Quality Assurance and Safety of Crops & Foods, 2023; 15(3): 36–42

Ultrasensitive and highly selective determination of iodate by reversed-phase ion pair HPLC–amperometric detector after online electrochemical reduction to iodide

Qingwei Jiang[1], Tao Wang[2*], Gao Li[1*]

[1]*Key Laboratory of Natural Medicines of the Changbai Mountain, Ministry of Education, Yanbian University College of Pharmacy, Yanji, P.R. China;* [2]*Sinovac Biotech Co. Ltd., Beijing, P.R. China*

***Corresponding Authors**: Gao Li, Key Laboratory of Natural Medicines of the Changbai Mountain, Ministry of Education, Yanbian University College of Pharmacy, Yanji, P.R. China. Email: gli@ybu.edu.cn. Tao Wang, Sinovac Biotech Co. Ltd., P.R. China. Email: wangtaojk2007@163.com

Received: 16 October 2022; Accepted: 18 May 2023; Published: 1 July 2023

RESEARCH ARTICLE

Abstract

We developed an ultrasensitive and highly selective method to quantify iodate (IO_3^-) in iodized salt and biological samples. In the high-performance liquid chromatography (HPLC) system, IO_3^- was injected with sampler and reduced to iodide (I^-) online using a Coulomb detector (CD) as a pre-column reactor, retained by C18 column with the help of ion pairs, and then determined by an amperometric detector (AD). For analysis of IO_3^-, several parameters and reactive conditions of Coulomb detector, including applied potential, pH value, and salt concentration, were optimized to obtain the best reduction efficiency. The optimized HPLC–CD-AD method was found to be linear over a wide range of IO_3^- concentration (0.05–0.25 μg/mL) with appreciable recovery rates (86.90–94.70%) of quality controls at excellent detection limits (100 pg) and acceptable variability. The analysis of IO_3^- in actual samples, such as urine of volunteers, iodized salt, and seawater, discovered that the method has high selectivity and little interference. It can be concluded that the proposed method was ultrasensitive and highly selective in the trace detection of IO_3.

Keywords: iodate; electrochemical reduction; high-performance liquid chromatography (HPLC); amperometric detector; Coulomb detector

Introduction

Iodine is one of the trace elements required by the human body. For the synthesis of human thyroid hormones, iodine is essential and affects human growth and development, especially the growth and development of the brain. Lack of iodine causes large thyroid gland and decreased intelligence (Delange and Lecomte, 2000). China is one of the major countries with iodine deficiency disorders. Fortunately, in the past few decades, the problem of iodine deficiency has been gradually solved by common use of iodized salt. However, in some economically developed areas of China, people have taken in excess of iodine to cause iodine-induced hyperthyroidism (Delange *et al.*, 1999). Both iodine deficiency and excess of iodine lead to changes in the morphology and functioning of the thyroid, causing various disorders. Therefore, it is necessary to find an accurate and precise determination method of iodine for quality control of iodized salt and monitoring human urine samples.

Nowadays, salt products in Chinese markets are "iodized" by the addition of potassium iodate (KIO_3). Thus, it is important to analyze iodate (IO_3^-) in salt and urine samples of patients.

In the literature, many analytical methods have been developed for determining IO_3^-, including spectrophotometry (Ford and Johnson, 1991), volumetric methods (Edmonds and Morita, 1998), mass spectrometry (Barnett *et al.*, 1999; Shin *et al.*, 1996), electrochemical analysis (Shabani *et al.*, 1999), atomic absorption spectroscopy, ion chromatography (Kumar *et al.*, 2000), and high-performance liquid chromatography (HPLC; Li *et al.*, 2001). Among the above-mentioned methods, ion chromatography is undoubtedly the most attractive method of choice. At the very beginning, ion chromatography equipped with conductivity detector was used to determine iodide (I^-) in seawater, urine, and other natural product samples, and then it was developed to a more sensitive stage, in which electrochemical detector (ED) was used.

Commercial ion chromatographs with various detectors are used to analyze various inorganic ions. However, some challenge still prevailed. First, high price hinders the application of ion chromatograph brand machine equipped with ion chromatography column in any China laboratory. Second, large amounts of matrix ions (chloride, sulfate, and other inorganic ions) impede the determination of target analysts by disturbing IO_3^- signals. Furthermore, with the development and fusion of liquid chromatography, boundaries between conventional ion chromatography and other HPLC techniques have blurred. For example, reversed-phase liquid chromatography, through the addition of ion-pairing reagents, can retain and separate periodate, IO_3^-, and I^- very well in C8 or C18 column without using a dedicated ion exchange chromatography column (Huang *et al.*, 2013; Sajonz, 2006). Ion chromatography columns are expensive and have a short lifetime. The iodine method is cumbersome to operate and cannot achieve online automated analysis; therefore, it is necessary to develop highly sensitive, inexpensive, and automatic instrumental analysis methods.

Our previous study reported a novel technique to determine IO_3^- in table salt by HPLC–ultraviolet (UV) detector after online electrochemical reduction to I^-. IO_3^- undergoes reduction on the electrodes of Coulomb detector (CD) to produce I^-: “$IO^{3-} + 6e + 6H^+ = I^- + 3H_2O$.” The UV response to I^- is nearly 10 times more than that of IO_3^-, thus amplifying the signal of IO^{3-} (Wang *et al.*, 2015).

In this study, we improved the above-mentioned method of online electrochemical reduction. An ultrasensitive result for quantification of IO_3^- was obtained by selecting amperometric detector (AD) to detect the resulting I^-. The sensitivity of amperometric detector is higher by two orders of magnitude than that of ultraviolet detector.

A schematic diagram of the method is illustrated in Figure 1. A Coulomb detector (Eicom, Japan) was linked on pre-column as an electrochemical reactor, and a high-voltage switching six-way valve (Shiseido, Japan) prevented the cell and electrode of Coulomb detector from high back pressure of column. The samples containing IO_3^- were injected by auto-sampler and transferred into Coulomb detector through mobile phase 1. In the reactor, IO_3^- was changed into I^- by electrochemical reduction at electrode, and the resulting I^- was collected in the peek loop.

The process would take 30 s to get ready for the next stage. At the next stage, the high-voltage six-way valve was immediately switched to INJECT position, and pump 2 transferred mobile phase 2 to inject I^- from the opposite end of peek loop to C18 column for subsequent isolation and analysis. Finally, amperometric detector was used to detect I^-, and ultrasensitive signal was obtained.

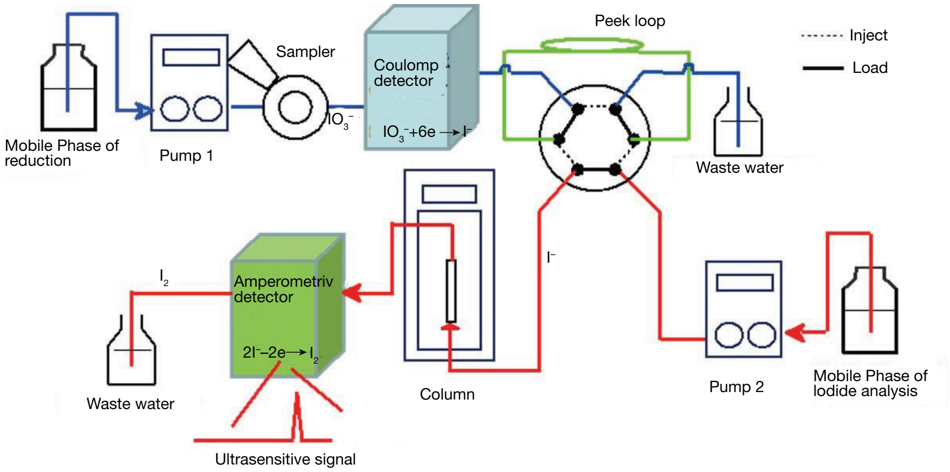

Figure 1. **Schematic diagram of HPLC–CD-AD system for determining IO_3^-.**

Materials and methods

Materials

Potassium iodate and potassium iodide (KI) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Potassium dihydrogen phosphate (KH_2PO_3), tetrabutylammonium chloride, and ethylenediaminetetracetic acid disodium (EDTA-2Na) were obtained from Fluka (Milwaukee, WI). Deionized water was purified through a water purification system (Millipore Milli-Q, Millipore, Bedford, MA). Methanol was of HPLC gradient grade and purchased from Mallinckrodt Baker (Deventer, the Netherlands). Different iodized salt samples were purchased from supermarkets in Beijing, China. Seawater samples were obtained from Bohai Seashore in Tianjin, China. Human urine samples were provided by volunteers. All chemicals were used without further purification.

Equipment and chromatographic conditions

In this study, the HPLC equipment consisted of two 3001 high-pressure pumps (Shiseido, Japan) equipped with a 3010 degasser, a HTEC-500 Coulomb detector (Eicom, Japan), a HTEC-500 amperometric detector (Eicom, Japan), a high-voltage switching six-way valve (Shiseido, Japan), and a 3004 column oven (Shiseido, Japan). A MG II C18 column (5 µm, Φ 4.6 × 250 mm; Shiseido, Japan) was used for analysis of I^-. The operating conditions of HPLC system are given in Table 1.

Table 1. Operating conditions for HPLC system.

Items	Conditions
Mobile phase of reduction	200 mmol L^{-1} phosphate buffer solution (pH 1.0)
Flow rate	0.2 mL·min^{-1}
Applied potential of CD	–2,000 mV
Peek loop volume	200 µL
Switch internal time	30 s
Cell temperature	35°C
Mobile phase of I^- detection	Aqueous solution (20 mmol/L $KH2PO_3$, 10 mmol/L tetrabutylammonium chloride, 1 mmol/L EDTA-2Na, pH 4.5): methanol = 90:10
Stationary phase	MG II C18
Flow rate	0.3 mL·min^{-1}
Column temperature	35°C
Applied potential of amperometric detector	+50 mV

Operating conditions optimization of online electrochemical reduction system

Clearly, the reduction efficiency of IO_3^- in Coulomb detector is critical to the sensitivity and accuracy of determination. Reduction efficiency (RE) was calculated by using the following equation:

$$\text{Reduction efficiency (RE)} = (\text{Amount of producing } I^-/\text{amount of total } IO_3^-) \times 100\%$$

In order to obtain high reduction efficiency, four operating factors of condition that could influence reduction efficiency, including applied potential, pH, salt concentration, and flow rate of mobile phase 1, were investigated in detail.

Sample preparation

We prepared 1 ug·mL^{-1} of I^- and IO_3^- standard solutions by diluting their stock solutions (100 ug mL^{-1}) with water (Watsons, HK). Then IO_3^{-1} was analyzed by HPLC–CD-AD system in the form of I^- with different conditions listed in Table 1.

Samples of Sseawater, iodized salt, and human urine samples were diluted and filtered through a 0.22-µm filter membrane for detection under the above-mentioned chromatographic conditions.

Statistical analysis

For methodological validation, the data were calculated as mean and relative standard deviation (RSD). Statistical differences between groups were assessed using Student's *t*-test. For measurements, $P < 0.05$ was considered statistically significant between groups.

Results

Operating conditions optimization of online electrochemical reduction system

The effect of applied potential on reduction efficiency was investigated; the results are shown in Figure 2A. More is the negative applied potential, more is the intensive reduction that takes place at electrode. In the present study, eight levels of applied potential were designed, namely –300, –500, –1,000, –1,300, –1,500 –1,800, –1,900, and –2,000 mV. Reduction efficiency with –1,900 and –2,000 mV were 95.61% and 97.44%, respectively. The effect of pH on the reduction efficiency of IO_3^- is shown in Figure 2C. It was indicated that the reduction efficiency of IO_3^- were very low, within the range

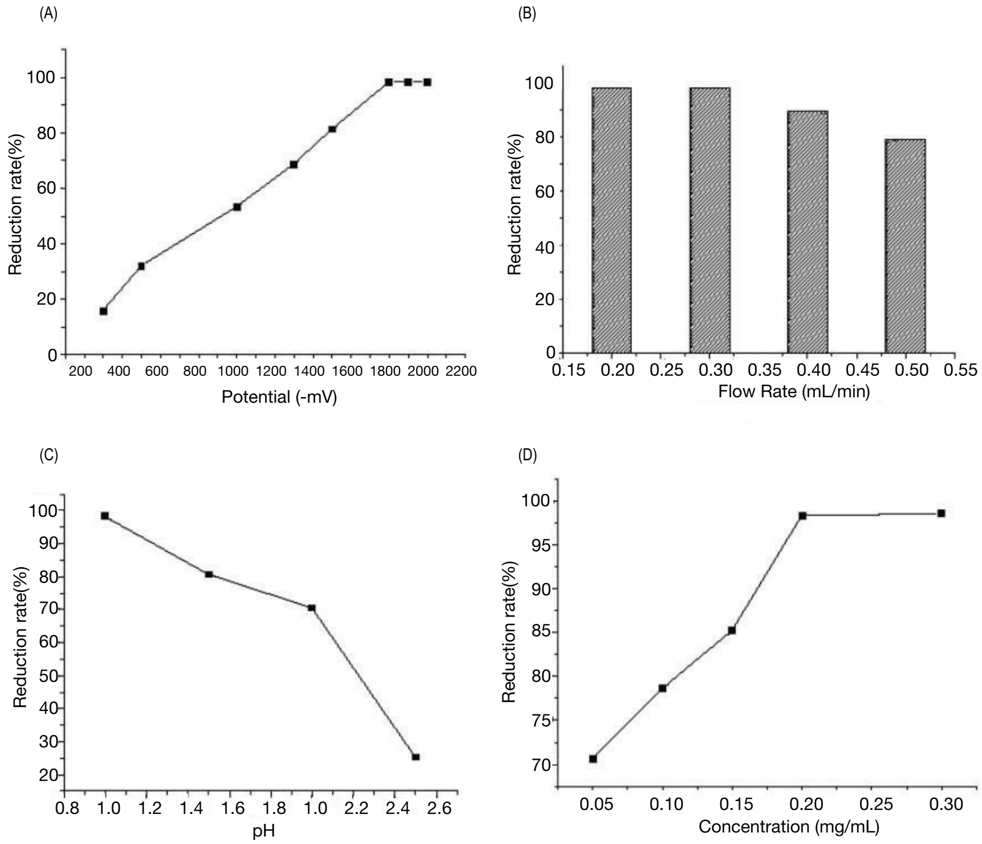

Figure 2. (A) Effect of applied potential; (B) flow rate of mobile phase of reduction; (C) pH; and (D) salt concentration on reduction deficiency of IO_3^- (1.0 μg mL^{-1}).

of pH 4.0–7.5. When pH was adjusted within 2.0–1.0, reduction efficiency increased obviously and reached over 95%. Figure 2B shows the effect of flow rate of mobile phase 1 on the reduction efficiency of IO_3^-. The effect of flow rate on reduction rate was small, but when the flow rate was too large, reduction rate decreased significantly. If the salt concentration was changed in the range of 50–200 mmol·L^{-1}, the reduction efficiency of IO_3^- increased from 74.3% to 96.5% (Figure 2D). Ions increase the conductivity of the system and, therefore, promote redox reaction.

Calibration of method

As shown in Table 2, the recovery of the method was from 86.9% to 91.7% and RSD was in the range of 1.1–3.0%. The linearity of the curves, y = 5199651.8x–1538705.4, $r^2 = 0.9987$ and y = 1765541.126x-24738.1938, were in the range of 0.4–1.7 μg/mL and 0.05–0.25 μg/mL, respectively, with a correlation coefficient of 0.999 (Figure 3). Common anions (Cl^-, Br^-, NO_3^-, NO_2^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-}, and HCO_3^-) do not interfere with the determination of I^- ions, and the detection limit (LOD) was 100 pg (5 ng/mL, 20 μL was injected, S/N = 3) (Figure 4).

Table 2. Method accuracy and reproducibility.

Samples	Added (μg/mL)	Test (μg/mL)	Recovery (%)	RSD (%)
1	0.3	0.275	91.7	3.0
	0.3	0.277	92.3	
	0.3	0.262	87.3	
2	1.2	1.112	92.7	2.3
	1.2	1.136	94.7	
	1.2	1.085	90.4	
3	2	1.766	88.3	1.1
	2	1.773	88.7	
	2	1.738	86.9	

RSD: relative standard deviation.

Analysis of iodized salt, seawater, and human urine samples

Iodate in iodized salt was determined by HPLC–CD-AD system (Table 1) and results are given in Table 3. All results were consistent with those obtained by titration (Chinese Pharmacopoeia Commission, 2015; Ministry of Housing and Urban-Rural Development of PR China, 2012). The results obtained for urine samples (samples

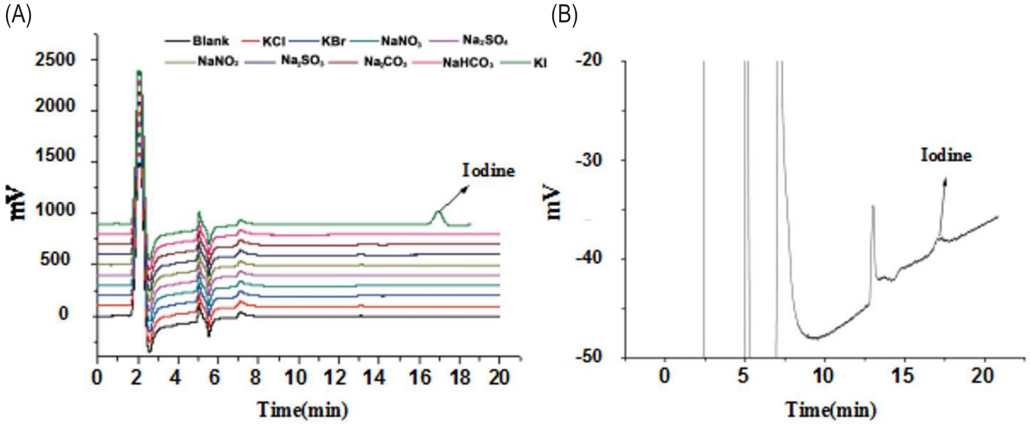

Figure 3. Calibration linearity of method in the range of (A) 0.4–1.7 μg/mL, and (B) 0.05–0.25 μg/mL.

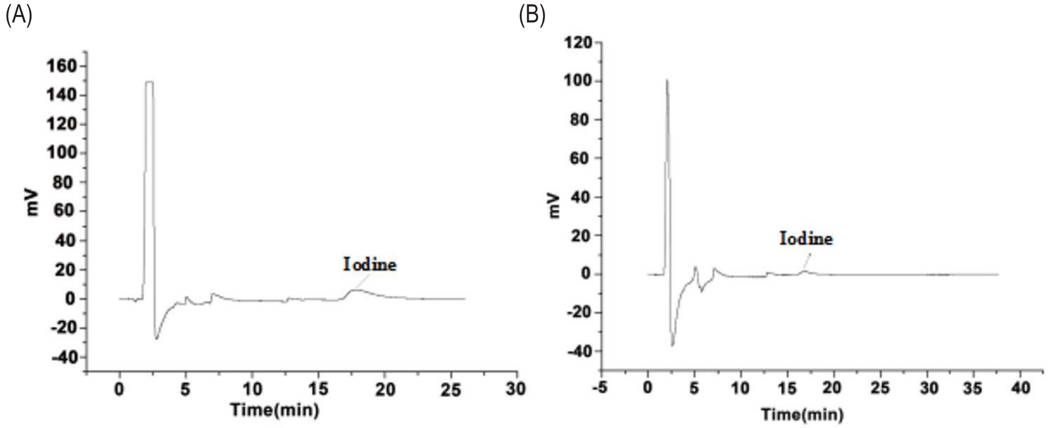

Figure 4. Ultrasensitive and highly selective determination of IO_3^-: (A) Common anions do not interfere with the determination of I^- ions; and (B) detection limit (LOD) (S/N = 3,100 pg).

Table 3. Detection of iodized salt in Chinese market.

Samples	Results by standard method (mg/kg)	Results by new HPLC–CD-AD (mg/kg)
1	1.98	2.07
2	67.5	70.65
3	nd	nd
4	37.3	37.7
5	nd	nd
6	8.56	8.8

were filtered with a 0.22-μm filter membrane) showed the presence of IO_3^- ions in only one sample (Figure 5). The seawater samples were collected from Bohai Bay in Tianjin and filtered with a 0.22-μm filter membrane. The result of IO_3^- ions determination are shown in Figure 5A.

Discussion

The Coulomb carbon electrode is more suitable for the analysis of organic matter, rather than inorganic ions. However, because of large adsorption surface area of carbon material, it has high reaction efficiency as a full reaction electrode, so it is indispensable for online reduction and detection of IO_3^-. In order to improve the reduction efficiency of IO_3^-, we adjusted the pH value of the mobile phase to a harsher acidity. Fortunately, it was within the tolerance range of the instrument, and the ideal experimental results were obtained.

According From to Faraday's law of electrolysis, the mass of produced substance at an electrode, because of electrochemical reaction, is proportional to the mass of electrons transferred to the electrode. IO_3^- transferred from the bulk solution to the electrode surface was affected by flow rate, diffusion coefficient, and viscosity of mobile phase 1. And the appropriate flow rate of mobile phase 1 is beneficial for diffusion. Therefore, 0.2 mL·min^{-1} of flow rate was chosen in the present study.

Naturally, the most important influencing factor is the applied voltage of electrode, which is the driving force of electrolytic reaction. As shown in Figure 4, reduction

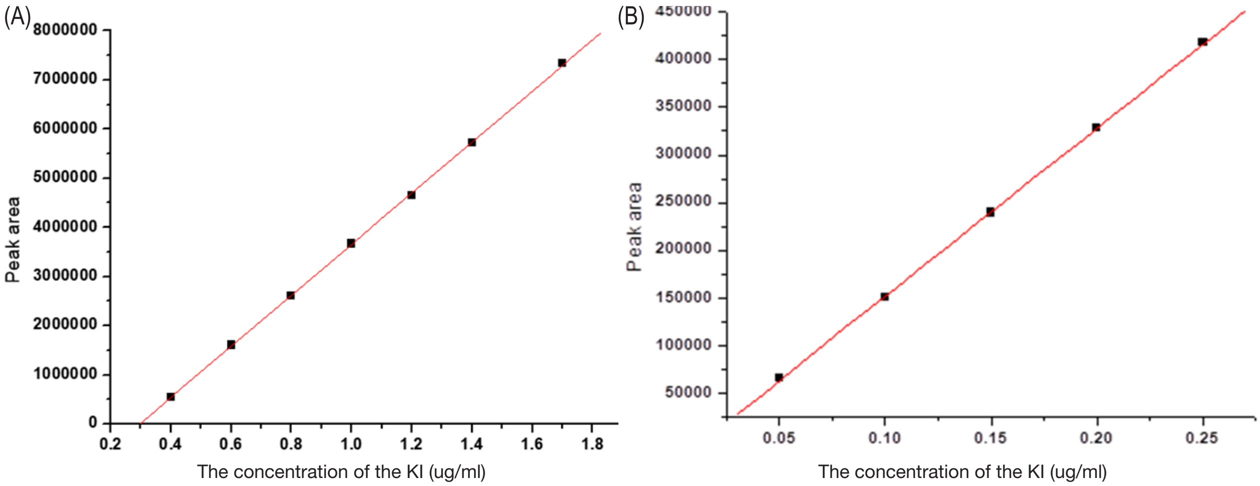

Figure 5. Chromatogram of samples: (A) Seawater sample from Bohai Bay; and (B) urine sample of volunteer A.

of IO_3^- was very weak with applied potential of –600–1,100 mV. The amount of I^- increased significantly with increase of applied potential from –1,100 to –1,900 mV.

The selected operating conditions in the present work were: 200 $mmol\cdot L^{-1}$ sodium dihydrogen phosphate (pH 1.0) of mobile phase 1, –1,900 mV of applied potential, and 0.2 $mL\cdot min^{-1}$ of flow rate. The efficiency of IO_3^- reduction to I^- ion could be more than 95%, which satisfied the needs of high-sensitivity detection.

Because the electrode potential required for the oxidation of iodine ions to iodine element is extremely low, the ampere detector only needs an operating voltage of +50 mV to obtain a strong response signal so that the detection result with low interference and high sensitivity is obtained. Detection limit and quantity limit (LQD) were 9 ng and 20 ng, respectively.

Conclusion

A novel method based on HPLC–CD-AD for obtaining ultrasensitive signal of IO_3^- by online reduction to I^- ion was described. The proposed method was simple and highly sensitive with good precision. In the future, the HPLC–CD-AD system could be used widely for IO_3^- detection.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contributions

Gao Li and Tao Wang designed the study. Qingwei Jiang and Tao Wang performed the experiments and collected the data. Qingwei Jiang and Tao Wang interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

References

Barnett, D.A., Guevremont, R. and Purves, R.W., 1999. Determination of parts-per-trillion levels of chlorate, bromate, and iodate by electrospray ionization/high-field asymmetric waveform ion mobility spectrometry/mass spectrometry. Applied Spectroscopy 53(11): 1367–1374. https://doi.org/10.1366/0003702991945984

Chinese Pharmacopoeia Commission, 2015. Chinese pharmacopoeia: appendix. 194–195. China Medical Science Press, Beijing, China

Delange, F., Benoist de B. and Alnwick D., 1999. Risks of iodine-induced hyperthyroidism after correction of iodine deficiency by iodized salt. Thyroid 9: 545–556. https://doi.org/10.1089/thy.1999.9.545

Delange, F. and Lecomte, P., 2000. Iodine supplementation: benefits outweigh risks. Drug Safety 22: 89–95. https://doi.org/10.2165/00002018-200022020-00001

Edmonds, J.S. and Morita, M., 1998. The determination of iodine spices in environmental and biological samples. Pure and Applied Chemistry 72: 1567–1584. https://doi.org/10.1351/pac199870081567

Ford, H.C. and Johnson, L.A., 1991. Ascorbic acid interferes with an automated urinary iodide determination based on the ceric-arsenious acid reaction. Clinical Chemistry 37: 759. https://doi.org/10.1093/clinchem/37.5.759

Huang, Z.P., Subhani, Q., Zhu, Z.Y., Guo, W.Q. and Zhu, Y.A., 2013. Single pump cycling-column-switching technique coupled with homemade high exchange capacity columns for the determination of iodate in iodized edible salt by ion chromatography with UV detection. Food Chemistry 139: 144–148. https://doi.org/10.1016/j.foodchem.2013.01.070

Kumar, S.D., Maiti, B. and Mathur, P.K., 2001. Determination of iodate and sulphate in iodized common salt by ion chromatography with conductivity detection. Talanta 53: 701–705. https://doi.org/10.1016/S0039-9140(00)00504-X

Li, H.B., Chen, F. and Xu, X.R., 2001. Determination of iodide in seawater and urine by size exclusion chromatography with iodine–starch complex. Journal of Chromatography A 918: 335–339. https://doi.org/10.1016/S0021-9673(01)00776-2

Liu, L., Li, X., Wang, H., Cao, X. and Ma, W., 2017. Reduction of iodate in iodated salt to iodide during cooking with iodine as measured by an improved HPLC/ICP–MS method. Journal of Nutritional Biochemistry, 42, 95–100. https://doi.org/10.1016/j.jnutbio.2016.12.009

Ministry of Housing and Urban-Rural Development of PR China, 2012. General test method in salt industry – determination of iodide ion. GB/T 13025.7-2012, the Chinese national standard. China Environment Science Press, Beijing, China.

Sajonz, P., 2006. Separation of periodate, iodate and iodide on a C-18 stationary phase. Dependence of the retention on the temperature and solvent composition. Monitoring of an oxidative cleavage reaction. Chromatographia 64: 635–640. https://doi.org/10.1365/s10337-006-0082-9

Shabani A.M.H., Ellis P.S. and Mckelvie I.D., 2011. Spectrophotometric determination of iodate in iodised salt by flow injection analysis. Food Chemistry 129(2): 704–707. https://doi.org/10.1016/j.foodchem.2011.04.077

Shin, H.S., Oh-Shin, Y.S., Kim, J.H., Ryu, J. K., 1996. Trace level determination of iodide, iodine and iodate by gas chromatography-mass spectrometry. Journal of Chromatography A 732(2): 327–333. https://doi.org/10.1016/0021-9673(95)01281-8

Wang, T., Lin, W.M., Dai, X.L., Gao, L.J., Wang, B. and Quan, D.Q., 2015. Determination of iodate by HPLC-UV after on-line electrochemical reduction to iodide. Journal of Chromatographic Science 53(2): 280–284. https://doi.org/10.1093/chromsci/bmu053