Simultaneous Quantification of Iodine and Other Elements in Infant Formula by ICP-MS Following an Acid Digestion with Nitric Acid and Hydrogen Peroxide

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A method for quantifying iodine in infant formula is described. Nitric acid and hydrogen peroxide converted iodine into iodate in microwave-assisted digestion and prevented iodine volatilization and memory effects. Acetic acid as a carbon source was added to both the sample and standard solutions as a countermeasure against carbon charge transfer to iodine and the addition of acetic acid helped to enhance the sensitivity. The instrument limit of quantification was 0.1 ng mL⁻¹ and the relative standard deviation was less than 3%. The spike recoveries were between 94.8 and 106%. Good agreement with the values obtained using the tetramethylammonium hydroxide method was obtained for infant formula sold in several countries. This method permitted the simultaneous determination of iodine and 12 other important elements (Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se and Mo) in infant formula.

Keywords ICP-MS, iodine, infant formula, simultaneous determination, carbon charge transfer

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Table 1. Plasma conditions and measurement parameters for ICP-MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
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<tbody>
<tr>
<td>ICP-MS 7500ce</td>
<td></td>
</tr>
<tr>
<td>Plasma condition</td>
<td></td>
</tr>
<tr>
<td>RF power</td>
<td>1.55 kw</td>
</tr>
<tr>
<td>Plasma gas flow rate</td>
<td>15 L/min</td>
</tr>
<tr>
<td>Makeup gas flow rate</td>
<td>0.3 L/min</td>
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<tr>
<td>Carrier gas flow rate</td>
<td>0.8 L/min</td>
</tr>
<tr>
<td>Sampling depth</td>
<td>8.0 mm</td>
</tr>
<tr>
<td>Data acquisition</td>
<td></td>
</tr>
<tr>
<td>Data points</td>
<td>3 points/peak</td>
</tr>
<tr>
<td>Dwell time</td>
<td>100 ms/point</td>
</tr>
<tr>
<td>Repetition</td>
<td>3 times</td>
</tr>
<tr>
<td>Collision gas</td>
<td>H2 (5 mL/min)</td>
</tr>
<tr>
<td>Internal standard</td>
<td>Te 125</td>
</tr>
</tbody>
</table>

Experimental

Instrumentation

A TOP wave microwave digestion system (Analytik Jena, Jena, Germany) was used to digest infant formula; the pressure vessels were made up of polytetrafluoroethylene. A graphite heating block system (DigiPREP Jr., SCP SCIENCE, Champlain, NY) was used for alkaline dissolution. Measurements were made on an ICP-MS 7500ce (Agilent Technologies, Santa Clara, CA) equipped with an Agilent I-AS autosampler and a collision reaction cell. Separate tubing on the peristaltic pump and a Y connector were used to add Te as an internal standard. The measurement parameters and plasma conditions are shown in Table 1.

Reagents, standard and samples

Water was purified (18 MΩ cm) using a Milli-Q Gradient A10 system (Merck Millipore, Billerica, MA). Nitric acid (for electronics use) and acetic acid (for atomic absorption spectrometry) were obtained from Kanto Chemical (Tokyo, Japan). Hydrogen peroxide (for atomic absorption spectrometry) was obtained from Wako Pure Chemical (Tokyo, Japan). Potassium iodate (for atomic absorption spectrometry) was obtained from Tama Chemicals (Tokyo, Japan). Molecular iodine adsorbs onto glass, causing memory effects from 0 to 12%. Iodide forms molecular iodine in the presence of nitric acid. The sensitivity of ICP-MS for elements with a high ionization potential, such as As and Se, due to electron transfer;16–18 this is called carbon charge transfer (CCT). Iodine and tellurium used as internal standards also have high ionization potentials, and their sensitivity of detection can be similarly enhanced. Carbon in the sample solution can cause differences in sensitivity between the working standard and the sample solution; also, CCT contributes to inaccurate measurements of the concentrations of elements with a high ionization potential. This effect can be suppressed by adding excess acetic acid to both the working standard and sample solution, because acetic acid is a carbon source, which effect of the enhancing sensitivity. To optimize the concentration of acetic acid needed to compensate for iodine and tellurium signal enhancement, various concentrations of acetic acid were added to both the working standard and the sample solution. The signals of iodine and tellurium as well as the sensitively ratio of iodine/tellurium were measured in the range of the acetic acid concentration from 0 to 12% (Fig. 1). The sensitivity of ICP-MS for iodine and tellurium was increased by 5% by adding acetic acid, resulting in a stable sensitively ratio between iodine and tellurium in more than 5% acetic acid. Thus, the amount of added acetic acid was set in order to 5% to improve both the precision and accuracy.

Alkaline dissolution

Infant formula (0.1 – 0.5 g) was accurately weighed in tubes. Then, Milli-Q water (5 mL) and TMAH (1 mL) were added, as described by Fecher et al.13 The tubes were then closed and placed in a DigiPREP Jr. at 90 °C. After 3 h, the samples were cooled and diluted to 50 mL with Milli-Q water. Undissolved residues were removed by centrifugation (1500g, 10 min). Measurements were conducted on the clear supernatant. The working standard solution was based on 0.5% TMAH, but had to be adapted to the amount of TMAH in the sample solution.

Results and Discussion

Countermeasure against carbon charge transfer

Organic carbon significantly enhances the signal intensity in ICP-MS for elements with a high ionization potential, such as As and Se, due to electron transfer;16–18 this is called carbon charge transfer (CCT). Iodine and tellurium used as internal standards also have high ionization potentials, and thus their sensitivity of detection can be similarly enhanced. Carbon in the sample solution can cause differences in sensitivity between the working standard and the sample solution; also, CCT contributes to inaccurate measurements of the concentrations of elements with a high ionization potential. This effect can be suppressed by adding excess acetic acid to both the working standard and sample solution, because acetic acid is a carbon source, which effect of the enhancing sensitivity. To optimize the concentration of acetic acid needed to compensate for iodine and tellurium signal enhancement, various concentrations of acetic acid were added to both the working standard and the sample solution. The signals of iodine and tellurium as well as the sensitively ratio of iodine/tellurium were measured in the range of the acetic acid concentration from 0 to 12% (Fig. 1). The sensitivity of ICP-MS for iodine and tellurium was increased by 5% by adding acetic acid, resulting in a stable sensitively ratio between iodine and tellurium in more than 5% acetic acid. Thus, the amount of added acetic acid was set in order to 5% to suppress the CCT effect. Also, adding acetic acid enables highly sensitive analysis for iodine, better than not adding.

Memory effect

Iodide forms molecular iodine in the presence of nitric acid. Molecular iodine adsorbs onto glass, causing memory effects and background problems during ICP-MS. Removing the
memory effect and regaining a stable baseline requires washing with dilute acid for an extended period of time. However, Larsen et al.\textsuperscript{15} reported that oxidized species, such as iodate, do not exhibit memory effects, or adhere on inside surface of the tubing, nebulizer and spray chamber in the ICP-MS instrument. Accordingly, we converted iodine into iodate by oxidation with \textit{H}_2\textit{O}_2, and compared the results obtained if \textit{H}_2\textit{O}_2 was added or not.

Figure 2 shows the stability of the signals with and without added \textit{H}_2\textit{O}_2. The addition of \textit{H}_2\textit{O}_2 resulted in a stable iodine signal for at least 90 s, whereas in the absence of \textit{H}_2\textit{O}_2, the iodine signal increased constantly; after 60 s, the signal increased to 140% of the signal in the presence of \textit{H}_2\textit{O}_2. Figure 3 shows the effect of washing with dilute acid if the sample had or had not been treated with \textit{H}_2\textit{O}_2, which shows a significant difference. Adding \textit{H}_2\textit{O}_2 resulted in a rapid depletion of the iodine signal during washing. The signals of those samples washed for 30 and 60 s were 4.3 and 2.1% of that before washing, respectively. On the other hand, not adding \textit{H}_2\textit{O}_2 to samples resulted in a slow depletion of the signals. The signals of the sample washed for 30 and 60 s were 12 and 5.5%, respectively. These results indicate that the presence of \textit{H}_2\textit{O}_2 stabilizes the iodine signal during the measurement, and decreases signal quickly during the rinse in the instrument, because iodine was kept from adhesion on the instrument surfaces by converting iodine into iodate. We thus conclude that the addition of \textit{H}_2\textit{O}_2 is effective for eliminating the memory effect.

### Table 2

<table>
<thead>
<tr>
<th>Infant formula</th>
<th>Present analytical method/mg kg(^{-1})</th>
<th>Recovery, %</th>
<th>Alkaline dissolution method/mg kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.25 ± 0.01</td>
<td>98.9</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>B</td>
<td>0.35 ± 0.02</td>
<td>106</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>C</td>
<td>0.94 ± 0.07</td>
<td>97.3</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>D</td>
<td>1.13 ± 0.06</td>
<td>94.8</td>
<td>1.13 ± 0.03</td>
</tr>
</tbody>
</table>

### Linearity of the calibration curve and limit of quantification

Calibration curves for iodine were made by using 0, 1, 5, 7.5 and 10 ng mL\(^{-1}\) working standard solutions. The correlation coefficients ranged from 0.9995 to 1.000. The data were collected on 7 separate days. The instrument limit of quantification (LOQ) was 0.1 ng mL\(^{-1}\). The sample weight should be increased if the concentration of an element in the sample solution is below the instrument LOQ. The blank test values on 3 separate days were below the instrument LOQ.

### Method validation

The performance of the analytical method incorporating \textit{H}_2\textit{O}_2 treatment was tested using NIST 1849a. The analytical result (1.30 ± 0.04 mg kg\(^{-1}\), \(n = 8\)) obtained by the present method agreed with the certified value (1.29 ± 0.11 mg kg\(^{-1}\)) of NIST SRM 1849a within the uncertainty. The relative standard deviation (including microwave digestion and determination of iodine) was less than 3%. These results indicate that iodine is quantitatively recovered during sample digestion, and that the formation of volatiles and signal instability due to memory effects are suppressed.

### Sample analysis

The concentration of iodine in infant formula varies widely worldwide. The present method was successfully applied to a wide variety of infant formulas chosen to demonstrate the applicability of the methodology to infant formula sold in several countries. The samples had a wide range of iodine concentrations, from 24.0 to 113 \(\mu\)g 100 g\(^{-1}\) (Table 2). Infant formula A and B are sold in Japan, C is sold in Pakistan and D is sold in Korea. The values of iodine obtained using the present analytical method were in good agreement with those obtained using the alkaline dissolution method. The relative standard deviation of separate ICP-MS determinations of iodine is below 10% as containing a determination procedure in the present analytical method and an alkaline dissolution method. Between 94.8 to 106% of iodine (125 ng, the equivalent amount with a sample) spiked into marketed infant formula was recovered, showing that this method is useful for analyzing wide concentrations of iodine in infant formula.

### Simultaneous determination

The present analytical method and the alkaline dissolution method were used to simultaneously quantify 12 elements plus iodine in NIST 1849a. The results of both methods are compared in Table 3 with the certified values. The range of values obtained with the alkaline dissolution method was between 33 – 646% of that of the certified values. The values of elements other than Cr, Cu, Se, Mo and I were low due to incomplete dissolution, and the value of Cu was high, likely due to interference of hydrogen, carbon and nitrogen from TMAH and sample solutions. In contrast, the values obtained with the

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Fig. 2 Stability of the iodine signal with and without the addition of hydrogen peroxide.

Fig. 3 Removal of the memory effect of the iodine signal with and without the addition of hydrogen peroxide using a washing solution.
present analytical method were 100 ± 10% that of the certified values. Taken together, these results indicate that the method developed in this study would be satisfactory for simultaneous efficient determinations of these 12 elements plus iodine by ICP-MS.

In the future we will consider applying this simultaneous determination method to a wide variety of foods. Also, we expect that this method can be used to quantify halogens, such as Cl and Br.

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References