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ARTICLES

Determination of Iodine in Foods by Inductively Coupled Plasma Mass Spectrometry after Tetramethylammonium Hydroxide Extraction

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Abstract—In this study, the determination of iodine in 123 different types of foods was conducted. Iodine-selective electrode, microwave digestion/inductively coupled plasma mass spectrometry and tetramethylammonium hydroxide extraction/ inductively coupled plasma mass spectrometry (**TMAH/ICP-MS**) methods were tested to achieve the highest recoveries for the determination of iodine. Among them, TMAH/ICP-MS method was the most effective with respect to analytical recoveries (90–99%), limits of detection (0.013 μg/kg) and quantification (0.04 μg/kg) values. The intra- and inter-day relative standard deviations for iodine SRM were <4.8% and <10.8%, respectively. Iodine content in food samples showed a wide range of variations: 0.4–4 μg/100 g for fruits and vegetables, 4 μg/100 g for cereals and grain products, 15 μg/100 g for meat, 36 μg/100 g for milk, 137 μg/100 g for infant formula and 2.3–394 mg/100 g for seafood.

Keywords: iodine, tetramethylammonium hydroxide extraction, inductively coupled plasma mass spectrometry, method validation, certified reference material

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Iodine is one of the major components of the thyroid hormone involved in body growth and development [1]. Iodine concentrations in foods of all types vary considerably. In Korea, people consume enough iodine because of the relatively abundant seaweed intake; iodine deficiency disorders such as cretinism, multiple goiters and thyroid cancer [2] were less reported than in other countries [3–6]. However, not only iodine deficiency is a problem, but also its overdose. Excessive intake of iodine may lead to thyroiditis, hyperthyroidism and hypothyroidism, iodine intake plays an important role in the development of autoimmune thyroid disease as well [7]. The nutritional intake standard for iodine issued by the Codex and World Health Organization/Food and Agriculture Organization of the United Nations guidelines on food fortification with micronutrients is set at a recommended daily dose of 150 μg for adults and 200 μg for pregnant and lactating women [8, 9]. However, Korean Nutrition Society recommended for pregnant and lactating women to take 90 and 190 μg, respectively, more than the adult dose. For pregnant or lactating women, taking seaweed soup with high iodine content for a certain period after giving birth is an example of good practice in Korea [10].

Although the data on iodine content in food are needed, it is difficult to quantify accurately due to low iodine content, sample preparation and influence of other substances present in food [11]. Furthermore, the content of iodine in food varies from low to high. Ion selective electrode (**ISE**) method is widely applied in foods with high iodine content such as health functional foods and seaweeds and is also listed as the official method in Korea Food Code [12] and American Society for Testing and Materials (**ASTM**) [13]. The ISE method has many advantages: responsive over a wide concentration range, not affected by color or turbidity of sample, rugged and durable, rapid response time, real time measurements, low cost to purchase and operate and easy to use. However, it is insensitive to low concentration in foods, and hard to be applied to various food matrices [6, 14].

Inductively coupled plasma mass spectrometry (**ICP-MS**) with minimal sample preparation is the most widely used technique due to its high accuracy, precision and sensitivity. The most critical step in

iodine determination by ICP-MS is the extraction procedure. Current microwave digestion (MD) method and wet digestion using concentrated acids are not widely applicable due to iodine losses by volatilization as HI or I_2 that result in non-quantitative recoveries [15]. In addition, iodine-containing samples are usually decomposed with strong oxidizing acids, typically nitric acid. Memory effects in the ICP-MS introduction system have been often reported. According to many research papers, the extraction procedure by nitric acid can increase iodine signal and establish severe memory effects [16]. In this sense, sample preparation approaches have been developed using sample dilution or dispersion in alkaline solution such as ammonia, sodium hydroxide, tetramethylammonium hydroxide (**TMAH**) and water-soluble tertiary amines solution [17, 18]. Alternative procedures using oxidizing acid mixtures can be used, whereby the oxidizing potential must be high enough for oxidation of iodide into non-volatile iodate. However, these procedures are time-consuming, reagents generally present high blank values or can cause interferences in the further determination step [18]. Among the sample preparation methods, extraction of iodine by TMAH solution at high temperatures in a closed vessel is most widely used sample treatment method. In this study, optimized method for iodine determination in the food was selected by comparing ISE, MD/ICP-MS and TMAH/ICP-MS. After confirming the effectiveness of the method, the iodine content in the food was determined.

EXPERIMENTAL

Materials and chemicals. Nitric acid and hydrogen peroxide were of electronic grade (Dongwoo fine chem., Seoul, Korea), TMAH was purchased from Alfa Aesar (electronic grade, MA, USA) and other reagents of HPLC grade were purchased from Sigma-Aldrich (MO, USA). Iodine standard solution was purchased from AccuStandard Inc. (New Haven, CT, USA). Standard reference materials (**SRM**) 1849a infant formula and 1548a typical diet were purchased from National Institute of Standards and Technology (Gaithersburg, MD, USA) for the validation of procedure for iodine determination. In addition, 123 different food samples including infant formula, green laver, sea mustard and kelp were purchased from the market. Powder samples (infant formulas, sweeteners, SRM 1849a and SRM 1548a) and liquid samples (milk, vegetable oil, seasonings) were used without homogenization. Dried seaweeds (lavers, sea mustards and kelps) and cereals were pulverized with a grinder and passed through 100 mesh for analysis. The meats, cooked foods, beans, vegetables and fruits were ground in a homogenizer (Omni mixer homogenizer 17106, Kennesaw, GA, USA) at 10000 rpm for 5 min. The samples were stored at −20°C during analysis.

Preparation for iodine-selective electrode. The weighed sample was dissolved in 10 mL of water. 2 mL of 3% acetic acid was added and thoroughly mixed with water to a final volume of 50 mL for 10 min. The mixture was sonicated again for 10 min and filtered.

Microwave digestion. 8 mL of nitric acid and 2 mL of hydrogen peroxide were added to a microwave (Multiwave ECO, Anton Paar, Austria) digestion teflon beaker containing ca. 1 g of sample and the lid was closed. The sample was heated to 100°C for 10 min and further decomposed at 180°C for 30 min. After cooling down to room temperature, the solution was transferred into a 50 mL volumetric flask. The container was then rinsed with 50 mL of 5% aqueous ammonia to fill up to the mark, and then the solution was filtered.

Tetramethylammonium hydroxide extraction. The sample was weighed into a Perfluoroalkoxy tube and 4.5 mL of water and 1 mL of 25% TMAH were added in the tube. After closing the lid, the tube was placed in a 90°C dry oven for 3 h. After cooling, the mixture was diluted with 25 mL of water and centrifuged at 3000 rpm for 15 min. The supernatant was used as the test solution.

Preparation of standards. For ISE method, the standard solution at a concentration of 1000 mg/kg was diluted to 0.1, 1, 10 and 100 mg/kg. 1 M KNO_3 was used as the ionic active solution. For MD method, the standard was diluted in 2% nitric acid to a concentration of 100 mg/kg. For TMAH method, the standard was diluted to 1, 5, 25 and 50 μg/kg by dilution in 1% TMAH.

Instrumental analysis. The ISE was a Seven Excellence Multiparameter (Mettler Toledo, Switzerland), and the ionic electrode was immersed in an electrode electrolytic 1 mg/kg iodine standard for stabilization. The temperature of the test solution was maintained at 25°C with stirring. Calibration curves were prepared using standard materials.

The ELAN DRC-e ICP/MS (Perkin-Elmer Shelton, CT, USA) equipped with a single-channel mass flow controller was used. The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin-Elmer AS-90 autosampler and aspirated in the argon plasma. The instrument was run at normal resolution and set to detect the iodine signal intensity at 127 m/z in the quantitative and graphics data acquisition modes, which allowed quantitation and recording of the signal intensity vs. time, respectively. Further details of the instrumental settings were: 1500 W RF power, 20 L/min plasma gas flow, 0.98 L/min nebulizer gas flow and 1.2 L/min auxiliary gas flow.

Method validation. Assay validation was performed according to the Food and Drug Administration (**FDA**) guidance for industry Q2B procedure [19, 20]. The following equations were used for the limits of detection (**LOD**) and quantification using standard solutions based on the slope of the calibration curve

Table 1. Determination of iodine in SRM 1849a and SRM 1548a by MD/ICP-MS and TMAH/ICP-MS methods

SRM	Certified value, mg/kg ± *Uc*[a]	Sample amount, g	MD/ICP-MS ($n = 3$)		TMAH/ICP-MS ($n = 3$)	
			mean ± *Uc*, mg/kg	*En*[b]	mean ± *Uc*, mg/kg	*En*
SRM 1849a	1.29 ± 0.11	0.1	1.8 ± 0.4	1.30	1.16 ± 0.10	−0.87
		0.5	1.52 ± 0.02	2.06	1.18 ± 0.07	−0.81
		1.0	1.38 ± 0.12	0.55	1.27 ± 0.13	−0.12
SRM 1548a	0.76 ± 0.10	0.1	1.1 ± 0.7	0.49	0.82 ± 0.05	0.54
		0.5	0.45 ± 0.10	−2.19	0.72 ± 0.03	−0.38
		1.0	0.62 ± 0.02	−1.37	0.71 ± 0.01	−0.50

[a] *Uc* is measurement uncertainty, [b] *En* is *En* score.

obtained from the standard deviation and linearity tests. Precision and accuracy were measured with SRM 1849a taking 0.1, 0.5 and 1.0 g of samples. The experiment was repeated 3 times a day for 4 days. The daily precision was calculated and reported as relative standard deviation (**RSD**, %).

Measurement of uncertainty and *En* score. The final result and its 95% confidence interval were estimated by the Eurachem procedure [21]. The ISO 13528 standard for proficiency testing defines the following *En* score (Eq. (1)):

$$En_i = \frac{X_i - X_{\text{ref}}}{\sqrt{u_x^2 + u_{\text{ref}}^2}}, \quad (1)$$

with X_{ref}, u_{ref} and u_x denoting the assigned value, the standard uncertainty of the assigned value and the laboratory's estimate of the standard uncertainty of its result, respectively. When the coverage factor is 2, a critical value of 1 for the *En* score is equivalent to a critical value of 2 for a z-score [22].

RESULTS AND DISCUSSION

Optimization of iodine determination method. To check accuracy of the methods, SRM 1849a and 1548a were used. Table 1 shows the results of iodine determination by ISE, MD/ICP-MS and TMAH/ICP-MS methods for 0.1, 0.5 and 1.0 g of SRM, respectively.

Iodine was not detected by ISE in both SRM 1849a and 1548a due to low iodine content. ISE are widely used in analytical chemistry and biochemical or biophysical research, where measurements of ionic concentration in an aqueous solution are required [23, 24]. The use of ISE in environmental analysis offers several advantages over other methods of analysis because the cost of initial setup is relatively low. However, sensitivity is quite low and measurement range is from 1 μM to 1 M. This result revealed that ISE is not suitable for the determination of iodine in low concentration food samples.

The MD/ICP-MS method was not suitable for iodine determination as well because a large variation was observed comparing to certified values. The reason may consist in volatility of iodide and interference effect due to the remaining solvents in the spray chamber after each analysis.

On the other hand, there was no significant difference between the results of TMAH/ICP-MS method and that of certified values. The *En* score was calculated for the MD/ICP-MS and TMAH/ICP-MS methods except the ISE method with low sensitivity. The MD/ICP-MS method showed a low reliability for the experimental method with the *En* score ranging from 0.49 to 2.19, but the TMAH/ICP-MS method showed good results with the *En* score less than 1 (0.12–0.87). These results reveal that the TMAH/ICP-MS is an effective method for iodine determination, and the validity of the method was verified.

Validation of inductively coupled plasma mass spectrometry procedure after tetramethylammonium hydroxide extraction. The method validation was performed by TMAH/ICP-MS using SRM 1849a. The linearity and sensitivity of iodine quantification by ICP-MS are presented below:

Linear range, μg/kg	Correlation coefficient (r^2)	LOD, μg/kg	LOQ, μg/kg
1–50	0.9990	0.013	0.04

As seen, the linearity was confirmed in the concentration range of 1–50 μg/kg using iodine standard. The correlation coefficient r^2 was statistically significant (>0.9990) for the linear curve consisting of 4 points. The limits of detection and quantification were 0.013 and 0.04 μg/kg, respectively.

As shown in Table 2, the accuracy and precision were performed by TMAH/ICP-MS using 0.1, 0.5, and 1.0 g of SRM 1849a, respectively. According to the results, intra- and inter-day RSD values were from 0.9 to 4.8% and from 7.9 to 10.8%, respectively. The recovery showed good results from 90 to 99%, this intra-day, inter-day and recovery values were very successful.

Table 2. The precision and accuracy for TMAH iodine extraction method for SRM 1849a using ICP-MS

Sample amount, g	RSD, %		Recovery,%
	intra-day ($n = 3$)	inter-day ($n = 4$)	
0.1	4.8	7.9	90 ± 6
0.5	0.9	5.7	92 ± 6
1.0	4.1	10.8	99 ± 10

Table 3. Determination of iodine by TMAH/ICP-MS in foods from the market

Sample	*n*	Range	Mean ± SD[a]
Content, μg/100 g			
Milk	1	35.8 ~ 36.1	35.9 ± 0.2
Infant formula	2	109.0 ~ 150.3	137.1 ± 1.3
Meat	2	14.4 ~ 15.7	15.0 ± 0.5
Cooked food	8	<LOD ~ 4.1	1.5 ± 0.1
Vegetable oil	1	4.1 ~ 4.3	4.2 ± 0.2
Seasoning	2	<LOD ~ 4.34	2.2 ± 0.5
Cereals and grain products	17	<LOD ~ 12.5	4.1 ± 0.2
Bean	13	0.5 ~ 3.4	2.5 ± 0.1
Vegetable	58	<LOD ~ 62.3	3.8 ± 0.2
Fruit	6	<LOD ~ 1.5	0.4 ± 0.0
Sweetener	3	<LOD ~ <LOD	<LOD
Content, mg/100 g			
Laver	3	1.6 ~ 2.9	2.3 ± 0.2
Sea mustard	3	7.5 ~ 11.0	9.5 ± 0.3
Kelp	4	302.3 ~ 447.0	394 ± 15

[a]SD – standard deviation.

Quantification of iodine in foods. The iodine contents in 123 commercially available foods were determined using the TMAH/ICP-MS method. The results are shown in Table 3. As a result of iodine quantification in foods, the group with the highest iodine content was seaweeds. Among them, iodine content in kelp was 302–447 mg/100 g, 8–11 mg/100 g in while sea mustard and 1.6–2.9 mg/100 g in laver. Several studies have reported similar results that kelp contains 179.1–430.0 mg/100 g, sea mustard contains 8.7–28.0 mg/100 g and laver contains 2.0–3.7 mg/100 g of iodine [25, 26]. Iodine contents in cooked foods, vegetable oils, seasonings, beans and fruits were detected to be less than 5 μg/100 g. However, some reports show higher or lower results comparing to this research. Judprasong et al. [27] reported iodine contents of 52.1–1304.0 μg/100 g in cooked foods. Another study reported the values of 0.4–3600.2 μg/100 g in seasonings, <LOD–2.5 μg/100 g in beans and 29.8–311.3 μg/100 g in fruits [28, 29]. In cereals and grain products, iodine contents were <LOD–12.5 μg/100 g but another report was 0.2–39.3 μg/100 g [28, 30–32]. Iodine contents in milk, infant formulas and meat in this study were 35.8–36.1 and 109.0–150.3, 14.4–15.7 μg/100 g, respectively. However, they were 31.0–110.7, 21.1–109.8 and <LOD–48.0 μg/100 g, respectively, in another research [28, 30, 33]. The difference of iodine content in each food group was estimated to be the result of sample treatment and different sensitivity of analyzer as well as conditions due to regional characteristics, growth environment, sample collection time, etc.

CONCLUSIONS

In this study, ISE, MD/ICP-MS and TMAH/ICP-MS methods were compared for iodine quantification in foods. The ISE method has many advantages and can be applied to foods with high iodine content such as health functional foods and seaweeds but is insensitive to foods with its low concentration and hard to be applied to various food matrices. The MD/ICP-MS method that showed a large variation comparing to certified values may not be adequate owing to the memory effect due to the remaining solvents in the spray chamber after analysis. The TMAH/ICP-MS method showed no significant difference between the results of certified values with the *En* score less than 1. In addition, the results of validation showed that the linearity range for iodine, detection and quantification limits, precision within day, the accuracy of daily precision and the confirmation of accuracy were all in conformity with the guideline for validation proposed by FDA. These facts reveal the TMAH/ICP-MS can be confirmed as an effective method for iodine quantification.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Glinoer, D., *Thyroid*, 2006, vol. 16, no. 10, p. 947.
2. Vanderpas, J., *Annu. Rev. Nutr.*, 2006, vol. 26, p. 293.
3. Assey, V.D., Greiner, T., Mzee, R.K., Abuu, H., Mgoba, C., Kimboka, S., and Peterson, S., *Food Nutr. Bull.*, 2006, vol. 27, no. 4, p. 292.
4. Dillon, J.C. and Milliez, J., *Br. J. Obstet. Gynaecol.*, 2000, vol. 107, no. 5, p. 631.

5. Mason, J.B., Deitchler, M., and Gilman, A., *Food Nutr. Bull.*, 2002, vol. 23, no. 3, p. 292.
6. Shelor, C.P. and Dasgupta, P.K., *Anal. Chim. Acta*, 2011, vol. 702, no. 1, p. 16.
7. Zimmermann, M.B., Jooste, P.L., and Pandav, C.S., *Lancet*, 2008, vol. 372, no. 9645, p. 1251.
8. *CodexAlimentarius, Guidelines on Nutrition Labelling CAC/GL2-1985*, World Health Organization, Food and Agriculture Organization, 2013.
9. *Vitamin and Mineral Requirements in Human Nutrition*, World Health Organization, Food and Agriculture Organization, 2nd ed., 2004. http://www.who.int/nutrition/publications/micronutrients/9241546123/en. Accessed April 28, 2018.
10. Paik, H.Y., *Asia Pac. J. Clin. Nutr.*, 2008, vol. 17, no. S2, p. 416.
11. El-Ghawi, U.M. and Al-Sadeq, A.A., *Biol. Trace Elem. Res.*, 2006, vol. 111, nos. 1–3, p. 31.
12. Korea food code, Seoul: Ministry of Food and Drug Safety. http://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=312. Accessed August 2017.
13. *ASTM D3869-15: Standard Test Methods for Iodide and Bromide Ions in Brackish Water, Seawater, and Brines*, ASTM International, 2015, Sections 26–34.
14. Julshamn, K., Dahl, L., and Eckhoff, K., *J. AOAC Int.*, 2001, vol. 84, no. 6, p. 1976.
15. Gelinas, Y., Iyengar, G.V., and Barnes, R.M., *Fresenius' J. Anal. Chem.*, 1998, vol. 362, no. 5, p. 483.
16. Vanhoe, H., *J. Trace Elem. Electrolytes Health Dis.*, 1993, vol. 7, no. 3, p. 131.
17. Knapp, G., Maichin, B., Fecher, P., Hasse, S., and Schramel, P., *Fresenius' J. Anal. Chem.*, 1998, vol. 362, no. 6, p. 508.
18. Fecher, P.A., Goldmann, I., and Nagengast, A., *J. Anal. At. Spectrom.*, 1998, vol. 13, no. 9, p. 977.
19. *Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology*, Rockville: Food and Drug Administration (FDA), 1996. http://www.gmp-compliance.org/guidemgr/files/1-12-5.PDF. Accessed February 2017.
20. Validation and verification guidance for human drug analytical methods (Appendix 1), Food and Drug Administration, Document no. ORA-LAB.5.4.5, version no. 1.6, p. 17, 2012. http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM092147.pdf. Accessed January 2017.
21. *Eurachem/CITAC Guide CG4: Quantifying Uncertainty in Analytical Measurement*, 2000, 2nd ed. www.eurachem.ul.pt. Accessed March 2017.
22. *ISO: Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons*, International Organization for Standardization, 2005.
23. Bard, A.J. and Faulkner, L.R., *Electrochemical Methods: Fundamentals and Applications*, New York: Wiley, 2001, 2nd ed.
24. Archer, M.D., in *Electrochemistry, Past and Present*, Washington, DC: Am. Chem. Soc., 1989, vol. 390, ch. 8, p. 115.
25. Moon, S.J., Kim, J.Y., Chung, Y.J., and Chung, Y.S., *J. Korean Nutr. Soc.*, 1998, vol. 31, no. 2, p. 206.
26. Yeh, T.S., Hung, N.H., and Lin, T.C., *J. Food Drug Anal.*, 2014, vol. 22, no. 2, p. 189.
27. Judprasong, K., Jongjaithet, N., and Chavasit, V., *Food Chem.*, 2016, vol. 193, p. 12.
28. Chung, S., Chan, A., Xiao, Y., Lin, V., and Ho, Y.Y., *Food Addit. Contam., Part B*, 2013, vol. 6, no. 1, p. 24.
29. Salau, B.A., Ketiku, A.O., Ajani, E.O., Ezima, E.N., Idowu, G.O., and Soladoye, M.O., *Afr. J. Biotechnol.*, 2008, vol. 7, no. 23, p. 4328.
30. Haldimann, M., Alt, A., Blanc, A., and Blondeau, K., *J. Food Compos. Anal.*, 2005, vol. 18, no. 6, p. 461.
31. Longvah, T., Toteja, G.S., and Upadhya, A., *Food Chem.*, 2013, vol. 136, no. 2, p. 384.
32. Shinonaga, T., Gerzabek, M.H., Strebl, F., and Muramatsu, Y., *Sci. Total Environ.*, 2001, vol. 267, nos. 1–3, p. 33.
33. Tracnicek, J., Herzig, I., Kursa, J., Kroupova, V., and Navratilova, M., *Vet. Med.*, 2006, vol. 51, no. 9, p. 448.