Notes

Speciation Analysis of 6 Arsenic Species in Sea Mustard Using IC-ICP-MS

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Arsenic is the 51st most abundant element in the earth's crust. It has been used in a wide range of areas such as medicine, agriculture, industry, and military. Differences occur in arsenic's physical and chemical properties depending on its oxidation state or combined elements.[1] There are two types of arsenic: organic and inorganic. Inorganic arsenic, arsenite (As(III)) and arsenate (As(V)), is more toxic than organic arsenic. Dimethylarsonic acid (DMA) and monomethylarsinic acid (MMA) are moderately toxic. Arsenobetaine (AsB) and arsenocholine (AsC) are considered to be nontoxic.[2] The human exposure to arsenic is predominantly due to foods such grain and rice, as well as seafood. The pathways of exposure for humans are predominantly foods such grain and rice, but also seafood. AsB and arsenosugars (derviatives of dimethylarsinoylribosides and trimethylarsonioriboside) are mainly found in algae which live in uncontaminated marine ecosystems, where low concentrations of DMA, MMA, and AsC are also found.[3] Many nutrients are found in seaweed. Among them, in particular, iodine is an essential element for the maintenance of normal human growth and development. Seaweed is consumed by coastal people, particularly in East Asia.[4] Selective and highly sensitive techniques are required to carry out speciation in environmental or biological matrices without species change. Inductively coupled plasma mass spectrometry (ICP-MS) is the most sensitive and robust elemental detector. The ICP-MS technique has spectral interferences which are generally presented by polyatomic species. The most commonly appearing interference is a polyatomic combination of argon and chloride encountered during the analysis

of the total arsenic. A polyatomic connection ($^{40}Ar^{35}Cl$) is created by the interaction of gaseous plasma, reagents, and a sample matrix. Spectral interferences can be eliminated by the analysis of the isotopes of other elements. Extracting arsenic from matrices without any changes in concentration of the species is a crucial step. Shaking and sonication extraction methods have been used effectively for a long time.[5,6] Instruments for this type of extraction, which are found in many laboratories, are cheap and can be used for various purposes.

Ion chromatography (IC) is the method most widely used for arsenic species analysis.[7] In particular, an anion-exchange column is most commonly used. Two drawbacks of this process are its short life-span and high cost. In many studies, nitrate, carbonate, and phosphate buffers are used alone or in mixture as mobile phases.[6–11] But using of phosphate or carbonate solvents can result in potential clogging problems of instrument and column. They are also not good for columns due to the reduction of the capability of columns that results from conducting an operating analysis separation at a high pH. Thus, it is imperative to study different solvents such as acids[12–15] and others[16] in order to find the most effective solvents for the best conditions of the column. Using acid solvents as a mobile phase is good for connecting to ICP-MS and it can prevent metal precipitation in the columns. If the operating conditions have a relatively low pH, however, it is possible that this could reduce the column life. Many scientists have studied analysis methods using acid. However, the analysis method with nitric acid has not been used much even though it has several advantages.[12–15] In

this study, optimized method can analysis more arsenic species in shorter instrument run time.[14] Also, the resolution increase to As(III) and other organic species.[15] So, we were able to optimize effective methods by IC-ICP-MS.

The purpose of this work consisted of (1) The development of arsenic speciation analysis methods using nitric acid as a mobile phase. (2) The establishment of an ultra-sonication extraction method in sea mustard for arsenic speciation. (3) The validation of the methods through confirming the selectivity, stability, accuracy, precision, LOD, and LOQ.

Three different extract solvents, deionized water, 50% (v/v) MeOH and 100% MeOH were tested for their extraction efficiency using spiked sea mustard samples on 30 minutes extraction time (*Fig.* 1a). When 100% MeOH was used as an extraction solvent, two arsenic species (the recovery of AsB and AsC were obtained at 83.3% and 89.2% respectively) showed acceptable efficiency, but other species had quite high and low efficiencies: MMA-134.0%, As(III)-25.7%, DMA-34.1%, and As(V)-24.8%. Therefore, the extract solvent proved inappropriate for use in the analysis of arsenic species. The results produced did not agree with the data of a previously reported study.[17]

When 100% deionized water and 50% (v/v) MeOH were used as extraction solvents, the recovery efficiencies of the six arsenic species extracted were similar: the recovery efficiencies using 100% deionized water were 88.3–107% and that of 50% (v/v) MeOH were 90.1–98.8%. Using 100% deionized water (RSD: 0.7–1.7%) yields a better reproducibility than 50% (v/v) MeOH (RSD: 0.5–7.9%) as the extraction solvent (n=3). Three samples for each extract solvent were used to calculate the RSD, applying the entire extraction process to respective samples. 100% deionized water proved the optimal extraction solvent. When 100% deionized water was used to extract arsenics from the samples, the process of extraction was simpler and more suitable for long-term analysis with ICP-MS.[18]

Fig. 1b and *Fig.* 1c show the results of tests comparing extraction time and extraction numbers. Tests to identify optimal extraction time were conducted for 30, 60 and 90 minutes using one time extraction. Because, with the exception of AsB, among the arsenic species extracted, extraction efficiencies according to the extraction time were not significantly different, tests of 30 minutes were used to optimize the extraction number. *Fig.* 1c shows the increasing of extraction efficiency (88–107%) produced by increasing the extraction number from one to two. When the extraction number was increased to three, the extraction efficiency was very good (91–120%). However, the increase in the quality of the results with three repetitions was not significant enough to justify the extra time and effort, so we decided that repeating the extraction two times was suitable for analysis.

In our study, we validated the extraction method and the effectiveness of arsenic speciation analysis for sea mustard samples. The validation factors were selectivity, stability, linearity, accuracy, precision, LOD, and LOQ.

Nitric acid 0.5 mM (pH 3.4) and 500 mM (pH 1.4) were optimized and used on the eluents with 1% (v/v) MeOH. *Fig.* 2 shows a good resolution and completed speciation analysis within 5 minutes. The retention order was similar to the results of Wangkarn & Pergantis.[19] We speculate that the retention time difference between the DMA and MMA was due to the difference of the concentration of the mobile phase B in gradients. The retention times of As(III), DMA, MMA, As(V), AsB, and AsC were 80, 129, 200, 218, 250,
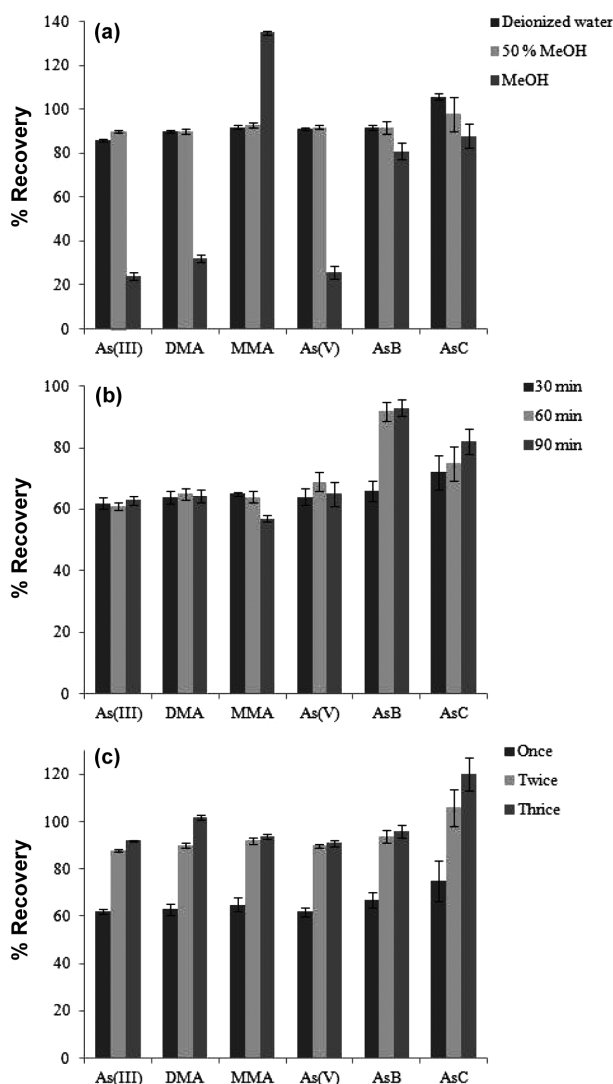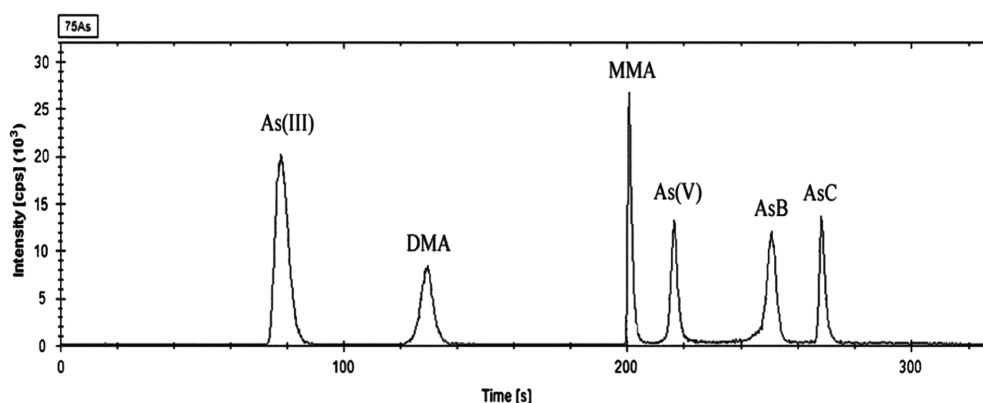

Figure 1. The dependence of spiked sample recovery upon (a) extraction solvent; (b) extraction time; and (c) number of extraction.

Figure 2. Chromatogram of the six arsenic species (10 μg L^{-1}).

and 275 sec, respectively (n=10). The effect of the interference of chloride in the seaweed samples can considerably affect the arsenic speciation analysis. It is important to reduce misidentification related to the interferences of the $^{75}As^+$ signal due to the presence of the polyatomic ArCl species ($^{38}Ar^{37}Cl^+$ and $^{40}Ar^{35}Cl^+$) formed from the argon gas and chloride of the matrix.[20] The ICP-MS used in this work enables kinetic energy discrimination (KED), which can eliminate interference effects such as polyatomic interference.

To ensure reliable results, stability of the arsenic species during the sample extraction process is essential.[21] We confirmed whether or not the arsenic species were stable during the extraction procedure. Some sea mustard samples were spiked with a mixture of the six arsenic species and other sea mustard samples were spiked with one of the six species. The spiked samples were processed by the extraction method previously described and analyzed using IC-ICP-MS. No change in the arsenic species during the extraction procedure was found. A linearity was performed with standard solutions of different concentration levels (10–100 μg kg^{-1}, 10 points). Good linearity (r^2 > 0.99) of the six arsenic species was obtained. In order to check for possible matrix effects and accuracy, recoveries of 20 μg L^{-1} spikes were measured in the sea mustard samples with a sample concentration of 10 mg kg^{-1}. The spiked samples were held on a table for one day after they were spiked and then processed in the same way as the un-spiked samples.

When 100% deionized water was used as the extract sol-

vent, only AsC showed high extraction efficiency (118.9%). Inorganic arsenic species did not have good extraction efficiency when 100% deionized water was used for the extraction. However, we obtained fairly good extraction efficiency (92.0–118.9%) and precision (0.7–1.7%) from all arsenic species. Certified Reference Material (CRM) such as NMIJ 7405-a was used to measure the extraction efficiency. The certified values in NMIJ 7405-a were 35.8 ± 0.9 mg kg^{-1} (total arsenic) and 10.1 ± 0.5 mg As kg^{-1} (As(V)).

The LOD and LOQ were defined as three and ten times the standard deviation of the average from the blank samples (n=10). The LOD values were higher than those previously obtained.[22] Reports that many kinds of seaweed contain ppm units of arsenics are widespread.[23] We conclude that this study had a low enough LOD (0.01–0.07 μg kg^{-1}) and LOQ (0.03–0.23 μg kg^{-1}) to be used in identifying and quantifying arsenic species in real samples (*Table* 1).

EXPERIMENTAL

A Dionex ICS-5000 high performance ion chromatography consisting of a binary pump and thermal compartment was used to carry out the analysis with ICP-MS measurements (iCAP Q, Thermo scientific). The ICP-MS was equipped with a perfluoroalkoxy (PEA) micro flow concentric nebulizer and a cyclonic chamber. An IonPac AS7 (250×4 mm, 10 μm) anion exchange column was used for the separa-

Table 1. Figures of merit of optimized speciation method

	As (III)	DMA	MMA	As (V)	AsB	AsC
Recovery (%)	92.0	93.6	98.5	101.5	100.6	118.9
RSD (%)	0.7	0.7	1.1	1.7	1.5	1.1
LOD (μg kg^{-1})	0.010	0.023	0.030	0.068	0.055	0.052
LOQ (μg kg^{-1})	0.032	0.076	0.100	0.226	0.184	0.174

tion of arsenic species with an IonPac AG7 (50×4 mm, 10 μm) guard column connected. Nitric acid 0.5 mM and 500 mM were optimized and used on the eluents with 1% (v/v) MeOH. Thanks to nitric acid eluents, the compatibility of ICP-MS was increased further than when using the buffer solution as the mobile phase. A lot of works reported that adding methanol to the eluents would enhance the element ionization and arsenic response.[16,24] An improved peak and intensity could be obtained due to the effect of MeOH. The flow rate was 1.20 mL/min and a gradient elution method was used for optimized analysis. The kinetic energy discrimination (KED) mode was used to eliminate interference effects, like $^{75}ArCl^+$ (m=74.93124). A 1 μg L^{-1} tuning solution (indium was especially used for optimization) was used daily to optimize the ICP-MS sensitivity before carrying out the arsenic speciation analysis.

Total arsenic analysis

The prepared sea mustard samples (0.2 g), nitric acid (4 mL) and hydrogen peroxide (2 mL) were combined in vessels for overall arsenic analysis. At 600 W power, the temperature was increased to 190 °C for 15 minutes and maintained at 190 °C for 15 minutes. After the vessels cooled down, the gas was released from the high-pressure vessels. The residue solution inside the vessels was diluted to an adequate mass per volume with deionized water and large particles were filtered with filter paper. The arsenic standard solution calibration curve was used at 10, 50 and 100 μg L^{-1} concentrations.

Arsenic speciation analysis

Dried sea mustard samples (0.2 g) and deionized water (10 mL) were added to a 50 mL Teflon centrifuge tube. The samples and solvents were stirred homogeneously and placed in an ultrasonic bath for 30 minutes and centrifuged for 10 minutes (4 °C, 3500 rpm). The supernatants 6 mL were transferred into a clean tube and 10 mL deionized water was added to the residues. The ultra-sonication and centrifugation steps were repeated one more time with the same conditions. The supernatants 10 mL were transferred into a tube that had 6 mL supernatants. The combined supernatants were diluted with deionized water to an adequate concentration and filtered with a 0.45 μm nylon syringe filter to eliminate the matrices. Arsenic speciation analysis was conducted with the IC-ICP-MS.

In this study, a method for the qualitative and quantitative determination of six arsenic species in sea mustard samples using IC-ICP-MS was developed and validated. In addition, we analyzed four real samples using validated

method. Deionized water only used to extract arsenics from the samples and ultra-sonication extraction was used. This extraction method was cheap, simple and effective. Also, ultra-sonicated extraction method using water as the solvent provided good extraction efficiency and precision results by twice to extraction step. We were able to produce solutions to two problems remaining in previously studies. First, the use of buffer solutions has shortcomings due to the decrease in the sensitivity and reproducibility of ICP-MS detector caused by clogging. Second, metal can be precipitated in the inner column at high pH. Automatically, another problem, that of As(III) and DMA peaks sometimes overlapping from repeated analysis, was also resolved using this method. Using deionized water as the extraction solvent and a nitric acid gradient as a mobile phase is most compatible with the long-term stability of the IC-ICP-MS system.

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