

AOAC Official Method 2012.15
Total Iodine in Infant Formula
and Adult/Pediatric Nutritional Formula
Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)
First Action 2012
Final Action 2015

ISO/IDF-AOAC Method*

[Applicable to the measurement of total iodine in infant formula and adult/pediatric nutritional formula from 0.5 to 1500 µg/100 g reconstituted final product and for ready-to-feed (RTF) products from 2.5 to 1000 µg/100 g using ICP-MS. This method is not applicable to products containing FD&C Red Dye No. 3 (erythrosine). The iodine from erythrosine is also quantitatively determined by this method; thus, accurate quantification of fortified levels of iodine is not possible.]

See Table 2012.15A for results of the interlaboratory study supporting acceptance of the method.

Caution: Refer to Material Safety Data Sheets (MSDS) for safety precautions when using chemicals. Use personal protective equipment recommended in MSDS.

A. Principle

Digestion occurs using a potassium hydroxide (KOH) solution in an oven or open-vessel microwave system. Iodine is stabilized with ammonium hydroxide and sodium thiosulfate after digestion. The solution is brought to volume followed by filtration. The filtrate is analyzed directly or after dilution by ICP-MS.

B. Safety Considerations

(a) Use only ovens and microwave ovens specifically designed for laboratory use.

(b) The method involves the use of strong bases and concentrated acids. Avoid spills, inhalation, and exposure to human tissues.

(c) Oven and microwave digestion procedures involve moderately elevated temperatures. Carefully remove samples and allow cooling before removing the lids from the digestion vessels.

C. Chemicals and Reagents

(a) *KOH pellets*.—Certified ACS grade (Fisher Scientific, Fairlawn, NJ, USA). (*Note:* KOH may contribute background levels of iodine.)

(b) *Ammonium hydroxide 28–30% (NH₄OH)*.—Certified ACS PLUS (Fisher Scientific).

(c) *Sodium thiosulfate (Na₂S₂O₃)*.—≥99.99% metal basis (Fisher Scientific).

(d) *Surfactant (e.g., Triton® X-100)*.—Sigma (St. Louis, MO, USA).

(e) *Nitric acid concentrated (HNO₃)*.—OPTIMA (high purity; Fisher Scientific).

(f) *Perchloric acid 70% (HClO₄)*.—Reagent ACS (Fisher Scientific).

(g) *Purified water*.—18 MΩ/cm.

Note: Equivalent chemicals and reagents may be substituted.

D. Apparatus

(a) *Polypropylene (PP) tubes*.—Assorted sizes, use as received; 50 mL PP DigiTUBES® (Part No. 010-500-261), 100 mL PP DigiTUBES (Part No. 010-501-263); SCP Science (Montreal, Canada).

(b) *Oven (i.e., warming/drying oven)*.—Isotemp oven Model 6921 (Fisher Scientific).

(c) *Open-vessel microwave digestion unit (optional)*.—MARS 5 or MARS 6 (CEM Corp., Matthews, NC, USA).

(d) *Analytical and top-loader balances*.—Sensitive to 0.0001 and 0.01 g, respectively (Sartorius, Goettingen, Germany).

(e) *ICP-MS system*.—ELAN DRC II (PerkinElmer, Waltham, MA, USA).

(f) *Autosampler for ICP-MS*.—SC4-DX (Elemental Scientific, Inc., Omaha, NE, USA).

(g) *Adjustable (electronic or manual) volumetric pipets*.—Eppendorf (Hamburg, Germany). Capable of volumes 100–5000 µL.

(h) *Re-pipet volumetric dispensers*.—Adjustable volume.

(i) *PP or Teflon bottles for storage of reagents*.

(j) *Disposable plastic syringes*.—e.g., 10 mL with LuerLok.

(k) *Syringe filters with 1 µm membrane*.—Nonsterile glass fiber B (Part No. SLPBDZ5NK; EMD Millipore, Corp., Billerica, MA, USA).

(l) *Beakers*.—Assorted sizes.

(m) *Stir bars*.—7.9 × 50 mm, assorted sizes (VWR, Chester, PA, USA).

(n) *Stir plate*.—Adjustable speed, Corning (Corning, NY, USA) or equivalent.

Table 2012.15A. Statistical data

| Sample name | Average | S _r ^a | RSD _r | S _R ^b | RSD _R | No. of outlier laboratories ^c | HorRat | No. of laboratories used |
|------------------------------------------------|---------|-----------------------------|------------------|-----------------------------|------------------|------------------------------------------|--------|--------------------------|
| NIST SRM 1849a, mg/kg | 1.24 | 0.010 | 0.77 | 0.067 | 5.42 | 1 | 0.35 | 12 |
| Infant formula RTF, milk based-1 ^d | 5.48 | 0.262 | 4.78 | 0.507 | 9.25 | 0 | 1.06 | 13 |
| Infant formula powder, soy based ^d | 12.4 | 0.313 | 2.53 | 0.945 | 7.62 | 2 | 0.98 | 11 |
| Infant formula powder, milk based ^d | 18.5 | 0.693 | 3.75 | 1.39 | 7.54 | 2 | 1.03 | 11 |
| Infant formula RTF, milk based-2 ^d | 5.45 | 0.226 | 4.16 | 0.626 | 11.5 | 0 | 1.31 | 13 |
| Child formula powder ^d | 3.47 | 0.135 | 3.87 | 0.278 | 8.01 | 2 | 0.85 | 11 |
| Adult nutritional powder, low fat ^d | 7.03 | 0.137 | 1.94 | 0.503 | 7.15 | 2 | 0.85 | 11 |

^a S_r = SD for repeatability.

^b S_R = SD for reproducibility.

^c Values from laboratories with outliers were not used in statistical calculations.

^d Results expressed as µg/100 g reconstituted final product.

(o) *Pump tubing*.—Peristaltic, black/black two-stop polyvinyl chloride (PVC), 0.76 mm id (SCP Science, Champlain, NY, USA), used for introducing carrier solution.

(p) *Pump tubing*.—Peristaltic, orange/green two-stop PVC pump tubing, 0.38 mm id (SCP Science), used for introducing internal standard (IS) solution.

Notes: Equivalent apparatus may be substituted.

All laboratory plasticware should be single-use whenever possible. If reuse is necessary, wash using 10% HNO₃, then rinse thoroughly with purified water prior to use. When needed, general laboratory acid-washed glassware may also be used.

Filter membranes <1 µm (e.g., 0.25 or 0.45 µm) may be used.

Adherence as close as possible to the recommended ids of the pump tubing is critical. The ratio of the pump tubing id (0.76 mm) used for the carrier solution to the pump tubing id (0.38 mm) used for the IS solution may be used as a guideline (0.76/0.38 = 2). For best performance, the ratio should remain as close to 2 as possible. Vast differences in id between the carrier solution pump tubing and the IS solution (e.g., 1.02/0.19, respectively) may result in poor accuracy.

E. Instrument and Parameters

(a) *Instrument*.—ICP-MS PerkinElmer ELAN DRC II, or equivalent.

(b) *Mode*.—Standard (STD).

(c) *Gas*.—Argon (≥99.998%, high purity).

(d) *Rinse*.—0.1% Triton/1% NH₄OH in purified water.

(e) *Sweeps/readings*.—20.

(f) *Readings/replicate*.—One.

(g) *Replicates*.—Three.

(h) *Nebulizer gas flow*.—Optimized daily.

(i) *Auxiliary gas flow*.—1.2 L/min.

(j) *Plasma gas flow*.—15.00 L/min.

(k) *Lens voltage*.—Optimized daily.

(l) *ICP radio frequency power*.—1500 watts.

(m) *Peristaltic pump*.—Rate optimized.

Notes: Parameters of other manufacturer's instruments may be optimized accordingly to ensure the instrument's minimum daily performance requirements are met.

All analyses must be performed using the STD mode. (Use of a reaction or collision gas is not required or allowed.)

F. Reference Standards

(a) *Iodide 1000 ppm standard solution in H₂O*.—SPEX CertiPrep (Metuchen, NJ, USA).

(b) *Iodide 1000 ppm standard solution in 1% triethanolamine (TEA)*.—Inorganic Ventures (Christiansburg, VA, USA).

Notes: Either stock iodide reference solutions may be used for intermediate and working standard solutions preparation. The remaining source may be used as a continuing calibration verification (CCV) standard.

Equivalent reference standards may be substituted.

"Iodide" may be referred to as "iodine" throughout this method.

G. Internal Standard

Praseodymium 10 ppm standard solution in 5% HNO₃.—Inorganic Ventures.

Notes: Individual values of iodine will be reported for each test sample using praseodymium as the IS. Equivalent stock IS solutions may be substituted.

H. Procedure

(a) *Reagent solutions preparation*.—*Note:* Prepare all reagent solutions as recommended by either weight/volume (w/v) or volume/volume (v/v). Adjusting for purity and/or concentration is not required.

(1) *5% KOH solution*.—Dissolve 25 g KOH pellets in an appropriate amount purified water, then dilute to 500 mL with purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(2) *Stabilizer concentrate*.—Dissolve 5 g Na₂S₂O₃ in an appropriate amount purified water, add 50 mL NH₄OH, then dilute to 500 mL with purified water. The resulting concentration is 10% NH₄OH and 1% Na₂S₂O₃ in purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(3) *Wash solution (rinse)*.—Dissolve 2 g Triton X-100 in an appropriate amount of purified water, add 20 mL NH₄OH, then dilute to 2 L with purified water. The resulting concentration is 1% NH₄OH and 0.1% Triton X-100 in purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(4) *Diluent*.—Dissolve 10 g KOH pellets and 0.4 g Na₂S₂O₃ in an appropriate amount of purified water, add 4 mL NH₄OH, then dilute to 2000 mL with purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date. Alternatively, for a smaller volume, dilute 50 mL 5% KOH and 10 mL stabilizer concentrate to 500 mL with purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

Note: The resulting concentrations for both preparations are 0.5% KOH, 0.2% NH₄OH, and 0.02% Na₂S₂O₃ in purified water.

(5) *Conditioning solution*.—Prepare by aliquoting 25 mL 5% KOH solution, then diluting to 250 mL with purified water. This solution is used to prepare the instrument for analysis. The resulting concentration is 0.5% KOH. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(6) *Carrier solution*.—Equivalent to the wash solution. The carrier solution is used to deliver the sample solution to the nebulizer through the ICP-MS autosampler introduction system. The carrier solution is introduced via a peristaltic pump using black/black two-stop PVC pump tubing (0.76 mm id). Store this solution at room temperature. Reagent expires 6 months after preparation date.

(b) *Standard solutions preparation*.—*Notes:* Stock solutions are stable until the date indicated on the certificate of analysis. Intermediate, calibration, continuing calibration verification, and IS solutions are stable at room temperature until the earliest expiration date of all components used to prepare the solution.

All calibration standards, continuing calibration verification, continuing calibration blank, and IS solutions are analyzed as prepared. Do not carry these solutions through sample preparation or digestion.

(1) *Stock iodine and praseodymium solutions*.—Purchase of stock iodine and praseodymium standard solutions with accompanying certificates of analysis is recommended.

(2) *Intermediate stock standard (ISS) iodine solutions*.—Prepare the ISS iodine solutions according to Table 2012.15B.

(3) *Calibration standard (CS) iodine solutions*.—Prepare the solutions according to Table 2012.15C.

Table 2012.15B. Preparation of intermediate stock standard (ISS) iodine solutions^a

| Iodine standard solution ID | ID of solution used for preparation | Initial iodine concentration, ng/mL | Aliquot volume, mL | Final volume, mL | Final iodine concentration, ng/mL |
|-----------------------------|-------------------------------------|-------------------------------------|--------------------|------------------|-----------------------------------|
| 10000 (ISS) | Stock | 1000000 | 0.5 | 50 | 10000 |
| 1000 (ISS) | 10000 (ISS) | 10000 | 5 | 50 | 1000 |
| 10.0 (ISS) | 1000 (ISS) | 1000 | 0.5 | 50 | 10.0 |

Aliquot the appropriate amount of iodine standard solution into a single use 50 mL DigiTUBE® and add 5 mL of stabilizer concentrate, fill to the 50 mL mark on the tube with water, cap the tube, and then mix thoroughly. The resulting matrix concentration is 1% NH₄OH and 0.1% Na₂S₂O₃ in water.

^a ISS solutions are used for calibration standard preparation and are typically prepared according to the table. The ISS concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis, determine the exact concentration of each ISS. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 µL, is recommended.

(4) *Intermediate continuing calibration verification (ICCV), continuing calibration verification (CCV) iodine solutions, and continuing calibration blank (CCB).*—Prepare the ICCV, CCV standard solutions, and CCB blank according to Table 2012.15D.

Note: A CCV must be prepared from a second source stock solution (e.g., purchased from another vendor) other than that used for the CS solutions.

(5) *IS solutions.*—Prepare the IS solution according to Table 2012.15E. The IS concentration typically used for analysis is 30 ppb praseodymium (Pr).

Notes: Ideally, the intensity generated for the IS should be similar to the intensity of iodine standard at the mid-point of the standard curve.

As some ICP-MS instruments provide greater sensitivity, the concentration of Pr may be adjusted accordingly to provide intensities similar to the intensity generated by the 50.0 ppb iodine standard.

(c) *Reconstitution.*—*Note:* All powdered samples, with the exception of NIST SRM 1849a, are required to be analyzed on a reconstituted basis. Do not reconstitute RTF samples.

Accurately weigh approximately 25 g powdered test sample into an appropriate vessel (e.g., 400 mL beaker) and record the weight. Without zeroing the balance, add water to make approximately 225 g. Record the sample + water weight. Place a stir bar in the mixture and stir

on a stir plate to form a homogeneous slurry/suspension. Proceed to *Sample preparation (d)*.

Note: This reconstituted solution should be discarded after 24 h.

(d) *Sample preparation.*—Weighing [after weighing all materials, proceed to *Addition of reagents, (e)*].

(1) *Reconstituted material.*—Accurately weigh an aliquot of approximately 6 g reconstituted test sample into a 50 mL DigiTUBE® or 12 g into a 100 mL DigiTUBE.

(2) *NIST SRM 1849a.*—Accurately weigh approximately 0.5 g NIST SRM 1849a into a 50 mL DigiTUBE or 1 g into a 100 mL DigiTUBE.

(3) *RTF material.*—Accurately weigh approximately 1 g of the RTF test sample into a 50 mL DigiTUBE or 2 g into a 100 mL DigiTUBE.

Note: The remaining RTF material should be transferred to a sealed, brown PP container and held at refrigerated conditions between 2 to 8°C. These solutions should be discarded after 5 days.

(4) *Blank.*—Designate at least one 50 mL or 100 mL DigiTUBE digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples.

(e) *Addition of reagents [after adding all reagents and mixing, proceed to Oven digestion, (f), or Open vessel microwave digestion, (g)].*—(1) *Water.*—Add 10 mL purified water to each 50 mL DigiTUBE or 20 mL to each 100 mL DigiTUBE.

Table 2012.15C. Preparation of calibration standard (CS) iodine and calibration blank (CB) solutions^a

| Iodine standard solution ID | ID of solution used for preparation | Initial iodine concentration, ng/mL | Aliquot volume, mL | Final volume, mL | Final iodine concentration, ng/mL |
|-----------------------------|-------------------------------------|-------------------------------------|--------------------|------------------|-----------------------------------|
| 100 (CS) | 1000 (ISS) | 1000 | 5 | 50 | 100 |
| 50.0 (CS) | 1000 (ISS) | 1000 | 2.5 | 50 | 50.0 |
| 10.0 (CS) | 1000 (ISS) | 1000 | 0.5 | 50 | 10.0 |
| 1.00 (CS) | 10.0 (ISS) | 10.0 | 5 | 50 | 1.00 |
| 0.500 (CS) | 10.0 (ISS) | 10.0 | 2.5 | 50 | 0.500 |
| 0.250 (CS) | 10.0 (ISS) | 10.0 | 1.25 | 50 | 0.250 |
| Blank (CB) | NA ^b | NA | NA | 50 | 0 |

Aliquot the appropriate amount of iodine standard solution into a single-use 50 mL DigiTUBE and add 5 mL of 5% KOH and 1 mL of stabilizer concentrate, fill to the 50 mL mark on the tube with water, cap the tube, and then mix thoroughly. The resulting matrix concentration is 0.5% KOH, approximately 0.2% NH₄OH, and approximately 0.02% Na₂S₂O₃ in water.

^a Typical CS standard concentrations are nominally 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb iodine and are typically prepared according to the table. The CB is the zero point of the curve. The curve type used, if using a PerkinElmer ICP-MS system with ELAN software, should be linear through zero. If using an Agilent or Thermo ICP-MS system, force the curve through the calibration blank. The calibration curve must have a correlation coefficient (r) of ≥0.998 to be acceptable. Determine the exact concentration of each CS (traceable back to the certificate of analysis) and assign these values to the curve points used to generate final results. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 µL, is recommended.

^b NA = Not applicable.

Table 2012.15D. Preparation of intermediate continuing calibration verification (ICCV) and continuing calibration verification (CCV) iodine solutions and continuing calibration blank (CCB) solution^a

| Iodine standard solution ID | ID of solution used for preparation | Initial iodine concentration, ng/mL | Aliquot volume, mL | Final volume, mL | Final iodine concentration, ng/mL |
|-----------------------------|-------------------------------------|-------------------------------------|--------------------|------------------|-----------------------------------|
| 10000 (ICCV) | Stock | 1000000 | 0.5 | 50 | 10000 |
| 1000 (ICCV) | 10000 (ICCV) | 10000 | 5 | 50 | 1000 |
| 10.0 (CCV) | 1000 (ICCV) | 1000 | 0.5 | 50 | 10.0 |
| Blank (CCB) | NA ^b | NA | NA | 50 | 0 |

Aliquot the appropriate amount of iodine standard solution into a single-use 50 mL DigiTUBE, fill to the 50 mL mark on the tube with diluent, cap the tube, and then mix thoroughly. The resulting matrix concentration is 0.5% KOH, approximately 0.2% NH₄OH, and approximately 0.02% Na₂S₂O₃ in water. For the blank (CCB), fill a single-use 50 mL DigiTUBE to the 50 mL mark on the tube with diluent, cap the tube, and then mix thoroughly.

^a ICCV solutions are used for preparation of the CCV standard solution and are typically prepared according to the table. The ICCV and CCV concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis (from the second source), determine the exact concentration of each ICCV. With this information, determine the exact concentration of the CCV standard. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 µL, is recommended.

^b NA = Not applicable.

(2) *5% KOH*.—Add 5 mL 5% KOH if material was weighed into a 50 mL DigiTUBE or add 10 mL of 5% KOH if material was weighed into a 100 mL DigiTUBE.

(3) *Mixing*.—Seal the vessels and swirl or use a vortex apparatus to mix. Avoid inverting as this may allow sample to adhere to the inner walls of the vessel above the level of the digestion solution.

(f) *Oven digestion*.—(1) *Digestion/extraction*.—Digest samples in an oven set to maintain 105 ± 5°C until the dissolution of iodine is complete, approximately 1 h.

Notes: The digestion vessels may either be tightened completely or loosened slightly while in the oven.

Carefully swirl by hand each digestion vessel approximately halfway through the digestion/extraction procedure.

(2) *Addition of stabilizer*.—After removal of samples from the oven, add 1 mL of stabilizer concentrate to the 50 mL DigiTUBE samples or add 2 mL if material was weighed into a 100 mL DigiTUBE. Allow samples to cool to room temperature.

Note: Alternatively, allow samples to cool to room temperature first, and then add the stabilizer concentrate.

(3) *Final volume*.—If 50 or 100 mL vessels were used for digestion, bring samples to a final volume of 50 or 100 mL respectively, with purified water.

(4) *Capping/mixing*.—Cap all vessels, and then invert to mix thoroughly.

(g) *Open vessel microwave digestion*.—(1) *Digestion/extraction*.—Place the digestion vessels into the carousel of the open-vessel microwave digestion unit. If less than the maximum capacity is to be digested, distribute the vessels evenly throughout the carousel. Digest the samples in the microwave unit until the dissolution of iodine is complete. See Table 2012.15F for suggested open-vessel microwave digestion parameters.

Note: Vessel caps should be loosened slightly (from fully tightened) during the digestion procedure. *Use caution*: Ensure

vessels do not completely seal (bursting hazard) or overheat (melting may occur). Alternatively, instead of just loosening the caps, drill small holes (approximately 3 mm) in the caps. This way the caps can be tightened, but venting (thus the “open” vessel) can occur. Caps may be reused after acid washing.

(2) *Addition of stabilizer*.—After removal of samples from the oven, add 1 mL stabilizer concentrate to the 50 mL DigiTUBE samples or add 2 mL if material was weighed into a 100 mL DigiTUBE. Allow samples to cool to room temperature.

Note: Alternatively, allow samples to cool to room temperature first, and then add the stabilizer concentrate.

(3) *Final volume*.—If 50 or 100 mL vessels were used for digestion, bring samples to a final volume of 50 or 100 mL, respectively, with purified water.

(4) *Capping/mixing*.—Cap all vessels, and then invert to mix thoroughly.

(h) *Sample filtering*.—(1) *Filtering*.—Filter each sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, and then filter an adequate amount (e.g., at least 5 mL) into an appropriate vessel (e.g., 15 mL PP centrifuge tube or autosampler vial) to be used for analysis.

Notes: Samples may be difficult to filter. Use of multiple filter membranes may be required. To ease filtration, allow the inverted sample digestates to rest for a period of time (e.g., 1 h) before filtering.

Digested sample solutions may be stored at ambient temperature. Samples may be stored at ambient temperature indefinitely, as long as the results for the applicable digest blank(s) and/or control sample(s) are acceptable when analyzed.

(i) *Sample dilution*.—Aliquot 5 mL of each sample’s filtrate into an appropriate volumetric vessel and then bring to a final volume of 10 mL with diluent.

Note: Analyze all samples diluted 5 to 10 mL as directed above.

Table 2012.15E. Preparation of internal standard (IS) solution^a

| Standard solution ID | ID of solution used for preparation | Initial concn, ng/mL | Aliquot volume, mL | Final volume, mL | Final concn, ng/mL |
|----------------------|-------------------------------------|----------------------|--------------------|------------------|--------------------|
| 30.0 (Pr) | Stock | 10000 | 1.5 | 500 ^b | 30.0 |

^a The IS concentration typically used for analysis is 30 ppb. The table outlines a typical preparation scheme.

^b After aliquoting the 10000 ppb Pr into the 500 mL vessel, add approximately 100 mL water, 10 mL HNO₃, 0.5 mL HClO₄, 0.05 g Triton® X-100, and then bring to volume with water and mix thoroughly. The resulting concentration is 2% HNO₃, 0.1% HClO₄, and 0.01% Triton® X-100 in water.

Table 2012.15F. Open-vessel microwave digestion parameters^a

| Six 50 mL vessels | | |
|----------------------------------|----------|---------|
| Wattage | Power, % | Minutes |
| 400 | 10 | 5 |
| 400 | 20 | 6 |
| 400 | 20 | 7 |
| Twelve to eighteen 50 mL vessels | | |
| 400 | 25 | 10 |
| 400 | 40 | 10 |
| Twenty-four 50 mL vessels | | |
| 400 | 25 | 10 |
| 400 | 40 | 10 |
| 400 | 65 | 10 |

^a Microwave used: CEM MARS 5 or CEM MARS 6. *Use caution:* Ensure vessels do not completely seal (bursting hazard) or overheat (as melting may occur). *Note:* Using AOAC Method 2012.15, the parameters, with the corresponding number of vessels, produced acceptable results for NIST SRM 1849a infant/adult nutritional formula. For each number of vessel's range, if fewer vessels than the minimum are placed in the microwave, overheating may occur resulting in loss of sample or injury. If greater than the suggested number of vessels is placed in the microwave, the digestion may not be complete.

I. Determination (Instrument and Parameters; see Section E)

Notes: All analyses must be performed using the STD mode. (Use of a reaction or collision gas is not required or allowed.)

Prior to conditioning, calibration, and sample analysis, ensure the instrument is optimized to meet the manufacturer's minimum daily performance requirements.

(a) *Conditioning.*—Condition the ICP-MS sample introduction system. Analyze the conditioning solution while concomitantly introducing IS solution online (e.g., through a mixing block or T) until conditioned (approximately 1 h). The IS solution is introduced via a peristaltic pump using orange/green two-stop PVC pump tubing (0.38 mm id). After conditioning, begin to aspirate carrier solution while continuing to add IS. Analyze samples using ICP-MS. Ensure the wash solution (rinse) is available and ready for use to rinse out the sample lines and introduction system between each analysis.

Notes: If acidic sample solutions are typically analyzed on the ICP-MS system, perform a thorough cleaning of the entire sample introduction system prior to conditioning. Background counts for both iodine and the IS should be relatively stable (e.g., not ascending or descending).

A dedicated set of cones (sampler and skimmer), if possible, is recommended. Analysis of acid-type (e.g., HNO₃) matrixes with the same set of cones used for iodine analysis may increase conditioning time or produce elevated background levels.

Analyzing several (e.g., at least six) digested samples prior to calibration is recommended. Introducing and analyzing actual digested sample solutions increases conditioning efficiency.

Possible additional maintenance: Due the nature of the digestion/extraction solution (i.e., KOH) and the amount of organic material in the sample solutions, additional maintenance may be required (as compared to typical acid matrix digestions/analysis). Lenses in instruments and/or lens stack assemblies may require more frequent cleaning. Once cleaned, a period of reconditioning may be required.

(b) *Calibration.*—In addition to a calibration blank, working standards of 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb are used. Calibrate the ICP-MS system using an autosampler or manually.

Notes: The curve type used should be linear, forced through the calibration blank.

All standards must be included in the calibration curve.

The 0.250 ppb signal must be ≥ 1.5 times the calibration blank signal. Consistent background throughout the entire analytical run is imperative for a successful analysis. This will be evident based on the results obtained for the CCB.

(c) *Sample analysis.*—Analyze a 5 to 10 mL dilution of each digested filtered sample using ICP-MS.

Notes: A 5 to 10 mL dilution is preferable and required in order to achieve a reporting limit of 0.5 $\mu\text{g}/100\text{ g}$ as reconstituted final product or the limit of 2.5 $\mu\text{g}/100\text{ g}$ for RTF samples.

Diluting the samples reduces the matrix load on the plasma and may reduce frequency of maintenance (e.g., cleaning cones).

For other applications, samples digested with 5% KOH solution may be analyzed directly or diluted (if necessary) so that the iodine concentration will fall within the calibration range. Alternative volume aliquots may be prepared by placing an aliquot of the filtrate into an appropriate volumetric vessel, and then diluting to an appropriate final volume with diluent. Greater dilutions, such as 1 to 18 mL, would achieve a higher upper reporting limit (e.g., 1500 $\mu\text{g}/100\text{ g}$ reconstituted final product).

(d) *Data acceptability.*—The calibration curve must include a calibration blank (as a calibration point). The calibration curve must have a correlation coefficient (r) ≥ 0.998 to be acceptable.

The individual back-calculated calibration standard concentrations must be within 90–110% of the theoretical concentrations to be acceptable.

The 0.250 ppb signal must be ≥ 1.5 times the calibration blank signal. Consistent background throughout the entire analytical run is imperative for a successful analysis. This will be evident based on the results obtained for the CCB.

ACCB is analyzed after calibration, at least every 10 samples, and after the last sample in the analysis batch to monitor background. A CCB should be of the same matrix as the standards used for calibration. Iodine levels $\leq 30\%$ of the lowest calibration standard are considered acceptable.

With each batch of samples, at least one digest blank should be prepared in the same manner as the samples. An iodine result of $\leq 30\%$ of the lowest calibration standard is considered acceptable.

A CCV standard solution containing iodine from a source other than that of the calibration standards is used to verify acceptable calibration and to evaluate the ongoing performance of the instrument. The CCV should be analyzed after calibration, at least every 10 samples, and after the last sample in the analysis. A CCV should be of the same matrix as the standards used for calibration. A CCV result is considered acceptable when the result is within 90–110% of theoretical.

J. Calculations

If a reconstitution was performed, use the following equation:

$$\{(C \times V) \times D\} / \text{WRA} \div 10 = S$$

where C = sample concentration (ng/mL, sample solution reading on the curve); V = volume (mL, final volume after digestion); D = dilution factor (if not applicable, enter 1); WRA = weight (g) of reconstitution aliquoted during *sample preparation*, (d); and S

= sample concentration of iodine (µg/100 g reconstituted “as fed” basis).

If a reconstitution was not performed, use the following equation:

$$\{[(C \times V) \times D]/W\}/10 = S$$

where C = sample concentration (ng/mL, where sample solution reads on the curve); V = volume (mL, final volume after digestion); D = dilution factor (if not applicable, enter 1); W = sample size (g); and S = sample concentration of iodine (µg/100 g).

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* Method adopted by AOAC as an *Official Method of Analysis*SM and by the International Organization for Standardization and International Dairy Federation as ISO 20647 | IDF 234:2015.

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