Determination of Total Iodine in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS): A Collaborative Study of AOAC Official First Action Method 2012.15

Final Report

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Abstract

A collaborative study was conducted to determine total iodine in infant formula and adult/pediatric nutritional formula by inductively coupled plasma - mass spectrometry (ICP-MS). The method determined total “iodide” and may be referred to as “iodine” throughout this report.

Result Summary

The results demonstrate that the method is fit-for-purpose to determine iodine in infant formula and adult/pediatric nutritional formula and the Study Director recommends that it be adopted Final Action Official Method status.

Introduction

The AOAC INTERNATIONAL Official Method 2012.15 entitled, “Determination of Total Iodine in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS),” was selected by the AOAC Expert Review Panel (ERP) as the most appropriate method for the determination of total iodine in infant formula and adult/pediatric nutritional formula. The single laboratory validation (SLV) and the multi-laboratory testing (MLT) of this method have been completed.

Collaborative Study

Purpose

The purpose of this study was to evaluate the method’s intra-laboratory and inter-laboratory performance and submit the results to the AOAC INTERNATIONAL for adoption as an Official Method for the determination of total iodine in infant formula and adult/pediatric nutritional formula.

Study Design

This study evaluated the method performance for determination of total iodine in infant formula and adult/pediatric nutritional formula in seven fortified test materials, one of which is National Institute of Standards & Technology (NIST) Standard Reference Material (SRM) 1849a. Random identification numbers were assigned to each of the seven fortified test materials.
**Test Sample Preparation**

Test samples used in this study were obtained from commercial sources and provided by AOAC INTERNATIONAL.

Upon successful completion of two qualification samples, individually prepared test kits including seven test samples and their blind duplicates were provided to each collaborator. All powdered samples, with the exception of NIST SRM 1849a1, were required to be analyzed on a reconstituted basis where approximately 25 grams of material was diluted with approximately 200 grams of de-ionized water resulting in a total weight of approximately 225 grams. Once the test sample was in solution and well mixed, an accurately weighed aliquot of approximately 6 or 12 grams (depending on final transfer volume) was sub-sampled (while continuously stirring) for analysis. This reconstituted solution was discarded after 24 hours. Approximately 0.5 or 1 gram (depending on final transfer volume) of the NIST SRM 1849a was weighed for analysis. For ready to feed (RTF) samples, the laboratory weighed approximately 1 or 2 grams (depending on final transfer volume) for analysis. The remaining RTF solutions were transferred to a sealed, brown polypropylene container and held at refrigerated conditions between 2°C to 8°C. These solutions were discarded after five days.

The test samples were shipped at ambient temperature. Collaborators were asked to store the samples at room temperature before and during analysis with the exception of the RTF samples, which were refrigerated after the initial sampling.

Bulk standards were to be stored as directed on the certificate of analysis/receipt paperwork. Laboratories were directed to follow instructions in the method for storage and shelf-life of solutions.

**Standards**

The following reference and internal standards (or equivalent materials may be substituted) were purchased by each collaborator:

**Reference standards**
- Iodide 1,000 ppm standard solution in H2O, (SPEX CertiPrep, Metuchen, NJ)
- Iodide 1,000 ppm standard solution in 1% TEA, (Inorganic Ventures, Christiansburg, VA)

Note:Either may be used for calibration standard solutions preparation. The remaining source may be used as a continuing calibration verification (CCV) standard.

**Internal standard**
- Praseodymium 10 ppm standard solution in 5% HNO3, (Inorganic Ventures, Christiansburg, VA)
**Laboratory Qualification**
Two qualification samples were provided. These qualification samples were used to optimize each participant’s instrument and ensure iodine free reagents/chemicals. Before proceeding with the full collaborative study, each participant’s qualification results were sent to and approved by the study director.

**Data Reporting**
Participants were asked to record all observations and any potential method deviations. In addition they were to investigate any potential aberrant results, for example incorrect calculations, use of wrong units, transposition errors, incorrect standard preparation or contamination. It was also a requirement to have all the results and calculations reviewed by a peer, laboratory supervisor or manager.

**Data Analysis**
The Study Director reviewed and compiled all the data submitted by the participants. Statistical analysis was conducted using the AOAC spreadsheet for blind duplicates [1]. The following tests were utilized in the AOAC spreadsheet for blind duplicates [1] to determine outliers: (i) Cochran test for removal of laboratories showing significantly greater variability among replicate (within-laboratory) analyses than the other laboratories for a given material and (ii) Grubbs tests for removal of laboratories with extreme averages. The spreadsheet was used to calculate the following:
- mean analyte concentrations
- standard deviation ($S_r$) relative standard deviation (RSD$_r$) for repeatability (for blind duplicate data)
- standard deviation ($S_R$) and relative standard deviation (RSD$_R$) for reproducibility
- number of valid data points
- HorRat value (RSD$_R$/predicted RSD$_R$)
AOAC Official Method 2012.15
Determination of Total Iodine in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)
First Action 2012

(Applicable to the measurement of total iodine in infant formula and adult/pediatric nutritional formula from 4 mcg to 1500 mcg/100 g reconstituted final product and for ready to feed products from 2.5 mcg to 1000 mcg/100 g using ICP-MS.)

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

C. Chemicals and Reagents
(a) KOH pellets.—Certified ACS grade (Fisher Scientific, Fairlawn, NJ).
   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).
(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. 50mL PP DigiTUBES® (Part number 010-500-261), 100 mL PP DigiTUBES® (Part number 010-501-263), SCP Science (Montreal, Canada).

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

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

(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).

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

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

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

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

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

(k) **Syringe filters with 1 μm membrane** (e.g., GMF-150 Filter Media or PTFE).

(l) **Beakers.**—Assorted sizes.

(m) **Stir bars.**—7.9 x 50 mm, VWR assorted sizes.

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

**Notes:**
- Equivalent apparatus may be substituted.
- 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. 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.**—Twenty.

(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 analysis must be performed using STD Mode. (Use of a reaction or collision gas is not required or allowed.)

F. Reference Standards

Reference standards
(a) Iodide 1,000 ppm standard solution in H₂O, (SPEX CertiPrep, Metuchen, NJ).
(b) Iodide 1,000 ppm standard solution in 1% TEA, (Inorganic Ventures, Christiansburg, VA).

Notes:

- Either stock iodide reference solutions may be used for intermediate and working standard solutions preparation. The remaining source may be used as a CCV standard.
- Equivalent reference standards may be substituted.
- “Iodide” may be referred to as “iodine” throughout this method.

Internal standards
(a) Praseodymium 10 ppm standard solution in 5% HNO₃, (Inorganic Ventures, Christiansburg, VA)

Notes:

- Individual values of iodine will be reported for each test sample using praseodymium as the internal standard. If another internal standard (e.g., tellurium) is preferred and/or typically used, results obtained using both Pr and the other internal standard must be reported.
- Equivalent stock internal standard solutions may be substituted.

G. Procedure

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.

(a) 5% KOH Solution.—Dissolve 25 g KOH pellets in an appropriate amount of 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.
(b) Stabilizer Concentrate.—Dissolve 5 g Na₂S₂O₃ in an appropriate amount of 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.
(c) 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.

(d) Diluent.—Dissolve 10 g KOH pellets and 0.4 g Na$_2$S$_2$O$_3$ in an appropriate amount of purified water, add 4 mL NH$_4$OH, 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% NH$_4$OH, and 0.02% Na$_2$S$_2$O$_3$ in purified water.

(e) 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 this solution at room temperature. Reagent expires 6 months after preparation date.

(f) 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 this solution at room temperature. Reagent expires 6 months after preparation date.

**Standard Solutions Preparation**

**Notes:**
- Stock solutions are stable until the date indicated on the certificate of analysis. Intermediate, calibration, continuing calibration verification, and internal standard 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 internal standard solutions are analyzed as prepared. **Do not** carry these solutions through sample preparation or digestion.

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

(b) Intermediate Stock Standard (ISS) iodine solutions.—Prepare the intermediate stock standard iodine solutions according to Table 2012.15A.

(c) Calibration Standard (CS) iodine solutions.—Prepare the calibration standard solutions according to Table 2012.15B.

(d) Intermediate Continuing Calibration Verification (ICCV), Continuing Calibration Verification (CCV) iodine solutions, and Continuing Calibration Blank (CCB).—Prepare the intermediate continuing calibration verification, continuing calibration verification standard solutions, and continuing calibration blank according to Table 2012.15C.

Note: A CCV must be prepared from a second source (e.g., another vendor) other than that used for the CS solutions.
(e) *Internal Standard (IS) solutions.*—Prepare the internal standard solution(s) according to Table **2012.15D**. IS concentrations typically used for analysis are 30 ppb Pr and 500 ppb Te.

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

**Reconstitution**

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

(a) Accurately weigh approximately 25 grams of 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”.

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

**Sample Preparation**

Weighing (after weighing all materials, proceed to “Addition of Reagents”)

(a) *Reconstituted Material:* Accurately weigh an aliquot of approximately 6 g of the reconstituted test sample into a 50 mL or 12 g into a 100 mL DigiTUBE®.
(b) *NIST SRM 1849a:* Accurately weigh approximately 0.5 g of the NIST SRM 1849a into a 50 mL or 1 g into a 100 mL DigiTUBE®.
(c) *Ready to Feed (RTF) Material:* Accurately weigh approximately 1 g of the RTF test sample into a 50 mL or 2 g into a 100 mL DigiTUBE®.

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

(d) *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.
Addition of Reagents (after adding all reagents and mixing, proceed to “Oven Digestion” or “Open Vessel Microwave Digestion”)

(a) Water: Add 10 mL of purified water to each 50 mL DigiTUBE® or 20 mL to each 100 mL DigiTUBE®.
(b) 5% KOH: Add 5 mL of 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®.
(c) 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.

Oven Digestion
(a) Digestion/Extraction: Digest samples in an oven set to maintain 105 ± 5°C until the dissolution of iodine is complete, approximately 1 hour.

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

(b) 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.

(c) Final Volume: If 50 mL or 100 mL vessels were used for digestion, bring samples to a final volume of 50 mL or 100 mL (respectively) with purified water.
(d) Capping/Mixing: Cap all vessels, and then invert to mix thoroughly.

Open Vessel Microwave Digestion
(a) 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 until the dissolution of iodine is complete. See Table 2012.15E for suggested open vessel microwave digestion parameters.

Note: The 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 (as melting may occur).

Alternatively: Instead of just loosening the caps, drill small holes (approx. 3 mm) in the caps. This way the caps can be tightened, but venting (thus the “open” vessel) can occur. The caps may be reused after acid washing.
(b) **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.

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

(d) **Capping/Mixing:** Cap all vessels, then invert to mix thoroughly.

**Sample Filtering**

(a) **Filtering:** Filter each sample solution by filling a disposable syringe with the digested sample solution, attach a 1 μm membrane filter, then filter an adequate amount (e.g., at least 5 mL) into 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 hour) 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.

**Sample Dilution**

(a) **Diluting:** 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 mL to 10 mL as directed above.

**H. Determination**

*Instrument parameters (see Appendix B, Section E).*

Notes:
- All analysis must be performed using 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 manufacturer’s minimum daily performance requirements.
Conditioning
Condition the ICP-MS sample introduction system. Analyze the Conditioning Solution while concomitantly introducing internal standard solution online (e.g., through a mixing block or T) until conditioned (approximately 1 hour). 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. Ensure the Wash Solution (rinse) is available and ready for use to rinse out the sample lines and introduction system between each analysis.

Notes:
- Background counts for both iodine and the internal standard 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., HNO$_3$) matrices 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 (to further conditioning) prior to calibration is recommended.

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 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 $\geq$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 Continuing Calibration Blank (see “Data Acceptability”).

Sample Analysis
Analyze a 5 mL to 10 mL dilution of each digested filtered sample using ICP-MS.

Notes:
- For the purpose of this collaborative study, a 5 mL to 10 mL dilution of the sample filtrates must be analyzed. (see Appendix D “Instructions to Collaborators” for additional information.)
- 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, then diluting to an appropriate final volume with Diluent.
Data Acceptability

The calibration curve must include a calibration blank (as a calibration point). The calibration curve must have a correlation coefficient (r) of $\geq 0.998$ 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 $\geq 1.5$ 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 Continuing Calibration Blank (see “Data Acceptability”).

A continuing calibration blank (CCB) is analyzed after calibration, midway through the sample analysis 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 continuing calibration verification (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, midway through the sample analysis, 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.

I. Calculations

If a reconstitution was performed, use the following (updated) formula.

$$\frac{((C \times V) \times D)}{WRA} \times 10 = S$$

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 of reconstitution aliquoted during Sample Preparation (g)
$S =$ Sample concentration of iodine (mcg/100 g reconstituted “as fed” basis)
If a reconstitution **was not** performed, use the following formula.

\[((C \times V) \times D) / W\] / 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)  
S = Sample concentration of iodine (mcg/100 g)

**References:**  
*J. AOAC Int.* **95**, 195(2012)  
*J. AOAC Int.* **96**, 493(2013)  
DOI: 10.5740/jaoacint.13-014  
AOAC SMPR 2012.008  
*J. AOAC Int.* **96**, 486(2013)  
DOI: 10.5740/jaoac.int.SMPR2011.008
Table 2012.15A. Preparation of Intermediate Stock Standard (ISS) Iodine Solutions

Notes:
- ISS solutions are used for calibration standard preparation and are typically prepared according to the table below.
- 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 5000μL, is recommended.

<table>
<thead>
<tr>
<th>Iodine Standard Solution ID</th>
<th>ID of Solution Used for Preparation</th>
<th>Initial Iodine Concentration (ng/mL)</th>
<th>Aliquot Volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Final Iodine Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 (ISS)</td>
<td>Stock</td>
<td>1,000,000</td>
<td>0.5</td>
<td>50</td>
<td>10,000</td>
</tr>
<tr>
<td>1,000 (ISS)</td>
<td>10,000 (ISS)</td>
<td>10,000</td>
<td>5</td>
<td>50</td>
<td>1,000</td>
</tr>
<tr>
<td>10.0 (ISS)</td>
<td>1,000 (ISS)</td>
<td>1,000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
</tbody>
</table>

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.
Table 2012.15B. Preparation of Calibration Standard (CS) Iodine and Calibration Blank (CB) Solutions

Notes:

- 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 below. The CB is the zero point of the curve. The curve type used, if using a PerkinElmer ICP-MS with ELAN software, should be linear through zero. If using an Agilent or Thermo ICP-MS, 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.
- NA=Not applicable.

<table>
<thead>
<tr>
<th>Iodine Standard Solution ID</th>
<th>ID of Solution Used for Preparation</th>
<th>Initial Iodine Concentration (ng/mL)</th>
<th>Aliquot Volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Final Iodine Concentration (ng/mL)</th>
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<tbody>
<tr>
<td>100 (CS)</td>
<td>1,000 (ISS)</td>
<td>1,000</td>
<td>5</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>50.0 (CS)</td>
<td>1,000 (ISS)</td>
<td>1,000</td>
<td>2.5</td>
<td>50</td>
<td>50.0</td>
</tr>
<tr>
<td>10.0 (CS)</td>
<td>1,000 (ISS)</td>
<td>1,000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
<tr>
<td>1.00 (CS)</td>
<td>10.0 (ISS)</td>
<td>10.0</td>
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<td>50</td>
<td>1.00</td>
</tr>
<tr>
<td>0.500 (CS)</td>
<td>10.0 (ISS)</td>
<td>10.0</td>
<td>2.5</td>
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<td>0.500</td>
</tr>
<tr>
<td>0.250 (CS)</td>
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<td>10.0</td>
<td>1.25</td>
<td>50</td>
<td>0.250</td>
</tr>
<tr>
<td>Blank (CB)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

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, and approximately 0.2% NH₄OH and approximately 0.02% Na₂S₂O₃ in water.
Table 2012.15C. Preparation of Intermediate Continuing Calibration Verification (ICCV), Continuing Verification (CCV) Iodine Solutions, and Continuing Calibration Blank (CCB) Solution.

Notes:
- ICCV solutions are used for preparation of the CCV standard solution and are typically prepared according to the table below.
- 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.
- NA=Not applicable.

<table>
<thead>
<tr>
<th>Iodine Standard Solution ID</th>
<th>ID of Solution Used for Preparation</th>
<th>Initial Iodine Concentration (ng/mL)</th>
<th>Aliquot Volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Final Iodine Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 (ICCV)</td>
<td>Stock</td>
<td>1,000,000</td>
<td>0.5</td>
<td>50</td>
<td>10,000</td>
</tr>
<tr>
<td>1,000 (ICCV)</td>
<td>10,000 (ICCV)</td>
<td>10,000</td>
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<td>50</td>
<td>1,000</td>
</tr>
<tr>
<td>10.0 (CCV)</td>
<td>1,000 (ICCV)</td>
<td>1,000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
<tr>
<td>Blank (CCB)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

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.

Table 2012.15D. Preparation of Internal Standard (IS) Solution

Note:
- IS concentrations typically used for analysis are 30 ppb Pr and 500 ppb Te. The table below outlines a typical preparation scheme.

<table>
<thead>
<tr>
<th>Standard Solution ID</th>
<th>ID of Solution Used for Preparation</th>
<th>Initial Concentration (ng/mL)</th>
<th>Aliquot Volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Final Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0 (Pr)</td>
<td>Stock</td>
<td>10,000</td>
<td>1.5</td>
<td>500*</td>
<td>30.0</td>
</tr>
<tr>
<td>500 (Te)</td>
<td>Stock</td>
<td>10,000</td>
<td>25</td>
<td>500*</td>
<td>500</td>
</tr>
</tbody>
</table>

* After aliquoting the 10,000 ppb Pr and 10,000 ppb Te into the same 500 mL vessel, add approximately 100 mL of water, 10 mL of HNO₃, 0.5 mL of HClO₄, 0.05 g of 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 2012.15E. Open Vessel Microwave Digestion Parameters
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 Iodine 2012.15, the following 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.

### Six - 50 mL vessels:

<table>
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<tr>
<th>Wattage</th>
<th>Power</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>10%</td>
<td>5</td>
</tr>
<tr>
<td>400</td>
<td>20%</td>
<td>6</td>
</tr>
<tr>
<td>400</td>
<td>20%</td>
<td>7</td>
</tr>
</tbody>
</table>

### 12 to 18 - 50 mL vessels:

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<th>Wattage</th>
<th>Power</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
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<tr>
<td>400</td>
<td>40%</td>
<td>10</td>
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</table>

### 24 - 50 mL vessels:

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<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>25%</td>
<td>10</td>
</tr>
<tr>
<td>400</td>
<td>40%</td>
<td>10</td>
</tr>
<tr>
<td>400</td>
<td>65%</td>
<td>10</td>
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Results and Discussion

Laboratory Qualification Phase

Invitations to participate in the Collaborative Study of AOAC Official First Action Method 2012.15 were sent to thirty eight laboratories. Twenty four expressed interest in participating. Qualification samples were sent to twenty laboratories after four laboratories decided not to participate (i.e., too busy). Six labs did not meet acceptance criteria. The resulting fourteen laboratories went on to analyze seven test samples. Note: Thirteen laboratories submitted test sample data.

Results

MLT Results

Table 1.1 Laboratory Results

<table>
<thead>
<tr>
<th>NIST SRM 1849a</th>
<th>Adult Nutritional RTF High Fat</th>
<th>Infant Formula Powder Soy Based</th>
<th>Infant Formula Powder Milk Based</th>
<th>Adult Nutritional RTF High Protein</th>
<th>Child Formula Powder</th>
<th>Adult Nutritional Powder Low Fat</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VJKY373</td>
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<td></td>
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<td></td>
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</tr>
<tr>
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<tr>
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<tr>
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<th>2</th>
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</table>

<table>
<thead>
<tr>
<th>Lab</th>
<th>Iodine Results (mg/kg)</th>
<th>Iodine Results (mcg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.19 1.17</td>
<td>5.32 4.92</td>
</tr>
<tr>
<td>B</td>
<td>1.25 1.24</td>
<td>5.43 5.45</td>
</tr>
<tr>
<td>C</td>
<td>1.10 1.10</td>
<td>4.95 4.33</td>
</tr>
<tr>
<td>D</td>
<td>1.17 1.16</td>
<td>5.12 4.83</td>
</tr>
<tr>
<td>E*</td>
<td>1.29 1.30</td>
<td>6.18 6.15</td>
</tr>
<tr>
<td>F</td>
<td>1.25 1.11</td>
<td>5.20 4.83</td>
</tr>
<tr>
<td>G</td>
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<td>6.14 6.07</td>
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<td>5.54 4.92</td>
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<td>K</td>
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<td>L</td>
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<td>5.95 5.29</td>
</tr>
<tr>
<td>M</td>
<td>1.25 1.27</td>
<td>5.87 5.61</td>
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</tbody>
</table>

* - Reconstituted powder data not included for statistical analysis. See Discussion.

a - NIST SRM 1849a results presented as mg/kg
b - mcg/100 g reconstituted final product

2012.15_IODINE MLT
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Table 1.2 Laboratory Results with Outliers Removed

<table>
<thead>
<tr>
<th>NIST SRM 1849a</th>
<th>Adult Nutritional RTF High Fat</th>
<th>Infant Formula Powder Soy Based</th>
<th>Infant Formula Powder Milk Based</th>
<th>Adult Nutritional RTF High Protein</th>
<th>Child Formula Powder</th>
<th>Adult Nutritional Powder Low Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>EKVJ578</td>
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<td>XKIP216</td>
<td>MGN284</td>
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<table>
<thead>
<tr>
<th>Lab</th>
<th>Iodine Results (mg/kg)</th>
<th>Iodine Results (mcg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.19</td>
<td>1.17</td>
</tr>
<tr>
<td>B</td>
<td>1.25</td>
<td>1.24</td>
</tr>
<tr>
<td>C</td>
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<td>1.16</td>
</tr>
<tr>
<td>E*</td>
<td>1.29</td>
<td>1.30</td>
</tr>
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<td>F</td>
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<td>1.28</td>
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<tr>
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<td>1.31</td>
</tr>
<tr>
<td>K</td>
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<tr>
<td>L</td>
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<td>1.20</td>
</tr>
<tr>
<td>M</td>
<td>1.25</td>
<td>1.27</td>
</tr>
</tbody>
</table>

* - Reconstituted powder data not included for statistical analysis. See Discussion.

\(a\) - NIST SRM 1849a results presented as mg/kg

\(b\) - mcg/100 g reconstituted final product
# Table 1.3 Statistical Data

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Average</th>
<th>S&lt;sub&gt;r&lt;/sub&gt;</th>
<th>RSD&lt;sub&gt;r&lt;/sub&gt;</th>
<th>S&lt;sub&gt;R&lt;/sub&gt;</th>
<th>RSD&lt;sub&gt;R&lt;/sub&gt;</th>
<th>No. of outlier labs&lt;sup&gt;c&lt;/sup&gt;</th>
<th>HorRat</th>
<th>No. of labs used</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST SRM 1849a&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24</td>
<td>0.029</td>
<td>2.34</td>
<td>0.070</td>
<td>5.63</td>
<td>0</td>
<td>0.36</td>
<td>13</td>
</tr>
<tr>
<td>Adult Nutritional RTF, High Fat&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.48</td>
<td>0.262</td>
<td>4.78</td>
<td>0.507</td>
<td>9.25</td>
<td>0</td>
<td>0.37</td>
<td>13</td>
</tr>
<tr>
<td>Infant Formula Powder, Soy Based&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.4</td>
<td>0.31</td>
<td>2.53</td>
<td>0.94</td>
<td>7.62</td>
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<td>0.35</td>
<td>11</td>
</tr>
<tr>
<td>Infant Formula Powder, Milk Based&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.5</td>
<td>0.69</td>
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<td>1.39</td>
<td>7.54</td>
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<td>0.37</td>
<td>11</td>
</tr>
<tr>
<td>Adult Nutritional RTF, High Protein&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.45</td>
<td>0.226</td>
<td>4.16</td>
<td>0.626</td>
<td>11.5</td>
<td>0</td>
<td>0.46</td>
<td>13</td>
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<tr>
<td>Child Formula Powder&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.47</td>
<td>0.135</td>
<td>3.87</td>
<td>0.278</td>
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<td>11</td>
</tr>
<tr>
<td>Adult Nutritional Powder, Low Fat&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.03</td>
<td>0.137</td>
<td>1.94</td>
<td>0.503</td>
<td>7.15</td>
<td>2</td>
<td>0.30</td>
<td>11</td>
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</table>

S<sub>r</sub> = Standard deviation for repeatability.
RSD<sub>r</sub> = Relative standard deviation for repeatability.
S<sub>R</sub> = Standard deviation for reproducibility.
RSD<sub>R</sub> = Standard deviation for reproducibility.

<sup>a</sup> - Results expressed as mg/kg.
<sup>b</sup> - Results expressed as mcg/100 g reconstituted final product.
<sup>c</sup> - Values from outlier laboratories were not used in statistical calculations.
**Discussion**

Seven samples were analyzed by thirteen independent laboratories. These laboratories were from industry, contract research organizations and government institutions. Laboratories were located in North America, Europe, and Asia. The seven samples for the collaborative study were selected to represent varying levels of iodine in a variety of applicable matrices. The matrices include a standard reference material, an adult nutritional high fat RTF, an adult nutritional high protein RTF, a child powder formula, an adult nutritional low fat powder, soy based infant formula powder, and milk based infant formula powder.

All of the laboratories’ results are presented in Table 1.1. Table 1.2 presents the results with outliers removed. Table 1.3 shows the statistical evaluations for all the samples analyzed in this MLT study. The RSDr ranged from 1.94 - 4.78% and the RSDr ranged from 5.63 - 11.5%. The HorRat values for all results were excellent, ranging from 0.30 - 0.46. Results for all seven of the samples met all of the Standard Method Performance Requirements (SMPR) guidelines. All thirteen laboratories’ data was included for statistical analysis for the NIST SRM 1849a and both RTF samples. Outliers for the powdered reconstituted samples were removed prior to performing statistical analysis based on the Dixon’s Outlier Test. Comparison of the outlier data suggests a calculation error related to the reconstitution.

The overall results demonstrate that the method is fit-for-purpose to determine iodine in infant formula and adult/pediatric nutritional formula and the Study Director recommends that it be adopted Official Final Action.

**Recommendations**

It is the recommendation of the Study Director the method is fit-for-purpose in determining total iodine in infant formula and adult/pediatric nutritional formula by inductively coupled plasma - mass spectrometry (ICP-MS) and that it be adopted as an AOAC Final Action Official Method status.
Additional Information
Note: Shows the technique used for sample digestion and the make/model of the instrument used for analysis.

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<thead>
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<th>Laboratory Code</th>
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<th>Instrument</th>
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<td>Yes</td>
<td>No</td>
<td>Thermo iCAP Q</td>
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</tbody>
</table>

Collaborator’s Comments
Note: Study Director responses are italicized.

Laboratory A
1) % RSD was < 5% for all samples and CCVs. % RSD for CCBs was > 5%. Study Director reviewed/approved – no impact to data.
2) Samples 1-7 were digested on 12/18/14 and analyzed on 12/19/14. Samples 8-14 were digested and analyzed on 12/19/14.

Laboratory B
1) The Digested Blank result is 0.086 ng/ml, slightly higher than 0.075 ng/mL. Study Director reviewed/approved – no impact to data.
2) Samples were digested on 05Jan15 afternoon, dilution was conducted on 06Jan15, and injected on 06Jan15 at about 10am.

Laboratory C
1) No comments provided.
Laboratory D

1) 17-50 days between digestion and analysis (*response provided when asked if samples digested on the same day as analysis*) Study Director reviewed/approved – no impact to data.

Laboratory E

1) No comments provided.

Laboratory F

1) The samples were originally digested and tested on 12/30/2014 but was repeated on 1/6/2015 due to recalibration.
2) 55 ml Digestion Vessels were used instead 50 ml Digitubes. The digestion vessels were sealed and digested in our microwave. Study Director reviewed/approved – no impact to data.
3) The samples were then transferred to a final volume of 50 ml in another container. 0.25 micron syringe filters were used instead of 1 micron syringe filters. Study Director Note: This deviation from protocol reviewed and approved - no impact to data.

Laboratory G

1) Less than 24 hours (*response provided when asked if samples digested on the same day as analysis*)

Laboratory H

1) No comments provided.

Laboratory I

1) In the method, the CS standard concentrations are 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb, but in our laboratory we added 5.00 ppb and deleted 100 ppb working standard in the preparation of calibration curve. Study Director (SD) Note 1: The curve did not appear to be forced through the blank as stated in the protocol. SD reviewed/approved - no impact to data. Note 2: Dropping the top calibration standard and adding a 5.00 ppb curve point. SD reviewed/approved - no impact to data.
Laboratory J
1) Lowest curve point was 0.282, higher than 0.275 allowed, but continued since it was as close as could be obtained for that point. Study Director reviewed/approved – no impact to data.
2) Digested on 1-9-15, analyzed 1-13-15
3) After not performing the method for a long period of time (two months), the instrument was plagued by poor RSDs and internal standard drift and spikes. It seems that by repeatedly running samples through it, it was able to condition itself to finally test samples.
4) Study Director observation during review of data: Low calibration standard was 113% of theoretical. All other instrument back calculated curve point concentrations were 90-110%. The last CCB (at end of run) was -0.081 (ideally no lower than -0.075 ng/mL). Study Director reviewed/approved – no impact to data.

Laboratory K
1) The samples were digested late afternoon on Jan. 14, and analyzed early morning on Jan. 15. They were held less than 24 h.

Laboratory L
1) GEUH577, Just One Injection RSD exceeded 5% (regarding internal standard RSD associated with this sample) Study Director reviewed/approved – no impact to data.
2) Study Director observation during review of data: Low calibration standard was 81.1% of theoretical. All other instrument back calculated curve point concentrations were 90-110% of theoretical. The mid-run CCB was 0.078 (ideally no higher than 0.0750 ng/mL). The last CCV (at end of run) was 8.84 ppb (ideally no lower than 9.00 ng/mL). All other QC during run was acceptable. Study Director reviewed/approved – no impact to data.

Laboratory M
1) Individual 0.25 mg/L was 84% (of theoretical for the low calibration standard) Study Director reviewed/approved – no impact to data.
2) Study Director observation during review of the data: The low calibration standard was 83.3% of theoretical. All other instrument back calculated concentrations were 90-110% of theoretical. Study Director reviewed/approved – no impact to data.
**Author's Comments**

The following provides suggested clarifications to include in the final action method.

1) When performing oven digestion, swirl the vessels approximately half way through digestion.

2) Even though it is currently not stated in the method, include a statement that “protecting standard and sample solutions from light is not necessary”.

3) After aliquoting sample filtrate for a dilution, bring to volume by accurately aliquoting the Diluent (i.e., if using centrifuge tubes).

4) Include a cautionary statement about the low end of the calibration curve and stress the importance of acceptable instrument back calculated concentrations.

5) Emphasize the importance of achieving and maintaining the lowest background possible (i.e., ensuring iodine free chemicals and reagents).

6) Include a cautionary statement about the contribution of iodine from FDA Red Dye # 3 (erythrosine). Although considered to be non-bioavailable, the iodine from this source will also quantitatively be determined by this method.

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AOAC SPIFAN Single Laboratory Validation for Iodine Analysis in Infant Formula and Adult Nutritionals, Covance Laboratories, Inc., February 2013.