U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

Elemental Analysis Manual

for Food and Related Products

4.13 INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRIC DETERMINATION OF IODINE IN FOOD USING TETRAMETHYL AMMONIUM HYDROXIDE EXTRACTION

Current Validation Status
AOAC/ASTM: No
SINGLE LAB VALIDATION: YES
MULTI-LAB VALIDATION: YES – Level III

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GLOSSARY

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4.13.1 SCOPE AND APPLICATION

This method describes a procedure for the determination of iodine in foods and dietary supplements by alkaline extraction followed by inductively coupled plasma-mass spectrometric detection (ICP-MS).

This method should only be used by analysts familiar with the determination of trace elements and experienced in the use of ICP-MS. The method has been validated for food, beverage and some dietary supplement matrices.

4.13.2 SUMMARY OF METHOD

An analytical portion (0.5 to 5.0 g dependent on food composition) is mixed with tetramethyl-ammonium hydroxide (TMAH) and a hot block extraction system at 85 °C is used to extract the available iodine. The supernatant contains extractable iodine in 1% TMAH at pH>9. The analytical solution is analyzed using an ICP-MS. The iodine mass fraction is quantified using an external calibration and quality controls are incorporated to ensure data quality.

Typical analytical limits were calculated per EAM §3.2 and are listed in 4.13 Table 1 but will vary depending on the specific instrumentation, analytical portion mass, blank contamination, sensitivity and operating conditions.

4.13 Table 1. Typical Analytical Limits

Element	Symbol	MBK_L	MBK_C	ASDL	ASQL	LOD	LOQ
Iodine	^{127}I	0.085	0.110	0.043	0.381	4.1	36.5

Based on 0.5 g analytical portion and 50 g analytical solution, 24 method blanks from 8 extractions analyzed over 2 months (see EAM §3.2). All analytical limits are in µg/kg.

4.13.3 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-mass spectrometer (ICP-MS)—Capable of scanning the mass-to-charge (m/z) range 5 – 240 amu with a minimum resolution of 0.9 amu at 10% peak height, mass flow controller for nebulizer gas.

(2) Nebulizer and spray chamber—Concentric quartz (Meinhard part number #ME2040-54), Glass Expansion concentric (Glass expansion part number # ARG-1-QSS1) or Glass Expansion MicroMist (part number #AR35-1-FM02E) and 20 mL cyclonic spray chamber (Meinhard part number # ML151018ES, or Glass expansion part number # 20-

809-0261HE)

(3) Hot block extraction system—Requires temperature control to at least 85 ± 3 °C (as measured on the hot block center point). Recommended equipment is SCP science DigiPrep MS (part number #010-500-205).

(4) Laboratory centrifuge – suitable for 50 mL centrifuge tubes and capable of 3000 rpm.

(5) Labware—All reusable laboratory ware must be sufficiently clean for trace metals analysis. The recommended cleaning procedure for all laboratory ware includes washing in special clean-rinsing laboratory detergent such as Micro-90, reagent water rinse, soaking in 10% nitric acid and final reagent water rinse immediately before use. Glass should not be used for dilution or storage of sample or standard solutions because of possible contamination.

(6) Plastic labware—This includes disposable plastic laboratory ware such as autosampler tubes and capped centrifuge tubes. Plastic bottles for solution storage should be tested for contamination before using a particular lot with 1% TMAH rinse immediately before use. Items can also be cleaned, dried and stored in a dust-free environment for later use. FEP, PFA, PP, LDPE or HDPE are recommended materials for bottles and tubes. FEP, FEP coated or polypropylene spatulas should be used for sampling food portions. Becton Dickinson polypropylene Falcon centrifuge tubes (blue cap) and Fisher 8 mL polypropylene culture tubes (Fisher part number # 14-956-7a) can be used for sample preparation and analysis.

(7) Gloves—Use powder-free vinyl or nitrile. Do not use powdered gloves or latex because of possible contamination. Gloves manufactured for clean room use that are free from trace metals contamination are suggested. It is good practice to put on gloves and then rinse with reagent water to remove residual plasticizers or releasing agents before handling clean laboratory ware or samples.

(8) Analytical balance—Capable of measuring to 0.1 mg.

(9) Top Loading balance—Capable of measuring from 0.01 g to 2500 g.

(10) Micropipettes—Air displacement micropipettes with metal-free colorless disposable plastic tips. Do not use colored tips due to possible contamination. If applicable, remove metal tip ejector to avoid potential contamination.

(11) Clean air hood/canopy—Class 100 polypropylene metal-free hoods/canopies are recommended for sample handling.

(12) Peristaltic pump tubing—The recommended sample and internal standard peristaltic pump tubing are orange:green (0.38 mm inner diameter). At 0.2 rev/s approximately 100 µL/min sample and 100 µL/min internal standard solutions are delivered to the nebulizer. For higher flow nebulizers, Glass Expansion concentric, black:black (0.76 mm inner diameter) at 0.1 rev/s, delivers approximately 350 µL/min sample and 350 µL/min internal standard.

(13) Drain tubing—The recommended drain tubing is yellow:blue (1.52 mm i.d.) or larger Fluran tubing which drains >1000 µL/min from the spray chamber. Using less than 1.52 mm i.d. drain tubing will result in spray chamber flooding and instrument damage.

(14) Optional plastic syringes – general use and nonsterile, 5 or 10 mL, Luer-Loc tip.

(15) Optional PTFE syringe filter (Pall Gelman Acrodisk PTFE 1um, part number Z259926-1PAK, or Environmental express Filtermate PTFE plunger filter).

4.13.4 REAGENTS AND STANDARDS

Reagents might be significantly contaminated with iodine. Use high purity or trace metals grade reagents at all times. Non-quantifiable (<ASQL) blank levels can be obtained by utilizing best laboratory practices and high-purity reagents. Blank levels <ASQL are the objective for all labs and analysts.

Safety Notes: Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents.

Exercise caution when handling and dispensing concentrated strong bases. Bases are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

Reagents

(1) Reagent water—Water processed to meet specifications for ASTM Type-I water[1]. Method validation was done using 18.2 MΩ-cm deionized water (example is Millipore Milli-Q Element system).

(2) Argon supply for instrument—High purity (99.99%) liquid argon. Argon compressed gas tanks can also be used but are more expensive than liquid argon.

(3) High purity tetramethyl-ammonium hydroxide —25% (m/m), electronics grade (99.9999% purity). For the method validation Alfa Aesar part number # 20932 was used.

(4) High purity isopropanol—Electronic grade or equivalent. For the validation Fisher LC-MS Optima grade isopropanol was used.

(5) Triton X-100 (ACS grade)

Solutions

(1) TMAH 5% (m/m)—Dilute 100 g electronic grade 25% TMAH to 500 g with reagent water.

Recommendation: Use an empty Teflon bottle originally used for concentrated nitric acid. To minimize contamination, dilute gravimetrically on a top-loading balance with a capacity of a least 1000 g. Tare bottle. Fill with approximately 300 mL reagent water. Note mass. Add 100 g TMAH while pouring slowly from the stock bottle. Add reagent water until a total solution mass of 500 g is reached (400

g water + 100 g TMAH). Place bottle cap on and mix.

(2) TMAH 1% (m/m)—Dilute 80 g high purity TMAH to 2,000 g with reagent water.

Recommendation: Use an empty Teflon bottle originally used for concentrated nitric acid. To minimize contamination, dilute gravimetrically on a top loading balance with a capacity of a least 2500 g. Tare bottle. Fill with approximately 1000 mL reagent water. Note mass. Add 80 g TMAH while pouring slowly from the stock bottle. Add reagent water until a total solution mass of 2000 g is reached (1920 g water + 80 g TMAH). Place bottle cap on and mix.

(3) Internal standard solution (ISTD)—Multi-element solution prepared by diluting an appropriate mass of stock standard. ISTD matrix is 1% TMAH, 6% isopropanol, 0.01% triton X-100. The presence of isopropanol will help equalize iodine sensitivity due to residual carbon remaining in solution after the extraction. The dilution factor of the internal standard solution is 1:1 if the autosampler and internal standard peristaltic pump tubes have equal inner diameters. The analytical solution pumped into the nebulizer will be approximately 3% isopropanol.

a. The exact mass fraction is not as important as maintaining the same mass fraction over an analytical run. Since the element mass fraction is only approximate, the solution may be prepared volumetrically.

b. ISTD elements and suggested mass fractions: 2±0.5 μg/kg Rh, 20±2 μg/kg Te.

c. ISTD solution must be prepared daily because of instability of Rh in alkaline solutions longer than 48 h.

(4) Suggested Tuning Solution — 1 μg/kg iodine solution in 1% TMAH is used to tune and optimize instrument. Typical sensitivity for 1 μg/kg I should be around 50,000 cps/ppb.

The method specifies sample tubing and internal standard tubing to be of equal diameter, diluting tune solution by half.

Calibration Standard Solutions

(1) Analyte stock standard solutions — commercially prepared single element NIST traceable standard solutions prepared specifically for plasma mass spectrometric or ion chromatography analysis should be used. Due to the low mass fractions of solutions required for ICP-MS standardization, starting from 10 mg/kg stock solutions is recommended to minimize the number of dilutions and intermediate solutions.

a. Standards can be purchased on a mass/mass basis to eliminate density correction factors. If standards are mass/volume, a density correction will be necessary (refer to EAM §3.4.4 for gravimetric standard solution preparation).

b. Stock standard solutions must be used prior to manufacturer's expiration date (typically 12 – 18 months from time of purchase). Be aware that stock solutions may slowly become more concentrated over time due to transpiration of water vapor through the bottle material and loss while the bottle is uncapped.

(2) Standard solutions—Dilute stock standard with 1% TMAH to prepare iodine standards.

Depending on the mass fraction of the stock standard the use of serial dilutions is recommended in preparing the calibration standards. Store in Teflon® FEP, PP or HDPE bottles. A minimum of 4 calibration standard levels should be used for calibration (4.13 Table 2).

4.13 Table 2. Example of calibration curve standard mass fractions

Analyte	Level 0 (µg/kg)	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 3 (µg/kg)	Level 4 (µg/kg)	Level 5 (µg/kg)	Level 6 (µg/kg)	Level 7 (µg/kg)
I	0	0.010	0.050	0.200	1.0	5.0	20.0	50.0

(3) Standard blank— 1% TMAH.

(4) Initial (independent) calibration verification (ICV) — Dilute an appropriate volume of stock iodine solution obtained from a different (second) source gravimetrically with 1% TMAH so that the analyte mass fraction will be at the approximate midpoint of the calibration curve.

(5) Continuing calibration verification (CCV) — Use a mid-level standard.

4.13.5 EXTRACTION PROCEDURE

Terms and definitions:

(1) An “extraction batch” is defined as digests from the vessels in a single tray in the same extraction program at the same time. The SCP Science DigiPrep extraction batch will have up to 48 vessels.

(2) An “analytical run” is defined as the total number of analytical solutions analyzed during a single sequence following tuning and optimization and with one calibration. An analytical run may contain analytical solutions from more than one extraction batch.

The following operations should be performed in a clean environment to reduce contamination. An exhausting hood must be used when working with TMAH.

Typically, only the edible portions of foods are analyzed. However, if an assignment requires mass fractions as a function of dry mass, dry a minimum of 10 g of the homogenized, ground samples in a laboratory oven at 85 °C until a constant mass is obtained. Standard reference materials (SRM) should be dried according to the manufacturer’s recommendations. Calculate the moisture content of the original sample. Store dried samples in a desiccator.

Food preparation and homogenization procedures are found in EAM §2.1 through EAM §2.2.2.

Extraction Procedure using DigiPrep hot block

(1) Weigh each 50 mL centrifuge tube and record mass with cap.

(2) In each extraction batch, a minimum of two method blanks must be included to check contamination from the vessels. The method blanks should be placed in random vessels.

(3) Place 0.5 g analytical portion into a clean centrifuge tube and record mass of tube and sample.

a. Less than 0.5 g should be used for samples high in salt content.

b. For most beverage and liquid samples, use an analytical portion mass of 5 g. Exceptions include thick liquids such as maple or corn syrups. Use a smaller mass (2-3 g) for thick syrups.

c. Use 0.5 g reagent water for method blanks (MBK) and optional fortified method blanks (FMB).

d. For dry samples and dry SRM materials adding 1 g of reagent water can help control reactions during the extraction.

(4) Add 10 mL of 5% TMAH into centrifuge tube, washing down any material on walls. Using a bottle top dispenser or tilting dispenser is suggested.

(5) Vortex each centrifuge tube containing sample and TMAH until mixed (approximately 1 min).

(6) Place capped samples on hot block. The hot block extraction of iodine temperature program contains a 150 min hold at 85 °C.

(7) After vessels have cooled to less than 30 °C (approximately 2 h if the samples are left to cool on the hot block) remove tubes and vortex until mixed (approximately 1 minute).

(8) Add reagent water to 50 mL mark, vortex until mixed (approximately 1 minute), weigh samples and record final mass.

(9) Centrifuge samples at 3000 rpm for 3 minutes. Collect supernatant and analyze by ICP-MS using the procedure listed below.

(10) (optional step) If particulates are still visible in the supernatant after centrifuging, filtering can prevent clogging of the nebulizer or sample probe. Uptake 5 mL of supernatant using a disposable polypropylene syringe and filter into a clean sample tube for analysis. If filtering, treat quality controls (blanks, RMs, etc) in the same way.

4.13.6 DETERMINATION PROCEDURE

The method was developed using Agilent 7700x and Thermo Element 2 mass spectrometers and has been validated on Agilent 7900, Agilent 8800 and Thermo iCAPq mass spectrometers. As there are no significant interferences for iodine at m/z 127, the quadrupole instruments are used in “no cell gas” or vented mode and the Thermo Element 2 is used in low resolution mode. Internal standards are used to help compensate for matrix effects and general instrumental drift. References to sensitivity, cps and % RSD refer to the Agilent 7700x instrument.

Instrument Setup

(1) See EAM §3.6.1.4 for additional details on ICP-MS.

Use a separate sample introduction system (sample probe, peri-pump tubing, nebulizer, spray chamber, injector, torch and cones) for alkaline solutions (TMAH). The sample

introduction system needs to be rinsed with 1% TMAH or 1% optima grade NH_4OH for a minimum of 4 hours before the first use for iodine measurements in order to wash out iodine from all glass and plastic components. Once the ICP-MS is switched to alkaline sample introduction it is recommended that several batches are run before switching to acid solution mode. Frequent acid to base switching may require a prolonged iodine washout in order to obtain the above mentioned iodine count rates.

(2) Perform manufacturer recommended instrument start up procedure or laboratory-specific procedures.

May include the following checks: Ar supply pressure, backing pump oil condition, sufficient exhaust flow, and peristaltic pump tubing condition.

(3) Ignite plasma and perform initiation procedures as instructed in the owner's manual.

a. Fill a rinse bottle with 1% TMAH. Send autosampler probe to the rinse bottle while instrument is warming up. Place internal standard line in tube containing reagent water during warm up. Rinse and warm up the instrument for a minimum of 1 hour.

b. Program autosampler sequence table to run standards and samples of the batch.

(4) Set up method to include analytes and internal standard elements as shown in 4.13 Table 3.

4.13 Table 3. ICP-MS Instrument Parameters

Element	Monitored Isotope	Internal Standard	Recommended isotope for reporting	Minimum Integration Time (sec)	Analysis Mode
Iodine	^{127}I	^{103}Rh or ^{125}Te	^{127}I	0.3	No gas
Rhodium	^{103}Rh	—		0.3	No gas
Tellurium	^{125}Te	—		0.3	No gas

a. Use 3 points per peak and at least 3 replicates for integration. Use the mean of the integrations for reporting.

b. Be sure there is adequate rinse time programmed in between samples. Program the autosampler probe to go to the rinse station for at least 10 seconds after analyzing an analytical solution and then to the rinse bottle filled with 1% TMAH. The rinse time must be great enough so that a standard blank solution produces stable iodine baseline signal. A minimum of a three minute rinse is recommended.

c. An “intelligent rinse” or “smart rinse” feature may be used if so equipped. Analyte levels must return to within 10% RPD of the calibration blank cps levels before moving onto the next analytical solution.

(5) Optimize instrument

a. Configure the tune to monitor ^{103}Rh, ^{125}Te and ^{127}I. Introduce calibration blank solution. Pump speed during tuning and analyses should be set at 0.1 rev/s. Typical

sensitivity to be achieved in an Agilent 7700x system is 300,000 cps for ^{103}Rh, and 100,000 cps for ^{125}Te. The background for iodine in a blank solution should be at or below 6,000 cps.

Note: During tuning, the internal standard tubing is placed in the ISTD solution containing 1% TMAH, 0.01% triton-x100 and 6% IPA.

b. Tune for highest stability while maintaining optimal sensitivity for the m/z 125 and 103, and lowest cps at m/z 127.

c. Save updated and optimized tune file.

d. Check instrument performance using a 1 μg/kg I standard. Typical sensitivity to be achieved on Agilent 7700X at 0.1 rev/s, black/black tubing is 50,000 cps with 1-2% RSD.

e. Precision Check: Demonstrate instrument stability by analyzing a midrange iodine standard solution (*e.g.* CCV). The resulting relative standard deviation (RSD) of ion signals must be ≤10%. If RSD >10%, determine and correct problem before standardization. Stability problems are usually related to sample introduction.

f. Measure iodine signal stability using two 1% TMAH blank samples separated by a minimum of 30 minutes. If iodine cps deviation between the two blank samples is more than 5%, continue rinsing the system with 1% TMAH until the iodine cps deviation is below 5%.

Determination of Analyte Mass Fraction Using External Standard Calibration Curve

An example of a typical analytical sequence is shown in 4.13 Table 4

Calibrate using the standard blank and at least four iodine standards. A calibration blank is used as a point on the calibration curve (0 μg/kg calibrant). Additionally, a high standard check at or around 50 μg/kg iodine should be analyzed as a sample to ensure linearity to 50 μg/kg iodine. The high standard linearity check should be within 10% of the calculated mass fraction.

(1) Use linear regression with blank offset and either weighted or unweighted calibration.

(2) Check standardization performance

a. Linear regression correlation coefficient (r) (intensity - (analyte counts/sec):(internal standard counts/sec)) versus mass fraction) is ≥ 0.9975.

b. Analyze initial calibration verification (ICV) to verify standardization. Recovery must be 100 ± 10%.

c. Analyze a high standard check at or around 50 μg/kg iodine as a sample to ensure linearity to 50 μg/kg iodine. The high standard linearity check should be within 10 % of the calculated mass fraction.

(3) Check instrument measurement performance and analyze analytical solutions

a. Interpolate analyte mass fraction from standard curve. Start samples analysis

sequence and analyze the highest standard, standard blank and ICV in that order. This will verify proper autosampler rinse time and valid calibration curve.

b. Continuing calibration verification (CCV) must be analyzed at every 10 samples and at the end of the analytical run. Recovery must be 100 ± 10%.

c. RSD of the measurements of replicate integrations must be ≤ 10% for all solutions when instrument response >ASQL.

d. Continuing calibration blank (CCB) analyzed at a frequency of 10% and at the end of the analytical run. CCB solutions should be ≤ASQL.

e. Analytical solutions producing mass fractions which are greater than the high linearity check solution should be diluted with 1% TMAH and re-analyzed at a level falling between the lowest non-zero standard and the high standard.

(4) Suppression or enhancement of internal standard isotope response may indicate a matrix effect is present. Monitor internal standard signals and dilute any analytical solution where the internal standard signal differs by more than 40% from the standard blank. Use 1% TMAH for diluent. Rh is suggested to be used as a primary internal standard element.

(5) Elevated internal standard isotope response may indicate the presence of the internal standard element in the sample or an interference on the internal standard isotope. If the internal standard signal is greater than 140% of the standard blank, choose a different internal standard and reprocess the data.

(6) Analyze duplicate analytical portions every 10 samples. The duplicate analytical portions must have relative percent difference <20% when analyte mass fractions are >LOQ. If it fails, repeat analysis of the duplicate portion. If it fails again, re-digest and re-analyze. Analyze duplicate analytical portions for each sample type in a run (if all samples have similar compositions, only one duplicate portion must be analyzed for every 10 samples). It is highly recommended that duplicate analytical portions be analyzed for every food sample if feasible.

(7) At least one fortified analytical portion (FAP) should be included in each extraction batch and if more than 10 samples are extracted, an FAP should be included for every 10 samples. Fortification recoveries are described in EAM §3.4.1. The marginal method of calculating percent recovery is used for fortification recovery calculations[2].

a. FAP preparation: Spike 50-300% of the native elemental mass fraction,

FAP % marginal recovery: 80 - 120%. If it fails, re-analyze one time. If the FAP fails again, re-digest and re-analyze.

4.13 Table 4. Example of Typical Analytical Sequence

Grouping	Solution	QC Criteria
	tune report	RSD, sensitivity
	precision check (n > 10)	≤10% RSD
Calibration	calibration standards	r ≥ 0.9975
	standard blank	≤ASQL
	high standard solution	90% - 110% of calculated mass fraction
	standard blank	≤ASQL (memory check)
	ICV	90% - 110% recovery
	MBK 1	≤MBK_C
	MBK 2	≤MBK_C 2/3 of MBKs ≤MBK_C
	MBK 3	≤MBK_C
	SRM	80% - 120% recovery
Unknowns - Set 1	sample 1	≤10% instrument RSD, < high cal. std
	sample 1 duplicate	
	sample 1 FAP	80% - 120% recovery
	sample 2	
	sample 3	
	sample 4	
	sample 5	
	sample 6	
	sample 7	
	CCV	90% - 110%
	CCB	≤ ASQL
Unknowns - Set 2	sample 8	≤ 10% instrument RSD, < high cal. std
	sample 8 duplicate	
	sample 8 FAP	
	sample 9	
	sample 10	
	sample 11	
	sample 12	
	sample 13	
	sample 14	
	CCV	90% - 110%
	CCB	≤ ASQL
Precision required: All solutions must be ≤10% RSD when analyte ≥ASQL.		

4.13.7 CALCULATIONS

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

$$Mass\ fraction\ \left(\frac{\mu g}{kg}\right) = [(S \times DF) - MBK_L] \times \frac{M}{m \times MCF}$$

where

S = mass fraction of analyte in analytical solution (or diluted analytical solution) (μg/kg)

MBK_L = laboratory MBK (mg/kg) (subtract if MBK is greater than ASQL) (EAM §3.6.2)
DF = dilution factor (1 if analytical solution not diluted) (EAM §3.4.3)
MCF = mass correction factor (1 if no water or other solvent was added to aid homogenization) (EAM §3.4.6)
M = Mass (g) of analytical solution (usually 50 – 100 g)
m = mass of analytical portion (g)

Round calculated mass fraction to at most 3 significant figures. Mass fractions may be converted to other convenient units (*e.g.*, µg/kg, ng/g for solids or ng/L for liquids).

Calculate the marginal recovery (%) in the fortified analytical portion according to the formula

$$\%\ Recovery = \left[\frac{C_{x+s} - C_x}{\frac{C_s M_s}{M_x}}\right] \times 100$$

where

C_{x+s} = concentration determined in spiked sample (µg/kg)

C_x = concentration determined in unspiked sample (µg/kg)

C_s = concentration of spiking solution (µg/kg)

M_s = mass of spiking solution added to analytical portion (g)

M_x = mass of analytical portion (g)

4.13.8 QUALITY CONTROL

The following is the minimum number of quality control samples analyzed with each analytical run:

- 1 certified reference material (SRM/CRM)

  Match reference material matrix to the food matrix. In-house RMs are acceptable only if no traceable RM is available and the in-house RM is well characterized.
- 1 fortified analytical portion (FAP) per sample type per every 10 samples
- 2 method blanks (MBKs)
- 1 duplicate sample preparation for every 10 samples
- Replicate analytical portions should be analyzed for each sample whenever sample non-homogeneity may be an issue.

Reference Material

Control limits for True Value recovery of reference materials are 100 ± 20% or within mass fraction uncertainty (converted to percent relative uncertainty) supplied on certificate, whichever is greater. Traceable standard or certified reference materials (CRM/SRM) should be used when available, for example NIST 1549a (non-fat milk), NIST 1566a (oyster tissue).

FAP Recovery

Control limit for FAP marginal recovery is 80 – 120%.

Method Blanks (MBK)

Minimum of 2 MBKs analyzed in an analytical run and mass fraction of both MBKs must be $\leq MBK_C$. If 3 or more MBKs are analyzed then at least two-thirds of MBKs must be $\leq MBK_C$.

Relative Percent Difference (RPD) of Replicate Analytical Portions

Control limit for RPD is 20% for analyte levels >LOQ. (EAM §3.4.5)

4.13.9 REPORT

Report results only when all the quality control criteria for a batch have been satisfied. Report results that are ≥LOQ as the mass fraction determined followed by the units of measurement. Report results that are ≥LOD and <LOQ as the mass fraction determined followed by the units of measurement and the "Trace" data qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification. Trace values are documented by a "TR" after the result. 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 = 10 µg/kg; LOD =3 µg/kg. Levels found for three different samples were 11 µg/kg, 5 µg/kg and 2 µg/kg.

11 µg/kg is ≥LOQ; report 11 µg/kg

5 µg/kg is ≥LOD but also <LOQ; report 5 µg/kg (TR)

2 µg/kg is <LOD; report 0 µg/kg (ND)

If an analytical portion is analyzed in duplicate and one replicate mass fraction is >LOD but <LOQ and the other replicate is >LOQ, average the two results and report the measurements using the rules shown above.

4.13.10 METHOD VALIDATION

Single lab validation

EAM 4.13 was validated following the guidelines set forth in FDA's *Guidelines for the Validation of Chemical Methods for the FDA Foods Program*[3] and exceeds the standard method performance requirements approved by the AOAC Stakeholder Panel on Strategic Food Analytical Methods[4].

The method was validated by analyses of reference materials and fortified analytical portions for accuracy and replicate portions for precision. Reference materials used for validation are listed in the single laboratory validation publication[5].

Analyses were performed on 21 different foods that were similar to those collected in FDA's Total Diet Study purchased from local grocers. Foods were prepared as described and analyzed several ($N \geq 3$) times. Fortified analytical portions (3 spike levels each) were also prepared and analyzed. Method validation results are shown in Todorov et al 2016[5].

Interlaboratory trial.

EAM 4.13 has undergone a level 3 multi-laboratory validation (MLV) as described in the FDA's *Guidelines for the Validation of Chemical Methods for the FDA Foods Program*[3]. Six FDA laboratories participated in the study using four different ICP-MS models. The method evaluation included determination of limits of detection and quantification, analysis of National Institute of Standards and Technology standard reference materials (SRMs), unknown samples, blinded SRMs, and fortified analytical portions by all six collaborators. The samples were chosen to represent all sectors of the AOAC food triangle and additionally included pet food and multivitamin tablets. The means obtained from the study, SRM certificate values and uncertainties, repeatability, reproducibility, HorRat values, z-score ranges, and spike recoveries are summarized in 4.13 Table 5. Of the 175 analysis results, 174 of the z-scores were below 2 and one was between 2 - 3. The analytical results for the unknown samples test materials that were prepared in house (seven foods, one prenatal vitamin and one pet food) are summarized in 4.13 Table 6. The repeatability and reproducibility ranges were 1.8 – 11.4% and 3.6 –13.7%, respectively; the calculated HorRat values were between 0.17–1.18. The fortified analytical portion samples had recoveries of 102–105%, indicating acceptable method performance. The MLV results were reviewed by the FDA Chemical Methods Validation Subcommittee and were published in Journal of AOAC International[6].

4.13 Table 5. SRM analysis

	Average, ng/g	Certificate, ng/g	Repeatability[a]		Reproducibility[b]		HORRAT value	z-score range	Spike recoveries
			s(r), ng/g	RSD(r), %	s(R), ng/g	RSD(R), %			
NIST 3280 Multivitamin tablets	127000	132700 ± 6600	5470	4.3	6670	5.3	0.68	-1.1 to 0.2	
NIST 1549a Whole milk powder	3310	3340 ± 300	185	5.6	185	5.6	1.18	-1 to 0.6	
NIST 3290 Dried cat food	3690	3380 ± 540	197	5.3	233	6.3	0.48	-0.2 to 1	
NIST 1845a Egg powder	2820	3030 ± 100	312	11.1	313	11.1	0.81	-1.5 to 2.8	
NIST 1849a Infant formula (blind)	1140	1290 ± 110	41.6	3.7	58	5.1	0.33	-0.9 to 0.6	103 ± 8
NIST 3252 Protein drink mix (blind)	2120	1840 ± 200	227	10.6	252	11.8	0.83	-1.5 to 1.8	

[a]Within collaborator uncertainty

[b]Uncertainty between collaborators

4.13 Table 6. Unknown sample analysis

	Average, ng/g	Repeatability		Reproducibility		HORRAT value	z-score range	# labs above LOD	# labs above LOQ	Spike recoveries
		s(r), ng/g	RSD(r), %	s(R), ng/g	RSD(R), %					
Salad dressing	169	3	1.8	6.12	3.6	0.17	-0.4 to 0.5	6	6	
Chocolate chips	4.6	0.4	8.36	0.54	11.73	0.33	-1.3 to 1.6[a]	5	1[b]	
Processed meat product	868	39.9	4.6	48.1	5.5	0.34	-1.2 to 0.6	6	6	102 ± 7
Hard-boiled eggs	913	38.4	4.2	45.7	5	0.31	-1 to 0.5	6	6	
Enriched bread	14	1.6	11.4	1.92	13.7	0.45	-1.4 to 2.8[a]	6	4[b]	
Canned oysters	3990	210	5.3	328	8.2	0.63	-1.5 to 0.8	6	6	
Canned fish	782	50.3	6.4	340	44	2.62	-3.2 to 3.9[c]	6	6	103 ± 8
Prenatal vitamin	152000	9990	6.6	10700	7	0.94	-1.1 to 1.2	6	6	105 ± 8
Canned dog food	7970	179	2.2	429	5.4	0.46	-0.8 to 0.3	6	6	102 ± 8

[a] All analysis above LOD used for z-score calculation

[b] All analysis for enriched bread and chocolate chips above LOD were included in the statistical analysis

[c] Bimodal distribution likely due to non-homogeneity

Uncertainty.

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

4.13.11 REFERENCES

(1) ASTM International (2006) ASTM D 1193-06, Standard Specification for Reagent Water. Available from ASTM (http://www.astm.org).

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(4) AOAC International (2013) AOAC SMPR 2012.007 – Standard Method Performance Requirements for Heavy Metals in a Variety of Foods and Beverages, *J. AOAC Int.* **96**, 704.

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