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*Research Article*

# Trypsin Digestion Pretreatment of Kelp Samples and the Iodine Speciation Analysis of Iodate, Iodide, MIT, and DIT by HPLC-ICP-MS

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The analysis of iodine content in kelp is mainly focused on determining total iodine content, which lacks a rapid and sensitive analytical method for iodine species corresponding to iodate, iodide, monoiodotyrosine (MIT), and diiodo-tyrosine (DIT). Based on exploring the pretreatment methods of enzymatic hydrolysis of kelp, a rapid determination method of high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP-MS) for different iodine species existed in kelp samples was established. The pretreatment method is trypsin hydrolysis of kelp samples treated at 50°C for 10 hours, then the extraction solution was obtained by centrifugation, and filtration was proved for the first time. A high-efficiency separation anion-exchange column (Dionex IonPac AS-14) for iodate, iodide, MIT, and DIT with 0.1 mol·L$^{-1}$ $(NH_4)_2CO_3$ solution at pH = 9 as a mobile phase, a flow rate of 0.8 mL·min$^{-1}$ for the first 1500 s, and 1.2 mL·min$^{-1}$ for the last 1800 s was confirmed. The linear ranges of the standard calibrations on four iodine species are 1.0–150 $\mu$g·L$^{-1}$, and the detection limits are 1.62, 1.02, 1.59, and 2.55 $\mu$g·L$^{-1}$, respectively, while the RSD values of four iodine species are all less than 2%. Under the optimized conditions, the proposed method was successfully applied for the speciation analysis of iodine in kelp samples.

## 1. Introduction

As an essential trace element for human life activities, iodine mainly exists in the iodide or iodate in nature, and a small part exists in the organic iodine in seaweed [1]. Iodine can only be obtained from the outside world, and the iodine content in food is extremely low. The resulting iodine deficiency causes goiter and affects the intelligence and physical development of the human body [1–3]. The bioavailability and toxicity of different forms of iodine are different, and the toxicity of iodate is more significant than iodide. So far, potassium iodide and potassium iodate are the main iodine additives in the iodized salt. Excessive consumption fails to supplement iodine and leads to adverse reactions such as iodine poisoning [4]. Organic iodine is an ideal scientific iodine supplement product, which can be directly absorbed by the human body, not affected by the absorption of iodine due to insufficient protein intake. Therefore, the speciation analysis of iodine in kelp is essential for producing iodized table salt.

Due to the complex and diverse forms of iodine, the characteristics of instability and easy sublimation greatly increase the limitation of the determination of iodine species. Kelp, as a biological sample, has a complex matrix, so it is hugely challenging for speciation analysis of trace and ultratrace iodine. At present, there are the following

measurement methods of ultraviolet spectrophotometry (U.V.) [5], capillary electrophoresis (C.E.) [6, 7], liquid chromatography and inductively coupled plasma mass spectrometry (LC-ICP-MS) [2, 4, 8–12], and ion chromatography (I.C.) [13], and so on, for the determination of total and speciation of iodine. Nunes et al. [5] used a U.V. spectrophotometer to measure iodine in kelp, which is simple but easily affected by substances such as protein. Sun et al. [6] successfully separated $IO_3^-$, $I^-$, MIT, and DIT by UV electrophoresis. Xu et al. [7] completed the separation of various iodine forms using surfactant-coated multiwalled carbon nanotubes as the stationary phase in electrophoresis. However, the separation conditions were complicated and challenging to operate. Liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) has high sensitivity, low detection limit, and low negative interference by other ions [2, 4, 8–12] and is able to separate various iodine species and simultaneously measure iodine content. Therefore, this paper selects the HPLC-ICP-MS combined method for iodine speciation analysis and determination.

Before sample determination, it is necessary to extract the iodine in kelp into the solution. At present, the common pretreatment methods include dry alkali incineration [5], ionic surfactant extraction [14], ultrasonic microwave-assisted extraction [6, 10, 15], alkali digestion [4, 12], and enzymatic hydrolysis [10, 16]. Wang et al. [14] treated kelp samples using microwave assistance with zwitterionic surfactants as extractants, and $I^-$, MIT, and DIT could be measured. However, the surfactants are expensive and the high temperature of microwaves can destroy partial iodine species. Alkaline digestion is mostly used as a pretreatment method for the total iodine content determination in kelp samples and cannot extract organic iodine in the samples. Romaris-Hortas et al. [10] comparatively studied the enzymatic hydrolysis properties of cellulase, $\alpha$-amylase, $\beta$-glucose, lipase, pepsin, pancreatin, and mixed enzymes on kelp under ultrasound, and experimental data showed that the highest content of organic iodine (MIT and DIT) was obtained by trypsin extraction. In this work, we systematically compared the enzymatic hydrolysis performance of trypsin, pancreatin, protamex, and neutral protease. Finally, we first establish a pretreatment method for kelp samples using trypsin. Analysis method of iodine speciation ($IO_3^-$, $I^-$, MIT, and DIT) was established by using liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS), and it was implemented in kelp samples.

## 2. Experimental

### 2.1. Reagents and Instrumentation

*2.1.1. Chemicals and Reagents.* Ultrapure water (resistivity, 18.2 $M\Omega \cdot cm^{-1}$) obtained from a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA) was used for this work. Ammonium carbonate (≥99.999%, Rhawn Reagent Co., Ltd.), ammonium nitrate (≥98.5%, Shanghai Aladdin Biochemical Technology Co., Ltd.), and ammonium dihydrogen phosphate (A.R., Sinopharm) were used as the selected mobile phase. Potassium hydroxide (A.R., Shanghai Merrill Chemical Technology Co., Ltd.) was added to standard solutions as the preservative medium. Sodium chloride (A.R., Sinopharm Chemical Reagent Co., Ltd.) was purchased for the interference experiment. Pancreatin (activity 1 : 4000, Nanjing Herbal Source Biopharmaceutical Co., Ltd.), trypsin (activity 1 : 250, Shanghai McLean Biochemical Technology Co., Ltd.), protamex (120 $u \cdot mg^{-1}$, Shanghai Yuanye Biotechnology Co., Ltd.), and neutral protease (50 $u \cdot mg^{-1}$, Beijing Jinxin Tianyou Biotechnology Co., Ltd.) were used for enzymatic hydrolysis of kelp. Sodium dihydrogen phosphate (≥99%, Tianjin No. 1 Chemical Reagent Factory) and sodium hydroxide (≥96%, Shanghai McLean Biochemical Technology Co., Ltd.) were used to prepare the buffer.

The stock solutions of 1 $g \cdot L^{-1}$ of $IO_3^-$ and $I^-$ and 100 $mg \cdot L^{-1}$ of MIT and DIT (all from Shanghai Aladdin Biochemical Technology Co., Ltd.) were used to prepare 0, 10, 20, 50, 80, 100, 150 $\mu g \cdot L^{-1}$ $IO_3^-$, $I^-$, MIT, and DIT mixed standard solutions [12].

*2.1.2. Instrumentation.* An iCAP Q ICP-MS (Thermo Scientific, USA) was used for quantitative analysis of iodine speciation. In this study, a Thermo U3000 HPLC system was successfully used to separate iodine species with an injection volume of 20 $\mu$L. Separation of iodine species was achieved using an IonPac AS-14 anion column (4 mm × 250 mm, Thermo Scientific, USA). The pH value was measured by a high-precision pH meter (WTW PH-7310, Shanghai Precision Scientific Instruments Co., Ltd., China) with an uncertainty of ±0.003.

*2.2. Sample Preparation.* The kelp samples were rinsed twice with deionized water and dried in an oven at 40°C. Grind dried kelp to powder, seal, and store in a cool and dry place for later use. 0.04 g of trypsin and 0.2 g of dried kelp samples were weighed and sonicated in 20 mL of $NaH_2PO_4$/NaOH (pH = 8) buffer solution at 50°C for 10 h [9, 16]. The obtained solution was centrifuged and filtered repeatedly, and the volume was fixed in a 50 mL volumetric flask and then stored at low temperature and protected away from light. All solutions were filtered through 0.45 $\mu$m microporous membranes before use.

*2.3. Experimental Conditions.* The operating conditions of the HPLC-ICP-MS are shown in Table 1.

*2.4. Sample Analysis and Determination.* The sample to be tested was diluted and injected into the sampling system with an injection needle. Under optimal instrument operating conditions, a gradient elution method with 100 $mmol \cdot L^{-1}$ $(NH_4)_2CO_3$ (pH = 9) as mobile phase at a flow rate of 0.8 $mL \cdot min^{-1}$ from 0 to 1500 s and a flow rate of 1.2 $mL \cdot min^{-1}$ from 1500 s to 3300 s was used.

Table 1: Instrumental operating conditions.

| Parameter | Operation setting |
|---|---|
| *Experimental conditions for HPLC* | |
| Column | IonPac AS-14 anion-exchange column (4 mm × 250 mm, 10 $\mu$m) |
| Mobile phase | 100 mmol·L$^{-1}$ $(NH_4)_2CO_3$, pH = 9 |
| Elution mode | Multistep gradient: 0–1500 s: 0.8 mL·min$^{-1}$、1500–3300 s: 1.2 mL·min$^{-1}$<br>1500 s–3300 s: 1.2 mL/min |
| Column temperature | 28°C |
| Injection volume | 20 $\mu$L |
| *Experimental conditions for ICP-MS* | |
| Forward power | 1550 W |
| Plasma gas flow | 14.000 L·min$^{-1}$ |
| Auxiliary gas flow | 0.8000 L·min$^{-1}$ |
| Nebulizer gas flow | 1.0150 L·min$^{-1}$ |
| Sampling depth | 5.0 mm |
| Analytical model | KED (kinetic energy discrimination) |
| CCT flow | 5.198 mL·min$^{-1}$ |
| Dwell time | 0.2 s |

## 3. Results and Discussion

*3.1. Pretreatment Method Establishment.* Different biological enzymes' biological activities vary, and the corresponding active sites of enzymatic hydrolysis are various, resulting in different peptide chain structures and lengths obtained by enzymatic hydrolysis. Considering that 3-iodo-L-tyrosine (MIT) and 3,5-diiodo-L-tyrosine (DIT) are obtained by enzymatic hydrolysis of kelp protein, the experiment mainly explored hydrolytic characteristics of trypsin, pancreatin, protamex, and neutral protease.

When the solid-liquid ratio was 1 : 20, and the amount of enzyme added was 0.2%, ultrasonication was performed at pH = 7-8 and 50°C for 10 h, respectively. The obtained solution was centrifuged and filtered and then brought to volume by deionized water in a 50 mL volumetric flask to be measured. The contents of diverse iodine species measured after treatment with different enzymes are shown in Table 2. In addition, the chromatogram of the hydrolysis effects of the four enzymes is shown in Figure 1.

It can be seen from Figure 1 that the four enzymes all showed a certain enzymatic hydrolysis effect on the protein of kelp. The experimental results show that the hydrolysis effect of neutral protease and pancreatin is poor and that only iodide can be obtained. Both trypsin and protamex can hydrolyze $I^-$ and MIT. However, the content of two kinds of iodine in kelp hydrolyzed by trypsin was higher than that of protamex. It can be seen from Table 3 that after adding $I^-$ and MIT to the kelp samples and enzymatic hydrolysis, the recovery rates of standard additions are all between 98% and 103%. Hence, *trypsin* was selected for the pretreatment of kelp samples due to its best hydrolysis effect on the protein of kelp without damaging the iodine form.

*3.2. Optimization of Enzyme Dosage.* The enzyme concentration is a key parameter affecting the extraction concentration of organic iodine (MIT and DIT). In order to explore the effect of enzyme solvent concentration on the hydrolysis

Table 2: Iodide, MIT, and DIT concentrations released by ultrasound-assisted enzymatic hydrolysis with different enzymes.

| | $I^{-b}$ ($\mu$g $L^{-1}$) | $MIT^b$ ($\mu$g $L^{-1}$) | $DIT^b$ ($\mu$g $L^{-1}$) |
|---|---|---|---|
| Trypsin | 3159.21 | 156.36 | —[a] |
| Pancreatin | 3522.03 | —[a] | —[a] |
| Protamex | 2743.01 | 77.27 | —[a] |
| Neutral protease | 2931.74 | —[a] | —[a] |

[a]Undetected; [b]determined by HPLC-ICP-MS.

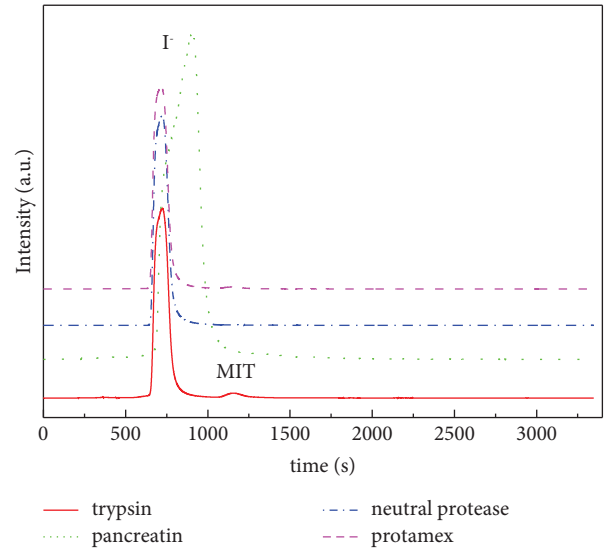


Figure 1: Effects of different enzymes on the hydrolysis degree of kelp protein.

of kelp protein, 0.02, 0.04, and 0.10 g of trypsin were weighed and dissolved in 10.0 mL of pH = 8 buffer (0.2 mol·L$^{-1}$/0.2 mol·L$^{-1}$ sodium dihydrogen phosphate/sodium hydroxide), and a blank control group was set. The *trypsin* concentrations of the buffer were 0, 2.0, 4.0, and 10.0 mg·mL$^{-1}$, respectively. From the data in Figure 2 and Table 4, it can be seen that there was no organic iodine

Table 3: Results of recoveries and real sample analysis.

| | Content of iodine[a] ($\mu$g $L^{-1}$) | | Spiked ($\mu$g $L^{-1}$) | | Found[a] ($\mu$g $L^{-1}$) | | Recoveries (%) | |
|---|---|---|---|---|---|---|---|---|
| | $I^-$ | MIT | $I^-$ | MIT | $I^-$ | MIT | $I^-$ | MIT |
| Trypsin | 3159.21 | 156.36 | 1000.00 | 100.00 | 4148.01 | 258.70 | 98.88 | 102.34 |
| Pancreatin | 3522.03 | —[b] | 1000.00 | 100.00 | 4518.33 | 99.52 | 99.63 | 99.52 |
| Protamex | 2743.01 | 77.27 | 1000.00 | 100.00 | 3750.51 | 176.97 | 100.75 | 99.70 |
| Neutral protease | 2931.74 | —[b] | 1000.00 | 100.00 | 3953.94 | 102.22 | 102.22 | 102.22 |

[a]Direct determining data by HPLC-ICP-MS. [b]Not detected.

leaching when no *trypsin* was involved in the hydrolysis, indicating that the buffer would not extract MIT and DIT from the kelp under ultrasonic assistance. When the trypsin was 0~0.04 g, with the increase of enzyme concentration, the degree of hydrolysis increased, and the concentration of iodide and monoiodothyronine increased. When the *trypsin* was more than 0.04 g, the concentrations of iodine and monoiodothyronine decreased to varying degrees. It was speculated that due to the excessive concentration of enzyme, kelp protein was hydrolyzed into iodine amino acids and then continued to hydrolyze, resulting in the decrease of monoiodothyronine.

*3.3. Optimization of Enzymatic Hydrolysis: pH.* The pH value is the main parameter affecting the enzymatic hydrolysis process. The change of pH will affect the stability of the protein structure and has a significant effect on the enzymatic hydrolysis effect. Trypsin has a suitable pH. When the pH is not reached, the trypsin activity is not high and the contact with the substrate is less. Trypsin is easily inactivated at pH 8.0 or more and the substrate cannot be digested. Above this pH, the structure of trypsin was destroyed, the activity decreased, and the degree of hydrolysis decreased. Therefore, in this experiment, buffers (0.2 mol·$L^{-1}$/0.2 mol·$L^{-1}$ sodium dihydrogen phosphate/sodium hydroxide) with pH of 6.0, 7.0, and 8.0 were prepared. The same quality of kelp samples and 0.04 g trypsin was weighed to explore the effect of pH on trypsin hydrolysis. According to the experimental data, the histogram is shown in Figure 3. When the buffer pH = 8.0, the trypsin activity is the highest, indicating that the enzyme is in sufficient contact with the substrate kelp sample, and the kelp protein can be fully hydrolyzed, so the released iodine content is the highest.

*3.4. Optimization of Enzymolysis Time.* When using trypsin to hydrolyze kelp protein, it is necessary to determine the appropriate enzymatic hydrolysis time. Too short time will make inadequate enzymolysis; too long time will not only cause excessive hydrolysis of kelp protein but also unnecessary resource waste. In this experiment, 0.2 g kelp sample and 0.04 g trypsin were weighed to explore the effect of enzymolysis time. In order to destroy the cell membrane and wall of seaweeds more efficiently and rapidly and promote the direct contact between trypsin and intracellular structures, ultrasound was used as an auxiliary means in the hydrolysis process. According to the data of Table 5, the histogram can be drawn as shown in Figure 4. The total

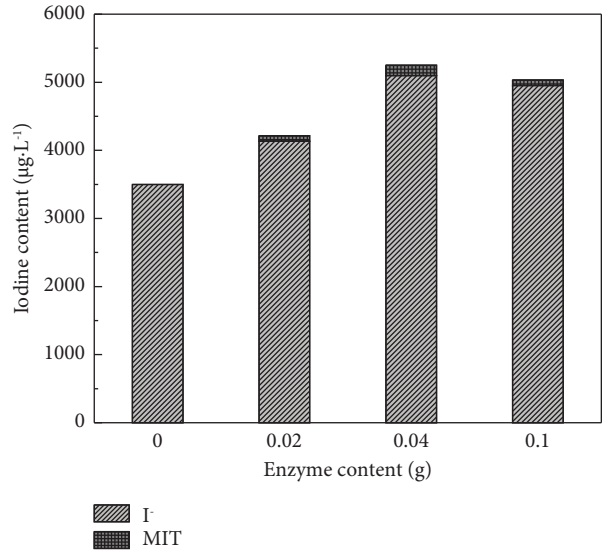


Figure 2: Effects of different enzyme solvent concentrations on the hydrolysis degree of kelp protein.

Table 4: Effects of different enzyme solvent concentrations on the hydrolysis degree of kelp protein.

| Concentration of enzyme (g) | MIT ($\mu$g·$L^{-1}$) | $I^-$ ($\mu$g·$L^{-1}$) |
|---|---|---|
| 0 | 0 | 3500.55 |
| 0.02 | 80.57 | 4133.28 |
| 0.04 | 150.22 | 5098.95 |
| 0.10 | 79.63 | 4953.03 |

iodine amount of enzymatic hydrolysis increases with the prolongation of time in the range of 6–10 h. When the enzymolysis time was more than 10 h, the total iodine did not change significantly and decreased slightly. It is speculated that monoiodothyronine is not stable in this case. Prolonging the hydrolysis time may cause the decomposition of iodine amino acids, and too long time will also cause the loss of iodide. Therefore, the enzymatic hydrolysis time was controlled at 10 h to ensure the highest content of iodide and monoiodide.

*3.5. Selection of Separation Conditions.* In order to obtain the best separation effect, the separation conditions such as type, concentration, pH, and flow rate of the mobile phase were optimized. $NH_4H_2PO_4$, $(NH_4)_2CO_3$ [8], and $NH_4NO_3$

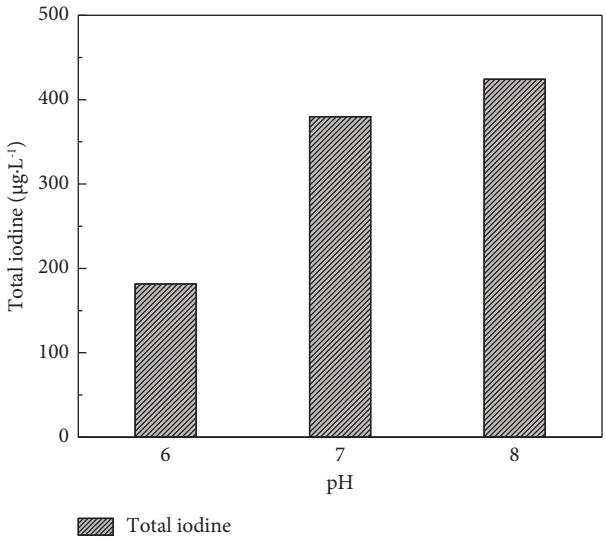


Figure 3: Effects of pH of buffer solution on the hydrolysis degree of kelp protein.

Table 5: Effects of enzymatic hydrolysis time on the hydrolysis degree of kelp protein.

| Concentration of enzyme (g) | Enzymatic hydrolysis time (h) | Total iodine ($\mu g \cdot L^{-1}$) |
|---|---|---|
| 0.04 | 6 | 383.52 |
| | 8 | 404.90 |
| | 10 | 521.47 |
| | 12 | 523.71 |

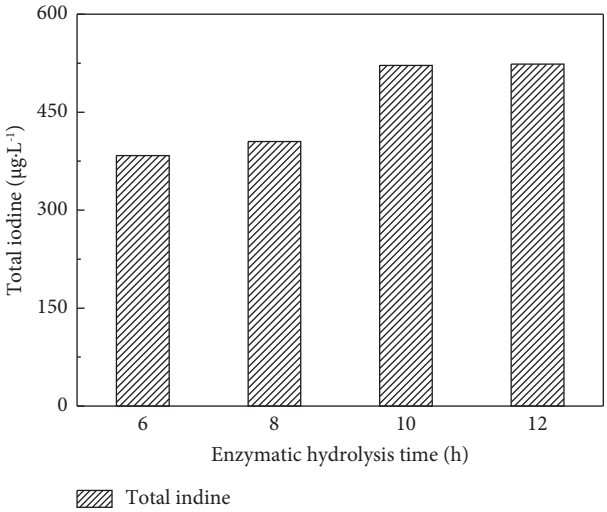


Figure 4: Effects of enzymatic hydrolysis time on the hydrolysis degree of kelp protein.

[9, 10] as eluent were primarily compared. Under the isocratic elution, the mobile phase of $NH_4H_2PO_4$ and $NH_4NO_3$ can only separate $IO_3^-$, $I^-$, and MIT, except DIT. Conversely, $(NH_4)_2CO_3$ can completely elute these four iodine species and separate them nicely. The mobile phase concentration greatly influences the retention time of iodine. And the continuous injection of high-salinity eluent into the ICP-MS system for a long time will lead to the accumulation of salt in the sampling cone, affecting the experiment's accuracy and the instrument's service life. In this work, the elution of 50, 100, and 150 $mmol \cdot L^{-1}(NH_4)_2CO_3$ mobile phase was investigated and is shown in Figure 5. It could be seen that the peak of DIT disappears when the mobile phase concentration is low, and when the mobile phase concentration increases to 150 $mmol \cdot L^{-1}$, the retention time is advanced, but the area of peaks becomes smaller, which means that the detection sensitivity is reduced. Therefore, the whole iodine species can be measured within 3000 s by using 100 $mmol \cdot L^{-1}$ $(NH_4)_2CO_3$ as the mobile phase. The pH of the mobile phase affects the degree of dissociation and distribution ratio of ions, which accordingly affects the retention time of target components on the column. 2% nitric acid and 5% ammonia were used to adjust the pH of the mobile phase. It was seen from Figure 6 that $IO_3^-$, $I^-$, MIT, and DIT were well separated when pH of the mobile phase was 9, while the peak overlapped and MIT could not be eluted when pH of the mobile phase was 8 and 10, respectively. A large flow rate can cause an increase in column pressure, which will affect the service life of the column. The excessively low flow rate will prolong elution time, resulting in excessive peak width. 1.2 $mL \cdot min^{-1}$ and 1.5 $mL \cdot min^{-1}$ of flowrates were studied for better separation. It was found that $IO_3^-$, $I^-$, MIT, and DIT could be eluted and separated well both in two flow rates. However, during the measurement of kelp samples, it was found that the peak shapes of $IO_3^-$ and $I^-$ occurred tail dragging, so the flow rate was reduced to 0.8 $mL \cdot min^{-1}$. After the complete separation of $IO_3^-$ and $I^-$, increasing the flow rate to 1.2 $mL \cdot min^{-1}$ can appropriately shorten the entire elution time and maintain good peak shape. Therefore, considering the separation efficiency, detection sensitivity, and column lifetime, a mobile phase containing 100 $mmol \cdot L^{-1}(NH4)_2CO_3$ at pH 9 and a flow rate of 1.2 $mL \cdot min^{-1}$ was applied.

*3.6. Interference Research of $Cl^-$ on Iodine Species.* Kelp contains a small amount of sodium chloride. In order to explore the effect of $Cl^-$ in iodine speciation analysis, a mixed standard solution of 150 $g \cdot L^{-1}$ $IO_3^-$, $I^-$, MIT, and DIT containing 100 $mg \cdot L^{-1}$ $Cl^-$ was prepared under the optimal experimental conditions. The experimental results have shown that a small amount of $Cl^-$ in the sample will not affect the iodine speciation analysis of the kelp, and its chromatogram is shown in Figure 7.

*3.7. Analytical Performances.* The mixed standard solutions of $IO_3^-$, $I^-$, MIT, and DIT with linear ranges of 0, 10, 20, 50, 80, 100, and 150 $\mu g \cdot L^{-1}$ were determined by HPLC-ICP-MS [8, 12]. Table 6 summarizes all the analytical performance characteristics of the optimal method. The detection limits of $IO_3^-$, $I^-$, MIT, and DIT were 1.62, 1.02, 1.59, and 2.55 $\mu g \cdot L^{-1}$, respectively. The 50 $\mu g \cdot L^{-1}$ mixed standard solution was measured five times in parallel, and the precisions of the four iodine species were all between 1.08% and 1.71%. Figure 8 shows that HPLC-ICP-MS can effectively separate four

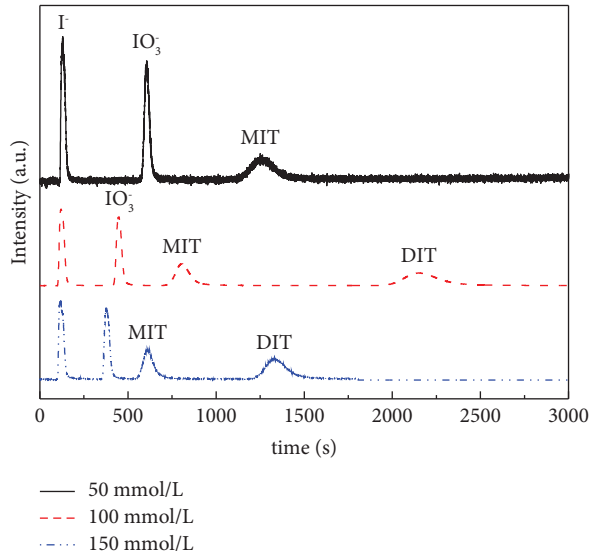


Figure 5: Influences on the chromatogram spectra peaks and retention time by the concentration of $(NH_4)_2CO_3$ elution solution in the mixing standard solution of each 150 $\mu$g/L iodate, iodide, MIT, and DIT.

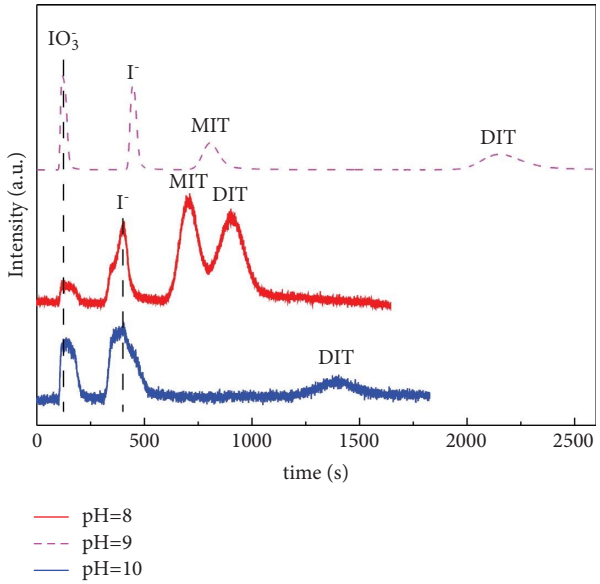


Figure 6: Chromatogram spectra for iodate, iodide, MIT, and DIT at 150 $\mu$g/L for each ion using 100 mmol/L $(NH_4)_2CO_3$ at pH = 8, 9, and 10, respectively.

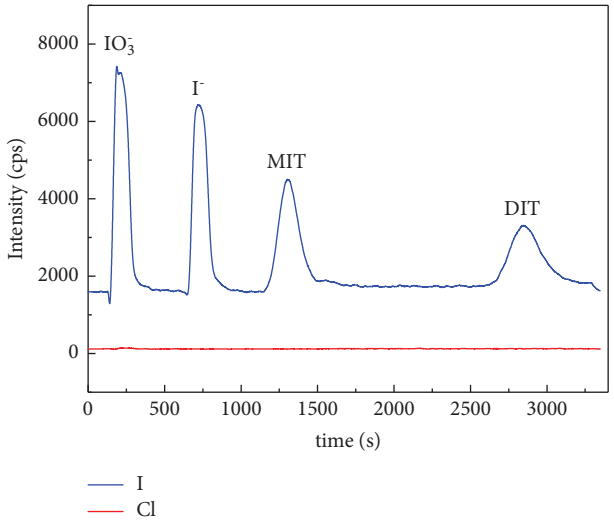


Figure 7: Chromatogram spectra of iodate, iodide, MIT, and DIT containing 100 mg/L $Cl^-$.

iodine species of $IO_3^-$, $I^-$, MIT, and DIT. The working curves of the four iodine species are shown in Figure 9. The linear range is wide, the linear relationship is reasonable, and the correlation coefficients are all greater than 0.999.

*3.8. Recovery of Iodine in Kelp.* To verify the accuracy of this method, the kelp samples were spiked and determined. As shown in Table 7, the experimental results show that $I^-$ and MIT recovery rates are between 99% and 103%, which shows that the experimental method is accurate and reliable.

*3.9. Iodine Content of Kelp.* In this paper, the method of ultrasonic-assisted trypsin was used for pretreatment, and the established method of HPLC-ICP-MS was used for determination. The iodine content in the kelp sample is shown in Figure 10 and Table 8. It can be seen from Table 8 that the kelp sample only contains $I^-$ and MIT, which are $7.90 \times 10^3$ mg·kg$^{-1}$ and 390.91 mg·kg$^{-1}$, respectively, and the organic iodine content accounts for 4.72% of the total iodine.

Table 6: Analytical performances of iodate, iodide, MIT, and DIT analyses by HPLC-ICP-MS.

| Ions | Concentration ($\mu$g/L) | Calibration curve | $r$ | LOD ($\mu$g/L) | LOQ ($\mu$g/L) | Precision ($n = 5$ RSD%) |
|---|---|---|---|---|---|---|
| $IO_3^-$ | 0–150 | $y = 4258.3\ x - 941.4$ | 0.9997 | 1.62 | 5.40 | 1.08 |
| $I^-$ | 0–150 | $y = 4187.0\ x - 460.9$ | 0.9995 | 1.02 | 3.40 | 1.71 |
| MIT | 0–150 | $y = 1659.7\ x - 3301.1$ | 0.9992 | 1.59 | 5.30 | 1.08 |
| DIT | 0–150 | $y = 1601.4\ x - 49.3$ | 0.9998 | 2.55 | 8.50 | 1.70 |

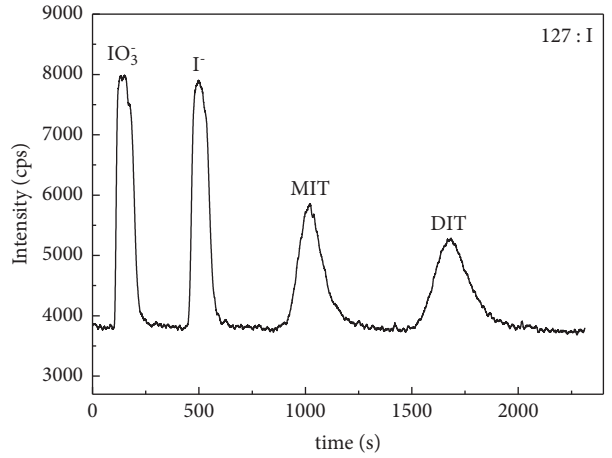


Figure 8: Chromatogram spectra peaks of iodate, iodide, MIT, and DIT at 150 $\mu$g/L using 100 mmol/L $(NH_4)_2CO_3$ as eluent.

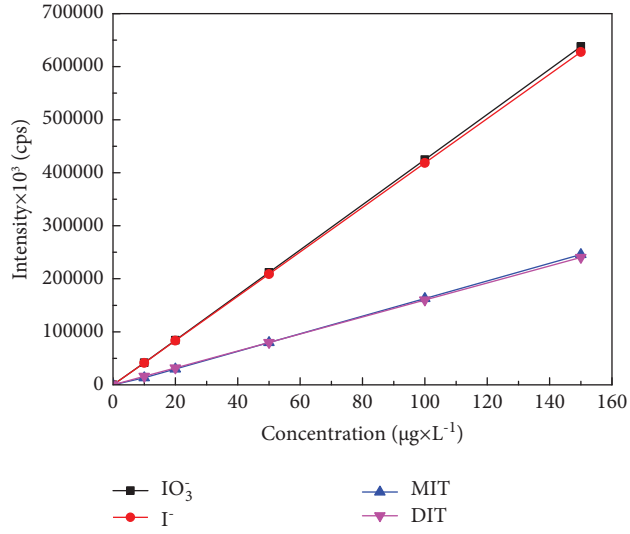


Figure 9: Working curves of iodate, iodide, MIT, and DIT.

Table 7: Concentration and recoveries of iodine in kelp sample.

| Ions | Concentration ($\mu$g/L) | Spiked ($\mu$g/L) | Found[a] ($\mu$g/L) | Recoveries (%) |
|---|---|---|---|---|
| $I^-$ | 3159.21 | 3000.00 | 6226.41 | 102.24 |
| MIT | 156.36 | 50.00 | 205.88 | 99.02 |

[a]Determined by HPLC-ICP-MS.

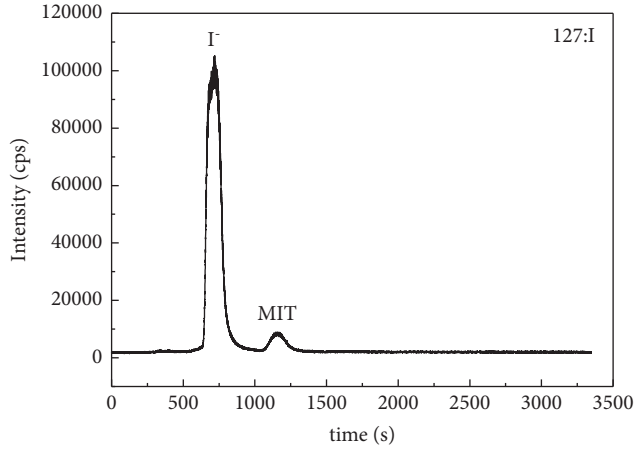


Figure 10: Chromatogram spectra of iodide and MIT in kelp.

Table 8: Analysis of iodine in samples.

| Sample | Total iodine (mg/kg) | Content of iodide (mg/kg) | Content of MIT (mg/kg) | Percentage of MIT (%) |
|---|---|---|---|---|
| Kelp | 8288.93 | 7898.01 | 390.91 | 4.72 |

## 4. Conclusions

In this work, the optimum pretreatment conditions of kelp samples using trypsin with ultrasound were explored for the first time. A technique combining high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to establish a sensitive and accurate quantitative analysis speciation method of iodine in kelp. This method can achieve the separation and quantitative analysis of four iodine species ($IO_3^-$, $I^-$, MIT, and DIT) with high accuracy and low detection limit and can be widely used in the analysis and determination of different iodine species in kelp and table salt.

## Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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