Determination of Iodine in Seafood by Inductively Coupled Plasma/Mass Spectrometry

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A method was developed for determination of total iodine content in different standard reference materials (SRMs) and seafood products by inductively coupled plasma/mass spectrometry (ICP/MS). If iodine is present as iodide and nitric acid is used in the wet digestion system, the observed signal is not stable when iodine is measured by ICP/MS at m/z 127. To stabilize the iodine signal, 3% ammonia solution (1 + 1, v/v) was added to the digest. The limit of quantitation of the method, defined as 6 times the standard deviation in the blank solution (n = 20) was estimated to be 15 mg/kg (using 0.2 g dry mass and a dilution factor of 50). The precision, expressed as repeatability of the iodine concentration, varied between 3.2 and 12% in SRMs, with concentrations of 4.70–0.17 mg/kg dry matter. The described method was compared with a method using tetramethylammonium hydroxide extraction. Both methods showed good precision and trueness by analyses of SRMs. The 2 methods were used to determine iodine in seafood from the Barents Sea, the Norwegian Sea, and the North Sea. The results showed great variation between different fish species as well as between individuals within a species. The lowest values of iodine were recorded in muscle of ling (Molva molva) with a mean of 0.07 mg/kg fresh weight and a variation between 0.03 and 0.11 mg/kg fresh weight. The highest values were found in cod (Gadus morhua) from the Barents Sea, with a mean of 2.5 mg/kg and a variation between 0.7 and 12.7 mg/kg fresh weight.

The role of iodine as an essential trace element has been known since 1922. It has been estimated that 1.5 billion people are at risk for developing iodine deficiency diseases, i.e., 29% of the world’s population, including 655 million (12%) who already have goiter (1). Iodine deficiency leads to goiter disease, and several other dysfunctions known as iodine deficiency disorders (IDD). In Europe, only 6 countries are known to have goiter and IDD’s under control Austria, Finland, Iceland, Norway, Sweden, and Switzerland (1). The concentration of iodine in food needs to be monitored, because the recommended daily intake for adults, estimated internationally as well as in the Nordic countries, is 150 μg/day (2, 3).

The most important natural source of iodine in the human diet is from marine fish and other seafood products. However, the main source of dietary iodine in Norway comes from milk and dairy products because of the addition of iodine to cow’s fodder, which is done to prevent cows from having goiter. Iodized table salt also contributes to dietary iodine intake in the Nordic countries, except Iceland.

A variety of analytical methods including spectrophotometry (4) and neutron activation analysis (5) are used to determine iodine in food. Due to its high selectivity and sensitivity, inductively coupled plasma/mass spectrometry (ICP/MS) is commonly used for the quantitative determination of iodine in biological samples (6–14).

The main challenge, however, in the determination of iodine in various foodstuffs using ICP/MS, is to stabilize iodide ions in the sample solutions before the ICP/MS determination. Different approaches have been used to stabilize iodine. Perchloric acid in combination with nitric acid was used successfully to digest cream and cheese in PTFE-lined steel bombs (11). The acid mixture oxidized potentially volatile iodine species present in the sample to nonvolatile species such as iodate. In contrast to iodide, these oxidized species did not exhibit any memory and adhesion effects in the ICP/MS instrument. Other analytical approaches applying ICP/MS are based on alkali extraction either combining potassium hydroxide and tetramethylammonium hydroxide (TMAH) to determine iodine in milk by flow injection ICP/MS (9), or using TMAH as a strong alkali at elevated temperature to determine iodine in dietetic samples supplemented with iodine (13).

Baumann (6) and Vanhoe et al. (7) reported a rapid and sensitive determination of iodine in fresh milk, milk powder, and human serum using a digestion system involving 0.5% (v/v) ammonia solution in a microwave oven. For human serum, accurate and precise results were obtained by using nitric acid or an ammonia solution. The ammonia solution stabilized iodine species present in the milk and milk products.

This report describes the development and evaluation of an analytical method for determining iodine in seafood. The method is based on microwave oven wet digestion and use of ammonia as a stabilizer for iodine prior to the ICP/MS determination. The decomposition procedure was compared with an alkaline extraction procedure using TMAH. The 2 decom-
position procedures were used to determine iodine concentrations in fish fillets of species caught mainly in the Barents Sea, the Norwegian Sea, and the North Sea.

**Experimental**

**Apparatus**

All measurements were made using a Perkin-Elmer SCIEX Elan 5000A ICP/MS (Toronto, Canada) equipped with standard configuration, with a single-channel mass flow controller. The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin-Elmer AS-90 autosampler and aspirated into the argon plasma. The instrument was run at normal resolution and set to detect the iodine signal intensity at m/z 127 in the quantitative and graphics data acquisition modes, which allowed quantitation and recording of the signal intensity versus time, respectively. Further details of the instrumental settings are given in Table 1.

The biological samples were wet-digested using a Milestone MLS-1200 microwave oven (Sorisole, Italy) equipped with a rotor for 10 sample vessels (MDR-300/10). The Teflon vessels (tetrafluorine methoxil, TFM; Product No. 33802, 100 mL) are suitable for pressures up to 30 bar. The microwave digestion unit has a blow-off valve, which opens at a

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**Table 1. Instrumental parameters for ELAN 5000A**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma RF power</td>
<td>1050 W</td>
</tr>
<tr>
<td>Argon flow rates</td>
<td></td>
</tr>
<tr>
<td>Plasma flow</td>
<td>15.0 L/min</td>
</tr>
<tr>
<td>Auxiliary</td>
<td>0.8 L/min</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>0.91 L/min</td>
</tr>
<tr>
<td>Mass-to-charge ratio detected m/z</td>
<td>127</td>
</tr>
<tr>
<td>Conventional sample aspiration</td>
<td>0.75 mL/min</td>
</tr>
<tr>
<td>Wash time between samples (water)</td>
<td>120 s</td>
</tr>
<tr>
<td>Sample delay</td>
<td>90 s</td>
</tr>
<tr>
<td>Replicate time</td>
<td>3125 ms</td>
</tr>
<tr>
<td>Dwell time per mass</td>
<td>25 ms</td>
</tr>
<tr>
<td>Sweeps per reading</td>
<td>125</td>
</tr>
<tr>
<td>No. of replicates</td>
<td>7</td>
</tr>
<tr>
<td>Points across peak</td>
<td>1</td>
</tr>
<tr>
<td>Resolution</td>
<td>Normal</td>
</tr>
</tbody>
</table>

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**Figure 1. Map of Barents Sea, Norwegian Sea, and North Sea with sampling sites for different fish species analyzed.**
pressure of 30 bar. The pH was measured by a standard procedure with an electrode from Orion Research, Inc. (Cambridge, MA).

Reagents and Vessels

All solutions were prepared with analytical reagent grade chemicals and ultra pure water (18 MΩ/cm) generated by ROPure reversed osmosis and a NANOpure ion exchange unit (Barnstead, Dubuque, IA). Nitric acid was of suprapure quality (65%, s.g. 1.4 g/mL, Merck, Darmstadt, Germany); hydrogen peroxide was of pro-analysis quality (30%, s.g. 1.1 g/mL). The calibration standards were prepared from commercially available stock solutions. An aqueous standard stock solution of 1000 μg iodine/mL was prepared from potassium iodate (Merck) in 0.5% (v/v) ammonia. Additionally, a commercial 1000–0.5 mg iodine/L standard as sodium iodide in water was obtained from the producer (Teknolab Ldl 1, Drøbak, Norway). Working standard solutions of 10 μg/mL were prepared daily from these stock solutions by dilution with 0.5% (v/v) ammonia. Tellurium, used as internal standard, was prepared from a 1000 mg/L solution obtained from Teknolab. Working standard solutions of 5 μg/mL were prepared daily by dilution with water. Tellurium was selected as internal standard as it possesses a similar mass and a comparable first ionization energy as iodine. It, therefore, can be expected to behave similarly to the iodine in the plasma.

TMAH (25%) of high purity was obtained from Tama Chemicals Co., Ltd. (Kawasaki, Japan). Alkaline extraction with TMAH was performed in closed gas-tight tubes of glass (Sovirell tube) equipped with screw caps and PTFE-coated rubber sealing. Membrane filters with a pore size of 5 μm or alternatively a centrifuge (minimum 12 000 rev/min or 10 000 × g) were used to remove coarse particles; 0.45 μm membrane filters were used for final filtration.

Biological Samples

Standard reference materials (SRMs) produced by NIST (National Institute of Standards and Technology, Gaithersburg, MD) and BCR (Community Bureau of Reference, Brussels, Belgium) certified for iodine were analyzed. The samples were continuously stored in desiccators at room temperature. The residual water content in the SRMs stored under these conditions was ca 2–5%.

The following fish species were sampled by inspectors from the Directorate of Fisheries in Norway: North East Arctic cod (Gadus morhua), spawning North East Arctic cod (G. morhua), coastal cod (G. morhua), North Sea herring (Clupea harengus), spring spawning herring (C. harengus), Greenland halibut (Reinhardtius hippoglossoides), haddock (Mellanogrammus aeglefinus), ling (Molva molva), mackerel (Scomber scomber), saithe (Pollachius virens), Atlantic salmon (Salmo salar), redfish (Sebastes matinus), tusk (Brosme brosme), and minke whale (Balaenoptera acutorostrata). Sampling sites for the different fish species are shown in Figure 1.

Muscle samples of 25–50 fish of each species and 2 individuals of minke whale were individually homogenized and prepared for analysis.

Wet Digestion (Microwave Digestion and Ammonia Dilution)

Ten vessels were taken through the following procedure for each analysis. Two randomly selected vessels were filled with the digestion acids and taken through the entire procedure to monitor the average and variation of the iodine blank value. Subsamples of 0.1–0.25 g (dry mass) were weighed into the TFM digestion vessels; 2.0 mL nitric acid and 0.5 mL hydrogen peroxide were subsequently added. The sealed containers were placed in a microwave oven for 17 min and the samples were heated according to the temperature program given in Table 2. After digestion, the sample solutions were cooled 20 min to 20–30°C. The solutions were transferred quantitatively into 25 mL polyethylene calibrated flasks. Before dilution, 0.250 mL 5 mg/L tellurium solution was added as an internal standard for a final concentration of 50 ng/L. Tellurium was selected as it has similar mass, being next to io-

![Figure 2](image-url)
dine in the periodical system, and similar first ionization energy and is expected to behave similar to iodine in the plasma (6, 7). Immediately prior to the ICP/MS analysis, an aliquot of the sample solution was mixed with 3% ammonia (1 + 1, v/v), giving a pH between 9 and 10.

Alkaline Extraction (TMAH)

Subsamples of 0.1–0.5 g (dry mass) were weighed into Sovirell tubes followed by the addition of 5 mL water and then 1 mL TMAH (25%) as described by Fecher et al. (13). The sample and water were thoroughly mixed to obtain a homogeneous suspension before TMAH was added. The tubes were subsequently closed and placed in an oven at 90°C. After 3 h, the samples were cooled. The solutions were transferred quantitatively into 25 mL polyethylene calibrated flasks. Before dilution, 0.250 mL 5 mg/L tellurium solution was added as an internal standard for a final concentration of 50 ng/L. Undissolved particles were removed by centrifugation or filtration through a membrane filter (5 μm). To avoid clogging the nebulizer, final filtration was performed with a 0.45 μm membrane filter. The remaining solution was visually clear in most cases.

Optimization of settings was performed by using iodide standards of up to 50 ng/mL in dilute TMAH containing Te as an internal standard. The concentration of TMAH was ca 0.5% (v/v), but should be adapted to the amount of TMAH present in the individual extraction solution.

ICP/MS Determination of Iodine

The method of standard addition calibration was used for quantitation of iodine. Three spikes of iodine (as iodide) were added at 1, 2, and 4 ng/mL to sample solutions of each type of biological material, obtained from the treatment of wet digestion and ammonia addition as well as from extraction with TMAH. Iodide was used because it presumably occurs as iodide in the sample solution after microwave oven digestion, and was added at ca 100, 200, and 400% of the natural iodine content for optimum precision of the results.

Results and Discussion

Signal Behavior of Iodine in Nitric Acid and Ammonia Solution

To obtain precise and accurate results, the iodine signal intensities of the diluted sample solutions were recorded versus time in the graphic mode. The shape of the signal profile was used to judge the stability of the iodine species present in the sample solution. In general, with the conventional methodology for sample introduction, a stable and constant signal is observed approximately 40–60 s after uptake of an analyte solution. This is illustrated in Figure 2 for tellurium. In sample solutions obtained by microwave oven wet digestion using nitric acid, iodine is mainly present as volatile iodide compounds and needs to be converted to nonvolatile species and stabilized before ICP/MS analysis. The analysis of iodine with ICP/MS, in a sample solution of biological material digested with nitric acid, gives a signal intensity profile recorded in the graphics mode that does not reach steady-state even after several minutes. Thus, it is impossible to quantitate iodine accurately in a matrix of nitric acid without stabilizing volatile iodine species prior to ICP/MS analysis.

<table>
<thead>
<tr>
<th>CRM</th>
<th>Wet digestion (mg/kg; n = 10)</th>
<th>TMAH extraction (mg/kg; n = 10)</th>
<th>Certified value (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR 150 Milk Powder</td>
<td>1.32 ± 0.08 (6.1)</td>
<td>1.27 ± 0.05 (4)</td>
<td>1.29 ± 0.09</td>
</tr>
<tr>
<td>BCR 129 Hay Powder</td>
<td>0.172 ± 0.022 (12)</td>
<td>ND</td>
<td>0.167 ± 0.024</td>
</tr>
<tr>
<td>BCR 422 Cod Muscle</td>
<td>4.70 ± 0.15 (3.2)</td>
<td>4.60 ± 0.08 (1.7)</td>
<td>4.95 ± 0.49</td>
</tr>
<tr>
<td>NIST SRM 1572 Citrus Leaves</td>
<td>1.90 ± 0.06 (3.2)</td>
<td>ND</td>
<td>1.84 ± 0.03</td>
</tr>
<tr>
<td>NIST SRM 1566a Oyster Tissue</td>
<td>4.22 ± 0.17 (4.0)</td>
<td>ND</td>
<td>4.46 ± 0.42</td>
</tr>
<tr>
<td>NIST SRM 1549 Non-Fat Milk Powder</td>
<td>ND</td>
<td>3.39 ± 0.03 (0.9)</td>
<td>3.38 ± 0.02</td>
</tr>
</tbody>
</table>

a Uncertainty given as 95% confidence interval.

b Based on 6 determinations.

c ND = not determined.
We chose an ammonia solution as stabilizer and diluent for iodine present in digests together with nitric acid. The combination of nitric acid and hydrogen peroxide as acid mixture in microwave oven digestion was shown to be very efficient for determining different elements in biological materials (15, 16).

Several trials were performed to assess the effect of different amounts of ammonia on the stabilization of standards of iodine as iodide and iodate in nitric acid. A nitric acid concentration of 8% (v/v) was chosen, even though the oxidation of nitric acid during the digestion gives a residue of nitric acid <8% (v/v). Early in the study, results clearly demonstrated memory effect in the ICP/MS analysis related to high iodine concentrations (signals > 80 000 ions/s). Consequently, samples with high iodine concentrations were avoided.

For precise and accurate iodine analyses, a stable and low background signal was needed before the analysis. The use of ammonia to stabilize standards of iodide and iodate in nitric acid gave promising results. The experiments showed that iodide was the species of iodine most difficult to stabilize; hence, subsequent study only involved iodide.

Experiments were performed with a series of ammonia concentrations ranging from 0.5 to 4.0% (v/v) added to 20 ng I/mL standard solutions (1 + 1). The standard solutions also contained tellurium (50 ng/mL) and 8% (v/v) nitric acid. The ammonia solution and standard solutions were mixed (1 + 1) and analyzed by ICP/MS. The results for the solutions containing 1.5, 2.0, and 3.0% (v/v) ammonia are given in Figure 2, which shows that the iodine signal intensity versus time in the graphics mode was substantially improved when the ammonia concentration was increased from 1.5 to 3% (v/v). The iodine signal for the sample, containing iodide dissolved in nitric acid and 3% (v/v) ammonia solution, reached a stable steady...
The solution had a pH between 9 and 10 after ammonia was added. The stability of the iodine signal was not further improved by increasing the ammonia concentration from 3 to 4% (v/v; results not shown).

Figure 3 shows the iodine signal intensity versus time in the graphics mode when the described method was used for a biological sample, in this case muscle tissue from farmed salmon (Salmo salar). The iodine signals have an acceptable form when the signals reach a steady state within an acceptable period of time, and without any significant tailing, allowing quantitative analyses to be performed.

**Quality Assurance Data for Wet Digestion and Ammonia Method**

Precise and accurate ICP/MS analyses of iodine in biological samples require blanks with low iodine levels and small variation in concentrations among parallels of blanks. The limit of detection (LOD) and the limit of quantitation (LOQ) are highly dependent on low blanks, and blanks must be under control in terms of small variation in iodine concentration among parallels. The LOD and the LOQ were calculated as 3 and 6 times the standard deviation of the average of the blank samples (n = 20), respectively. Prior to analysis of the blanks, the system was washed carefully. Water, 1% (v/v) nitric acid, 0.5% (v/v) ammonia, and 0.5% (v/v) TMAH were tested as washing solutions. The washing solution made of 0.5% (v/v) TMAH was most efficient for our system.

The washing-out step was continued until the background dropped to an acceptable level (250 ions/s) before samples were run. Depending on the type of samples run on the ICP/MS before the iodine analysis, the washing time can last for more than 20 h before the iodine background signal has reached an acceptable and stable level. Severe memory effect appears when the instrument has been used for element analysis in seafood products. High levels of unstable and volatile iodine compounds are present in digests of seafood where nitric acid is used for decomposition. Those volatile iodine species contaminate vital parts of the ICP/MS instrument (spray chamber, cones, tubes, etc.) which need to be washed out before the iodine analysis can start. The LOD of the method was calculated to be 0.06 ng/mL, and the LOQ was 15 ng/g (dry mass) when 0.2 g sample was used for analysis, and with a dilution factor of 50. This corresponds to an LOQ value of approximately 3 ng/g for the equivalent amount of fresh sample. The trueness and the repeatability of the analytical method for iodine were established by analyzing certified reference materials (CRMs) which covered a variety of sample types and concentrations (Table 3). Before the quantitative analyses, all samples were run in the graphics mode to ensure that the ICP/MS signal was stabilized at a steady-state intensity (Figure 2). In addition, the standard addition calibration curve obtained for each sample type should not give a coefficient of correlation <0.999. The poorest repeatability, expressed as RSD%, was found in CRM of hay powder of 12%, and the best repeatability was found in the CRMs with the highest iodine concentrations (BCR 422 Cod Muscle and NIST SRM 1566a Oyster Tissue) of approximately 4%. The results are acceptable in relation to the iodine concentration in the samples compared with the LOQ. In the case of a nonsteady state signal intensity for iodine, the precision would increase markedly. The trueness obtained for iodine in CRMs (Table 3) is in good agreement with the certified values.
Quality Assurance Data for TMAH Extraction Method

Determination of iodine in food samples by ICP/MS after TMAH extraction was extensively described by Fecher et al. (13). The memory effect and the problems associated with low and stable iodine background signal require special care as described for wet digestion and ammonia procedure. The LOD for this method, defined as 3 times the standard deviation of the average iodine concentration in the blanks (n = 20), was calculated to be 0.1 ng/mL. The LOQ of the method, defined as 6 standard deviations of the average iodine concentration (n = 20), was estimated at 29 ng/g for sample weight of 0.2 g (dry mass) and a dilution factor of 25. This LOQ corresponds to 6 ng/g wet weight. The results for the LOQ were quite similar to those reported by others (13). The quantitative results achieved by the TMAH method under optimal instrumental conditions are shown in Table 3. The repeatability of the method defined as RSD is <2% for CRMs with concentrations of 3–4 mg/kg dry matter, and the trueness is also good compared with the certified values. The method using TMAH appears to extract iodine quantitatively from the animal samples, including muscle and organ tissues of rats and fish studied so far.

Sources of Error

Wet digestion and ammonia.—Digests of biological samples of calcium-rich tissues give suspension of fine particles when ammonia is added. Analyses of such sample solutions leads to clogging of the ICP system, resulting in decreased signal of the internal standard. Even if the samples do not have a particularly high calcium/phosphorus content, a white coating appears in the spray chamber, cones, etc., after sample solutions are run for several hours. The coating has to be washed off before the ICP/MS instrument can be used for analysis of other elements.

TMAH extraction.—Extraction of iodine with TMAH works well for samples with low levels of starch, but high starch levels result in high viscosity, which cannot be filtrated. Samples with high starch content can, however, be decomposed by the wet digestion and ammonia procedure before ICP/MS analysis.

Content and Variation of Iodine in Fish Fillets

The trueness and precision (given as the internal reproducibility) for wet digestion using ammonia as a diluent and TMAH extraction were assessed by analyzing BCR 422 Cod Muscle reference material in parallel with a series of fillet samples from different fish species. The standard addition calibration method was routinely used throughout the study. Figure 4 shows the control chart for the wet digestion procedure using ammonia as a diluent. Figure 5 shows the control chart for the TMAH extraction, and the warning and rejection limits defined as ± 1 and ± 3 standard deviations, respectively, are also indicated. The critical values used for the control chart as average and standard deviation (SD) were taken from the CRM certificate. If the value for the CRM was accepted, the analyses of unknown samples, which were performed in parallel with the CRM, were also accepted. The results given for the CRM in the control chart show that the analyses were accurate and precise. From the data obtained for wet digestion and ammonia addition (Figure 4), the average iodine in BCR Cod Muscle was calculated to be 5.04 mg/kg, and internal reproducibility, given as RSD%, was 6.1%. The figures for TMAH extraction (Figure 5) were calculated to be 4.93 mg/kg and 4.4%, respectively. No significant differences were found between the 2 analytical methods.

The results from the fish fillets (Table 4) are from a national program in Norway aimed at establishing a database of pollutants (metals, chlorinated hydrocarbons, and cesium 137) in seafood caught in the open seas, including the Barents Sea, the Norwegian Sea, and the North Sea. The program was started in 1994 and is expected to continue beyond 2008 (unpublished data). Cod (G. morhua) were sampled at 4 sites (Figure 1), 2 sampling sites in the Barents Sea, spawning cod from Lofoten, and coastal cod from More. The iodine content found in cod fillet from the Barents Sea showed mean values of 1.0 and 2.5 mg/kg, and the iodine concentration in individual fish varied between 0.34 and 3.5 mg/kg and between 0.7 and 12.7 mg/kg fresh weight, respectively. The iodine content in spawning cod varied between 0.45 and 7.0 mg/kg fresh weight, with an average value of 1.1 mg/kg fresh weight, whereas the iodine content in fillet of coastal cod varied between 0.18 and 1.0 mg/kg fresh weight, with an average value of 0.36 mg/kg fresh weight. The results for iodine in haddock (M. aeglefinus; Table 4) varied between 0.6 and 9.2 mg/kg fresh weight, with an average value of 1.9 mg/kg fresh weight. Fillets of cod and haddock showed average values of iodine >1 mg/kg fresh weight, except in fillets of coastal cod. Fillets of North Sea herring (C. harengus), mackerel (S. scomber), and saithe (P. virens) showed average iodine values between 0.5 and 1.0 mg/kg fresh weight, whereas the following fish species showed average iodine values between 0.1 and 0.5 mg/kg fresh weight: Coastal cod (G. morhua), spring spawning herring (C. harengus), farmed salmon (S. salar), redfish (S. matinus), and tusk (B. brosme). A few fish species also showed average iodine values <0.1 mg/kg fresh weight: Greenland halibut (R. hippocoglossoides), ling (M. molva), and minke whale (B. acutorostrata). Fish absorb iodine both from the seawater and the food they eat (17). The Institute of Marine Research, Bergen, Norway, monitored the stomach contents of cod from the Barents Sea in 1994 and found more than 250 different species of prey. The prey organisms most frequently found in cod were Euphausiacea, Pandalus borealis, Mallotus villosus, Gadidae, and G. morhua (Bugstad, personal communications).

Table 4 shows that the human iodine intake from fish depends on the type of fish species consumed. A meal of fish consisting of 200 g cod fillet with an average concentration of 1 mg/kg will provide 200 μg iodine, whereas a 200 g meal of salmon fillet with an average iodine concentration of 0.15 mg/kg will provide 30 μg, that is only 20% of the recommended daily intake of iodine at 150 μg/day (2, 3).
Acknowledgments

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References