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Development of Isotope Dilution LC-MS/MS Method for Accurate Determination of Arsenobetaine in Oyster Certified Reference Material[†]

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An isotope dilution liquid chromatography tandem mass spectrometry (ID LC-MS/MS) method has been developed and applied to the determination of arsenobetaine (AsB, $(CH_3)_3{}^+AsCH_2COO^-$) from oyster candidate certified reference material (CRM). The exact matching isotope dilution approach was adopted for accurate determination of AsB using $^{13}C_2$-labeled AsB as an internal standard. Efficiencies of different AsB extraction methods were evaluated using a codfish reference material and a simple sonication method was selected as the method of choice for the certification of the oyster candidate CRM. The hydrophilic interaction liquid chromatography (HILIC) combined with electrospray ionization tandem mass spectrometry (ESI/MS/MS) in selected reaction monitoring (SRM) mode was optimized for adequate chromatographic retention and robust quantification of AsB from codfish and oyster samples. By analyzing 12 subsamples taken from each 12 bottles systematically selected from the whole oyster CRM batch, the certified value of AsB was determined as 6.60 $mg{\cdot}kg^{-1} \pm 0.31\ mg{\cdot}kg^{-1}$ and it showed excellent between-bottle homogeneity of less than 0.42%, which is represented by relative standard deviation of 12 bottles from the CRM batch. The major source of uncertainty was the certified value of the AsB standard solution.

Key Words : Arsenobetaine, Speciation analysis, Certified reference material, Isotope dilution mass spectrometry, HILIC-LC-MS/MS

Introduction

The concentrations of naturally occurring arsenic in sea foods such as fishes and crustaceans are relatively high, often above the recommended level, and its chronic exposure can be a potential threat to human health.[1,2] Since the toxicity of arsenic species depends on their chemical forms, providing information only on the total content of arsenic in foods is often not sufficient to evaluate safety and toxicological effects as a consequence of the food consumption. The demands on species-dependent toxicology assessments attracted a lot of attention to the researches on speciation analysis including arsenic species.[1-11] Arsenobetaine (AsB), an arsenic analogue of betaine, is one of the most abundant arsenic species in seafoods and is believed to be originated from metabolic transformation of inorganic arsenicals in marine organisms.[1,2,12] Hence, the accurate determination of AsB is essential for the reliable toxicity assessment of arsenicals in food.

The use of certified reference material (CRM) has been recommended to analytical laboratories for the purpose of method validation and quality control. ISO/IEC 17025 also listed the use of matrix matched reference materials as a basic requirement for quality assurance,[13] if they are available. Therefore, the development of CRM for AsB analysis is very useful in the context of addressing the method validation and quality control issues associated with reliable determination of AsB. For the development of CRM, it has been recognized that a definitive reference method should be placed on the highest priority for assigning the certified values of CRMs to make them acceptable internationally beyond dispute. In general, isotope-dilution mass spectrometry (IDMS) has been widely accepted as a definitive reference method and many CRMs have been certified by IDMS with appropriate method validation. For the analysis of AsB, the majority of analytical methods applied in the field laboratories are based on the inductively-coupled plasma/mass spectrometry (ICP/MS) monitoring the elemental arsenic. In these methods, the analyte has been separated from other arsenic species and/or sample matrix by means of liquid chromatography (LC) and, then, the elemental arsenic has been monitored by ICP/MS. In some applications, hydride generation (HG) technique has been added in the sample introduction part to improve the performance.[1,3,4-9,14-16] However, it is impossible to apply isotope dilution (ID) approach in the case of arsenic, since arsenic has only one stable isotope.[4,8] Although these LC-ICP/MS methods provide an advantage to determine all arsenic species present in a sample with high sensitivity, it cannot take advantage of robustness and reliability of the isotope-dilution method. Neutron activation analysis (NAA) interface with liquid chromatography in offline can be another candidate for a definitive reference method and there is a report to measure AsB in natural waters.[17] But it is very hard to use HPLC-NAA for the analysis of AsB because of lack of the facility for neutron

[†]This paper is to commemorate Professor Myung Soo Kim's honourable retirement.

radiation source.

Ever since Corr and Larsen[18,19] have reported the use of ion-spray mass spectrometry for arsenic speciation analysis, electrospray ionization mass spectrometry (ESI/MS) has been also studied for the analysis of organoarsenic compounds with chromatographic separation.[20-26] LC-ESI/MS based methods allow the use of ID approach with stable isotope-labeling on organic carbons or hydrogens instead of mono-isotopic arsenic. Moreover, AsB can be identified and selectively quantified using LC-ESI/MS/MS even in the presence of complex matrix. In the case of LC-ICP/MS, in contrast, sufficient chromatographic separation of individual arsenic compounds in a sample is required to get unbiased quantification results.

Chromatographic separation of AsB compatible with electrospray ionization is another issue to be resolved. Because AsB has high polarity and typically presents as either zwitter ion or positive ion forms in solution ($pK_a = 2.2$), simple reversed-phase LC cannot provide sufficient chromatographic retention of AsB. Use of ion-paring reagents or ion exchange chromatographic method as an alternative also generates a problem related to incompatibility of eluents with analyte ionization in ESI interface. More recently developed hydrophilic interaction chromatography (HILIC) has been shown to aid analysis of higher polar compounds without using ion-pairing reagents.[27-33] Use of simple mobile phases possessing excellent compatibility with ESI, including acetonitrile and aqueous volatile buffers, and retention of highly polar analytes are expected to provide the optimum LC conditions for polar organoarsenic compounds, which are easily interfaced with ESI/MS methods.

In this work, an ID HILIC-ESI/MS method has been developed as a reference method for certification of AsB contents in seafood. For ID analysis, $^{13}C_2$-labeled AsB was used as the internal standard. The analytical method, including sample extraction, MS conditions, was optimized and evaluated using a codfish CRM for AsB analysis. Finally, the established method was applied for the certification of AsB content in the KRISS oyster CRM.

Experimental

Materials and Reagents. The raw material of oyster was obtained near the Tongyeong area in Korea and then processed through the standard production procedure of KRISS for preparation of candidate reference materials in a form of powder. The batch-produced oyster powder was divided into 838 bottles, packaged, and irradiated with 25 kGy of ^{60}Co gamma ray for sterilization (KRISS CRM 108-04-003). The cod fish CRM (NMIJ CRM 7402-a) used for method validation was obtained from National Metrology Institute of Japan (NMIJ). The AsB standard solution (NMIJ CRM 7901-a) used throughout this study was also obtained from NMIJ (Tokyo, Japan) and its certified value of AsB content was $24.40 \text{ mg·kg}^{-1} \pm 0.62 \text{ mg·kg}^{-1}$.[34] The working standard solution was freshly prepared just before sample preparation by diluting the stock solution with deionized water (18 MW·cm resistivity) from Milli-Q RG purification system (Millipore, USA). The $^{13}C_2$-labeled AsB (carboxymethyl-$^{13}C_2$, 99%) internal standard used for isotope-dilution mass spectrometry was synthesized by Cambridge Isotope Laboratories (Andover, MA, USA). HPLC grade methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). Ammonium acetate, ammonium formate, acetic acid, and formic acid were purchased from Sigma-Aldrich (Milwaukee, WI, USA). All the solvents and buffer solutions were filtered through the 0.45 mm membrane filter before use.

Preparation of Sample and Calibration Blends for Isotope-dilution Mass Spectrometry. In this study, exact matching isotope-dilution mass spectrometric approach was used for the analysis of AsB using one-point calibration and $^{13}C_2$–labeled AsB internal standard. For exact matching of isotopic peak ratios close to 1.0 in spiked samples (sample blends) and spiked standard solutions (calibration blends), preliminary measurement of AsB content with external calibration has been carried out for the oyster candidate CRM. For the isotope dilution LC-MS analysis, about 0.5 g of oyster or cod fish subsamples was weighed into a 50 mL conical tube and spiked with appropriate amount of the $^{13}C_2$-labeled AsB solution to make the final isotopic peak ratio of labeled and natural AsB to be approximately one. Typically, three subsamples were taken from a single bottle of sample for method validation purpose. In the case of certification, 12 subsamples were taken from each 12 bottles, which are selected systemically. Subsamples for microwave-assisted extraction were directly taken into Teflon medium-pressure microwave extraction vessel and spiked with the same labeled AsB solution. Then, the natural and $^{13}C_2$-labeled AsB spiked in sample blend was equilibrated and extracted following the methods described in the next section. Four calibration blends with the isotope ratio of around 1.0 were prepared by spiking the $^{13}C_2$-labeled AsB solution into four individual aliquots of the working calibration standard solution of AsB.

Evaluation and Optimization of Extraction Procedures. To evaluate the performance of different extraction methods and optimize the extraction efficiency, three extraction methods (mechanical shaking, ultrasonication, and microwave-assisted extraction) with varying extraction solvents and pH were tested. The sample was extracted using 10 mL of an extraction solvent (methanol:water = 90:10; v/v) in combination with different extraction methods. In the case of extraction by shaking, subsample taken in a 50 mL conical tube was soaked in an extraction solvent and shaken for 20 min using a mechanical shaker, Multi Reax (Heidolph, UK). After the mixture was centrifuged for 25 min at 4000 rpm, the supernatant was collected in a new conical tube. The residue was re-equilibrated with 10 mL of additional extraction solvent and the extraction procedures were triplicated. The combined extract was evaporated to dryness and reconstituted to 10 g with the mobile phase used for isocratic LC run. Finally, the reconstituted solution was filtered through 0.45 μm membrane to remove residual particulates. In ultrasonic extraction, all procedures are the same except that the equi-

librium between sample and extraction solvent was achieved by sonication of the mixture for 20 min instead of mechanical shaking. The microwave-assisted extraction was performed using ETHOS SEL MW extraction system (Milestone, Italy). The extraction solvent was added to the isotope-spiked sample in Teflon extraction vessel and the vessels with different extraction solvent were set to the microwave oven. The temperature was raised to 80 °C within 5 min. and maintained at that temperature for 30 min. After cooling to the room temperature, the extract was centrifuged for 25 min. at 4000 rpm. Then, the supernatants were recovered and evaporated to dryness. After the extract was reconstituted with 10 g of the mobile phase, it was filtered through 0.45 μm membrane filter. Microwave-assisted extraction at the higher temperature of 120 °C was also performed and the measured results were compared.

LC-MS and LC-MS/MS Analysis. An Agilent 6410 Triple Quad LC-MS system combined with Agilent 1200 series HPLC system (Agilent Technologies, USA) was used for the separation and quantification of the AsB. Luna® HILIC column (150 mm length, 2.0 mm i.d., 3 μm particle size) from Phenomenex (Torrance, CA. USA) has been employed to separate the compound. Ten μL of the prepared sample or calibration blends (with 1:1 isotope ratio) were injected onto the column using an autosampler. The analyte in the injected sample was separated using isocratic elution of the mobile phase which consisted of 90 % acetonitrile and 10% 50 mM aqueous ammonium formate buffer of pH 3 at a flow rate of 0.4 $mL·min^{-1}$. The LC eluent was introduced into MS through the electrospray ionization interface with probe voltage of 4 kV. The gas flow rate for nebulization was 11 L min^{-1} and the gas temperature was 350 °C.

Isotope ratios of the natural AsB and the $^{13}C_2$-labeled AsB were measured either by selected ion monitoring (SIM) or by selected reaction monitoring (SRM). In the SIM mode $[M+H]^+$ ions of natural AsB and $^{13}C_2$-labeled AsB at *m/z* 179 and 181, respectively, were used to generate ion chromatograms. Then, the ratio of integrated peak areas from the two ion chromatograms were used to calculate the experimental isotope ratio. When the SRM mode was applied, ion chromatograms were obtained from SRM transitions of *m/z* 179 → *m/z* 105, *m/z* 179 → *m/z* 120, and *m/z* 179 → *m/z* 161 for natural AsB, while transitions of *m/z* 181 → *m/z* 105, *m/z* 181 → *m/z* 120, and *m/z* 181 → *m/z* 163, respectively, were used for $^{13}C_2$-labeled AsB (see Figure 1). The collision energy for SRM transition was optimized as 26 eV and nitrogen was used as a collision gas to assist CID in the collision cell. Mass Hunter™ software was used to operate the system and for data acquisition and processing.

The Optimized Certification Procedure. After optimization of experimental conditions and method validation, 12 subsamples of candidate CRM were taken from each 12 bottles for certification of AsB content. Twelve bottles including the first and the last bottles from the batch of candidate CRM were systematically selected in regular interval. As a quality control (QC) sample, a bottle of cod fish CRM (NMIJ CRM 7402-a) was also used. One subsample for analysis and three subsamples for dry-mass correction from each bottle were taken. For dry-mass correction of oyster samples, three subsamples of about 0.5 g were exactly weighed into weighing bottles and dried for one week in a desiccator with P_2O_5 drying reagent. The weight changes were measured and the dry-mass of the oyster samples was determined for each bottle. In the case of cod fish samples,

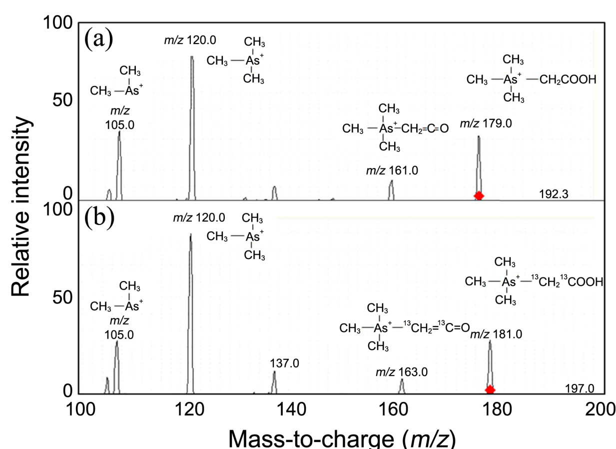

Figure 1. Tandem mass spectra of (a) AsB (*m/z* = 179) and (b) $^{13}C_2$-labeled AsB (*m/z* = 181) obtained from collision induced dissociation with 19 eV of collision energy. Structures and *m/z* of parent ions and major fragment ions are represented in the spectra. The parent ions are marked with the solid square.

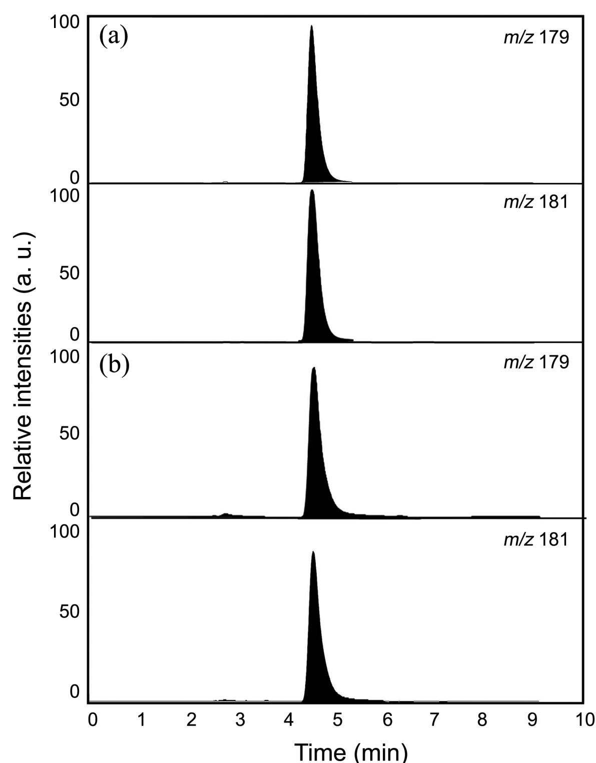

Figure 2. Selected ion chromatograms of natural AsB (*m/z*=179) and $^{13}C_2$-labeled AsB (*m/z*=181) from LC-MS analysis of (a) calibration blend and (b) sample blend of NMIJ 7402-a Cod fish using 0.4 $mL·min^{-1}$ of acetonitrile:50 mM aqueous ammonium formate (90:10, v/v) as an eluent in an Luna HILIC column (150 mm length, 2.0 mm i.d., and 3 μm particle size).

the dry-mass correction was carried out according to the guide in the certificate by drying in an oven at 102 °C for 6 hours. After sample blends and calibration blends were prepared by spiking the $^{13}C_2$-labeled AsB internal standard, sample blends including QC were undergone the extraction procedures. One procedure blank was prepared to monitor the blank level during the whole sample preparation procedure. Then, HILIC-ESI/MS/MS measurements were carried out using the SRM mode. Each sample run was triplicated. The measurement results from the 12 sample blends and 4 calibration blends were used to assign the certified value and to estimate combined standard uncertainty.

Results and Discussion

The isotope-dilution mass spectrometry (IDMS) has been widely used for the certification of reference materials. Although IDMS is potentially regarded as one of primary method of analysis, method validation based on ISO/IEC 17025, ISO Guide 34, and ISO Guide 35 is crucial to guarantee its robustness and reliability.[13,35,36] In the present study, IDMS method based on LC-ESI/MS was developed for determination of AsB in food matrices. For the purpose of method validation, the performances of major analytical steps including sample extraction and LC separation were carefully investigated using the cod fish CRM from NMIJ.

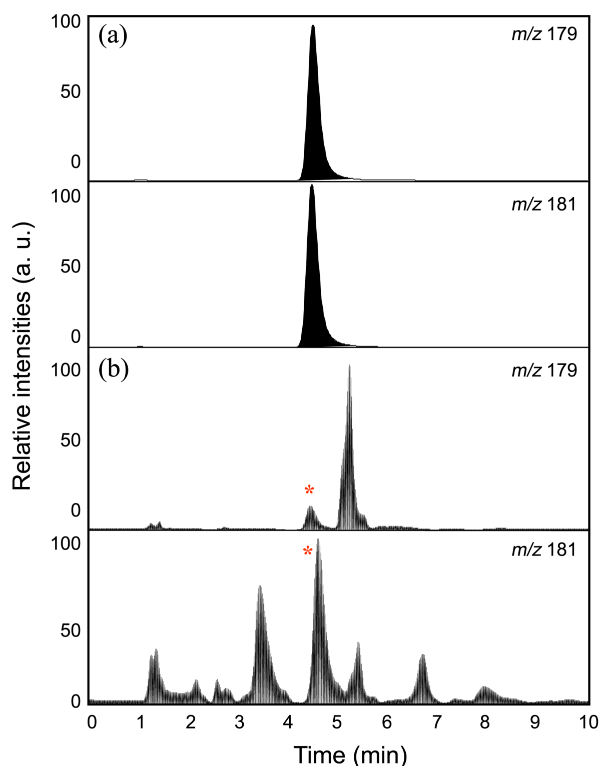

Figure 3. Selected ion chromatograms of AsB (*m/z* = 179) and $^{13}C_2$-labeled AsB (*m/z* = 181) from LC-MS analysis of (a) calibration blend and (b) oyster sample blend using 0.4 mL min^{-1} of acetonitrile:50 mM aqueous ammonium formate (90:10, v/v) as an eluent in an Luna HILIC column (150 mm length, 2.0 mm i.d., and 3 μm particle size). Peak of AsB in oyster sample blend is marked with asterisk.

LC-MS Optimization. Figure 2(a) and 2(b) show typical ion chromatograms of the natural AsB and $^{13}C_2$-labeled AsB at *m/z* 179 and 181 for calibration blend and Cod fish sample blend, respectively, obtained by positive ion SIM mode. The separation of AsB was successfully achieved using the hydrophilic interaction chromatography (HILIC) with 0.4 mL·min^{-1} of acetonitrile:50 mM aqueous ammonium formate (90:10, v/v) as an eluent. In the case of cod fish sample, LC-MS analysis using SIM mode was enough to determine AsB without significant interferences from chemical noises. The only noticeable peak observed around 4.5 min corresponds to either natural AsB (*m/z* 179) or isotope-labeled AsB (*m/z* 181). The isotope ratios calculated from the peak areas were in good agreement with the expected ratios of 1.0 and the measured results show acceptable values compared to the certified value of the CRM (data not shown).

Due to its high polarity, conventional reversed-phase (RP) stationary phase cannot provide enough chromatographic retention of AsB without the use of ion-pairing reagents, which usually result in adverse effects on ionization of analytes in the ESI interface.

As an alternative chromatographic method, cation-exchange chromatography, which is used frequently for the analysis of AsB, also suffers from limited choices of mobile phases due to incompatibility with ESI. Unique advantage of HILIC is excellent retention of highly polar AsB using ESI compatible eluent as shown in Figure 2. Moreover, chromatographic retention can be adjusted simply by changing the composition of acetonitrile and aqueous buffer.

The optimized separation condition for AsB was also applied to the analysis of AsB in oyster sample and Figure 3 shows the typical LC-MS chromatograms of AsB for the calibration blends and sample blends of the candidate reference material. While the ion chromatograms of calibration blends showed excellent peaks with appropriate retention,

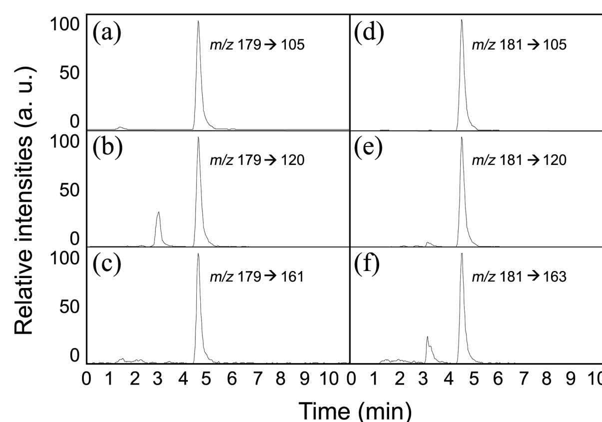

Figure 4. Selected ion chromatograms obtained from the oyster sample blend for each CID channels of the natural AsB and $^{13}C_2$-labeled AsB: (a) *m/z* 179 → *m/z* 105, (b) *m/z* 179 → *m/z* 120, (c) *m/z* 179 → *m/z* 161, (d) *m/z* 181 → *m/z* 105, (e) *m/z* 181 → *m/z* 120, and (f) *m/z* 181 → *m/z* 163 using 0.4 mL·min^{-1} of acetonitrile:50 mM aqueous ammonium formate (90:10, v/v) as an eluent in an Luna HILIC column (150 mm length, 2.0 mm i.d., and 3 μm particle size). Collision energies were in a range of 19 eV-26 eV corresponding to each CID channel.

those of the oyster sample blends showed many peaks due to chemical noises from oyster matrix. Even the potential overlaps of interfering peaks in AsB peaks cannot be excluded for oyster samples due to high levels of chemical noises. Therefore, LC-MS/MS approach was considered to improve the robustness and selectivity of the measurement method.

LC-MS/MS Optimization. For LC-MS/MS analysis of AsB with SRM mode, SRM transitions of m/z 179 → m/z 105, m/z 179 → m/z 120, and m/z 179 → m/z 161 were used for natural AsB, while those of m/z 181 → m/z 105, m/z 181 → m/z 120, and m/z 181 → m/z 163, respectively, were used for $^{13}C_2$-labeled AsB. The optimized collision energy for SRM transition was 26 eV. Figure 4 presents typical SRM chromatograms for the natural AsB and $^{13}C_2$-labeled AsB in oyster sample. The observed peak area ratio of each SRM channels was very closely to the expected isotope ratio (1.0). The relative standard deviation of the peak area ratios from triplicated LC-MS/MS run was in the range of 1-2% depending on the monitored channels. Although all of the six SRM channels exhibit much lower chemical noises compared with those in the Figure 3(b), the SRM channels of m/z 179 → m/z 105 and m/z 181 → m/z 105 were finally selected for the analysis since they showed lowest chemical noises with decent sensitivity.

Comparisons of Results from Different Extraction Methods. Quantitative extraction of AsB from complex matrices can be achieved only when the isotope-labeled AsB spiked in the sample is sufficiently equilibrated with natural AsB present in the sample. Incomplete equilibration may lead to a bias in the final result. Therefore, preliminary experiments were carried out to evaluate relative extraction efficiencies and degrees of equilibration of different extraction methods. A large number of studies on the extraction of organoarsenic compounds have been reported.[37-46] Although the extraction efficiencies were different depends on the matrices and extraction steps involved, most of them reported decent efficiencies between 90-110%. Mechanical shaking, sonication, and pressurized extraction including MW extraction have been used predominantly.

In the present study, the three popular extraction methods were evaluated using the cod fish CRM. Although the use of different solvent mixtures of methanol and water (from 0 to 100% of water) for AsB extraction showed almost equivalent efficiencies, methanol:water mixture (90:10, v/v) was chosen as the extraction solvent for further optimization considering the convenience of solvent evaporation step. As shown in Figure 5, mechanical shaking, sonication and microwave assisted extraction at two different temperature conditions (80 and 200 °C) were compared using the methanol: water mixture (90:10, v/v) as the extraction solvent. The AsB measurement results obtained with all four different extraction conditions were well-matched with the certified value, 84.37 mg·kg^{-1} ± 4.28 mg·kg^{-1}, of the codfish CRM. Additionally, effect of pH of the solvent used for extraction was also investigated. Since arsenobetaine has pK_a of 2.18 and exists in the forms of either zwitterion or cation depending on pH, the pH of extraction solvent may affect the chemistry of analyte extraction from matrix. However, the effect of pH on extraction efficiency turned out to be negligible as shown in Figure 5. It implies that the transition between zwitterionic and cationic forms of AsB doesn't induce significant changes in hydrophilicity of AsB significantly.

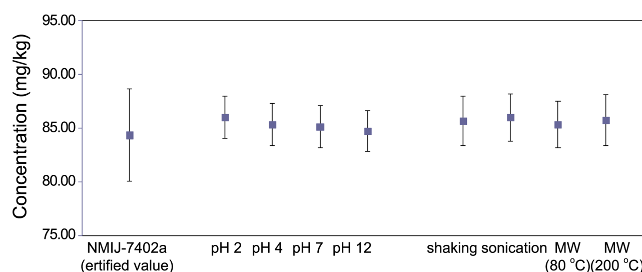

Figure 5. Measurement results for NMIJ CRM 7402-a Codfish tissue depending on the extraction methods. The certified value as arsenic of NMIJ CRM 7402-a was converted to the arsenobetaine content of 84.37 mg·kg^{-1}. MW represents the microwave assisted extraction method. Error bars represent the standard deviation of the results from three subsamples.

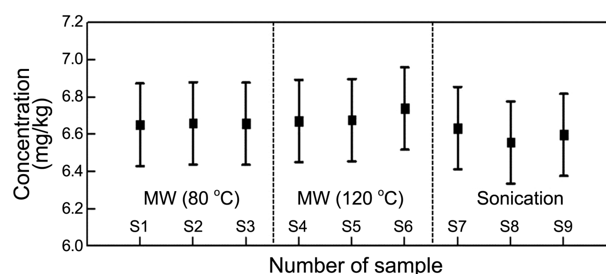

Figure 6. Measurement results for KRISS Oyster CRM depending on the extraction methods. MW represents the microwave assisted extraction method. Error bars represent estimated uncertainty of a single measurement result.

Although All the extraction methods evaluated using the codfish CRM showed almost equivalent result and matched with the certified value within the stated uncertainties. It should be noticed, however, that this agreement does not necessarily imply that the extraction was complete or the absolute extraction efficiencies were the same. It does tell us that the natural analyte in the sample was extracted equivalently to the isotope-labeled internal standard spiked in the sample.

Although the results obtained from codfish CRM show the robustness of the present methods based on the IDMS, the extraction methods has been further validated for oyster candidate CRM using different extraction methods as shown in Figure 6. The results obtained from three different extraction conditions were in excellent agreement with each other, indicating sufficient equilibration between the natural AsB and the isotope-labeled AsB spike was achieved. The ultrasonic extraction for 20 min using methanol:water mixture (90:10, v/v) as the extraction solvent was finally chosen to be the optimized extraction method used for certification of the candidate CRM due to its simplicity and convenience.

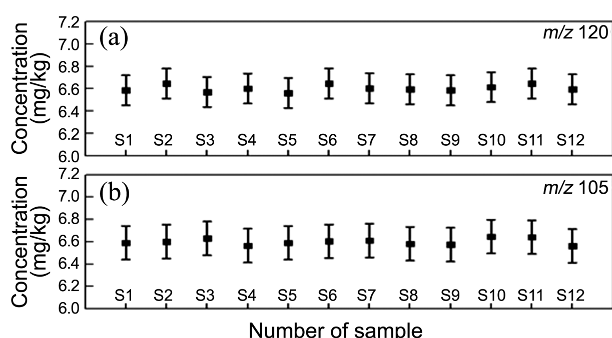

Figure 7. Measurement results of 12 bottles of the KRISS Oyster CRM batch obtained using SRM channels of (a) *m/z* 179 → *m/z* 105 and *m/z* 181 → *m/z* 105, (b) *m/z* 179 → *m/z* 120 and *m/z* 181 → *m/z* 120. Between-bottle homogeneity of the CRM was estimated from standard deviation of the measurement results.

Potential degradation or transformation into other species was tested for the optimized extraction method using calibration blends which underwent the same extraction procedures and it was found to be negligible.

Certified Value of AsB in Oyster CRM and its Uncertainty. The AsB content in oyster CRM was certified by analyzing subsamples taken from each 12 bottles systematically selected from the whole batch to take the effect of between-bottle homogeneity into account. The ultrasonic extraction using 9:1 mixture of methanol and water was used for sample preparation and the HILIC-ESI/MS/MS method with SRM mode was used for the IDMS analysis of AsB. Estimating from the standard deviation of the AsB measurement results of 12 bottles, the between-bottle homogeneity of AsB content in the oyster CRM is thought to be less than 0.42% (Figure 7). The certified value of AsB content in oyster CRM was 6.59 $mg{\cdot}kg^{-1} \pm 0.30$ $mg{\cdot}kg^{-1}$ ($k = 1.96$, 95% confidence level). The uncertainty of the certified value was calculated in accordance with ISO GUM (International Organization for Standardization Guide to the Expression of Uncertainty in Measurement) guidelines.[47] Major sources of uncertainty were certified value of the AsB standard solution used for calibration and the isotope ratio measurements of AsB and $^{13}C_2$-labelled AsB in sample blends. Considering the certification value for total arsenic, 10.39 $mg{\cdot}kg^{-1} \pm 0.34$ $mg{\cdot}kg^{-1}$ ($k = 2.23$, 95% confidence level), which was determined separately by INAA, AsB constitutes 26.7% of total arsenic in the oyster CRM. Although the SRM channels of *m/z* 179 → *m/z* 105 and *m/z* 181 → *m/z* 105 were used to determine to certified value as shown in Figure 7(a), results from the other two pairs of SRM channels were also equivalent and further confirmed the certified value. One of those examples is shown in Figure 7(b).

Conclusion

An ID HILIC-MS/MS method has been successfully developed and applied to the accurate quantification of AsB from oyster candidate certified reference material (CRM). For robust analysis of AsB from complex sample matrix, the exact matching isotope dilution approach was applied using $^{13}C_2$-labeled AsB as an internal standard. Individual analytical steps, including sample extraction, chromatographic separation, and MS/MS setup, were optimized and validated using a codfish CRM of NMIJ. Using the optimized analytical method, AsB content in the candidate oyster CRM was determined to be 6.60 $mg{\cdot}kg^{-1} \pm 0.31$ $mg{\cdot}kg^{-1}$ and it showed excellent between-bottle homogeneity of less than 0.42%. To the best of authors knowledge, this is the first report on the application of ID approach in AsB analysis using HILIC-ESI/MS/MS. The developed oyster CRM of KRISS is expected to be used for method validation and quality control of analytical procedures for arsenic speciation analysis.

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