Application Note

Trace Metals in Food

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Simultaneous Iodine and Bromine Speciation Analysis of Infant Formula by HPLC-ICP-MS

Determination of four halogen species in less than 6.5 minutes

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Introduction

Babies and young children often rely on infant formula for their nutritional requirements during early development, so regulators set high standards for the safety and nutritional value of these products. Most countries specify minimum levels for essential minerals in infant formulas, including iodine, to meet the nutritional needs of infants. If too little or too much iodine is consumed, babies and young infants are susceptible to developing thyroid problems (*1, 2*).

Regulatory values for iodine in infant formula manufactured from cow's milk or goat's milk proteins or protein hydrolysates relate to total iodine (Table 1). But the bio-availability of iodine depends on the chemical species present in a sample. Iodide (I^-) has a higher bio-availability compared to iodate (IO_3^-), which affects the nutritional status of iodine in food. For more information about the toxicity and health benefits of a food, elemental speciation is required to separate iodide and iodate from iodine (I_2) and organic forms of iodine.

In the case of bromine, its toxicity is species-dependent. Bromide (Br^-) has low toxicity compared to bromate (BrO_3^-) which is possibly carcinogenic to humans (*3*).

Table 1. Regulatory levels for iodine in infant formula.

Regulatory body	Minimum concentration µg/100 kcal	Maximum concentration µg/100 kcal	Reference
US FDA	5	75	(*4*)
European Union	15	29	(*5*)
China	10.5	58.6	(*6*)

In this study, four species, iodide (I^-), iodate (IO_3^-), bromide (Br^-), and bromate (BrO_3^-), were determined in four commercially available milk-based infant formulas using HPLC coupled to a triple quadrupole ICP-MS (ICP-QQQ). HPLC-ICP-MS methodology is a well-established analytical technique that has been used for the speciation analysis of arsenic in various food matrixes, including infant formula (*7, 8*). This application could also be done using a single quadrupole ICP-MS such as the Agilent 7800 or 7900 ICP-MS as the elemental detector.

Experimental

Reagents and Standards

Standards for bromide, bromate, iodide, and iodate were prepared from 1000 ppm stock solutions. The bromide ion standard solution was bought from Kanto Chemical Co., Inc., Tokyo. A 1000 ppm stock solution of bromate was made by dissolving $NaBrO_3$ (Wako Pure Chemical Corporation, Osaka) in water. Iodide and iodate stock solutions were prepared at 1000 ppm by dissolving KI and KIO_3 (Wako Pure Chemical Corporation, Osaka), respectively.

Standard reference material and samples

A milk-based standard reference material (SRM) NIST 1849a - Infant/Adult Nutritional Formula I (Gaithersburg, MD, USA) was used to validate the analytical method for the determination of total iodine.

Two of the four market basket infant formula samples were bought in Berkeley California, USA, and two were bought in Beijing, China. All four samples were in powder form.

Nitric acid (HNO_3, ≥ 65%, Sigma-Aldrich) was used for microwave digestion and standard/sample preparation. All dilutions were done using 18.2 MΩ·cm (Millipore, Bedford, MA, USA) de-ionized water (DIW).

Sample preparation

For total bromine determinations about 0.2 g of NIST 1849a and each sample were weighed and digested in 5 mL of HNO_3 by microwave digestion (Mars 6, CEM) using the program outlined in Table 2. The fully digested samples were then diluted to 40 mL with DIW. All samples and SRM were prepared in triplicate.

Table 2. Microwave digestion program.

Stages	Temp (°C)	Ramp (min)	Hold (min)
1	30	5	30
2	210	20	30
3	30	30	-

For I and Br speciation and total iodine determinations, about 0.2 g of each sample was weighed into 20 mL DIW and then put in a water bath at 50 °C for 1 h. The samples were then filtered using a syringe filter (0.45 µm pore size). Approximately 1 mL filtered solution was placed in 1 mL polypropylene HPLC vials (Agilent part number 5182-0567), ready for analysis.

Instrumentation

An Agilent 1260 Infinity II LC system comprising a quaternary pump was coupled to an Agilent 8900 Triple Quadrupole ICP-MS (ICP-QQQ) using the Agilent LC connection kit (part number G1833-65200). The Agilent Chromium Speciation Column for Drinking Water (part number G3268-80001) anion exchange column (4.6 mm internal diameter × 30 mm polyhydroxymethacrylate base resin) was used for separation of the iodine and bromine species. Details of the operating conditions and mobile phase are given in Table 3.

The Agilent 8900 ICP-QQQ (Advanced Applications configuration, #100) was fitted with the standard sample introduction system comprising a glass concentric nebulizer, quartz double-pass spray chamber, 2.5 mm injector quartz torch, and Ni interface cones. The ICP-QQQ was operated in single quadrupole (SQ) mode using helium cell gas, so the application could also be done using an Agilent 7800 or 7900 ICP-MS.

Table 3. HPLC and ICP-QQQ instrument operating conditions.

HPLC parameter	Setting
Mobile phase	5.0 mM NaH_2PO_4 / 15.0 mM Na_2SO_4 / 5.0 mM EDTA (pH 7.0)
Column	Agilent column for Cr speciation (part number G3268-80001)
Mobile phase flow (mL/min)	1.0
Injection volume (µL)	100
Temperature	Ambient
Run time (min)	8
ICP-QQQ parameter	**Setting**
Scan mode	Single quad mode
Sampling depth (mm)	8.0
Nebulizer gas flow (L/min)	1.10
Spray chamber temp. (°C)	2
Extract 1 (V)	0.0
Extract 2 (V)	-250
Octopole bias (V)	-20.0
Helium cell gas flow rate (mL/min)	2.0
Axial acceleration (V)	1.0
Energy discrimination (V)	3.0
Mass (*m/z*)	79 for Br, 127 for I
Integration time (sec/mass)	0.5

Results and discussion

Calibration

Calibration was carried out by analyzing solutions of iodide, iodate, bromide, and bromate, between 0 and 100 ppb. Figure 1 demonstrates calibration curves with excellent linearity over the calibration range, with calibration linearity equal to or better than 0.9999.

A chromatogram of the 5 ppb iodine and bromine standard is shown in Figure 2. All four species, IO_3^-, I^-, BrO_3^-, and Br^-, were separated and baseline-resolved in less than 6.5 minutes, all in the same run.

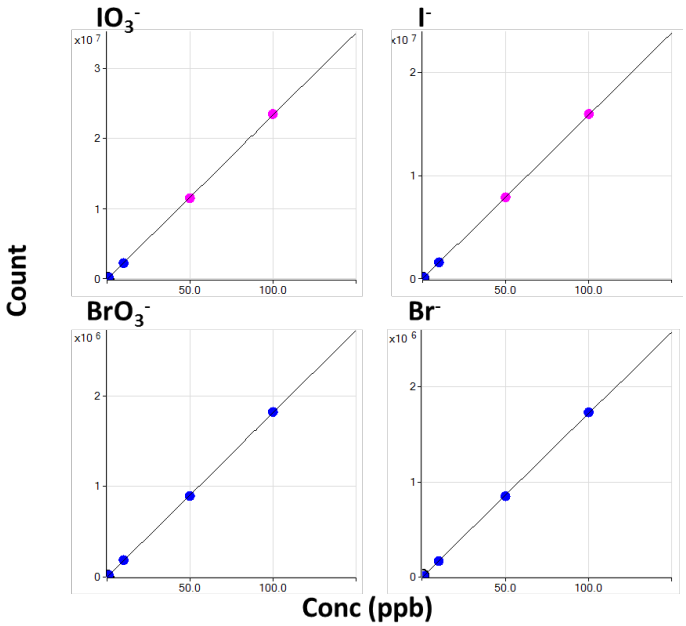

Figure 1. Calibration curves of IO_3^- (top left), I^- (top right), BrO_3^- (bottom left), and Br^- (bottom right).

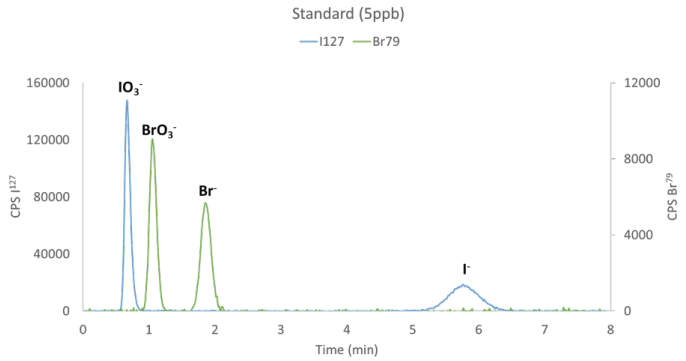

Figure 2. Chromatogram of 5 ppb standard showing IO_3^-, I^-, BrO_3^-, and Br^- in the same run.

Detection limits

Signal-to-noise (S/N) detection limits (DLs) were calculated from 3x the background noise divided by the signal for each peak of interest. The DLs of the four species were between 0.072 and 0.667 ppb as shown in Table 4.

Table 4. S/N DLs for iodate, bromate, bromide, and iodide.

Species	Standard concentration (ppb)	Signal	Noise	S/N	DL in solution (ppb)
IO_3^-	0.98	27781	676.6	41.06	0.072
BrO_3^-	1.07	1979	320.2	6.18	0.519
Br^-	0.9	1296	320.0	4.05	0.667
I^-	0.78	3379	677.2	4.99	0.469

Analysis of SRM

The four species were determined in the Infant/Adult Nutritional Formula SRM using LC-ICP-QQQ (seven sample preparation replicates, run in triplicate; n=21). The total iodine concentration was measured using the 8900 ICP-QQQ (without HPLC separation). The results for the four species, total iodine, and total bromine are shown in Table 5. No iodate or bromate was detected in the SRM. The measured concentrations for $^{127}I^-$, total iodine, and the certified concentration for total iodine are all in good agreement, suggesting a good level of method accuracy. Bromine isn't certified in the SRM.

Table 5. Results for the analysis of NIST 1849a Infant/Adult Nutritional Formula SRM*. n=21. Units: mg/kg.

$^{127}IO_3^-$	$^{127}I^-$	$^{79}BrO_3^-$	$^{79}Br^-$	Total I*		Total Br**
Measured				Reference	Measured	Measured
ND	1.31 ± 0.04	ND	0.74 ± 0.12	1.29 ± 0.11	1.33 ± 0.37 (103%)	0.77 ± 0.07 (96%)***

*ND = not detected. *Prepared using water bath extraction.*
***No certified value given for bromine. ***^{79}Br /Total bromine.*

Quantitative analysis of infant formula products

Extracts of the four powder infant formula samples were prepared in duplicate and analyzed in duplicate (n=4) using LC-ICP-QQQ. As shown in Table 6, no iodate or bromate was detected in any of the samples. The recoveries of iodide compared to the total iodine results range from 87 to 102%, suggesting the iodine present in the samples was in the form of iodide. The recoveries of bromine to the total bromine results ranged from 85 to 100%, suggesting the bromine present in the samples was in the form of bromide.

The total iodine measured in the four infant formula samples ranged from 1232 to 2592 µg/kg, which is equivalent to 25.9 to 54.5 µg/100 kcal. These concentrations are within the United States and China national standards for total iodine; however, some results fall outside the EU maximum limit for iodine of 29 µg/100 kcal.

Table 6. Infant formula sample analysis results. Units: µg/kg. The percentages are the recoveries of iodide and bromide compared to total iodine and bromine, respectively.

Sample ID	$^{127}IO_3^-$	$^{127}I^-$	$^{79}BrO_3^-$	$^{79}Br^-$	Total I*	Total Br**
1-USA	ND	1157 ± 11	ND	2993 ± 27	1333 ± 16 (87%)	3112 ± 151 (96%)
2-USA	ND	1455 ± 29	ND	10826 ± 141	1426 ± 50 (102%)	10815 ± 478 (100%)
3-China	ND	1240 ± 15	ND	25660 ± 230	1232 ± 23 (101%)	29850 ± 2392 (86%)
4-China	ND	2372 ± 24	ND	8529 ± 64	2592 ± 44 (92%)	9997 ± 389 (85%)

**Sample preparation with the water extraction method*
*** Sample preparation with microwave digestion method*

Spike recovery test

A spike recovery test was performed by spiking two infant formula samples with IO_3^-, BrO_3^-, Br^-, and I^- at 20 and 40 ppb, before extraction. Each spiked sample was prepared in triplicate and analyzed twice. Good recoveries for each of the four species in actual samples at 20 ppb and 40 ppb-level were achieved over the course of mulitple 100 µL injections (Table 7). The results indicate that the method could be used for the accurate determination of the four-halogen species in infant formula.

Table 7. Average spike recovery results for two infant formula samples spiked at 20 and 40 ppb with IO_3^-, BrO_3^-, Br^-, and I^-, n=12.

	$^{127}IO_3^-$	$^{79}BrO_3^-$	$^{127}I^-$	$^{79}Br^-$
Average recovery, %	96 ± 4	97 ± 5	98 ± 7	100 ± 5
Recovery range, %	90–100	89–101	88–104	94–105

Figure 3 shows the overlay of ^{79}Br and ^{127}I from the two infant formulas analyzed per Table 7. The infant formulas from the US and China were spiked at two levels (~20 and 40 ppb) and each level prepared in triplicate. The chromatograms show good repeatability for mulitple 100 µL injections.

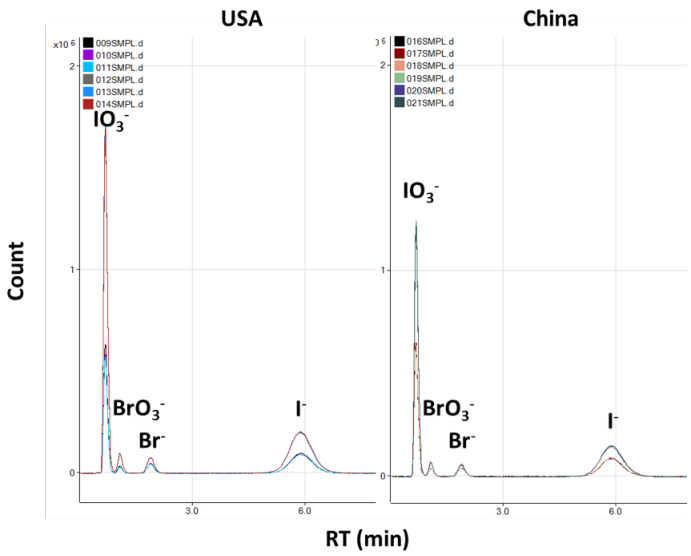

Figure 3. Overlay of ^{79}Br and ^{127}I spiked at two levels (~20 and 40 ppb) in the USA infant formula (chromatogram on the right) and a Chinese infant formula (chromatogram on the left). n=6 for each chromatogram.

Conclusion

For the first time, four halogen species were measured in the same run in infant formula samples using an Agilent 1260 Infinity II LC system coupled to an Agilent 8900 ICP-QQQ. Using an anion exchange column, baseline separations were achieved in around 6.5 minutes with detection limits for I^-, IO_3^-, Br^-, and BrO_3^- all less than or equal to 0.67 ppb.

Speciation analysis of infant formula provides valuable information on iodine bio-availability and the potential risk from bromate. Total elemental determinations of iodine and bromine were also performed using the 8900 ICP-QQQ. The measured value for iodine in an infant formula SRM was in good agreement with the certified value at 103% recovery. There was also good agreement between the measured concentration of $^{127}I^-$ with the certified value at 101% recovery. No certified value was provided with the SRM for bromine.

None of the four commercially available infant formula samples that were analyzed in the study contained iodate or bromate, only iodide and bromide. The total iodine content in the samples ranged from 25.9 to 54.5 µg/100 kcal. This range is within the US and China national standards for iodine in infant formula, but outside the EU maximum limit of 29 µg/100 kcal.

To test the suitability of the method for the accurate determination of low concentrations of the four species in infant formula samples, a spike recovery test was carried out at 20 and 40 ppb. Baseline separations were achieved, with good repeatability over the course of mulitple 100 µL injections.

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More Information

For a full account of this study, see Jennifer Nelson, Lawrence Pacquette, Shuofei Dong and Michiko Yamanaka, Simultaneous Analysis of Iodine and Bromine Species in Infant Formula using HPLC-ICP-MS, *JAOAC Int*., 102, **2019**, DOI: 10.5740/jaoacint.18-0352

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