium	0.69	<0.4 ^e	-	-	0.2 ^b	(0.79)	21	-	nv	<0.3 ^e	-	-
Cobalt	0.35 ^b	0.317	5	91	0.21	0.205	7	98	0.018 ^d	<0.03 ^e	-	-
Copper	63.0	63.0	2	100	158	149	3	94	2.4	2.5	1	105
Iron	195	184	3	94	194	175	3	90	7.4	7.6	1	103
Lead	0.48	(0.54)	10	-	0.135	<0.3 ^e	-	-	<0.010 ^d	< 0.2	-	-
Magnesium	1280	1150	2	90	600	554	3	92	560	483	1	86
Manganese	17.5	16.8	3	96	9.9	9.45	4	95	20.0	20.3	2	102
Molybdenum	0.20 ^b	(0.16)	2	-	3.5	3.69	3	105	1.46	1.62	1	111
Nickel	1.03	0.91	5	88	0.71 ^b	(0.30) ^e	21	-	nv	(0.31)	24	-
Phosphorus	7600 ^b	8170	3	108	11100	11600	4	105	1530	1750	3	114
Potassium	9690	8830	3	91	9960	8600	3	86	1280	1220	3	95
Selenium	2.1	2.8	7	134	0.71	(0.99)	44	-	0.38	<0.6	-	-
Sodium	5100	5140	2	101	2430	2370	2	98	6.6	<60	-	-
Strontium	10.36	10.7	2	103	0.138	<3	-	-	nv	<2	-	-
Thallium	≤0.005 ^d	<0.2	-	-	0.003 ^d	<0.3	-	-	nv	<0.2	-	-
Vanadium	2.6 ^b	2.44	3	94	0.099	(0.068)	25	-	0.007 ^d	<0.03	-	-
Zinc	852	788	4	92	123	115	4	93	19.4	19.3	4	99

4.4A Table 3. Reference Material Results Using Ultrasonic Nebulization^a (2 of 3)

Element	Total Diet (NIST 1548)				Whole Egg Powder (NIST 8415)				Dogfish Muscle (NRCC DORM-1)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	33 ^d	39	2	119	540	604	6	112	nv	11.4	7	-
Arsenic	0.104 ^b	<0.2	-	-	0.01 ^d	<0.2	-	-	17.7	18.6	3	105
Barium	nv	(1.3)	1	-	3 ^d	3.6	5	119	nv	<0.7	-	-
Boron	2.61 ^c	2.23	7	86	0.41	<0.4	-	-	nv	0.87	5	-
Cadmium	0.028	(0.041)	32	-	0.005 ^d	<0.04	-	-	0.086	0.103	5	120
Calcium	1740	1680	2	97	2480	2580	4	104	nv	1390	3	-
Chromium	0.5 ^b	<0.4	-	-	0.37	(0.78)	34	-	3.60	4.3 ^e	10	119
Cobalt	nv	(0.043)	12	-	0.012	(0.044) ^e	96	-	0.049	<0.03 ^e	-	-
Copper	2.6	2.68	2	103	2.70	2.86	5	106	5.22	5.21	11	100
Iron	32.6	30.6	3	94	112	104	3	93	63.6	59.0	1	93
Lead	0.05 ^d	<0.3	-	-	0.061	<0.3	-	-	0.40	(0.43)	17	-
Magnesium	556	494	2	89	305	295	4	97	1210	1070	4	88
Manganese	5.2	4.88	2	94	1.78	1.75	4	99	1.32	1.17	3	88
Molybdenum	0.27 ^d	0.30	8	110	0.247	0.26	9	105	nv	(0.18)	2	-
Nickel	0.43 ^b	(0.27)	32	-	nv	<0.2	-	-	1.20	0.81	13	68
Phosphorus	3240	3590	2	111	10010	10600	4	106	nv	11.0	3	-
Potassium	6060	5950	2	98	3190	3140	14	98	15900	13700	2	86
Selenium	0.245	<0.7	-	-	1.39	2.2	22	162	1.62	2.84	9	175
Sodium	6250	6560	2	105	3770	3900	8	103	8000	7930	2	99
Strontium	nv	(3.8)	5	-	5.63	(6.8)	6	-	nv	9.1	1	-
Thallium	nv	<0.3	-	-	nv	<0.3	-	-	nv	<0.2	-	-
Vanadium	0.49 ^b	(0.048)	17	-	0.459	0.536	12	117	nv	(0.044)	17	-
Zinc	30.8	29.9	3	97	67.5	66.6	5	99	21.3	18.0	5	84

4.4A Table 3. Reference Material Results Using Ultrasonic Nebulization^a (3 of 3)

Element	Mussel (NIES No.6)				Cocoa Powder (FDA CP)				Soy Powd. Infant Formula (FDA SPIF)			
	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)	Reference Value (mg/kg)	Mean Result (mg/kg)	RSD (%)	RM Rec (%)
Aluminum	220 ^d	218	5	99	50.1	45.2	6	90	12.5	12.6	8	101
Arsenic	9.2	9.6	1	105	nv	<0.2	-	-	nv	<0.2	-	-
Barium	nv	<0.8	-	-	nv	14.7	5	-	nv	<0.9	-	-
Boron	12.9 ^c	12.2	1	95	28.6	28.4	9	99	0.60	<0.3	-	-
Cadmium	0.82	0.849	1	104	0.390	0.366	12	94	nv	(0.081)	74	-
Calcium	1300	1260	2	97	1710	1520	13	89	6200	5640	7	91
Chromium	0.63	(0.88) ^e	85	-	0.92	<0.4	-	-	nv	(0.91)	52	-
Cobalt	0.37 ^d	0.307	6	83	1.164	1.06	13	91	nv	<0.03	-	-
Copper	4.9	5.31	12	108	41.7	43.6	5	104	9.3	8.77	7	94
Iron	158	147	4	93	131	113	10	86	100 ^d	95.8	6	96
Lead	0.91	0.83	11	91	0.117 ^d	<0.3	-	-	nv	<0.3	-	-
Magnesium	2100	1750	2	83	5990	5950	10	99	485	438	4	90
Manganese	16.3	15.0	3	92	54.2	46.7	11	86	2.67	2.28	7	85
Molybdenum	nv	0.84	3	-	nv	0.35	3	-	nv	0.29	13	-
Nickel	0.93	0.87	13	94	12.4 ^d	10.4	12	84	nv	(0.26) ^e	4	-
Phosphorus	7700 ^d	8220	2	107	8070	8220	11	102	3900 ^d	4310	4	111
Potassium	5400	5270	3	98	18750	18300	8	98	5660	5690	2	101
Selenium	1.5 ^d	2.3	9	152	nv	<0.6	-	-	nv	<0.7	-	-
Sodium	10000	9640	3	96	19	<60	-	-	2350	2400	4	102
Strontium	17 ^d	18.5	3	109	18.3 ^d	20	5	107	nv	(3.2)	16	-
Thallium	nv	<0.2	-	-	nv	<0.3	-	-	nv	<0.3	-	-
Vanadium	nv	0.562	3	-	0.096	(0.079)	11	-	0.161	0.172	12	107
Zinc	106	100	2	95	79.2	70.7	15	89	60 ^d	52.7	7	88

^aMean result based on 3 analyses except 4 analyses for Rice Flour, Whole Egg Powder, and Mussel. NIST=National Institute of Standards and Technology, NRCC=National Research Council of Canada, NIES=National Institute for Environmental Studies of Japan, FDA=Food and Drug Administration, nv=no value available. Cocoa Powder and Soy Powdered Infant Formula are FDA in-house reference materials. Mean results in () are "trace" levels and mean results below the LOD are listed as < LOD.

^bReference value not certified; consensus value⁷.

^cReference value not certified; value based on prompt gamma-ray activation analysis⁸⁻⁹.

^dReference value not certified; informational value provided by certifying organization.

^eExcluded one highly divergent analytical result due to spectral interference or environmental contamination.

4.4A.3 FOOD RESULTS

The results of replicate FAP analyses of foods were used to assess analyte recovery and matrix induced interference. Foods were analyzed using ultrasonic nebulization. All foods were portions of FDA Total Diet Study market basket 1994-4 prepared and analyzed for 14 elements under FDA's Total Diet Study program⁶ enabling comparison of analytical results for some elements.

FAP recovery

Results for 20 foods analyzed using ultrasonic nebulization are listed in 4.4A Table 4. FAPs were analyzed for all elements except calcium, magnesium, phosphorus, potassium and sodium. FAP recoveries for all foods were acceptable (80-120%) for arsenic, barium, cadmium, chromium, copper, iron, manganese, molybdenum, nickel, strontium thallium, and vanadium. Aluminum FAP recovery was low for 3 foods: peanut butter (44%), pancakes (62%), and broccoli (73%). Low FAP recoveries for pancakes and broccoli are attributed to fortification at less than 1 times the native level. Low FAP recovery for peanut butter cannot be explained with confidence but appears to be contamination or an instrument anomaly that caused the result of the unfortified analytical portion to be high (12.0 mg/kg). An aluminum concentration of 6.61 mg/kg for this peanut butter was determined by neutron activation analysis. Using this result for the unfortified analytical portion produces a 95% FAP recovery for the ICP-AES aluminum fortification result. This acceptable FAP recovery suggests that the unfortified aluminum results for peanut butter are erroneously high. Boron FAP recovery was high for 1 food: prune juice (123%). This high recovery may be attributed to fortification at less than 1 times the native level. Cobalt FAP recovery was low for 1 food: corn (67%). This low recovery cannot be explained. Lead FAP recovery was slightly low for 2 foods: pork bacon (74%) and broccoli (79%). These low recoveries cannot be readily explained. The high sodium concentration of the pork bacon may have hindered the ultrasonic nebulization but the other analyte FAP recoveries are acceptable. For broccoli, other analyte FAP recoveries are on the low end of the acceptable range (except selenium), which may indicate an inaccurate fortification. Selenium FAP recoveries are slightly high for 2 foods: spaghetti (122%) and haddock (121%). These high recoveries cannot be readily explained. A high bias on RM selenium results was also observed as mentioned above. In addition, most selenium FAP recoveries were on the high end of the acceptable range (average of acceptable FAP recoveries was 112%). These results indicate that insufficient correction of spectral background emission may be the cause of the slightly high bias on selenium results. Zinc FAP recovery was low for 1 food: fruit flavored cereal (69%). This low recovery may be attributed to fortification at less than 1 times the native level.

Precision

The precision of replicate analysis of unfortified portions (measured as RSD; 4.4A Table 4) was 15% or less for all element concentration measurements above LOQ except for cadmium in spaghetti (19%), calcium in haddock (30%), copper in cheddar cheese (16%), iron in cheddar cheese (19%) and mayonnaise (17%), nickel in prune juice (16%), potassium in mayonnaise (17%), and selenium in haddock (20%). Imprecision due to concentration measurements being near the LOQ and unknown sample homogeneity may explain the results for cadmium in spaghetti (LOQ 0.02 mg/kg), copper in cheddar cheese (LOQ 0.2), iron in mayonnaise (LOQ 2 mg/kg), and selenium in haddock (LOQ 0.8 mg/kg). Imprecision of calcium in haddock may be explained by non-homogeneity caused by haddock bone fragments and the relatively low level of calcium (LOQ 90 mg/kg). Imprecision due to levels being near the LOQ could not explain the slightly poor precision of iron in cheddar cheese (LOQ 0.7 mg/kg), nickel in prune juice (LOQ 0.05 mg/kg) or potassium in mayonnaise (LOQ 20 mg/kg). Non-homogeneity may be the cause

of the imprecision for iron in cheddar cheese. An extremely divergent aluminum result was obtained for 1 of the 3 replicates of unfortified pork bacon, beef, and haddock. These results ranged from a factor of about 2 to 5 higher than the other replicates and were excluded from the calculations. The cause of this variability was not thoroughly investigated but environmental contamination is suspected. Extremely divergent chromium results were obtained for 1 of 3 replicates of unfortified pork bacon, 1 of 3 replicates of fortified pork bacon, and 2 of 3 replicates of fortified tuna. Most of these results were negative values indicating spectral interference that may have affected background correction. An extremely divergent lead result was obtained for 1 of 3 replicates of unfortified pork bacon, tuna, and corn. These results ranged from a factor of about 70 to 300 higher than the other replicates and were excluded from the calculations. The cause of this variability was not thoroughly investigated but environmental contamination is suspected.

Comparison to Total Diet Study

FDA Total Diet Study results for market basket 1994-4 were available for comparison for all elements except barium, boron, chromium, molybdenum, strontium, and thallium. The Total Diet Study uses (1) a nitric-perchloric-sulfuric acid digestion and inductively coupled plasma atomic emission spectrometry to measure calcium, copper, iron, magnesium, manganese, phosphorus, potassium sodium and zinc, (2) the same digestion and hydride generation atomic absorption spectrometry to measure arsenic and selenium and (3) a dry ash mineralization and graphite furnace atomic absorption spectrometry to measure cadmium, lead, and nickel. Aluminum, cobalt, and vanadium results were only available for 3 foods (determined by instrumental neutron activation analysis as part of Total Diet Study independent quality assurance activity) and nickel was not available for 1 food. A majority of the results were in agreement with Total Diet Study results (4.4A Table 4). Direct comparisons were made when both results were above LOQ and results were considered in agreement if within $\pm 20\%$ of Total Diet Study result. No cobalt, lead, selenium or vanadium comparisons were available. Two of 3 aluminum results are in agreement and the high result for peanut butter discussed above is probably due to environmental contamination. The 2 arsenic comparisons and 1 cadmium comparison were in agreement. Fourteen of the 15 calcium comparisons agreed. The slightly low and imprecise calcium result for haddock is probably due to non-homogeneity and the relatively low concentration (LOQ 90 mg/kg). All 4 copper comparisons agreed. Eleven of 13 iron comparisons agreed. The iron results for prune juice and broccoli were about 25% lower than the Total Diet Study result. Both Total Diet Study results are near the LOQ (2 and 3 mg/kg, respectively), which may account for the discrepancy. Iron fortification recoveries for these foods were good. Seventeen of 18 magnesium comparisons agreed. The magnesium result for prune juice was about 25% lower than the Total Diet Study result. No reasonable explanation for this discrepancy was found. All 8 manganese comparisons agreed. Only 3 of 6 nickel comparisons agreed. Nickel results for peanut butter, pancakes, and fruit flavored cereal were 25% to 50% higher than Total Diet Study results. No reasonable explanation for this discrepancy was found. Nickel fortification recoveries for these foods were good. Eighteen of 19 phosphorus comparisons agreed. The phosphorus result for peanut butter was about 25% higher than the Total Diet Study result. No reasonable explanation for this discrepancy was found. Twelve of 20 potassium comparisons agreed. Potassium results for prune juice, broccoli, sweet potato, spaghetti, beer, beef and haddock were 21% to 27% lower than Total Diet Study results. No reasonable explanation for this discrepancy was found. Potassium result for mayonnaise was about 85% higher than the Total Diet Study result. The potassium level in mayonnaise was relatively low compared to other foods and measurement precision was at the high end of the acceptable range indicating that the LOQ for mayonnaise (20 mg/kg) may be under estimated. In addition, the Total Diet Study potassium result was near the estimated LOQ (40 mg/kg). All 19 sodium

comparisons agreed. Fifteen of 16 zinc comparisons agreed. The zinc result for prune juice was about 30% lower than the Total Diet Study result. The Total Diet Study zinc result was near the LOQ (1 mg/kg), which may explain the disagreement.

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (1 of 7)

Element	Evaporated milk (8)					Cheddar cheese (12)					Pork bacon (20)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	<0.2	-	2.6	88	-	(0.6)	28	7.3	95	-	(1.3) ^e	5	11	92	-
Arsenic	<0.05	-	5.3	97	<0.01	<0.2	-	15	93	<0.02	<0.2	-	23	87	<0.02
Barium	<0.3	-	1.1	103	-	(1.0)	18	2.9	93	-	< 1	-	4.5	101	-
Boron	0.71	8	5.3	90	-	(0.47)	20	15	96	-	(0.69)	42	23	95	-
Cadmium	<0.009	-	0.53	98	<0.002	<0.03	-	1.5	94	<0.003	<0.04	-	2.3	91	(0.004)
Calcium	1770	5	-	-	2040	6220	5	-	-	7480	(120)	13	-	-	84
Chromium	<0.2	-	2.6	91	-	<0.3	-	7.3	97	-	<0.5 ^e	-	11	92 ^e	-
Cobalt	<0.009	-	1.1	88	-	<0.03	-	2.9	90	-	<0.04	-	4.5	88	-
Copper	<0.02	-	2.6	89	<0.25	0.35	16	7.3	92	(0.401)	0.96	1	11	93	(0.886)
Iron	0.45	6	26	85	(0.713)	1.51	19	73	87	(2.53)	8.03	0.3	113	86	8.74
Lead	<0.07	-	5.3	95	<0.007	<0.2	-	15	85	<0.014	<0.3 ^e	-	23	74	(0.014)
Magnesium	157	8	-	-	194	238	7	-	-	283	(182)	10	-	-	181
Manganese	0.024	12	5.3	86	< 0.3	0.182	8	15	89	< 0.4	0.122	6	23	87	<0.4
Molybdenum	<0.02	-	1.1	92	-	(0.13)	23	2.9	92	-	<0.07	-	4.5	92	-
Nickel	<0.04	-	2.6	90	<0.025	<0.09	-	7.3	91	<0.05	<0.2	-	11	87	<0.05
Phosphorus	1900	6	-	-	1700	4890	3	-	-	4840	3420	3	-	-	3390
Potassium	2440	5	-	-	2860	733	9	-	-	736	3260	6	-	-	2940
Selenium	<0.2	-	11	114	0.040	<0.5	-	29	113	0.217	<0.8	-	45	102	0.295
Sodium	702	10	-	-	786	5340	7	-	-	6240	17400	6	-	-	17500
Strontium	(1.6)	20	2.6	95	-	6.2	11	7.3	98	-	< 3	-	11	100	-
Thallium	<0.07	-	11	87	-	<0.2	-	29	88	-	<0.3	-	45	85	-
Vanadium	<0.01	-	0.53	85	-	<0.03	-	1.5	93	-	<0.04	-	2.3	92	-
Zinc	6.34	1	11	91	6.90	32.3	9	29	86	36.0	19.8	6	45	83	22.3

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (2 of 7)

Element	Tuna (canned in oil) (32)					Eggs (boiled) (37)					Peanut butter (47)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	(0.9)	2	5.6	91	-	(0.38)	23	3	95	-	12	8	11	44	6.61 ^g
Arsenic	0.86	8	11	101	0.966	<0.07	-	6	98	<0.01	<0.2	-	21	102	(0.021)
Barium	<0.6	-	2.2	100	-	(0.64)	4	1.2	100	-	3.6	2	4.2	95	-
Boron	(0.6)	16	11	90	-	(0.2)	51	6	95	-	16.2	0.1	21	93	-
Cadmium	(0.028)	20	1.1	105	0.022	<0.02	-	0.6	105	<0.002	(0.099)	4	2.1	105	0.067
Calcium	(100)	15	-	-	78	523	2	-	-	506	592	4	-	-	499
Chromium	<0.3	-	5.6	118 ^f	-	(0.25)	29	3	102	-	<0.5	-	11	97	-
Cobalt	<0.02	-	2.2	97	-	<0.02	-	1.2	97	-	<0.04	-	4.2	81	0.031 ^g
Copper	0.45	1	5.6	90	(0.403)	0.58	1	3	95	(0.55)	4.66	1	11	92	4.48
Iron	7.93	3	56	92	8.38	16.6	1	30	94	16.9	19.9	1	106	95	19.5
Lead	<0.2 ^e	-	11	86	(0.011)	<0.1	-	6	95	<0.007	<0.3	-	21	106	<0.014
Magnesium	242	3	-	-	251	114	2	-	-	109	1650	1	-	-	1580
Manganese	0.265	4	11	94	<0.3	0.272	1	6	95	<0.3	13.9	1	21	94	13
Molybdenum	< 0.05	-	2.2	99	-	0.084	6	6	95	-	1.3	2	4.2	98	-
Nickel	(0.08)	23	5.6	96	(0.041)	<0.05	-	3	98	<0.025	1.24	7	11	98	0.931
Phosphorus	1610	4	-	-	1490	2080	2	-	-	1870	3980	5	-	-	3230
Potassium	1930	2	-	-	2070	1180	2	-	-	1170	5490	2	-	-	5500
Selenium	(0.88)	2	22	114	0.613	(0.48)	9	12	110	0.375	<0.8	-	42	116	0.118
Sodium	3200	3	-	-	3530	1200	2	-	-	1180	4260	3	-	-	4160
Strontium	<2	-	5.6	111	-	(0.86)	6	3	103	-	(5.5)	3	11	101	-
Thallium	<0.2	-	22	94	-	<0.1	-	12	95	-	<0.3	-	42	96	-
Vanadium	<0.03	-	1.1	97	-	<0.02	-	0.6	100	-	<0.04	-	2.1	98	<0.12 ^g
Zinc	4.45	9	22	94	4.4	12.2	2	12	91	12	26	1	42	87	25.2

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (3 of 7)

Element	Corn (54)					White bread (58)					Pancakes (68)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	(0.21)	6	1.8	91	<0.052 ^g	2.3	2	4.2	101	2.32 ^g	61.3	4	3.7	62	-
Arsenic	<0.04	-	3.7	95	<0.01	<0.1	-	8.5	100	<0.02	<0.09	-	7.3	100	<0.01
Barium	<0.2	-	0.73	101	-	(1.2)	2	1.7	103	-	(0.65)	5	1.5	99	-
Boron	0.61	3	3.7	93	-	(0.47)	12	8.5	99	-	1.4	4	7.3	98	-
Cadmium	(0.009)	24	0.37	102	(0.004)	(0.041)	6	0.85	103	0.021	<0.02	-	0.73	102	0.011
Calcium	(32)	8	-	-	23	577	2	-	-	578	1380	2	-	-	1330
Chromium	<0.1	-	1.8	93	-	<0.3	-	4.2	107	-	<0.2	-	3.7	103	-
Cobalt	<0.007	-	0.73	67	0.0011 ^g	<0.02	-	1.7	110	0.0073 ^g	<0.02	-	1.5	92	-
Copper	0.28	0.4	1.8	91	<0.25	1.07	3	4.2	96	(1.07)	0.9	3	3.7	95	(0.746)
Iron	2.4	6	18	90	(2.93)	29.5	2	42	91	31.5	14.2	2	37	90	14.1
Lead	<0.06 ^e	-	3.7	90	<0.007	<0.2	-	8.5	93	<0.01	<0.2	-	7.3	85	(0.012)
Magnesium	189	2	-	-	183	212	2	-	-	206	316	2	-	-	292
Manganese	0.884	2	3.7	92	(0.855)	4.36	2	8.5	94	4.46	3.06	1	7.3	90	2.8
Molybdenum	(0.031)	2	0.73	95	-	0.16	3	1.7	98	-	0.3	3	1.5	94	-
Nickel	(0.044)	33	1.8	93	<0.025	(0.17)	7	4.2	95	0.124	0.29	5	3.7	91	0.191
Phosphorus	683	2	-	-	613	1170	1	-	-	1040	4140	2	-	-	3540
Potassium	1600	1	-	-	1730	1150	1	-	-	1160	2340	1	-	-	2150
Selenium	<0.2	-	7.3	110	<0.01	<0.4	-	17	115	0.146	<0.4	-	15	116	0.123
Sodium	<20	-	-	-	<7	4900	1	-	-	5180	4620	1	-	-	4470
Strontium	<0.5	-	1.8	110	-	(2.5)	8	4.2	104	-	(1.3)	6	3.7	102	-
Thallium	<0.06	-	7.3	91	-	<0.2	-	17	94	-	<0.2	-	15	90	-
Vanadium	<0.008	-	0.37	96	<0.0047 ^g	<0.02	-	0.85	101	<0.12 ^g	(0.021)	28	0.73	96	-
Zinc	3.5	1	7.3	87	3.92	5.56	2	17	93	5.83	6.99	4	15	86	6.79

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (4 of 7)

Element	Fruit flavored cereal (72)					Prune juice (103)					Lemonade (105)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	2	5	7.2	102	-	0.44	10	1.3	97	-	(0.09)	11	0.76	100	-
Arsenic	<0.2	-	14	101	<0.01	<0.03	-	2.6	103	<0.01	<0.02	-	1.5	100	<0.01
Barium	<0.7	-	2.9	101	-	(0.28)	12	0.52	99	-	<0.08	-	0.3	110	-
Boron	<0.3	-	14	97	-	4.8	8	2.6	123	-	0.32	8	1.5	97	-
Cadmium	(0.057)	7	1.4	107	0.018	<0.005	-	0.26	109	<0.001	<0.003	-	0.15	105	<0.001
Calcium	703	4	-	-	645	83	11	-	-	98	32	1	-	-	28.8
Chromium	(0.38)	68	7.2	107	-	(0.17)	123	1.3	93	-	<0.04	-	0.76	109	-
Cobalt	(0.068)	9	2.9	99	-	<0.005	-	0.52	100	-	<0.003	-	0.3	100	-
Copper	1.01	5	7.2	98	0.96	0.17	8	1.3	96	(0.213)	0.06	1	0.76	97	<0.2
Iron	214	5	72	81	201	2.31	9	13	98	3.2	0.27	2	7.6	96	<0.5
Lead	<0.2	-	14	102	(0.01)	<0.04	-	2.6	96	<0.005	<0.03	-	1.5	97	<0.004
Magnesium	273	5	-	-	249	94	11	-	-	126	17	4	-	-	17.2
Manganese	6.91	3	14	95	6.56	0.66	9	2.6	100	(0.862)	0.041	1	1.5	97	<0.2
Molybdenum	0.23	3	2.9	100	-	(0.01)	24	0.52	99	-	<0.006	-	0.3	101	-
Nickel	0.55	4	7.2	98	0.441	0.1	16	1.3	101	0.113	<0.01	-	0.76	100	<0.014
Phosphorus	1370	3	-	-	1160	188	13	-	-	192	26	3	-	-	(22.5)
Potassium	1160	3	-	-	1090	1760	9	-	-	2350	229	4	-	-	262
Selenium	<0.6	-	29	110	0.055	<0.1	-	5.2	115	<0.01	<0.06	-	3	103	<0.01
Sodium	5140	3	-	-	5300	(16)	11	-	-	(16.9)	(14)	0.1	-	-	(12.9)
Strontium	<2	-	7.2	119	-	(0.7)	11	1.3	104	-	(0.25)	3	0.76	104	-
Thallium	<0.2	-	29	98	-	<0.04	-	5.2	97	-	<0.02	-	3	98	-
Vanadium	0.093	6	1.4	101	-	(0.008)	84	0.26	99	-	<0.003	-	0.15	104	-
Zinc	159	5	29	69	157	0.94	9	5.2	99	1.3	0.11	13	3	93	<0.2

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (5 of 7)

Element	Broccoli (113)					Sweet potato (140)					Spaghetti and meatballs (142)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	1.3	4	0.6	73	-	(0.4)	10	2.4	100	-	6.46	2	2	88	-
Arsenic	<0.02	-	1.2	90	<0.01	<0.06	-	4.8	94	(0.011)	<0.04	-	3.9	95	<0.01
Barium	0.34	4	0.24	83	-	2.2	3	0.97	97	-	(0.44)	2	0.78	96	-
Boron	1.7	3	1.2	91	-	1.5	2	4.8	93	-	0.84	4	3.9	97	-
Cadmium	0.013	10	0.12	88	0.014	(0.013)	39	0.48	100	0.01	0.024	19	0.39	97	0.019
Calcium	280	4	-	-	331	264	1	-	-	275	307	5	-	-	332
Chromium	<0.03	-	0.6	91	-	< 0.2	-	2.4	106	-	<0.09	-	2	102	-
Cobalt	0.017	2	0.24	83	-	(0.025)	10	0.97	94	-	<0.007	-	0.78	91	-
Copper	0.28	1	0.6	86	(0.295)	1.26	2	2.4	95	1.27	1.01	0.3	2	90	0.961
Iron	3.67	4	6	81	4.69	3.55	3	24	91	3.93	10.6	0.5	20	86	11.2
Lead	<0.02	-	1.2	79	<0.007	<0.08	-	4.8	91	<0.007	<0.06	-	3.9	88	<0.007
Magnesium	92	2	-	-	107	179	2	-	-	199	146	5	-	-	168
Manganese	1.34	5	1.2	86	1.6	8.4	2	4.8	92	8.85	1.65	5	3.9	87	1.8
Molybdenum	0.023	1	0.24	85	-	(0.046)	8	0.97	96	-	0.098	7	0.78	93	-
Nickel	0.17	2	0.6	84	0.167	0.19	6	2.4	94	0.127	0.11	9	2	91	(0.078)
Phosphorus	439	1	-	-	441	599	1	-	-	558	779	4	-	-	724
Potassium	858	3	-	-	1170	3470	4	-	-	4450	1600	4	-	-	2090
Selenium	<0.05	-	2.4	117	<0.01	<0.2	-	9.7	109	<0.01	< 0.2	-	7.8	122	0.094
Sodium	124	2	-	-	116	235	0.1	-	-	237	2020	3	-	-	2190
Strontium	2.4	1	0.6	85	-	2.8	4	2.4	100	-	(1.6)	2	2	98	-
Thallium	<0.02	-	2.4	81	-	<0.08	-	9.7	92	-	<0.06	-	7.8	87	-
Vanadium	(0.004)	34	0.12	89	-	<0.01	-	0.48	99	-	<0.008	-	0.39	97	-
Zinc	1.52	5	2.4	80	1.79	2.52	3	9.7	90	2.73	8.38	4	7.8	84	9.01

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (6 of 7)

Element	Mayonnaise (166)					Beer (198)					Beef (baby food) (205)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	(1.3)	8	12	96	-	(0.17)	2	0.76	84	-	(0.41) ^e	4	2.4	93	-
Arsenic	<0.3	-	24	100	<0.02	<0.02	-	1.5	94	<0.01	<0.05	-	4.8	93	<0.01
Barium	< 2	-	4.8	106	-	<0.09	-	0.31	103	-	<0.3	-	0.96	101	-
Boron	(1.1)	9	24	96	-	0.33	4	1.5	93	-	(0.28)	33	4.8	93	-
Cadmium	<0.05	-	2.4	107	<0.007	<0.003	-	0.15	101	<0.001	<0.009	-	0.48	104	<0.002
Calcium	<200	-	-	-	83.7	45	2	-	-	52.8	(52)	9	-	-	40.6
Chromium	<0.7	-	12	110	-	<0.04	-	0.76	108	-	<0.2	-	2.4	106	-
Cobalt	<0.05	-	4.8	100	-	<0.003	-	0.31	92	-	<0.009	-	0.96	96	-
Copper	(0.1)	19	12	97	<0.34	(0.018)	5	0.76	88	<0.17	0.42	2	2.4	92	(0.428)
Iron	2.26	17	121	96	(2.61)	(0.06)	13	7.6	88	<0.5	14.4	1	24	91	15
Lead	<0.4	-	24	97	<0.035	<0.03	-	1.5	87	<0.004	<0.08	-	4.8	94	<0.007
Magnesium	<70	-	-	-	(13.3)	50	1	-	-	62.9	124	1	-	-	141
Manganese	0.072	4	24	98	< 0.4	0.088	1	1.5	90	<0.2	0.052	2	4.8	93	<0.3
Molybdenum	<0.1	-	4.8	103	-	0.024	7	0.31	93	-	<0.02	-	0.96	97	-
Nickel	<0.2	-	12	100	-	<0.02	-	0.76	94	<0.014	<0.04	-	2.4	97	(0.06)
Phosphorus	261	2	-	-	276	159	1	-	-	159	1230	0.05	-	-	1170
Potassium	183	17	-	-	98.3	205	1	-	-	281	1670	1	-	-	2200
Selenium	<1	-	48	115	(0.029)	<0.07	-	3.1	115	<0.01	<0.2	-	9.6	115	(0.03)
Sodium	5190	2	-	-	4930	35	1	-	-	41.6	393	1	-	-	427
Strontium	<4	-	12	110	-	(0.2)	3	0.76	95	-	<0.7	-	2.4	104	-
Thallium	<0.4	-	48	98	-	<0.03	-	3.1	90	-	<0.07	-	9.6	94	-
Vanadium	<0.06	-	2.4	103	-	0.03	2	0.15	95	-	<0.01	-	0.48	98	-
Zinc	1.49	1	48	93	(1.98)	<0.01	-	3.1	84	<0.2	32.4	2	9.6	83	32.2

4.4A Table 4. Food Fortification Results Using Ultrasonic Nebulization^a (7 of 7)

Element	Haddock (243)					Pears (canned) (255)				
	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)	Unfort. Result ^b (mg/kg)	RSD (%)	Fort. Level (mg/kg)	Fort. Rec ^c (%)	TDS Result ^d (mg/kg)
Aluminum	0.85 ^e	7	3.3	87	-	(0.19)	5	1.1	97	-
Arsenic	5.91	2	6.7	95	6.59	<0.03	-	2.2	94	<0.01
Barium	<0.4	-	1.3	102	-	0.34	4	0.44	101	-
Boron	0.79	8	6.7	96	-	1.9	2	2.2	91	-
Cadmium	<0.02	-	0.67	101	<0.002	<0.004	-	0.22	103	<0.002
Calcium	204	30	-	-	269	46	3	-	-	45.2
Chromium	<0.2	-	3.3	105	-	(0.06)	59	1.1	105	-
Cobalt	<0.02	-	1.3	95	-	<0.004	-	0.44	98	-
Copper	0.25	9	3.3	94	<0.29	0.38	2	1.1	96	(0.413)
Iron	1.54	2	33	91	(1.64)	6.52	2	11	92	6.39
Lead	<0.1	-	6.7	90	<0.01	(0.041)	12	2.2	92	0.032
Magnesium	345	6	-	-	400	38	2	-	-	40
Manganese	0.34	10	6.7	92	(0.41)	0.979	2	2.2	94	1.03
Molybdenum	<0.03	-	1.3	97	-	<0.008	-	0.44	98	-
Nickel	(0.06)	28	3.3	93	<0.036	(0.044)	11	1.1	96	(0.042)
Phosphorus	2500	2	-	-	2300	67	3	-	-	68.4
Potassium	3060	6	-	-	3860	533	1	-	-	650
Selenium	(0.82)	20	13	121	0.503	<0.09	-	4.4	107	<0.01
Sodium	1440	1	-	-	1460	45	2	-	-	41.3
Strontium	(1.7)	40	3.3	102	-	(0.36)	9	1.1	103	-
Thallium	<0.09	-	13	93	-	<0.04	-	4.4	96	-
Vanadium	(0.034)	62	0.67	97	-	<0.005	-	0.22	101	-
Zinc	4.24	2	13	86	4.58	0.4	3	4.4	92	(0.447)

^aFoods from FDA Total Diet Study market basket 1994-4 with food number in () next to food name.

^bMean result based on 3 analyses (6 analyses for calcium, magnesium, phosphorus, potassium, and sodium) except 2 analyses (5 analyses for calcium, magnesium, phosphorus, potassium, and sodium) for evaporated milk or as indicated. Mean results in () are "trace" levels and mean results below the LOD are listed as <LOD.

^cMean result based on 3 analyses except 4 analyses for haddock or as indicated. Calcium, magnesium, phosphorus, potassium and sodium not fortified.

^dFDA Total Diet Study market basket 1994-4 results⁶ (n=1) except nickel results from FDA Kansas City District laboratory validation report¹⁰ or other source as indicated. Results in () are "trace" levels and results below LOD are listed as <LOD.

^eMean result based on 2 analyses.

^fResult of 1 analysis.

^gResult by neutron activation analysis¹¹.

4.4A.4 CONCLUSION

The in-house validation results for analysis of foods using microwave decomposition and element detection using ICP-AES indicate the following elements can be reliably measured at concentrations above LOQ: arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, sodium, strontium, vanadium and zinc. Thallium appears to be reliably measured but accuracy assessment is hindered by the lack of appropriate reference materials. The following elements appear prone to laboratory environmental contamination and therefore require extensive

assessment of contamination control: aluminum, chromium, and lead. Aluminum concentrations using the method do not account for aluminum bound to silicates. Selenium concentrations may have been biased high in this study. Use of ICP-AES for determination of selenium in foods requires a thorough assessment of that background correction was effective.

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