

# Determination of Iodide and Iodate in Soy- and Milk-Based Infant Formulas

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## Key Words

Ion Chromatography (IC), Pulsed Amperometric Detection (PAD), Silver Working Electrode, Nutrition, Thiocyanate

## Introduction

Iodine is an important micronutrient essential for the production of thyroid hormones that are involved in the regulation of many key biochemical reactions.<sup>1</sup> Iodine deficiency can lead to varying degrees of growth and developmental abnormalities in children and adults, including such illnesses as goiter and cretinism.<sup>2</sup> However, an excess of iodine can also lead to thyroid disorders, especially in infants.<sup>3</sup> As iodine is primarily absorbed from our diet, supplementation of iodine in food is a common practice.<sup>4</sup> The concentration of iodine in iodine-fortified foods is often regulated and monitored.

Infant formula is the most highly regulated consumer food product on the market today. The Infant Formula Act of 1980 specifies minimum and maximum amounts of several nutrients, and authorizes the U.S. FDA to create and enforce standards for commercial infant formulas. Based on the recommended dietary intake of iodine, infant formulas should have a minimum iodine concentration of 5 µg/100 kcal and no more than 75 µg/100 kcal.<sup>5,6</sup>

Accurate measurement of iodine in food matrices requires: (i) a robust iodine extraction method; and (ii) a sensitive analytical method for iodine quantification. Analytical methods that have been used for determining iodine in food matrices include: colorimetry based on catalytic reactions,<sup>7</sup> gas chromatography,<sup>8</sup> an ion-selective electrode method,<sup>9</sup> x-ray fluorescence spectrometry,<sup>10</sup> inductively coupled plasma-mass spectrometry/optical emission spectrometry (ICP-MS/OES),<sup>9,11,12</sup> radiochemical neutron activation analysis,<sup>13</sup> IC with UV detection,<sup>14</sup> electrochemical detection<sup>15-17</sup>, cathode stripping voltammetry,<sup>18</sup> and flame atomic absorption spectrometry.<sup>19</sup>



A few of these have been adopted as official methods: (i) AOAC Method 992.24, Iodine in Ready-to-Feed Milk-Based Infant Formula—Ion-Selective Electrode Method;<sup>20</sup> (ii) LMBG 49.00-6, the German Food Law's method for total iodine by ICP-MS (also sanctioned as the British-adopted European Standard for Determination of Iodine by ICP-MS as BS EN15111:2007);<sup>22</sup> and (iii) Determination of Iodide Content in Milk and Dried Milk Method Using High Performance Liquid Chromatography, BS ISO 14378:2000.

A number of iodine extraction methods have also been developed for milk and milk-based products—microwave digestion in open or closed vessels with perchloric acid/nitric acid/tetramethyl ammonium hydroxide, oxygen combustion, alkaline extraction, acid digestion (hydrochloric acid/acetic acid), precipitation with methanol/acetonitrile, and ultracentrifugation.<sup>14,15,22,24</sup> Please note that methods using perchloric acid and/or pressurized chambers can pose a safety risk.

Currently there is an initiative sponsored by the International Formula Council and AOAC International to evaluate methods in order to establish a standard method(s). A standard method should be capable of determining total iodine in all forms of infant, adult, and/or pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates).<sup>25</sup>

This application note includes the acetic acid digestion method for iodide extraction, coupled with an IC-PAD method for iodide detection first developed in an archived version of this application note. The IC method coupled with electrochemical detection allows for selective and sensitive determination of iodide in complex matrices.<sup>16,17</sup> The acid digestion procedure to extract iodide was optimized for milk- and soy-based infant formulas. In addition, sample preparation conditions to convert iodate to iodide for determining total iodine (i.e., iodide and iodate) are presented. This method is shown to be fast, robust, and sensitive.

## Goal

To create an IC-PAD-based method for determining iodide and iodate in soy- and milk-based infant formulas

## Experimental

### Equipment

- Thermo Scientific™ Dionex™ ICS-5000 IC system\* including:
  - Gradient Pump
  - DC Detector Chromatography Compartment
  - ED Electrochemical Detector without Cell (P/N 079830)
  - ED Electrode, Ag, with Gasket and Polishing Kit (P/N 079856)
  - Ag/AgCl Reference Electrode (P/N 061879)
  - AS or AS-AP Autosampler
- Thermo Scientific Dionex Chromeleon™ Chromatography Data System software

\*A Thermo Scientific™ Dionex™ ICS-5000+ IC system may also be used.

### Consumables

- Thermo Scientific Dionex OnGuard™ II RP Cartridges, 2.5 cc (P/N 057084)
- EO Eluent Organizer with two 2 L Plastic Bottles and Pressure Regulator
- Vial Kit, 0.3 mL Polyprop with Caps and Septa (P/N 055428)
- Micro Tubes 1.5 mL, Type D, without skirted base, screw cap assembled, sterile (Sarstedt™ P/N 72.692.005 or equivalent)
- Thermo Scientific Nalgene™ Narrow-Mouth Bottle, HDPE/PP, 1000 mL (P/N 2002-0032)
- Nalgene Polystyrene Lab Filter Unit, 500 mL Upper Capacity with 1000 mL Receiver Capacity 0.2 Micron, 75 mm Membrane Diameter (P/N 154-0020)

## Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better filtered through a 0.2 μm filter immediately before use
- Nitric Acid, 69.0-70.0% (J.T. Baker® P/N 9601-05)
- Sodium Iodide (Crystalline/Certified), (Fisher Scientific P/N S324)
- Sodium Iodate (Sigma-Aldrich® P/N S-4007)
- Ascorbic Acid (Powder/USP/FCC), (Fisher Scientific P/N A62-500)
- Acetic Acid, Glacial (J.T. Baker P/N 9515-03)

## Conditions

### Method

Columns:	Thermo Scientific Dionex IonPac™ AG11 Guard, 4 × 50 mm (P/N 44078) Dionex IonPac AS11 Analytical, 4 × 250 mm (P/N 44076)
Flow Rate:	1.5 mL/min
Injection Volume:	100 μL
Column Temp:	30 °C
Backpressure:	1000 psi
Flush Volume:	1000 μL
Detection:	PAD
Cell Temp:	30 °C
Background:	2–10 nC
Working Electrode:	Silver working electrode
Reference Electrode:	
Mode:	Ag/AgCl mode
Noise:	3–5 pC

### Silver IPAD Waveform

Time (s)	Potential (V)	Gain Region*	Ramp*	Integration
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.90	+0.1	Off	On	Off
0.93	–0.8	Off	On	Off
1.00	–0.3	Off	On	Off

\*Settings required in the Dionex ICS-3000/5000, but not used in older Dionex systems.

## Preparation of Solutions and Reagents

### 50 mM Nitric Acid

Add 3.125 mL of nitric acid to approximately 500 mL of degassed 18 MΩ-cm DI water in a 1 L volumetric flask. Dilute to the mark with degassed water.

### Iodide Standards

Prepare a 1000 mg/L standard by dissolving 1.31 g of potassium iodide in 1000 mL of DI water. This primary standard was used to prepare a 10 mg/L secondary standard, which was appropriately diluted for linearity studies. Both the primary and secondary standards were stored frozen. Because iodide is sensitive to light, minimize its exposure to light. Use all standards prepared from the 10 mg/L stock solution on the day they are prepared.

### Electrode Preparation

Polish the silver electrode with the white fine polishing compound. Rinse the electrode well with DI water and wipe with a damp paper towel. After this initial polish, only polish the electrode if it becomes discolored or if it has not been used for a month or longer.

### Sample Preparation

Five commercial infant formula samples were selected for testing. Four of the infant formulas (Brands 1–4) were milk based, and one (Brand 5) was soy based. Four of the samples (Brands 2–5) were labeled to indicate they used potassium iodide as a source of iodine.

### Dionex OnGuard II RP Cartridge Preparation

Pass 5 mL of methanol, followed by 10 mL of DI water, through the cartridge at a maximum flow rate of 4 mL/min. To save time, up to 12 cartridges can be prepared at one time using the Dionex OnGuard Sample Prep Workstation (P/N 039599).

### Infant Formula Sample Preparation

Prepare the infant formula as suggested for feeding. Pipet 10 mL of infant formula into a 50 mL centrifuge tube. Add 2 mL of 3% acetic acid and mix. Add 8 mL of DI water and mix. Centrifuge samples at 5000 rpm for 5 min to separate fats and proteins suspended in the sample. Pass the sample through a 0.2 µm syringe filter. Carefully pour the acid hydrolyzed sample into the syringe filter, and leave the precipitated proteins in the digestion tube. Pass 5 mL of sample through the Dionex OnGuard II RP Cartridge at 4 mL/min, discarding the first 3 mL of sample. Collect the remaining filtrate and inject an aliquot. Do not use an aliquot if it is cloudy.

For some samples, heating during acid digestion was found to be beneficial for iodide extraction. For these samples, incubate the centrifuge tubes in a water bath for 1 h at 70 °C. Allow the samples to cool to room temperature before centrifugation and subsequent sample preparation steps.

Iodide extraction can also be done by microwave digestion with acetic acid. Typical microwave digestion operating parameters are as follows:

100% Power (W)	1200
Ramp to Temperature (min)	15
Hold Time (min)	15
Temperature (° C)	100
Cool Down (min)	15

Following microwave digestion, allow the samples to cool to room temperature before centrifugation and subsequent sample preparation steps.

### Conversion of Iodate to Iodide

After acid hydrolysis, heat-assisted acid hydrolysis, or microwave heat-assisted acid hydrolysis and subsequent cooling, the iodate in samples can be converted to iodide. For this conversion, add 1 mL of ascorbic acid (5 g/L) and only 7 mL of DI water prior to centrifugation. Based on the iodate recovery (as iodide) with varying amounts (0.5–3 mL) of ascorbic acid (5 g/L), 1 mL was found to be optimum (data not shown). This is similar to recent reports of iodine determination in infant formula cereals.<sup>26</sup>

### Precautions

The Dionex IonPac AS11 column is packed with sodium hydroxide solution; therefore flush it with water for at least 30 min before equilibrating with the nitric acid eluent. If there is a decrease in iodide retention time and peak efficiency, wash the column with a stronger nitric acid eluent. The AS11 column is stable in the 0–14 pH range, and a strong base can also be used for column cleaning. Disconnect the column set from the detector during column cleaning. Install a 4 L eluent bottle (P/N 039164) to maximize unattended operation. Replace the Ag/AgCl Reference Electrode every six months.

### Results and Discussion

Figure 1 shows the separation of 0.1 mg/L iodide on a Dionex IonPac AG11/AS11 column set using 50 mM nitric acid eluent. Iodide elutes in less than 4 min and is well resolved from the void volume. Other anions—such as fluoride, chloride, and bromide—elute well before iodide. Chloride elutes at approximately 1.5 min. The baseline dip at approximately 6 min is due to dissolved oxygen from the previous injection (elution time of approximately 19 min) and varies from column to column. A 13 min run time places the dip where it does not interfere with iodide. After installing a new column, ensure that 13 min is an appropriate cycle time.

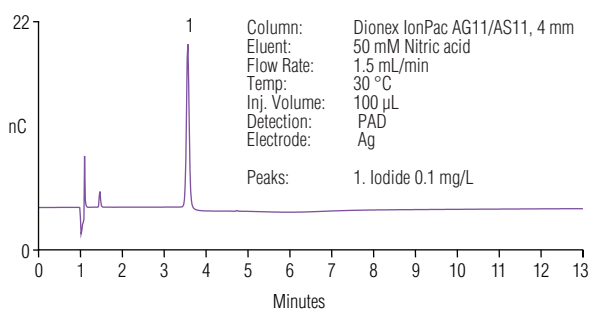


Figure 1. Iodide standard (0.1 mg/L).

Figure 2 shows that the peak area response of iodide is linear over the concentration range of 0.005–10 mg/L (with a 0.9998 coefficient of determination). The lower limit of quantitation was determined by injecting a standard at a concentration that resulted in a signal-to-noise ratio of 10. Typical baseline noise for this method was 3–6 pC. Figure 3 shows a chromatogram of 0.005 mg/L iodide, which is about 10 times the signal-to-noise ratio. When analyzing samples with low concentrations of iodide, check a blank (DI water) injection to confirm that there is no carryover. An autosampler rinse volume of 1000  $\mu$ L minimizes carryover. The retention time (RT) and peak area RSDs over 800 injections of standards and infant formula samples were < 0.6 and < 6%, respectively.

Typical chromatograms of milk- and soy-based infant formula are shown in Figure 4. In Figure 4, A is a 0.04 mg/L iodide standard, B–E are milk-based infant formulas, and F is a soy-based infant formula. The milk-based formulas contain iodide (Peak 1) and thiocyanate (Peak 2). The assignment for thiocyanate is based on reference 14 and coelution with a thiocyanate standard. The thiocyanate comes from cow's milk, a major ingredient of infant formula. When cows eat plants from the cruciferae family (e.g., broccoli, cabbage, and cauliflower), they ingest glucosinolates which are enzymatically hydrolyzed to thiocyanate and passed on in their milk. There is interest in determining thiocyanate in milk-based products as it is a competitive inhibitor of iodide uptake by the thyroid.

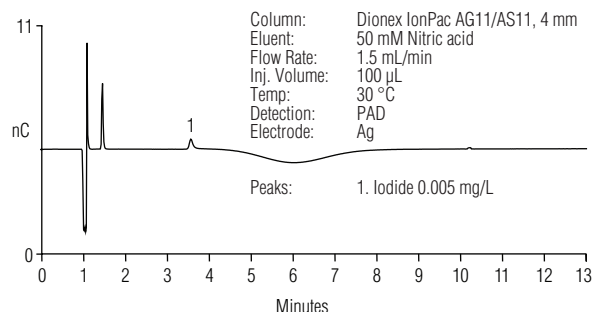


Figure 2. Low-level iodide standard (0.005 mg/L).

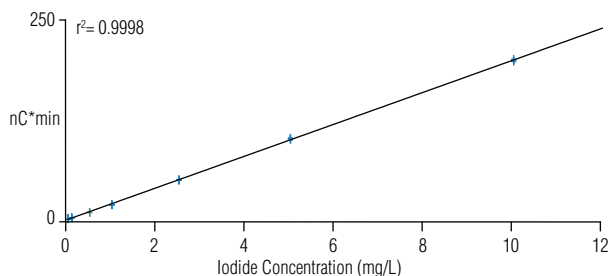


Figure 3. Iodide linearity 0.005–10 mg/L.

Column:	Dionex IonPac AG11/AS11, 4 mm	Samples:	Iodide (mg/L)
Eluent:	50 mM Nitric acid	Standard	0.040
Flow Rate:	1.5 mL/min	Infant Formula Brand 1	0.027
Temp:	30 °C	Infant Formula Brand 2	0.042
Inj. Volume:	100 $\mu$ L	Infant Formula Brand 3	0.023
Detection:	PAD	Infant Formula Brand 4	0.035
Electrode:	Ag	Soy Infant Formula	0.046

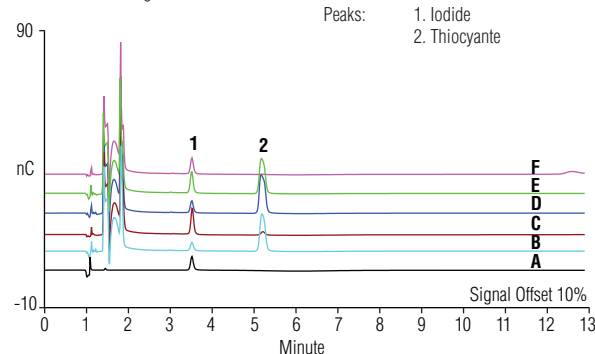


Figure 4. Determination of iodide in (A) DI water, (B–E) milk-based infant formulas, and (F) soy-based infant formula.

Table 1 summarizes the measured iodide concentrations of the milk- and soy-based formulas. The reported concentrations are relative to a calibration curve of external iodide standards and are not adjusted for sample dilution. The label value of iodine is also listed. This value was adjusted for sample dilution. When samples were subjected to the acid digestion at RT (to remove fat and protein), the iodide concentrations for the soy and three of the milk-based formulas (Brands 2–4) were in agreement with the label value for iodine. The measured iodide value for Brand 1 is about 50% of the labeled amount of iodine. Based on the ingredients listed, iodate (also a source of iodine) had not been added to this infant formula (nor the others tested), and thus could not account for the rest of the iodine in Brand 1.

Table 1. Iodide in infant formula—acid digest at RT vs 70 °C.

	Label Value <sup>a,b</sup> (mg/L)	Acid Digest—RT (mg/L)	Acid Digest (70 °C for 1 h)
Milk-Based Infant Formula # 1	0.0507	0.0270	0.0418
Milk-Based Infant Formula # 2	0.0406	0.0420	0.0693
Milk-Based Infant Formula # 3	0.0203	0.0229	0.0430
Milk-Based Infant Formula # 4	0.0338	0.0352	0.0688
Soy-Based Infant Formula	0.0505	0.0460	0.0484

<sup>a</sup> Reported as iodine

<sup>b</sup> Half of label value to account for dilution during sample preparation

Upon performing the acid digestion at an elevated temperature (1h at 70 °C), the measured iodide increased to 80% of labeled value for Brand 1. An overnight digest at 70 °C was needed to recover the labeled amount of iodine for Brand 1. In the other milk-based formulas, the amount of iodide in the extract from the 70 °C digests was twice that of the room temperature digest. For the soy-based formula, the 70 °C acid digest had similar iodide content as the room temperature digest. The recovery of iodide in a standard prepared in the same manner as the infant formula samples was 100%, indicating that there was no loss of iodide during the acid digestion at the 70 °C elevated temperature and subsequent sample preparation steps.

When iodine was extracted using microwave digestion, the iodide concentration in Brand 1 was 0.0913 mg/L (label 0.1014 mg/L), in Brand 2 it was 0.1450 mg/L (label value 0.0812 mg/L), and in the soy-based formula it was 0.0914 mg/L (label 0.101 mg/L). This suggests that microwave digestion with acetic acid can also be used for iodine extraction. Our work with microwave digestion was limited. More work may be needed to optimize for a particular sample and to evaluate the reproducibility of microwave digestion.

Recent studies on iodine speciation in milk and milk-based products suggest that iodine can be present in free and/or bound forms in infant formula.<sup>27</sup> Based on the measured iodide levels at different extraction conditions, the following can be postulated: (i) the room temperature acid digest extracts the free iodide and the label value for milk-based formulas (Brands 2–4) and the soy-based formula corresponds to free iodide; (ii) the acid digest at an elevated temperature (70 °C) also extracts the bound iodide from the matrix, and the label value of the milk-based formula Brand 1 corresponds to the free and bound forms of iodide; and (iii) the soy-based infant formula contains only free iodide, as the room and high temperature digests yielded similar iodide values.

#### Accuracy

The accuracy of this method was verified by determining recoveries of iodide in spiked milk- and soy-based infant formula samples over three consecutive days. The amount of iodide in infant formula samples ranged from 0.034–0.054 mg/L (Table 2). The samples were spiked with 0.05 mg/L iodide. Recoveries were calculated from the difference in response between the spiked and unspiked samples. The average recovery of iodide ranged from 82–115%. Recovery in samples that were spiked after the sample preparation steps ranged from 103–110%. This suggests that the matrix does not inhibit iodide detection after the sample preparation steps. The above results indicate that this method could be used for accurate determination of iodide in infant formulas.

Table 2. Iodide recoveries for spiked infant formula samples.

		Amount Present (mg/L)	Amount Spiked before Sample Treatment (mg/L)	Amount Detected (mg/L)	Recovery (%)
Milk-Based Infant Formula # 1	Day 1	0.036	0.050	0.086	98.7
	Day 2	0.034	0.050	0.083	97.0
	Day 3	0.034	0.050	0.084	99.1
Milk-Based Infant Formula # 2	Day 1	0.049	0.050	0.097	97.4
	Day 2	0.054	0.050	0.095	82.2
	Day 3	0.051	0.050	0.101	99.7
Soy-Based Infant Formula	Day 1	0.044	0.050	0.102	115
	Day 2	0.046	0.050	0.094	96.5
	Day 3	0.040	0.050	0.095	109

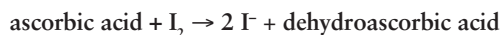
## Iodate

In some countries, potassium iodate is used as a source of iodine and is added to infant formula. The bioavailability of iodine from iodate and iodide is similar; therefore accurate determinations of iodate and iodide are needed to quantify total iodine in certain infant formulas.

The current IC-PAD-based method can also be used for measuring total iodine after converting iodate to iodide using ascorbic acid as the reductant (Figure 5). Under acidic conditions, iodate ( $\text{IO}_3^-$ ) and iodide ( $\text{I}^-$ ) undergo the following oxidation-reduction reaction (Equation 1):



The iodine formed by the Equation 1 reaction oxidizes ascorbic acid to dehydroascorbic acid and iodine is reduced to iodide ions, as follows (Equation 2):



Iodine formed in Equation 1 is immediately reduced to iodide as long as there is any ascorbic acid present. Remember that for this reaction to successfully convert iodate to iodide, the sample must already contain iodide.

Tables 3 and 4 summarize the recovery of iodate as iodide in the milk- and soy-based infant formulas.

Note: iodate was spiked into these samples at 0.06 mg/L (i.e., 0.04 mg/L as iodide). The results (Table 3) suggest

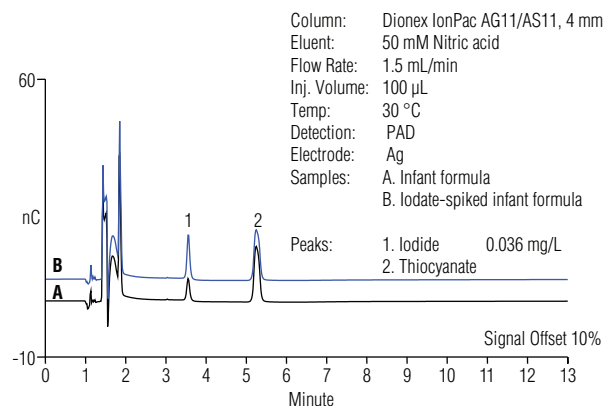


Figure 5. Chromatogram of iodide in (A) infant formula and (B) infant formula spiked with iodate.

that in the absence of iodate, ascorbic acid does not interfere with the detection of iodide. The recovery of iodate (Table 4) as iodide ranged from 94–103% for digests prepared at 70 °C, and 80–127% for digests prepared at room temperature. This shows that a combination of the above-mentioned optimized sample preparation conditions (for reducing iodate to iodide) and the analytical technique of IC-PAD, can be used for determining total iodine (from iodate and iodide) in milk- and soy-based infant formulas.

Table 3. Measuring iodate as iodide.

Sample	Iodide (mg/L) (average of 2 aliquots)	Recovery of Iodate as Iodide (%)
Milk-Based Infant Formula #2 (-iodate, -ascorbate)	0.072	—
Milk-Based Infant Formula #2 (-iodate, +ascorbate)	0.073	—
Milk-Based Infant Formula #2 (+iodate, +ascorbate)	0.119	103

Table 4. Recovery of iodate as iodide in milk- and soy-based infant formula.

Sample	Temperature of Acid Digestion	Recovery of Iodate as Iodide (%)
Milk-Based Infant Formula #1	RT	80.3
	70 °C	94.1
Milk-Based Infant Formula #2	RT	96.7
	70 °C	101
Milk-Based Infant Formula #3	RT	79.4
	70 °C	103
Milk-Based Infant Formula #4	RT	128
	70 °C	94.1
Soy-Based Infant Formula	RT	94.5
	70 °C	97.5

## Conclusion

Described here is a robust IC-PAD-based method for the accurate determination of iodide in milk-based infant formula from all major U.S. producers. This method was also accurate for determining iodide in one soy-based infant formula (additional soy-based formulas were not tested). The method uses a Dionex IonPac AG11/AS11 column set with nitric acid eluent and a silver working electrode. The sample preparation conditions were optimized for extracting the free and bound forms of iodide and also for reducing iodate to iodide to determine iodide and iodate (i.e., total iodine) in milk- and soy-based infant formulas. This method has broad linear range (0.005–10 mg/L), high precisions (RSD <0.6% for retention time and <6% for peak area for over 800 injections), and good recoveries.

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