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Development of a new method for analyzing free aluminum ions (Al^{3+}) in seafood using HPLC-ICP-MS

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This paper presents a novel method for simultaneous online examination of free aluminum ions (Al^{3+}) in seafood, using solid-phase extraction and high-performance liquid chromatography online with inductively coupled plasma mass spectrometry (SPE-HPLC-ICP-MS), without post-column reaction. The optimum conditions for chromatographic separation of Al^{3+} were achieved using an IonPac CS5A analytical column with an IonPac CG5A guard column. The mobile phase consisted of 0.040 mol/L LiOH, 0.0060 mol/L 2,6-pyridinedicarboxylic acid, and 0.090 mol/L CH_3COOH (pH 4.7). The free Al^{3+} ions in seafood were extracted by shaking with the mobile phase at 70°C for 2 h. SPE was conducted using an Oasis MCX, 3cc/60 mg, 30 μm column, which was activated and equilibrated with 2 mL of methanol and 4 mL of deionized water before use. HCl (0.075 mol/L, 2 mL) was used to wash inorganic Al from the SPE column. The standard recoveries of Al^{3+} were all above 89% and the relative standard deviations were all below 5%. The proposed method was successfully used for the examination of Al^{3+} in seafood samples, and the results were similar to those obtained using the static equilibrium method.

free aluminum ion, seafood, solid-phase extraction, HPLC-ICP-MS

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Aluminum (Al) was long considered to be an innocuous element [1]. It was not until the discovery in the 1970s of its implication in "dialysis encephalopathy" syndrome, which affected hemodialysis patients, that attention was drawn to its possible deleterious effects [2]. Public interest in Al has therefore been increasing in the last few decades. The 67th report of the Joint FAO/WHO Expert Committee on Food Additives established a provisional tolerable weekly intake for Al of 1 mg/kg body weight, which applies to all Al compounds in food, including additives.

Seafood (including fish, seaweed, shellfish, and sea cumber) is regarded as being a healthy and tasty food for humans, and human consumption of seafood in China has been increasing in recent decades. However, high concentrations of Al have been found in seafood, particularly seaweed, sea cucumber, and some shellfish. For example, the total Al concentration in seaweed is about 118–2715 mg/kg (dry weight) in *Porphyra haitanensis* and 340–1246 mg/kg (dry weight) in *Laminaria japonica*. In addition, the highest Al concentration in sea cucumber is about 1200 mg/kg (dry weight), and it is about 800 mg/kg (dry weight) in shellfish. There is therefore a serious problem with regard to the safety of seafood because of its high Al content, and this has attracted much attention. The current methods for determining the Al content of seafood almost all use concentrated acids, namely HNO_3, $HClO_4$ and H_2SO_4, to achieve digestion of all forms of Al to Al^{3+}, which is then measured using inductively coupled plasma mass spectrometry (ICP-MS) or ICP-atomic emission spectrometry (AES), so all the methods for determining the Al content of seafood measure the total Al.

It is well known that Al can form complexes with various organic compounds (e.g. humic and fulvic acids, and low-mass organic compounds) and inorganic ligands (e.g. fluo-

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ride, chloride, sulfate, and phosphate), most, but not all, of which are soluble [3]. Al in most of its forms does not harm living organisms. However, under certain conditions such as low pH, Al tends to form species that are potentially toxic to all living organisms, including humans [4]. The distribution of Al among its various organic and inorganic complexes influences its mobility in the environment, bioavailability, and toxicity [5,6]. Al is most toxic in its soluble ionic form (Al^{3+}), and Al bound in fluorides or organic complexes, phosphate or silicate Al polymers, and Al $(OH)_3$ are regarded as non-toxic [7]. Determination of the total concentration of Al therefore does not provide the full data concerning the processes that the element undergoes in the natural environment, and does not provide information on the actual toxicity, bioavailability, and accumulation in organisms and the environment [8].

Al speciation analysis has therefore evoked wide interest among researchers. One of the best-known and most commonly applied procedures for speciation analysis is Driscoll's method [9,10], but this method does not enable direct determination of particular speciation forms of Al, including fluoride complexes and Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)_2^+$ species [10,11]. The use of liquid chromatography (LC) provides many possibilities for separating particular forms of Al, both cationic and anionic [10,12–14]. Combinations of high-performance LC (HPLC) with ICP-MS and ICP-AES in online [15–18] as well as offline systems [13,19] have been used. However, methods based on separation and detection by ultraviolet spectrometry and ICP-AES are not sufficiently sensitive; compared with ICP-AES, ICP-MS can achieve very low detection limits for Al and a wide linear range of determination. Other advantages of online coupling of chromatography to ICP-MS are time saving resulting from minimization of labor-intensive steps and the speed of the separation procedures, which can prevent changes in sensitive species. Furthermore, trace contamination and analyte loss are minimized because of the closed instrumental system [20,21]. HPLC online with ICP-MS was therefore used to analyze Al speciation in the present research.

Previous research on Al speciation has almost all focused on environmental samples, vegetables, and biological fluids [19,22–24], and research has seldom been conducted on seafood. We previously proved that there were various forms of Al in seaweed, using the static equilibrium method. We found that labile organic Al accounted for 82.5%–87.6% and inorganic Al accounted for 3.75%–4.94% of the total Al [25]. However, the static equilibrium method cannot fully separate the Al complexes or be used to determine Al^{3+} qualitatively and quantitatively. So, the main aims of this study were (1) development of an extraction and clean-up method for Al^{3+} from seafood, (2) development and optimization of a new analytical method for Al^{3+}, and (3) application of the new analytical method to seafood samples.

1 Experimental

1.1 Samples and reagents

All samples, namely the seaweed *Porphyra haihanensis*, the scallop *Chlamys farreri*, and the sea cucumber *Stichopus japonicus*, were randomly bought from supermarkets and other markets. A standard Al^{3+} solution (100 μg/mL) was purchased from the National Standard Material Management Committee, China. LiOH and CH_3COOH were purchased from Alfa Aesar, USA. 2,6-Pyridinedicarboxylic acid (PDCA) was purchased from Sigma-Aldrich, USA. A solid-phase extraction (SPE) column (Oasis MCX, 3 mL/60 mg, 30 μm) was purchased from Waters, USA. A cation-exchange column (CS 5A 4 mm × 250 mm, CG 5A 4mm × 50 mm, Dionex IonPac, USA) was used for the Al speciation analysis.

1.2 Instruments

The Al species were analyzed using HPLC (Perkin-Elmer, Series 200, USA) coupled with inductively coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer, ELAN DRC II, USA). pH values were measured using a Mettler Toledo 320-S pH-meter (Mettler Toledo Co., China). Other equipments used were a temperature-consistent oscillating water-bath (SHA-B, GuoHua Co., China) and an Eppendorf 5810 centrifuge (Merck, Germany).

1.3 Al speciation analysis using HPLC-ICP-MS

Al speciation analysis was performed using HPLC-ICP-MS. The operating conditions of the ICP-MS and chromatographic systems are shown in Table 1. The Al^{3+} in the extracts was identified by matching the retention times with a standard. It should be noted that there were no Al species in

Table 1 Operating conditions of ICP-MS and chromatographic systems

Apparatus	Parameter setting
ICP-MS	
Radio frequency power	1300 W
Sampler and skimmer cones	Nickel
Plasma	15 L/min
Auxiliary	0.86 L/min
Nebulizer	0.92 L/min
Date acquisition mode	Graphics (signal intensity versus time)
Analytical mass (amu)	^{27}Al
Chromatography	
Guard column	IonPac CG 5A (50 mm×4 mm)
Analytical column	IonPac CS 5A (250 mm×4 mm)
Mobile phase	0.040 mol/L LiOH, 0.0060 mol/L PDCA, 0.090 mol/L CH_3COOH, pH 4.7
Flow rate	1.0 mL/min
Injection volume	50 μL

the standard other than Al^{3+}, so only Al^{3+} could be quantified in the present study. All unknown Al compounds were regarded as Al(X).

1.4 Sample extraction

About 0.5 g of dried or 2.0 g of fresh homogenized samples were weighed in 50-mL polypropylene tubes. The mobile phase (20 mL), which was used as the extractant, was added to each tube and entirely mixed with the sample using a vortex. Then the tubes were shaken at 70°C for 2 h, and the solutions were centrifuged for 10 min (8000 r/min). The supernatant was filtered using a 0.22-μm membrane and the percolate was kept for further use.

1.5 Clean-up method

In order to decrease interference from the large amount of organic substances in the seafood, solid-phase extraction (SPE) was used in the pretreatment. Methanol (2 mL) and 4 mL of deionized water were used to activate and equilibrate the SPE column. The percolate (1 mL) (described in Section 1.4) was passed through the column at a flow rate of 0.2 mL/min. Then 2 mL of HCl (0.075 mol/L) were used as the eluent to wash inorganic Al from the SPE column. The wash solution was then concentrated by evaporation at 100°C. Mobile phase (1 mL) was added to the concentrate and the solution was filtered using a 0.22-μm membrane. Al^{3+} in the percolate was analyzed using HPLC-ICP-MS.

2 Results and discussion

One of the main aims of the study was to create chromatographic conditions that would enable full separation of Al^{3+} and other forms of soluble Al, and their online detection using ICP-MS. Selecting an appropriate analytical column was therefore the first step. We examined several types of column, namely Mono Q (Bio-Rad, USA), Mono S HR (Amersham Pharmacia Biotech, Uppsala, Sweden), Supelcosil™ LC-SCX (Supelco, USA), and IonPac CS5A (Dionex, USA) columns. Experiments showed that separation of Al^{3+} from soluble Al was only achieved using the IonPac CS5A column (Figure 1), which was in accordance with previous research [26]. A mobile phase consisting of 0.040 mol/L LiOH, 0.0060 mol/L PDCA, and 0.090 mol/L CH_3COOH was selected according to Technical Note 27 from Dionex. Figure 1 shows that the analytical conditions using this column and mobile phase were successful for the analysis of Al^{3+}.

2.1 Optimization of extractant

Based on previous research on Al speciation analysis [25], different types of extractant, i.e. HCl (0.005–1 mol/L), 1 mol/L KCl, 0.2 mol/L ammonium citrate tribasic ($C_6H_{17}N_3O_7$), 1 mol/L CH_3COONH_4, 15 mmol/L $(NH_4)_2HPO_4$ (pH 6.0), deionized water, and the mobile phase described above (pH 4.7) were used to extract Al^{3+} and other Al forms. The results showed that the mobile phase was the best extractant because Al^{3+} and other soluble forms were successfully extracted, and different forms of Al were absorbed by the column. Al was not detected in any form with the other extractants. In addition, using the mobile phase as the extractant keeps the pH and ionic strength of the injected sample consistent with those of the mobile phase, which decreases the disturbance of the system peaks and prevents the Al forms from changing during the HPLC separation.

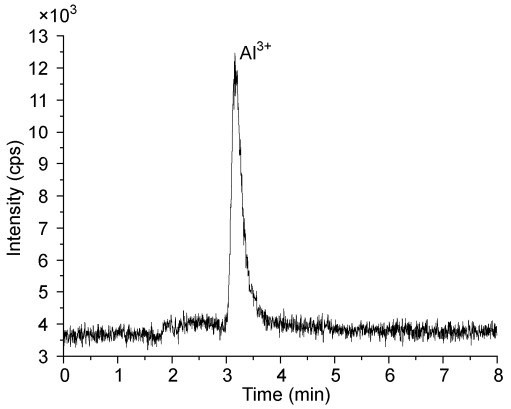

Figure 1 Chromatogram of standard Al^{3+} using HPLC-ICP-MS.

2.2 Optimization of extraction temperature and time

The *P. haihanensis* samples were extracted using the mobile phase by shaking at different temperatures for 1–9 h to determine the best extraction temperature and time. The temperatures used were 60, 70, and 80°C. The changes in the amount of total soluble Al are shown in Figure 2. It is easily seen that the total soluble Al content extracted at 60°C for 6 h was the same as that extracted at 70°C for 2 h. In addition, the chromatograms of the different Al speciations in both extract solutions are similar. However, when the samples were extracted at 80°C, the chromatogram of the Al speciation was significantly different from those for the extracts obtained at 60 and 70°C. This may be because the

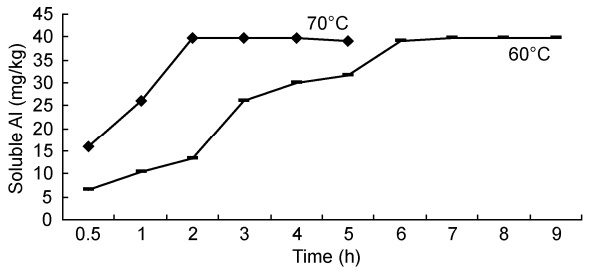

Figure 2 Changes in total soluble Al at different temperatures and times for *P. haihanensis*.

high extraction temperature changed the form of the soluble Al. Shaking at 70°C for 2 h was therefore selected as the best extraction method.

2.3 Al^{3+} extraction rate

There are at present no reference materials for Al^{3+}. We therefore used instant jellyfish, in which the Al almost all exists in the Al^{3+} form, as a reference. The extraction rates of Al^{3+} from instant jellyfish obtained using our new extraction method are shown in Table 2. The results show that the Al^{3+} extraction rates were all above 88%, and it could be concluded that this new extraction method is successful.

2.4 Role of clean-up in pretreatment

Because seafood samples such as fish, shrimp, and seaweed are enriched with soluble protein, soluble sugars, and other soluble organic compounds, matrix interference in the samples limits the maximum sensitivity that can be achieved. This constraint can be overcome by cleaning the sample with SPE products prior to further analysis. SPE not only simplifies the extraction procedure and shortens the analysis time, but may also improve the percentage recovery of the sample and reproducibility of the extraction. A weak cation-exchange SPE was used in the pretreatment. Figures 3 and 4 show the differences between the chromatograms of Al in *P. haihanensis* without and with SPE in the pretreatment. It was found that the Al forms were very complicated and Al^{3+} was seriously interfered by unknown forms of Al when SPE was not used in the pretreatment (Figure 3). However, after SPE, Al^{3+} could be easily separated and identified (Figure 4). A comparison of Figures 3 and 4 also proves that the clean-up method established in the present study successfully decreased interference from organic substances in the seafood. The clean-up in the pretreatment therefore played a crucial role in the identification and quantification of Al^{3+} in the seafood.

Table 2 Extraction rates of Al^{3+} from instant jellyfish

No.	Total Al content (mg/kg)	Al^{3+} content (mg/kg)	Extraction rate (%)
1	462.2	409.5	88.6
2	444.3	402.6	90.6
3	750.0	674.6	89.9
4	662.6	604.4	91.2

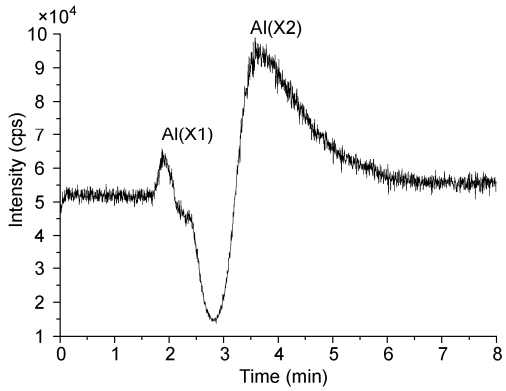

Figure 3 Chromatogram of Al in *P. haihanensis* without SPE pretreatment.

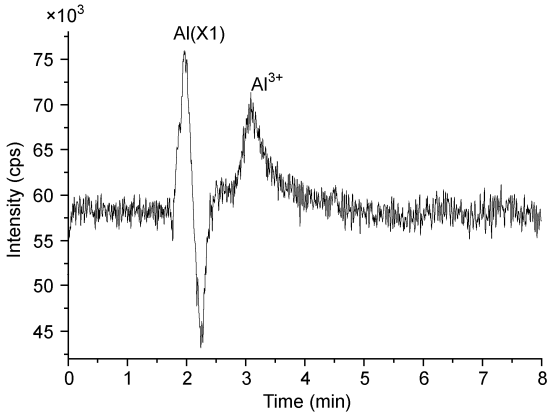

Figure 4 Chromatogram of Al in *P. haihanensis* with SPE in pretreatment.

2.5 Measurements

The Al^{3+} standard solution was prepared by diluting the above-mentioned stock standard solution (100 μg/mL). It was then diluted to 50, 100, 200, 400, and 800 ng/mL with the mobile phase. The chromatogram of the Al^{3+} standard is shown in Figure 5. Under the above experimental conditions, the retention time of Al^{3+} was 3.36 min, and the variations in the retention times of Al^{3+} in the samples were in the range ±5% compared with the standard solutions.

2.6 Analysis of samples

The Al^{3+} contents of seaweed (*P. haihanensis*), scallops (*C. farreri*), and sea cucumber (*S. japonicas*), which were randomly bought from markets, were determined using the

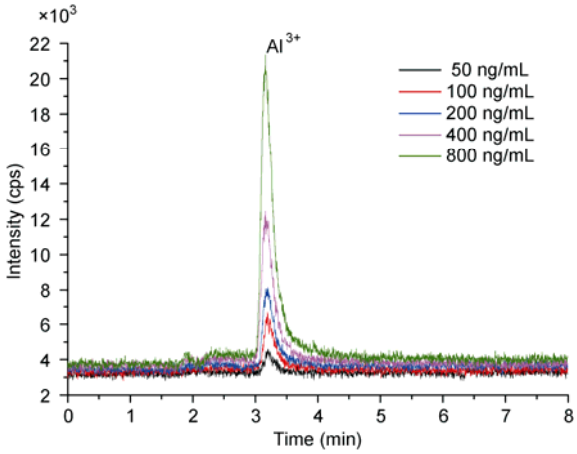

Figure 5 Chromatogram of standard Al^{3+} using HPLC-ICP-MS.

above optimized conditions. At the same time, the total Al contents of the samples were determined by ICP-MS. Table 3 shows the recoveries achieved and accuracy of the method. It is clearly seen that the standard recoveries of Al^{3+} from the three samples were all above 89%, and the relative standard deviations were all below 5%, indicating the feasibility of the proposed method for Al^{3+} detection in seafood.

The total Al and Al^{3+} contents were determined in a total of 15 *P. haihanensis* samples, 12 *S. japonicus* samples, and 12 *C. farreri* samples, which were collected from different origins. It should be mentioned that the data for both Al^{3+} and total Al content in *S. japonicus* and in *C. farreri* were based on fresh weight, whereas for *P. haihanensis*, the data were based on dry weight. The ranges and average Al^{3+} and total Al contents of all the samples, and the percentage of Al^{3+} in the total Al content, are shown in Table 4. Figures 6 and 7 show the chromatograms of Al^{3+} in the sea cucumbers and scallops, respectively. Al^{3+} in both *S. japonicus* and *C. farreri* was successfully separated and determined using the new method.

Table 4 shows that, based on fresh weight, the Al^{3+} content of *S. japonicus* ranged from 10.22 to 20.74 mg/kg and represented about 7.8%–10.2% of the total Al content. Compared with those of *S. japonicus*, the Al^{3+} contents of *C. farreri* were much lower, ranging from 2.86 to 4.28 mg/kg and representing about 6.2%–7.4% of the total Al content. Based on dry weight, the Al^{3+} content *of P. haihanensis* ranged from 9.62 to 25.8 mg/kg, and the percentage of Al^{3+} in the total Al was between 3.2% and 6.5%; these results are similar to previous results obtained using the static equilibrium method [25], which further proves the accuracy of the new method.

3 Conclusions

The new analytical method developed in the present study is the first to be used for the determination of Al^{3+} in seafood. The optimized determination conditions for the HPLC-ICP-MS system, using an IonPac CS5A (Dionex) analytical column and 0.040 mol/L LiOH, 0.0060 mol/L PDCA, and 0.090 mol/L CH_3COOH as the mobile phase, enabled full separation of Al^{3+} and other soluble Al forms to be achieved. The extractant, extraction temperature, and time were also

Table 3 Recoveries of Al^{3+} and accuracy of the method (n = 5)

Sample	Added Al^{3+} (mg/kg)	Al^{3+} recovery (%)	Average recovery (%)	Relative standard deviation (RSD)
P. haihanensis	10.0	90.2/86.6/96.2/88.6/92.2	89.76	3.67
	50.0	93.6/91.2/86.2/98.2/91.8	92.80	4.33
C. farreri	5.0	92.6/86.9/92.5/90.2/89.6	90.36	3.25
	25.0	100.0/90.6/89.9/93.7/97.2	94.14	4.78
S. japonicus	5.0	93.1/87.9/90.5/89.2/91.1	91.70	3.42
	25.0	101.0/96.9/92.5/89.2/95.2	94.96	4.70

Table 4 Al^{3+} and total Al contents of *S. japonicus*, *C. farreri*, and *P. haihanensis*

	Al^{3+} content (mg/kg)	Total Al content (mg/kg)	Percentage of Al^{3+} to total Al
S. japonicus	10.22–20.74 (14.90±2.94)	123.5–241.2 (173.6±39.2)	7.8%–10.2%
C. farreri	2.86–4.28 (3.34±0.46)	41.39–61.14 (48.62±6.25)	6.2%–7.4%
P. haihanensis	9.62–25.84 (20.31±4.60)	300.6–705.1 (492.0±131.6)	3.2%–6.5%

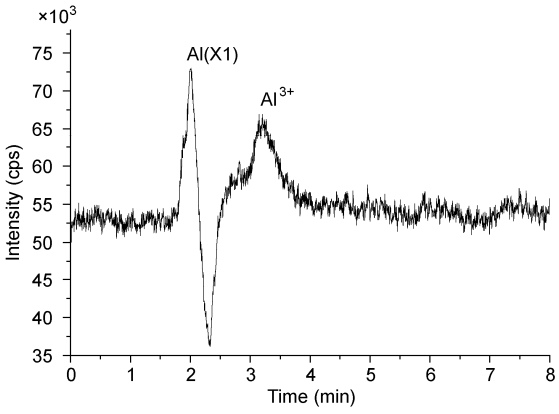

Figure 6 Chromatogram of Al^{3+} in *S. japonicas*.

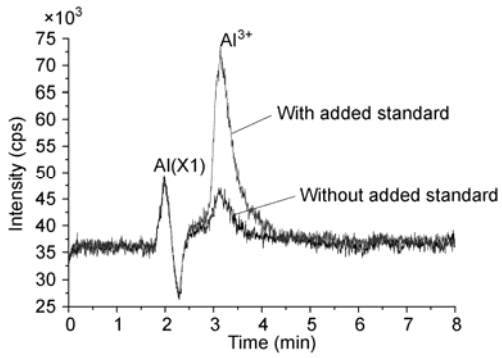

Figure 7 Chromatogram of Al^{3+} in *C. farreri*.

optimized. Using the mobile phase as the extractant keeps the pH and ionic strength of the injected sample consistent with those of the mobile phase, as far as possible, and thus keeps the Al forms unchanged during HPLC separation. Shaking at 70°C for 2 h achieved extraction of almost all of the Al^{3+} (above 88%) in the seafood. Additionally, in order to remove matrix interference and improve the sensitivity, a weak cation-exchange SPE was used to clean up the extracted sample before further analysis. An Oasis MCX, 3 mL/60 mg, 30 μm SPE column was selected and 2 mL of methanol and 4 mL of deionized water were used to condition and equilibrate the column before loading the sample. HCl (0.075 mol/L, 2 mL) was used as the eluent to wash inorganic Al from the SPE column. After clean-up, the Al^{3+} in the seafood was successfully separated and determined. The accuracy of the suggested method was high and satisfies the determination requirements. The results obtained using the present method showed that the toxic form of Al^{3+} in seafood represented less than 10% of the total Al. In addition, the results obtained using the SPE-HPLC-ICP-MS analytical system and those obtained using the static equilibrium method were in good agreement.

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