AOAC Official Method 2012.15
Total Iodine in Infant Formula and Adult/Pediatric Nutritional Formula
Inductively Coupled Plasma–Mass Spectrometry
First Action 2012

Caution: Use only ovens and microwave ovens specifically designed for laboratory use.

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

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

A. Chemicals and Reagents

(**a**) *KOH (KOH) pellets, certified ACS*.—KOH may contribute background levels of iodine.

(**b**) *KOH solution*.—50% (w/v).

(**c**) *Ammonium hydroxide (NH_4OH)*.—Certified ACS.

(**d**) *Sodium thiosulfate ($Na_2S_2O_3$)*.—99.99+% metal basis.

(**e**) *Surfactant (i.e., Triton® X-100)*.

(**f**) *Nitric acid (HNO_3)*.—High purity.

(**g**) *Perchloric acid ($HClO_4$)*.—High purity.

(**h**) *Purified water*.—18 MΩ/cm.

B. Reference Standards

Iodine stock standard solutions.—Certified ICP-MS or ICP-grade single- or multielement standard solutions (or other certified reference materials; CRM) are used to prepare calibration, calibration verification standards, internal standards, and spiking solutions.

Internal standards are prepared using certified ICP-MS or ICP-grade single- or multielement standard solutions whenever possible. When applicable, choose elements for internal standards within ±40 atomic mass units (amu) from the mass to be quantified. Likely choices for use as internal standards for iodine analysis are praseodymium (Pr), samarium (Sm), tellurium (Te), and rhodium (Rh). Concentrations used for analysis are 30.0 ppb Pr, Sm, Rh, and 500 ppb Te. The internal standard solution reagent's concentration is 2% HNO_3, 0.1% $HClO_4$, 0.01% Triton X-100, 0.25% KOH, 0.1% NH_4OH, and 0.01% $Na_2S_2O_3$ in purified water.

C. Reagent Solution Preparation

(**a**) *5% KOH solution*.—Dissolve 25 g KOH pellets in an appropriate amount of purified water, then dilute to 500 mL with purified water. Store at room temperature. Reagent expires 6 months after preparation date. Alternatively, dilute 50 mL 50% (w/v) KOH solution to a final volume of 500 mL with purified water. Store at room temperature. Reagent expires 6 months after preparation date.

(**b**) *50% KOH solution*.—Dissolve 250 g KOH pellets in an appropriate amount of purified water, then dilute to 500 mL with purified water. Store at room temperature. Reagent expires 6 months after preparation date. *Note*: Use caution when preparing this solution as a significant amount of heat is generated. Alternatively, use purchased 50% (w/v) KOH solution. Store at room temperature. Reagent expiration date may be found on the label. If no expiration is provided by the manufacturer, record the date opened and assign an expiration date of 6 months from the date of opening.

(**c**) *Stabilizer concentrate*.—Dissolve 5 g $Na_2S_2O_3$ in an appropriate amount of purified water, add 50 mL NH_4OH, then dilute to 500 mL with purified water. The resulting concentration is 10% NH_4OH and 1% $Na_2S_2O_3$ in purified water. Store at room temperature. Reagent expires 6 months after preparation date.

(**d**) *Wash solution (rinse)*.—Dissolve 2 g Triton X-100 in an appropriate amount of purified water, add 20 mL NH_4OH, then dilute to 2 L with purified water. The resulting concentration is 1% NH_4OH and 0.1% Triton X-100 in purified water. Store at room temperature. Reagent expires 6 months after preparation date.

(**e**) *Diluent*.—Dissolve 10 g KOH pellets and 0.4 g of $Na_2S_2O_3$ in an appropriate amount of purified water, add 4 mL NH_4OH, then dilute to 2000 mL with purified water. Store 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 at room temperature. Reagent expires 6 months after preparation date. *Note*: The resulting concentration for both preparations is 0.5% KOH, 0.2% NH_4OH, and 0.02% $Na_2S_2O_3$ in purified water.

(**f**) *Conditioning solution*.—Prepare by aliquoting 25 mL 5% KOH (2.5 mL 50% 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 at room temperature. Reagent expires 6 months after preparation date.

(**g**) *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 polyvinyl chloride pump tubing (0.76 mm id). Store at room temperature. Reagent expires 6 months after preparation date.

D. Apparatus

(**a**) *Polypropylene (PP) tubes*.—Assorted sizes, use as received.

(**b**) *Oven (i.e., warming/drying oven)*.

(**c**) *Open-vessel microwave digestion unit (optional)*.

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

(**e**) *ICP-MS system*.

(**f**) *Autosampler for ICP-MS*.

(**g**) *Adjustable (electronic or manual) volumetric pipets and pipet tips*.

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

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

(**j**) *Disposable plastic syringes*.

(**k**) *Syringe filters with 1 µm membrane*.

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

E. Sample Preparation

If the sample weight taken will be less than 1 g, use a balance accurate to 0.0001 g. If the sample weight taken will be more than 1 g, use a balance accurate to at least 0.01 g.

(**a**) *Oven digestion (preferred)*.—*Note*: The following oven digestion procedure is for a final volume of 100 mL. It is critical that the final reagent concentration in all vessels be equivalent to that of the calibration standards.

Samples expected to contain levels of iodine below 10 000 µg/kg may be digested using the 5% KOH solution. However, if samples are expected to contain >10 000 µg/kg iodine and are anticipated to be detectable after an appropriate dilution, the 50% KOH solution may be used. Vitamin/mineral dietary supplements or premixes or other certain matrixes should be digested using only the 50% KOH solution.

For the testing of vitamin/mineral tablets or premixes, it is recommended (due to potential homogeneity issues) that a reconstitution be performed. Unless a specific reconstitution procedure is required, use the following reconstitution procedure as a guide.

(**b**) *Suggested reconstitution procedure*.—Accurately weigh approximately 5.00 g of sample into an appropriate vessel (150 mL or 250 mL beaker) and record the sample weight. Without zeroing the balance, add water to make approximately 100 g. Record the sample + water weight. Place a stir bar in the mixture and stir on a stir plate to form a homogenous slurry/suspension.

While stirring, weigh 5–10 g of the slurry/suspension into an appropriate digestion vessel, add approximately 10 mL water, then proceed with the addition of the KOH as stated below.

For the testing of other matrixes, visually evaluate the sample for homogeneity before weighing an appropriate amount. If a sample does not appear to be homogenous, perform additional homogenizing (i.e., blending, grinding, etc.). If applicable, the sample may be reconstituted.

Accurately weigh or aliquot an appropriate amount (0.2500 to 2.50 g or 0.50 to 10 mL) of sample into a labeled 100 mL digestion vessel. Add 20 mL purified water to the vessel.

Accurately weigh an appropriate amount (0.2500 to 1.00 g) of an appropriate CRM, i.e., National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1549 or 3280, if applicable, in the same manner as the samples. SRM 1549 may be digested using either 5 or 50% KOH solution. SRM 3280 should be digested using only the 50% KOH solution.

Designate at least one digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples. If both the 5 and 50% KOH solutions will be used, prepare at least one blank with each concentration.

Place an aliquot of spiking solution (if applicable) into an appropriately labeled digestion vessel.

Add either 10 mL 5% KOH solution or 10 mL 50% KOH solution to each digestion vessel. Use the guideline previously mentioned for guidance on which solution to use. *Note:* If values well below 10 000 µg/kg are anticipated, add 5 mL 5% KOH solution, then dilute to 50 mL. 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. Digest samples in an oven set to maintain 105 ± 5°C until the dissolution of iodine is complete, approximately 1 h.

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

After removal from the oven, add 2 mL stabilizer concentrate, then allow the samples to cool before bringing to volume with purified water. Alternatively, allow samples to cool first, then add 2 mL stabilizer concentrate and bring to volume with purified water. *Note:* If the final volume will be 50 mL, add 1 mL stabilizer concentrate. Cap the vessels, then invert to mix thoroughly.

Filter the sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, then filter an adequate amount (i.e., several milliliters) into appropriate vessel (i.e., 15 mL PP centrifuge tube) to be used for analysis. Store samples 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.

(**c**) *Open vessel microwave digestion (optional)*.—*Note*: Use only 50 mL disposable PP centrifuge tubes. The following procedure is written for a final volume of 50 mL. It is critical that the final reagent concentration in all vessels be equivalent to that of the calibration standards. Follow the first several steps as outlined above in *Oven Digestion (preferred)*, then proceed as directed below. *Note*: For reconstitution of samples, prepare as outlined in *Oven Digestion (preferred)*, but weigh 5 g (instead of 5–10 g) of the slurry/suspension, and do not add additional water. Proceed with the addition of KOH as described below.

Accurately weigh or aliquot an appropriate amount (0.2500 to 1.00 g or 0.50 to 2 mL) of sample into a labeled microwave digestion vessel already containing 5 mL purified water.

Accurately weigh an appropriate amount (0.2500 to 1.00 g) of an appropriate CRM (i.e., NIST SRM 1549 or 3280), if applicable, in the same manner as the samples. SRM 1549 may be digested using either 5 or 50% KOH solution. SRM 3280 should be digested using only the 50% KOH solution.

Designate at least one digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples. If both the 5 and 50% KOH solutions will be used, prepare at least one blank with each concentration. Place an aliquot of spiking solution (if applicable) into an appropriately labeled microwave digestion vessel. Add either 5 mL 5% KOH solution or 5 mL 50% KOH solution to each digestion vessel. Use the guidelines previously described under *Oven digestion (preferred)* on which solution to use. *Note*: If values well below 500 µg/kg are anticipated, add 5 mL of 5% KOH solution.

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. 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 until the dissolution of iodine is complete. *Note*: The vessel caps should be loosened slightly (from fully tightened) during the digestion procedure.

After removal from the oven, add 1 mL stabilizer concentrate, then allow the samples to cool before bringing to volume with purified water. Alternatively, allow sample to cool first, then add 1 mL stabilizer concentrate and bring to volume with purified water. Cap the vessels, then invert to mix thoroughly.

Filter the sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, then filter an adequate amount (i.e., several milliliters) into appropriate vessel (i.e., 15 mL PP centrifuge tube) to be used for analysis. Store samples 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.

F. Sample Analysis

The digested samples are analyzed directly or diluted so that the iodine concentration will fall within the calibration range. Samples digested with 50% KOH solution must be diluted 1 to 10 mL to achieve the desired final concentration of 0.5% KOH. Aliquot 1 mL of the filtrate into an appropriate vessel (i.e., 15 mL PP centrifuge tube), add 0.18 mL stabilizer concentrate, then dilute to 10 mL with purified water. *Note*: If samples digested with 50% KOH solution

Table 2012.15. Isotope selection and interferences

Analyte	Mass, amu	Corrections	Potential interferences
Iodine	126.900	NA	None listed
Praseodymium	140.907	NA	None listed
Samarium	146.915	+1*Sm149	None listed
Rhodium	102.906	NA	None listed
Tellurium	146.915	NA	None listed

need more than a 1 to 10 mL dilution to obtain a reading on the calibration curve, an additional dilution must be prepared from the original 1 to 10 mL dilution. Aliquot the desired amount into an appropriate vessel (i.e., 15 or 50 mL PP centrifuge tube), then dilute to volume with diluent.

Samples digested with 5% KOH solution may be diluted (if necessary) by placing an aliquot of the filtrate into an appropriate vessel (i.e., 15 mL PP centrifuge tube), then diluted to an appropriate volume with diluent.

Condition the ICP-MS sample introduction system. Analyze conditioning solution while concomitantly introducing internal standard solution on-line through a mixing block until conditioned (approximately 1 h). The internal standard 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 internal standard. Analyze samples using ICP-MS.

G. Instrument and Parameters

Instrument.—ICP-MS PerkinElmer ELAN DRC II, or equivalent.

Gas.—Argon (high purity).

Rinse.—0.1% Triton/1% NH_4OH in purified water.

Sweeps/readings.—20.

Readings/replicate.—1.

Replicates.—3.

Nebulizer gas flow.—Optimized daily.

Auxiliary gas flow.—1.2 L/min.

Plasma gas flow.—15.00 L/min.

Lens voltage.—Optimized daily.

ICP radio frequency power.—1500 watts.

Isotope selection and interferences.—See Table **2012.15**.

Potential interferences are available for consideration from within the ELAN® instrument software once an analyte is selected. For the isotopes chosen, no potential interferences were listed. The results presented in this paper were obtained by using praseodymium as the internal standard. The other internal standards listed above were evaluated during method development; however, praseodymium was chosen over the others because it produced results closer to expected values (i.e., NIST reference materials).

Chemical and/or physical interferences from matrixes containing high levels of salt may create suppression or enhancement of signal resulting in erroneous values. Ruggedness of this method, with respect to high salt matrixes, was demonstrated by analyzing sodium chloride.

References: *J. AOAC Int*. **95**, 195(2012); (future issue)