JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLES

Quantitation of Toxic Arsenic Species and Arsenobetaine in Pacific Oysters Using an Off-line Process with Hydride Generation−Atomic Absorption Spectroscopy

TUNG-MING HSIUNG* AND CHIA-WEI HUANG

Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, Republic of China

An off-line process-based speciation technique was devised here to quantitatively determine toxic inorganic arsenic (iAs), methylarsonic acid (MA), dimethylarsinic acid (DMA), and the dominant, albeit virtually nontoxic, arsenobetaine (AB) in Pacific oysters (*Crassostrea gigas*). Oysters were extracted with fresh methanol−water (8+2), and this was replicated three times. They were then evaporated to near dryness and subsequently redissolved in pure water; defatting was then performed with a C18 cartridge. The trace hydride active arsenic species, that is, iAs, MA, and DMA, in the defatted solutions were determined with a sensitive hydride generation-packed coldfinger trap−atomic absorption spectrometric (HG-PCFT-AAS) coupled system. The arsenicals that were desorbed from the cation-exchange resin (Dowex 50W-X8) in the washings of 4 M NH_3 were categorized on the basis of AB + DMA. The total quantity of arsenic in the recovered AB + DMA was determined with a commercial hydride generation−atomic absorption spectrometric (HG-AAS) system, and finally, AB was calculated from (AB + DMA) − DMA. The average concentrations of iAs, MA, DMA, AB, and total arsenic (TAs) in the oysters collected from six aquacultural sites along the west coast of Taiwan were, respectively, 0.15, 0.06, 0.64, 6.93, and 13.74 mg kg^{-1} of dry weight. AB was the major species, whereas iAs (arsenite + arsenate) were the most toxic species, although the iAs made up only ∼1% of the TAs in the oysters. The lifetime target cancer risk, as determined by the concentration of iAs on a fresh weight basis in the oysters, was well below the ordinary health protection criteria (10^{-6}).

KEYWORDS: Oyster; inorganic arsenic; process-based; target cancer risk; Taiwan

1. INTRODUCTION

The biochemistry and biological fate of arsenic in marine organisms are both of considerable interest and concern because of the wide range of arsenic compounds and their various levels of toxicity. To be sure, all related issues take on great significance, especially when a risk assessment of their toxicological effects is performed (*1*−*5*). Many different patterns of arsenic compounds have been identified in different marine organisms. In marine algae, arsenosugars (AS) are the most abundant, whereas aesenobetaine (AB) is absent. In contrast, in fish and crustaceans, although AB is the major form of arsenical (*6*−*8*), its precise biotransformation path in the food chain is still unclear (*9*).

The charges of most water-soluble arsenic species can be neutral, anionic (the hydrolyzed species), or cationic (the protonated species), depending on pH. However, arsenocholine (AC), which may serve as a precursor to AB in organisms (*10*−*12*), carries a positive charge irrespective of pH (*13*). In earlier studies, when a Dowex 50 cation-exchange column was employed, arsenite [As(III)], arsenate [As(V)], and methylarsonic acid (MA) were not retained; dimethylarsinic acid (DMA) and AB were eluted in a 4 M NH_4OH fraction; and AC was eluted in a 4 M hydrochloric fraction (*7*, *14*−*16*). Ybanez et al. (*7*) reported that the recovery of AB from a defatted sample was definitively higher than that obtained from a sample which had not been defatted. Vilano and Rubio (*17*) demonstrated that pretreating oyster extract using a C18 cartridge resulted in a good recovery of AB. The C18 cleanup process to remove particulate matter and matrix components has since been widely adopted, and because of it, there is no fear of fouling the later cation-exchange characteristic of AB (*18*, *19*).

* Address correspondence to this at the Institute of Bioscience and Biotechnology, National Taiwan Ocean University, No. 2, Pei-Ning Rd., 202 Keelung, Taiwan, Republic of China (e-mail tmhsiung@mail.ntou.edu.tw; telephone 886-2-24622192, ext. 5515; fax 886-2-24634243).

10.1021/jf051181x CCC: $33.50
Published on Web 03/04/2006

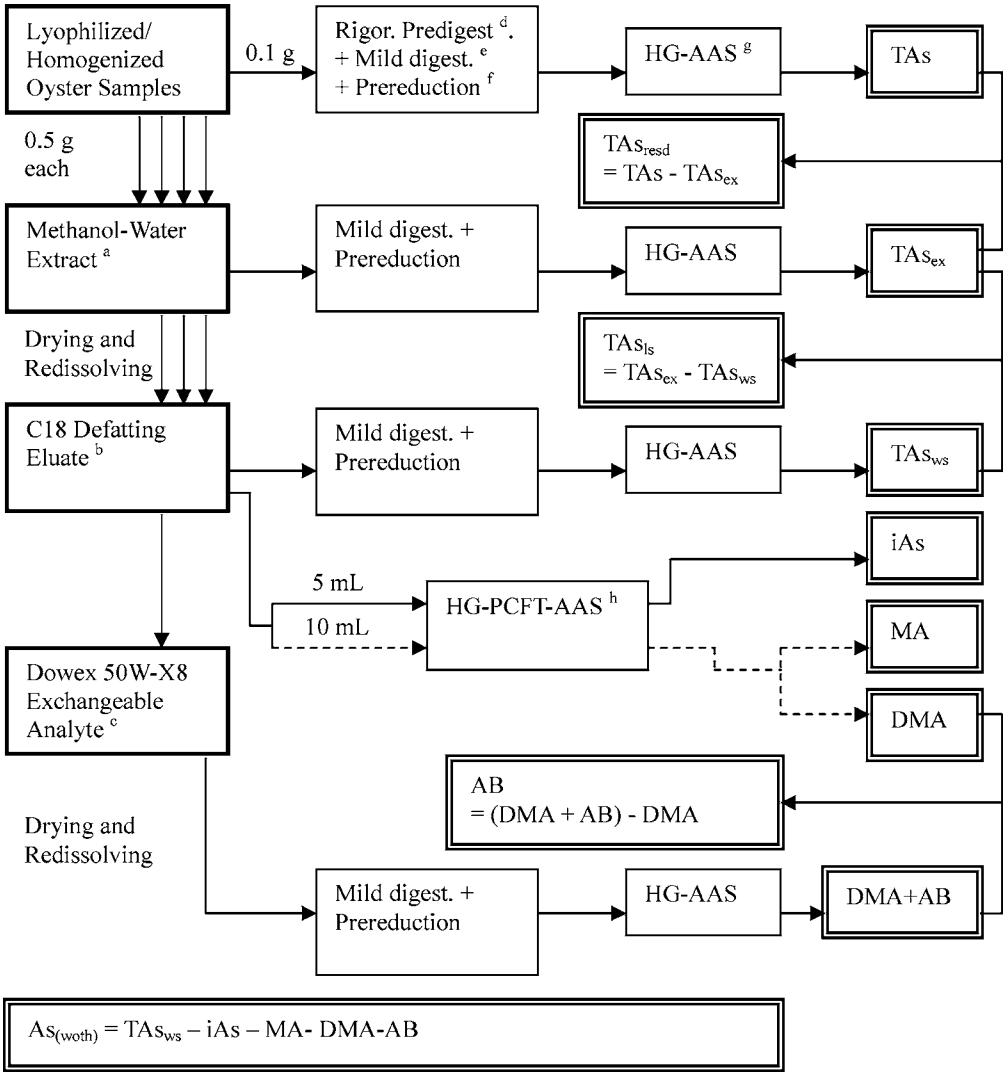

Figure 1. Flowchart outlining the principal steps in the process-based arsenic speciation analysis, as devised here. Abbreviations: TAs, total arsenic; TAs_{ex}, total extractable arsenic; TAs_{resd}, total residual arsenic; TAs_{ls}, total lipid soluble arsenic; TAs_{ws}, total water soluble arsenic; iAs, inorganic arsenic, arsenate + arsenite; MA, monomethylarsonic acid; DMA, dimethylarsinic acid; AB, arsenobetaine; As_{woth}, other water soluble arsenic. Established parameters: [a] extractant (MeOH–H_2O (8+2)); extraction temperature, 55 °C; three sequential extractions with 15 mL of extractant each time; combined and purged in nitrogen to dry; [b] redissolved in 5 mL of reagent water and then passed through a C18 cartridge; additional reagent water until 15 mL of eluate is collected; [c] 15 mL of defatted extract passed through a Dowex 50W-X8; addition of 30 mL of reagent water; recovered with 40 mL of 4 M NH_4OH; [d] as described in section 2.5.1; [e,f] as described in section 2.5.2; [g] as described in section 2.5.3; [h] as described in section 2.4.2.

What this study contributes to this line of research is that it devised a low-cost method which complies with the process-based definition (**Figure 1**) of arsenic speciation. The arsenic species of interest here are the most toxic arsenicals (iAs), the intermediate toxic arsenicals (MA and DMA), and the dominant, yet essentially nontoxic, arsenic species AB. For the validation of the method, first, it was necessary to determine the off-line sample preparation parameters, namely, the identity of the optimal extracting solvent, the preferred number of sequential extractions, and the optimal extraction temperature. After the samples had been prepared, total arsenic (TAs), total extractable arsenic (TAs_{ex}), total water-soluble arsenic (TAs_{ws}), and DMA + AB were analyzed using the hydride generation−atomic absorption spectrometric technique (HG-AAS). The hydride generation−packed coldfinger trap−atomic absorption spectrometric technique (HG-PCFT-AAS), first developed by Hsiung and Wang (*20*), was then used to detect the trace arsenic species (iAs, MA, and DMA). The arsenic species in the oyster samples (*Crassostrea gigas*) collected from aquacultural sites in western Taiwan were investigated, and a health risk assessment based on the average concentrations of the inorganic arsenic species, rather than the total amount of arsenic, was also conducted.

2. EXPERIMENTAL PROCEDURES

2.1. Experimental Materials. *2.1.1. Standards.* The calibration standards for TAs and inorganic arsenic (iAs) were prepared from a 1000 mg of As L^{-1} primary standard (in arsenite form, J. T. Baker). As(V), MA, DMA, and AB were respectively prepared from sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, J. T. Baker), sodium monomethylarsonate sesquihydrate [$(CH_3AsO(OH)(ONa) \cdot 1.5H_2O$, Chem Service PS-429], dimethylarsinic acid [$(CH_3)_2AsO(OH)$, Sigma C0125], and arsenobetaine [$(CH_3)_3As^+CH_2COO^-$, Fluka 11093]. Before quantitative analysis, the arsenic concentrations of these stock standards were calibrated in accordance with the primary arsenite standard (J. T. Baker) using inductively coupled plasma−mass spectrometry (ICP-MS).

2.1.2. C18 Cartridge and Dowex Cation-Exchange Resin. To defat the sample, an SPE C18 cartridge (particle size = 40 μm, sorbent mass = 1 g, volume = 6 mL, Mega Bond Elut, Varian) was first conditioned by passing 10 mL of methanol and then 10 mL of water through it at the flow rate of 1 mL min^{-1}. For the cation-exchange resin treatment,

5 g of Dowex AG 50W-X8 (100−200 mesh, H-form, Bio-Rad) was activated following the method of Persson and Iregum (*21*), and it was then packed in a 50 mm × 10 mm i.d. Econo-column (Bio-Rad). A fraction collector (model 2110, Bio-Rad) was used to collect the eluate for the defatting and ion-exchange processes.

2.1.3. Reagents. The reagent water used was distilled and deionized to a resistance of 18 MΩ cm. A methanol−water solution, which was used as extractant, was made by completely mixing methanol (LC grade, Fisons, Loughborough, U.K.) and reagent water. Other chemicals used in this study were all of reagent grade purchased from Riedel-deHaën. The prereduction reagent, 20% (w/v) KI for the analysis of total arsenic (TAs), was prepared with potassium iodide dissolved in reagent water. For continuous flow-hydride generation (CF-HG), the on-line acidified solution was 5% (v/v) hydrochloric acid. A 0.75% (w/v) sodium borohydride ($NaBH_4$) solution was prepared on a daily basis in 0.4% (w/v) sodium hydroxide.

2.2. Instrumentation. The resulting arsines of the hydride active arsenic species were detected using a Hitachi Z-8200 atomic absorption spectrophotometer (AAS) with a flame-heated quartz-tube atomizer (QTA) set at selected parameters: wavelength, 193.7 nm; band-pass, 1.3 nm; lamp current, 10.0 mA; air flow, 13.6 L min^{-1}; acetylene flow, 1.4 L min^{-1}; and time constant, 2.0 s.

2.3. Sample Preparation. A retail dried oyster sample was purchased from Ilan (on the east coast of Taiwan) and was used as the test sample to establish the optimized analytical parameters. Two certified reference materials, namely DORM-2/NRCC (dogfish muscle) and 1566b/NIST (oyster tissue), were used to validate the analytical procedures.

Fresh oyster (*C. gigas*) samples were collected at low tide in the months of March, May, August, and November of 2002 from six aquacultural sites located along the west coast of Taiwan: Hsianshan, Wanggung, Taishi, Budai, Tainan, and Dunggung (*22*). All were kept at 4 °C until they arrived at the laboratory, where they were immediately frozen at −20 °C. A composite sample of 20 oysters was collected from each individual sampling site. These laboratory samples were prepared by freeze-drying gross samples with a lyophilizer (Savant, Speedvac Plus SC110A) and by homogenizing them with agate mortars and pestles.

2.4. Measuring Concept for iAs, MA, DMA, and AB. *2.4.1. Extraction and Defatting.* The lyophilized oyster samples (0.5 g each) were weighed in separate 50-mL PP centrifuge tubes, each with a screw top and a conical base. Fifteen milliliters of methanol−water (optimal combination 8+2, as explained under section 3.2) solution was added to each, and the tubes were agitated at 100 rpm in a mechanical arm shaker water bath for 10 min.

The supernatant of the methanol−water extract was collected after centrifugation at 4000 rpm for 10 min, and it was evaporated to complete dryness by being purged in pure nitrogen for ∼12 h. The dry extract was then dissolved in 5 mL of water, and the total arsenic in the samples, measured using the HG-AAS, was defined as the total extractable arsenic (TAs_{ex}).

The defatted samples for the determination of total water-soluble arsenic (TAs_{ws}) were prepared by dissolving a duplicate of the dry extract in 5 mL of water, which was then passed through a previously conditioned C18 cartridge at the flow rate of 1 mL min^{-1}. Additional reagent water was added at the same flow rate until the total volume of the eluate was 15 mL. The total arsenic in this solution was also measured using the HG-AAS.

2.4.2. Measurement of iAs, MA, DMA. A HG-PCFT-AAS coupled system was previously employed to determine the trace arsenic species, namely iAs, MA, and DMA, in the water samples (*20*). Of particular importance here, the extended application of the HG-PCFT-AAS coupled system vis-à-vis its capability to measure hydride active arsenicals in the oyster samples was established in this paper. Although the HG-PCFT-AAS designation here is similar to the cryogenic trapping coupled system of the U.S. EPA method 1632 (*23*), it is considerably simpler. It is based on the premise that hydride-generated arsines are cryofocused with PCFT prior to fractional vaporization and that the detection of arsenicals can then be determined from the increase in their respective boiling point. In the present study, the best sensitivity for analyzing iAs [As(III) + As(V)], for example, is obtained from prereducing all of the inorganic arsenic into the As(III) form. This was prepared by adding 2 mL of concentrated HCl and 2 mL of 20% KI solution to each 5 mL of the aliquots of the defatted sample, and then this was digested in an 80 °C water bath for 1 h.

Worth bearing in mind is that the hydride generation efficiencies are very sensitive to acidity, which could perhaps be related to the pK_a values of their respective arsenicals (*20*, *24*, *25*). The corresponding AAS absorption signals of the arsenite-derived arsines increase with reaction acidity; however, the signals of the MA and DMA-derived arsines decrease when reaction acidity surpasses the critical value. Therefore, to analyze MA and DMA, in this study, another 10 mL portion of the defatted eluate without additional pretreatment was used.

A PCFT is a coldfinger with a round-bottom base packed with 10% OV-101 on Chromosorb (W-HP 60/80 mesh, Ohio Valley). In this study, the exterior of the PCFT was wrapped with 0.5 m of fiberglass heating cord (∼20 Ω), and the heating voltage was set at 5 V to raise the temperature to make the arsines evolve. The reagents for hydride generation have been described under section 2.1.3, and the parameters of AAS have been described under section 2.2. Details about operating the entire HG-PCFT-AAS have previously been reported by Hsiung and Wang (*20*).

2.4.3. Measurement of AB. A 15 mL replicate of defatted eluate was prepared following the same procedure as that described under section 2.4.1. This defatted eluate was adjusted to pH 2 and was then passed through an activated Dowex 50W-X8 column at the flow rate of 1 mL min^{-1}. Additional washing water (the optimal volume was 30 mL, as described under section 3.2) was passed through the column at the same flow rate, and the waste eluate was disposed of. The Dowex exchangeable arsenic species was recovered by eluting the Dowex resin with 4 M NH_4OH (the optimal volume was 40 mL, as established under section 3.2). The eluate containing the Dowex exchangeable arsenic species was collected and vaporized to near dryness on a conventional hot plate, and this was then redissolved in 25 mL of water. This solution was digested, as described under section 2.5.2, and this resulted in a combined value for AB plus DMA (AB + DMA). Finally, AB was calculated from AB = (AB + DMA) − DMA, where DMA was that which had already been determined, as described under section 2.4.2.

2.5. Measuring Concept for TAs, As_{ex}, As_{ws}, and AB + DMA. *2.5.1. Rigorous Predigestion for TAs Analysis.* A rigorous digestion pretreatment was applied for the analysis of total arsenic (*22*). The dried and homogenized oyster samples, 0.1 g each, were placed into separate 125 mL conical flasks into which 20 mL of concentrated nitric acid had been added. The flasks were placed on the hot plate until the solutions evaporated to near dryness, and then they were redissolved to 50 mL with reagent water.

2.5.2. Mild Digestion and Prereduction for TAs, TAs_{ex}, TAs_{ws}, and AB + DMA. The arsenicals from the oyster samples prepared after rigorous digestion, after methanol−water extraction, after C18 cartridge defatting, and after desorption from the Dowex exchange resin are defined as TAs, TAs_{ex}, TAs_{ws}, and AB + DMA, respectively. Four milliliters of 2.5 N sulfuric acid and 5 mL of 5% (w/v) potassium persulfate were added to the prepared solution, and this solution was then digested on the hot plate. When the solution was nearly dry, 8 mL of concentrated HCl and 10 mL of 20% (w/v) KI were added. Reagent water was then used to increase the final volume to 50 mL. The solution was then warmed in a 60 °C water bath for 1 h to ensure that the arsenicals were completely reduced to arsenite form.

2.5.3. Measuring TAs, As_{ex}, As_{ws}, and AB + DMA. A continuous HG-AAS system was used to measure the arsenic in the prereduced sample. The continuous flow hydride generator (model HFS-3, Hitachi) was equipped with a single peristaltic pump with a flow rate of 8.5 mL min^{-1}, and it delivered and mixed the sample solution with on-line 5% HCl and 0.75% $NaBH_4$. Arsine was separated from the liquid and carried to the AAS with auxiliary argon gas at the flow rate of 300 mL min^{-1}. The instrumental parameters of the AAS have been described under section 2.2.

3. RESULTS AND DISCUSSION

3.1. Extraction Parameters. Methanol exhibits both hydrophobic and hydrophilic characteristics in that it contains a methyl group and a hydroxyl group; it is easily removed through

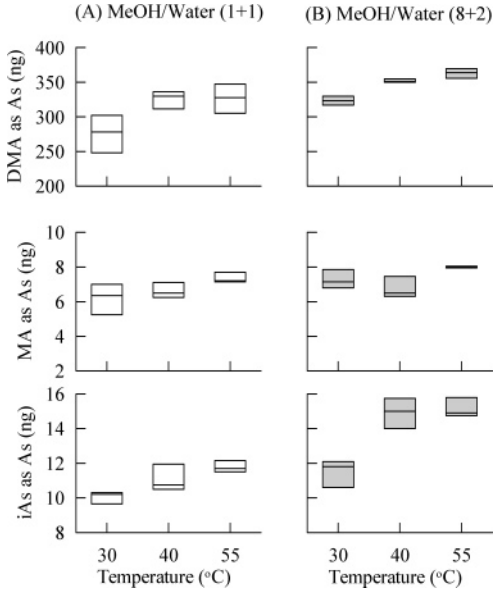

Figure 2. Arsenic species extracted from 0.5 g of the retail dry oyster using different combinations of methanol–water extractants and extraction temperatures ($n = 3$ for each test).

evaporation and is reportedly a poor solvent for extracting inorganic arsenic (*26*). Because almost all of the naturally occurring arsenic species identified so far are polar and hydrophilic, most researchers have used methanol−water for the extraction of arsenic species from a sample organism. Francesconi and Kuehnelt (*27*) claimed that varying the methanol−water ratio and the extraction temperature affects the efficiency of the extractability of arsenic from samples and that the optimal extraction parameters are dependent upon the type of sample composite.

The optimal extraction parameters in this study were evaluated by analyzing the retail dry oyster sample purchased from Ilan. The assigned values for the MeOH−H_2O extractant composition were 1+1 and 8+2, the same values as those that have customarily been used in the recent literature (*17*, *28*, *29*). Ackley et al. (*28*) had previously found that no other degradation or conversion was as high as that at the extraction temperature of 65 °C for the very same arsenic species of interest here. Thus, in this study, the maximum extraction temperature was set at 55 °C, 10 °C below the optimal extraction temperature of Ackley et al. (*28*), to ensure there was no degradation of these arsenic species. Here, three temperatures were used to determine the optimal extraction temperature: 30, 40, and 55 °C.

A 0.5 g sample was extracted with 15 mL of extractant by agitating it in a water bath for 10 min. After the sample was defatted with a C18 cartridge, the trace arsenic species iAs, MA, and DMA were determined using the HG-PCFT-AAS technique. The results from triplicate tests for each set are shown in **Figure 2**; notably, we found that MeOH−H_2O (8+2) coupled with the extraction temperature of 55 °C yielded the best extractability and the best reproducibility for all of the arsenic species. A duplicate of the previously described extract was centrifuged at the rate of 4000 rpm, and the residue was repeatedly extracted with another 15 mL of fresh extractant. The collected individual supernatants were measured, and the results are shown in **Figure 3**. The arsenic species we focused on in this study (iAs, MA, and DMA) were completely extracted after we sequentially triplicated the extraction process.

In summary, when the established extraction procedures outlined here were followed, 0.5 g of the oyster sample was extracted with 15 mL of MeOH−H_2O (8+2) at 55 °C; the number of replications was three. Three portions of the supernatants from each sample were combined and purged in nitrogen to dry, and then C18 defatting for the redissolved dry extract in reagent water was conducted.

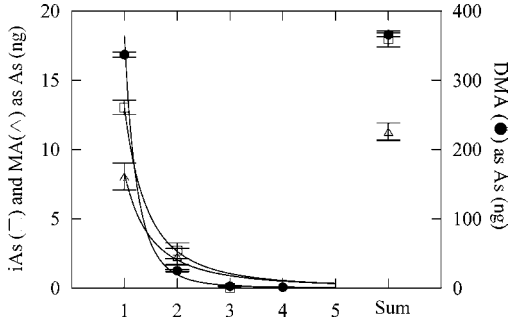

Figure 3. Hydride active arsenic species remaining after different numbers of extractions.

Table 1. Amount of Arsenic in the Individual Eluates during the Dowex 50 Exchange Process[a]

	spiked species				
	As(III)	As(V)	MA	DMA	AB
initial 20 mL sample (waste)	71.9	53.9	45.9	NA	NA
washing (H_2O)					
first 10 mL	30.3	36.8	47.1	NA	NA
second 10 mL	1.3	5.3	ND	NA	NA
third 10 mL	ND	ND	ND	NA	NA
fourth 10 mL	ND	ND	ND	NA	NA
fifth 10 mL	ND	ND	ND	NA	NA
recovering eluant (4 N NH_3)					
first 10 mL	NA	NA	NA	45.2	36.5
second 10 mL	NA	NA	NA	47.3	59.1
third 10 mL	NA	NA	NA	ND	5.9
fourth 10 mL	NA	NA	NA	ND	ND
fifth 10 mL	NA	NA	NA	ND	ND

[a] 100 ng as As was spiked in each test sample; NA, not available; ND, not detected; values expressed as ng.

3.2. Parameters To Recover the Dowex-Exchangeable Arsenic Species. Five 20 mL test sample solutions, each tube containing 100 ng of As in the respective form of As(III), As(V), MA, DMA, and AB in reagent water, were prepared from their respective stock standards, and they were then individually placed onto activated Dowex resin. A fraction collector at the flow rate of 1 mL min^{-1} was used to collect eluate. The first tube had 20 mL of eluate, and then each subsequent 10 mL of eluate was collected in the same way. H_2O was added as the washing solvent. The amount of arsenic collected in each tube was then measured using the HG-AAS system after acid digestion and prereduction, as described under section 2.5.2. As shown in **Table 1**, As(III), As(V), and MA were completely eluted after 30 mL of washings had been added, and DMA and AB were retained on the Dowex resin. Thereafter, 4 N NH_3 was added to the testing columns to recover DMA and AB, and 10 mL of eluate of each was collected in each. Each collection was also measured using the HG-AAS after acid digestion and prereduction. From **Table 1**, it is evident that DMA and AB were completely eluted with the addition of 20 and 30 mL of 4 N NH_3, respectively.

Fifteen milliliters of a simulated solution containing As(III), MA, DMA, and AB in 100 ng as As for each species was prepared by being passed though a C18 cartridge. To confirm the optimal parameters of the Dowex 50W-X8 exchange of ions, the C18 pretreated eluate was placed in the activated Dowex resin, and the volume of washing water was 30 mL; the volume

Table 2. Confirmation of the Design of the Parameters for the Dowex 50 Resin Treatment[a]

	species			
	iAs	MA	DMA	AB
spiked amounts (ng as As)	100	101	99	92
recovered amounts (ng as As)				
initial waste (20 mL)	87	66	ND	ND
H_2O (30 mL)	19	28	ND	ND
4 N NH_3 (40 mL)	ND	ND	90	95
total recovery (%)	106	93	91	103

[a] Spiked amounts were verified using ICP-MS calibration with a primary arsenite standard.

Table 3. Limits of Detection and Spike Recoveries

	LOD		
species[a]	ng or μg L^{-1}	mg kg^{-1}	spike recovery[e] (%)
iAs	0.98[a]	0.0020[c]	93.2
MA	0.75[a]	0.0015[c]	85.5
DMA	0.86[a]	0.0017[c]	92.3
AB	0.24[b]	0.024[d]	102.1

[a] 3 × SD of seven replicate analyses using the HG-PCFT-AAS of 2 ng (expressed as As) spiked reagent water, and iAs was spiked with As(III); expressed in ng. [b] 3 × SD of seven replicate analyses using the HG-AAS of 40 ng (expressed as As) spiked reagent water; expressed in μg L^{-1}. [c] Sample weight is assumed to be 0.5 g. [d] Sample weight is assumed to be 0.5 g, and the final prepared solution for HG-AAS detection is 50 mL. [e] Spiked amounts were 2 μg for AB, 0.2 μg for DMA, and 0.02 μg for MA and As(III) in 0.5 g of retail dry oyster (purchased from Ilan); results expressed as the average of four replicated analyses.

of 4 N NH_3 recovering eluate was 40 mL. For the collection of 4 N NH_3 eluate, pH was adjusted to neutral with 1 M HCl, whereas for the collection of the initial waste and washing, there was no treatment. Thereafter, iAs, MA, and DMA were measured in accordance with the HG-PCFT-AAS technique. Another portion of the eluate from the 4 M NH_4OH collection, which contained DMA and AB, was vaporized to near dryness on the hot plate and was then redissolved in 25 mL of water. This collection was then digested and prereduced with KI, and total arsenic was measured using the HG-AAS technique. This yielded the value of AB + DMA, and then from (AB + DMA) − DMA, AB was calculated.

Spiked amounts of these arsenic species were calibrated with the ICP-MS using an arsenic primary standard (in arsenite form, J. T. Baker). The results are given in **Table 2**. The total recoveries of iAs, MA, and DMA, which included the waste and washing, were 106, 93, and 91%, respectively. In the recovered eluate that resulted from passing 4 N NH_3 through Dowex resin, the net recovery of AB calculated from (AB+DMA) − DMA was 103%.

3.3. Method Performance. A set of fortified samples was prepared, with each sample being spiked with 2 ng as As and either As(III), MA, or DMA in 15 mL of reagent water. All of the procedures for the analysis of the three arsenic species starting in C18 defatting were followed, as shown in **Figure 1**; the optimized parameters are listed in the notes of the figure. The limits of detection (LODs) were calculated using 3 times the standard deviations of the analytical results ($n = 7$). As shown in **Table 3**, the absolute LODs were 0.98, 0.75, and 0.86 ng. These values are the equivalent of the concentration LODs of 0.0020, 0.0015, and 0.0017 mg kg^{-1} for iAs, MA, and DMA, respectively, which were obtained by substituting for the sample weight of 0.5 g for conversion.

Another set of samples was prepared by spiking 40 ng of AB in 15 mL of reagent water; then, all of the pretreatment procedures starting with C18 defatting for AB + DMA were followed. Because those samples contained only AB, the LOD of AB was directly calculated by 3 × SD, based on the HG-AAS analytical results, without subtracting the DMA value. This is equivalent to 0.024 mg kg^{-1}, which was converted from a 0.5 g sample and a final volume of 50 mL of the solution prepared for the HG-AAS measurements.

Also shown in **Table 3** are the results from the recovery experiments, which were conducted by spiking 0.5 g of retail dry oyster with 0.02 μg in the form of As(III), 0.02 μg of MA, 0.2 μg of DMA, and 2 μg of AB. After all of the established analytical procedures had been completed, the recoveries of iAs, MA, DMA, and AB were found to be 93, 86, 92, and 102%, respectively.

The repeatability of the analytical procedures was obtained by taking replicate measurements of the retail dried oyster sample ($n = 4$), which yielded relative standard deviations of 4.0, 11, 8.9, 6.9, and 9.1% for TAs, iAs, MA, DMA, and AB, respectively (**Table 4**).

For reference, several earlier data sets (*2*, *8*, *30*−*32*) of the measurements of various arsenic species in certified marine reference materials are also summarized in **Table 4**. The extraction efficiency (EE) of methanol−water (8+2) was calculated from (TAs_{ex}/TAs) × 100%. The resulting EEs in this study were 93.5 ± 1.3, 74.9 ± 2.3, and 83.0 ± 4.0% for DORM-2/NRCC, 1566b/NIST, and the retail dry oyster sample, respectively. As for the certified values, the TAs in DORM-2 and 1566b had recoveries of 102.4 and 104.7%, respectively, and the AB value of DORM-2 had a recovery of 97.6%. For DORM-2, our reported data for iAs and DMA were consistent with the findings of Kohlmeyer et al. (*8*); however, the average MA in this study was twice as high as that reported by Wahlen et al. (*32*). Total lipid soluble arsenic (TAs_{ls}) was calculated from $TAs_{ex} - TAs_{ws}$, but it had poor repeatability, which can be attributed to its low concentration in the samples.

The volatile AsH_3, CH_3AsH_2, $(CH_3)_2AsH$, and $(CH_3)_3As$ corresponded to the hydride active arsenicals iAs, MA, DMA, and trimethylarsine oxide (TMAO), which were reduced to arsines with $NaBH_4$ in an acidic solution. Schmeisser et al. (*33*) reported that arsenosugars produce volatile analytes, which can be measured from hydride generation in an HPLC coupled system. They also reported that it is unlikely that DMA degrades from arsenosugars and that the identity of the exact volatile analytes is still unclear.

The hydride generation−cold trap (HG-CT) coupled technique has reported several known and unknown volatile arsenic species at retention times longer than that of $(CH_3)_2AsH$ (*34*, *35*). We also experienced another peak at a retention time longer than that of $(CH_3)_2AsH$. However, in our routine procedures, once $(CH_3)_2AsH$ reached its peak, the temperature was elevated to clean up PCFT because our prime interest was iAs, MA, and DMA. It should follow, therefore, that if (1) the arsenosugars were not degraded to iAs, MA, or DMA in our prepared sample solution and (2) the arsenosugar-derived hydrides were not AsH_3, CH_3AsH_2, or $(CH_3)_2AsH$, then there should not have been any effect on our quantitative results for iAs, MA, or DMA.

It has been reported that the DMA and AB that were eluted with a 4 M NH_4OH fraction using the Dowex 50 cation-exchange technique in other studies could have been separated by a pH-independent, positively charged AC and tetramethylarsonium ion (TETRA) and the neutral form AS (*7*, *13*−*15*). It is suspected that trimethylarsoniopropionate (TMAP) was

Table 4. Performance of the Method Developed Compared with Relevant Referral Data[a]

sample	TAs	TAs_{ex}	EE (%)	TAs_{ws}	TAs_{ls}	iAs	MA	DMA	AB	As_{woth}	ref
NRCC DORM-2	18.00 (6)	NA	NA	NA	NA	NA	NA	NA	16.4 (7)	NA	certified value
dogfish	17.9	NA	NA	NA	NA	0.145	NA	NA	NA	NA	Munoz et al. (*30*)
muscle	NA	NA	NA	NA	NA	0.5	ND	0.3	13.5	NA	Londesborough et al. (*31*)
	17.4	17.4	100	NA	NA	0.10[b]	0.14	0.49	16.1	NA	Kohlmeyer et al. (*8*)
	19.63	NA	NA	NA	NA	0.006[c]	0.015	0.23	17.64	NA	Wahlen et al. (*32*)
	18.44 (2.3)	17.25 (3.1)	93.5 (1.3)	16.95 (3.0)	0.30 (22)	0.088 (7.1)	0.033 (9.1)	0.413 (4.0)	16.01 (2.8)	0.02 (35)	this study, $n = 4$
NIST SRM 1566a	14.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	certified value
oyster	14.60	NA	NA	NA	NA	0.55	0.66	0.79	2.63	NA	Chatterjee (*2*)
NIST SRM 1566b	7.65 (8)	NA	NA	NA	NA	NA	NA	NA	NA	NA	certified value
oyster	8.01 (1.1)	6.00 (3.3)	74.9 (2.3)	5.92 (3.7)	0.07 (31)	0.092 (9.7)	0.034 (8.4)	0.763 (4.1)	2.79 (9.9)	2.17 (7.4)	this study, $n = 4$
retail dry oyster	9.79 (4.0)	8.13 (3.9)	83.0 (4.0)	7.91 (3.7)	0.22 (32)	0.030 (11)	0.015 (8.9)	0.645 (6.9)	2.15 (9.1)	4.86 (6.4)	this study, $n = 4$

[a] Concentration unit is mg kg^{-1} of dry wt as As; EE, extraction efficiency; NA, not available; ND, not detected; relative standard deviation is in parentheses. Abbreviations of the arsenicals described are the same as those in **Figure 1**. [b] As(III) + As(V). [c] As(V) only.

Table 5. Arsenic Species in the Pacific Oysters (Milligrams per Kilogram of Dry Weight) Collected from the Six Aquacultural Sites in Western Taiwan[a]

sampling		moisture[b]		TAs			TAs_{ex}		TAs_{ws}				
site	month	(% w/w)	TAs	TAs_{ex}	TAs_{resd}	EE	TAs_{ws}	TAs_{ls}	iAs[c]	MA	DMA	AB	$As_{(woth)}$
Hsianshan	March	94.8	14.65	13.00	1.65	88.7	12.39	0.61	0.12 (0.006)	0.12	0.53	7.85	3.76
	May	88.6	16.73	14.25	2.48	85.2	13.63	0.62	0.29 (0.033)	0.18	0.65	8.29	4.21
	Aug	84.0	14.98	13.38	1.60	89.3	12.65	0.73	0.16 (0.026)	0.06	0.71	8.92	2.81
	Nov	89.8	14.36	11.00	3.36	76.6	10.92	0.08	0.15 (0.015)	0.09	0.59	8.29	1.80
Wanggung	March	96.9	14.36	12.44	1.92	86.6	10.85	1.59	0.15 (0.005)	0.05	0.62	7.26	2.77
	May	85.9	13.79	12.44	1.35	90.2	11.11	1.33	0.09 (0.013)	0.10	0.65	6.48	3.79
	Aug	84.3	11.94	7.50	4.44	62.8	6.78	0.72	0.04 (0.006)	0.06	0.85	3.96	1.87
	Nov	86.9	12.76	10.63	2.13	83.3	10.36	0.27	0.15 (0.020)	0.06	0.71	7.48	1.96
Taishi	March	89.5	15.81	12.88	2.93	81.5	11.11	1.77	0.09 (0.009)	0.02	0.79	6.46	3.75
	May	86.4	12.54	11.88	0.66	94.7	11.61	0.27	0.11 (0.015)	0.10	1.29	4.52	5.59
	Aug	87.2	11.88	6.88	5.00	57.9	6.73	0.15	0.21 (0.027)	0.03	0.44	4.25	1.80
	Nov	87.3	14.63	11.88	2.75	81.2	11.78	0.10	0.27 (0.034)	0.08	1.00	7.75	2.69
Budai	March	95.6	13.19	11.31	1.88	85.7	9.80	1.51	0.16 (0.007)	0.02	0.77	6.73	2.12
	May	88.2	15.24	12.81	2.43	84.1	11.75	1.06	0.13 (0.015)	0.02	0.55	7.83	3.22
	Aug	87.3	10.85	10.25	0.60	94.5	8.95	1.30	0.07 (0.009)	0.02	0.19	5.25	3.42
	Nov	90.1	11.24	10.50	0.74	93.4	9.40	1.10	0.08 (0.008)	0.06	0.42	7.39	1.45
Tainan	May	81.2	19.24	13.56	5.68	70.5	12.95	0.61	0.13 (0.024)	0.06	0.66	8.41	3.69
	Nov	82.3	14.19	12.38	1.81	87.2	11.81	0.57	0.11 (0.019)	0.01	0.35	7.59	3.74
Dunggang	March	95.8	14.39	11.94	2.45	83.0	11.70	0.24	0.16 (0.007)	0.06	0.58	9.17	1.73
	May	88.8	13.50	10.75	2.75	79.6	9.46	1.29	0.12 (0.013)	0.02	0.23	6.71	2.38
	Aug	85.8	8.27	6.75	1.52	81.6	6.50	0.25	0.25 (0.036)	0.03	0.64	4.05	1.53
	Nov	91.1	13.65	10.81	2.84	79.2	9.3	1.51	0.25 (0.022)	0.01	0.81	7.82	0.40
av		88.5	13.74	11.33	2.41	82.6	10.52	0.80	0.15 (0.017)	0.06	0.64	6.93	2.75

[a] Composite sample of each individual sampling site consisted of 20 oysters. [b] Courtesy of Professor S. M. Liu, Institute of Marine Biology, National Taiwan Ocean University, Taiwan. [c] In parentheses: mg kg^{-1} of fresh weight base.

coeluted in the 4 M NH_4OH fraction because of its structural analogue to AB. There was only a negligible amount of TMAP relative to AB in the oyster (*36*), however. Therefore, if TMAP had affected the measurements of AB, the quantitative error of AB caused by TMAP still could be negligible.

3.4. TAs, TAs_{resd}, TAs_{ex}, TAs_{ws}, and TAs_{ls} in *C. gigas*. The moisture and the concentrations of the arsenic species in *C. gigas* collected in March, May, August, and November of 2002 are shown in **Table 5**. It is clearly apparent that, on average, 82.5% of the arsenic species were MeOH−H_2O (8+2) extractable (TAs_{ex}) and that >90% of the extracted arsenic was a water-soluble species (TAs_{ws}). The results from previous studies (*3*, *8*, *17*, *37*) of arsenic concentrations in oysters worldwide are summarized in **Table 6**. In other countries, the TAs in oysters was reportedly as low as 5.69 mg kg^{-1} in Venice, Italy (*37*), but as high as 26.7 mg kg^{-1} in Arcachon Bay, France (*8*). Pertinent here is that the TAs in the oysters measured in this study ranged between 8.83 and 19.51 mg kg^{-1}, for an average of 13.74 mg kg^{-1}. The variations in our data appear to be insignificant when compared to those in the published data.

On the basis of an analysis of variance (ANOVA) test, the differences between the sampling sites were found to be insignificant at the 95% confidence level. Yet, the picture would be incomplete without recognizing that with the exclusion of the data from Hsianshan, which is suspected of being a source of toxin, as previously reported, the temporal and spatial variations show an exponential decaying relationship between the TAs of oysters and climate precipitation (*22*). In general, there appears to have been a trend whereby arsenic concentrations were high in the dry season but low in the wet season.

3.5. iAs, MA, DMA, and AB in *C. gigas*. Because the HG-PCFT-AAS coupled system is able to serve a preconcentration function, it is valuable for measuring trace hydride active arsenicals. In the field study here (**Table 5**), the average dry

Table 6. Comparison of the Concentrations of Arsenic Species (Milligrams per Kilogram of Dry Weight) in Oysters with Referral Data[a]

	iAs								
sampling site	As(III)	As(V)	MA	DMA	AC	AB	AS	TAs	ref
Atlantic coast, Spain	NA	NA	NA	NA	NA	NA	1.5–3.1	17.2	Sanchez-Rodas et al. (*3*)
Arcachon Bay, France	0.71	0.08	2.10	0.97	1.24	15.10	0.17	26.70	Kohlmeyer et al. (*8*)
Venice, Italy	NA	NA	NA	0.28	0.06	3.56	0.68	5.69	McSheehy et al. (*37*)
northwest coast, Spain	NA	NA	NA	0.46	NA	8.47	0.48	9.74	Vilano et al. (*17*)
west coast, Taiwan	0.15		0.06	0.64	NA	6.93	NA	13.74	this study

[a] AC (arsenocoline), AS (arsenosugars), and the other abbreviations of the arsenicals are the same as those given in **Figure 1**.

weight bases of iAs, MA, and DMA were, respectively, 0.04−0.29, 0.01−0.18, and 0.19−1.29 mg kg^{-1}, for averages of 0.15, 0.06, and 0.64 mg kg^{-1}, respectively. Thus, the concentration of the hydride active arsenic species follows the sequence DMA > iAs > MA.

The percentage of AB relative to TAs ranged between 36.0% (May, Taishi) and 65.7% (November, Budai), for a mean of 50.4%. The average of the other water-soluble arsenicals (As_{woth}), as estimated from As_{ws} − iAs − MA − DMA − AB, was most likely arsenosugars (AS) at the average of 2.75 mg/kg, with ∼20% contributed to total arsenic (TAs), a finding which is consistent with that reported in the literature (*3*, *17*).

3.6. Health Risk Assessment. The oyster, a sentinel organism, has the capability to serve as an indicator of the level of environmental pollution and, simultaneously, it is able to remove pollutants from a marine environment (*38*, *39*). Oyster farming is an important in-shore aquacultural industry on the west coast of Taiwan (*40*); however, with a rapid growth in population and a sharp increase in commercial activities in areas along the coast over much of the past few decades, several reported incidents of metal-contaminated oysters have arguably been a product of anthropogenic pollution (*41*). In examining this food category, the U.S. FDA (*42*) has indicated that fish and other seafood account for an alarming 90% of total food arsenic exposure, whereas all other foods merely account for the remaining 10%. In marine organisms, there are many species of arsenic, with the inorganic arsenic species unambiguously being more toxic than the organic arsenic species (*1*−*5*, *43*). Currently, the Food Standards Australia New Zealand (FSANZ) (*44*) sets a value of 1 mg kg^{-1} of fresh weight specifically for inorganic arsenic, rather than total arsenic, as the maximum level (ML) in molluscs. Our data show that the concentration of iAs in the oysters investigated here ranged from 0.006 to 0.033 mg kg^{-1}, for an average of 0.017 mg kg^{-1} on a fresh weight base. Strikingly important here is that this is only about $^1/_{60}$ of the FSANZ ML value.

The equation for carcinogen risk-based concentration (RBC) for edible fish, as developed by the U.S. EPA (*45*), is

$$\text{RBC (mg kg}^{-1}) = \frac{\text{TR} \times \text{BWa} \times \text{ATc}}{\text{EFr} \times \text{EDtot} \times \text{IRF} \times \text{CSFo}} \times 10^3 \quad (1)$$

where TR is the target cancer risk (1×10^{-6}); BWa is body weight, adult (kg); ATc is average time, carcinogens (25550 days); EFr is exposure frequency (350 days y^{-1}); EDtot is exposure duration, total (30 years); IRF is fish ingestion (g day^{-1}); and CSFo is the carcinogenic slope factor, oral (mg kg^{-1} day^{-1}). On the basis of the average of the data from their investigation, Han et al. (*46*) input BWa = 65 kg; CSFo = 1.5 mg kg^{-1} day^{-1}, which was reported by the U.S. EPA (*47*); IRF value = 18.6 g day^{-1}, which was an exceptionally high value in their survey; and the value of metal concentration in seafood (MCS) = 19.3 μg g^{-1} (dry weight basis) to replace RBC, and then they estimated the target cancer risk from the following equation:

$$\text{TR} = \frac{\text{EFr} \times \text{EDtot} \times \text{IRF} \times \text{CSFo} \times \text{MCS}}{\text{BWa} \times \text{ATC}} \times 10^{-3} \quad (2)$$

Their resulting TR value of 5.10×10^{-4} was 500 times higher than the criterion, 1×10^{-6} (*45*). Guo (*48*) contended that Han et al.'s (*46*) input for both IRF and MCS were misleading because most of the arsenic in shellfish is in the noncarcinogenic organic form.

In an attempt to be more reasonable, in this study, we determined (*49*−*51*) that if a person eats <1 kg of oysters per year, this should yield 2.7 g day^{-1} as the IRF value. As shown in **Table 5**, the average inorganic arsenic concentration based on the fresh weight is 0.017 μg g^{-1}, and this is considered the MCS value. The TR value is, therefore, estimated to be 4.4×10^{-7}, which is obviously well below the health protection criterion value of the lifetime risk assessment.

3.7. Conclusions. An off-line process-based analytical technique was devised, and this has allowed us to quantitatively measure iAs, MA, DMA, and AB in oysters. The optimal methanol−water ratio of the extractant, the extraction temperature, and the number of replicate extractions were evaluated using a retail dry oyster sample.

The HG-PCFT-AAS coupled system is widely considered to be a reliable tool for determining hydride active arsenicals, namely, iAs, MA, and DMA. Arsenic in the organic form is considered to be much less toxic than arsenic in the inorganic form; equally important, however, no consensus has ever been reached as to an acceptable ratio that can be used to convert total arsenic to inorganic arsenic in marine organisms. In this study, the average concentration of iAs in oysters collected from western Taiwan was 0.017 mg kg^{-1} of fresh weight, but this only made up ∼1% of total arsenic in the oysters. Because iAs is regarded as the most toxic species of all arsenic species, the average concentration of iAs was taken for our health risk assessment. The estimated target cancer risk value was 4.4×10^{-7}, which is well below the health protection criterion value.

Besides this, the process-based arsenic species, that is, TAs, TAs_{ws}, and AB, were also investigated here. The quantification of Dowex-exchangeable AB was validated with certified reference materials. AB is the dominant species, constituting ∼50% of the TAs in the oysters collected from the six aquacultural sites in western Taiwan that we sampled.

ACKNOWLEDGMENT

We thank three anonymous reviewers for providing invaluable suggestions and comments.

LITERATURE CITED

(1) Edmonds, J. S.; Shibata, Y.; Francesconi, K. A.; Rippingale, R. J.; Morita, M. Arsenic transformations in short marine food chains studied by HPLC-ICP MS. *Appl. Organomet. Chem.* **1997**, *11*, 281−287.

(2) Chatterjee, A. Determination of total cationic and total anionic arsenic species in oyster tissue using microwave-assisted extraction followed by HPLC-ICP-MS. *Talanta* **2000**, *51*, 303−314.

(3) Sanchez-Rodas, D.; Geiszinger, A.; Gomez-Ariza, J. L.; Francesconi, K. A. Determination of an arsenosugar in oyster extracts by liquid chromatography-electrospray mass spectrometry and liquid chromatography-ultraviolet photo-oxidation-hydride generation atomic fluorescence spectrometry. *Analyst* **2002**, *127*, 60−65.

(4) Le, X. C.; Lu, X.; Li, X. Arsenic speciation. *Anal. Chem.* **2004**, *76*, 26A−33A.

(5) Shiomi, K. Arsenic in marine organisms: chemical forms and toxicology aspects. In *Arsenic in the Environment. Part II: Human Health and Ecosystem Effect*; Nriagu, J. O., Ed.; Wiley: New York, 1994; pp 261−282.

(6) Cullen, W. R.; Reimer, K. J. Arsenic speciation in the environment. *Chem. Rev.* **1989**, *89*, 713−764.

(7) Ybanez, N.; Velez, D.; Tejedor, W.; Montoro, R. Optimization of the extraction, clean-up and determination of arsenobetaine in manufactured seafood products by coupling liquid chromatography with inductively coupled plasma atomic emission spectrometry. *J. Anal. At. Spectrom.* **1995**, *10*, 459−465.

(8) Kohlmeyer, U.; Kuballa, J.; Jantzen, E. Simultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high-performance liquid chromatography/inductively coupled plasma mass spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 965−974.

(9) Edmonds, J. S.; Francesconi, K. A. Organoarsenic compounds in the marine environment. In *Organometallic Compounds in the Environment*, 2nd ed.; Craig, P. J., Ed.; Wiley: New York, 2003; pp 195−222.

(10) Francesconi, K. A.; Edmonds, J. S.; Stick, R. V. Accumulation of arsenic in yelloweye mullet (*Aldrichetta forsteri*) following oral administration of organoarsenic compounds and arsenate. *Sci. Total Environ.* **1989**, *79*, 59−67.

(11) Francesconi, K. A.; Edmonds, J. S. Biotransformation of arsenic in the marine environment. *Adv. Environ. Sci. Technol.* **1994**, *26*, 221−261.

(12) Francesconi, K. A.; Kuehnelt, D. Arsenic compounds in the environment. In *Environmental Chemistry of Arsenic*; Frankenberger, Jr., W. T., Ed.; Dekker: New York, 2002; pp 51−94.

(13) Goessler, W.; Kuehnelt, D. Analytical methods for the determination of arsenic and arsenic compounds in the environment. In *Environmental Chemistry of Arsenic*; Frankenberger, Jr., W. T., Ed.; Dekker: New York, 2002; pp 27−50.

(14) Shiomi, K.; Orii, M.; Yamanaka, H.; Kikuchi, T. The determination method of arsenic compounds by high performance liquid chromatography with inductively coupled argon plasma emission spectrometry and its application to shellfishes. *Nippon Suisan Gakkaishi* **1987**, *53*, 103−108.

(15) Beauchemin, D.; Bednas, M. E.; Berman, S. S.; McLaren, J. W.; Siu, K. W. M.; Sturgeon, R. E. Identification and quantitation of arsenic species in a dogfish muscle reference material for trace elements. *Anal. Chem.* **1988**, *60*, 2209−2212.

(16) Velez, D.; Ybanez, N.; Montoro, R. Determination of arsenobetaine in manufactured seafood products by liquid chromatography, microwave-assisted oxidation and hydride generation atomic absorption spectrometry. *J. Anal. At. Spectrom.* **1997**, *12*, 91−96.

(17) Vilano, M.; Rubio, R. Determination of arsenic species in oyster tissue by microwave-assisted extraction and liquid chromatography-atomic fluorescence detection. *Appl. Organomet. Chem.* **2001**, *15*, 658−666.

(18) Benramdane, L.; Bressolle, F.; Vallon, J. J. Arsenic speciation in humans and food products: a review. *J. Chromatogr. Sci.* **1999**, *37*, 330−344.

(19) Vela, N. P.; Heitkempera, D. T.; Stewartb, K. R. Arsenic extraction and speciation in carrots using accelerated solvent extraction, liquid chromatography and plasma mass spectrometry. *Analyst* **2001**, *126*, 1011−1017.

(20) Hsiung, T.; Wang, J. Cryogenic trapping with a packed cold finger trap for the determination and speciation of arsenic by flow injection/hydride generation/atomic absorption spectrometry. *J. Anal. At. Spectrom.* **2004**, *19*, 923−928.

(21) Persson, J. A.; Irgum, K. Determination of dimethylarsinic acid in seawater in the sub-ppb range by electrothermal atomic absorption spectrometry after preconcentration on an ion-exchange column. *Anal. Chim. Acta* **1982**, *138*, 111−119.

(22) Hsiung, T. M.; Huang, C. W. Accumulation of arsenic in Pacific oysters, *Crassostrea gigas*, collected from aquaculture sites in western Taiwan. *J. Food Drug Anal.* **2004**, *12*, 342−346.

(23) U.S. EPA. Method 1632 (EPA-821-R-01-006), chemical speciation of arsenic in water and tissue by hydride generation quartz furnace atomic absorption spectrometry, revision A; U.S. GPO: Washington, DC, 2001.

(24) Shraim, A.; Chiswell, B.; Olszowy, H. Speciation of arsenic by hydride generation-atomic absorption spectrometry (HG-AAS) in hydrochloric acid reaction medium. *Talanta* **1999**, *50*, 1109−1127.

(25) Howard, A. G. (Boro) hydride techniques in trace element speciation. *J. Anal. At. Spectrom.* **1997**, *12*, 267−272.

(26) Edmonds, J. S.; Shibata, Y.; Prince, R. I. T.; Francesconi K. A.; Morita, M. Arsenic compounds in tissues of the leatherback turtle, *Dermochelys coriacea*. *J. Mar. Biol. Assoc. U.K.* **1994**, *74*, 463−466.

(27) Francesconi K. A.; Kuehnelt, D. Determination of arsenic species: a critical review of methods and applications, 2000−2003. *Analyst* **2004**, *129*, 373−395.

(28) Ackley, K. L.; B'Hymer, C.; Sutton, K. L.; Caruso, J. A. Speciation of arsenic in fish tissue using microwave-assisted extraction followed by HPLC-ICP-MS. *J. Anal. At. Spectrom.* **1999**, *14*, 845−850.

(29) Milstein, L. S.; Essader, A.; Murrell, C.; Pellizzari, E. D.; Fernando, R. A.; Raymer, J. H.; Akinbo, O. Sample preparation, extraction efficiency, and determination of six arsenic species present in food composites. *J. Agric. Food Chem.* **2003**, *51*, 4180−4184.

(30) Munoz, O.; Velez, D.; Montoro, R. Optimization of the solubilization, extraction and determination of inorganic arsenic [As(III) +As(V)] in seafood products by acid digestion, solvent extraction and hydride generation atomic absorption spectrometry. *Analyst* **1999**, *124*, 601−607.

(31) Londesborough, S.; Mattusch, J.; Wennrich, R. Separation of organic and inorganic arsenic species by HPLC-ICP-MS. *Fresenius' J. Anal. Chem.* **1999**, *363*, 577−581.

(32) Wahlen, R.; McSheehy, S.; Scriver, C.; Mester, Z. Arsenic speciation in marine certified reference materials. Part 2. The quantification of water-soluble arsenic species by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2004**, *19*, 876−882.

(33) Schmeisser, E.; Goessler, W.; Kienzl, N.; Francesconi, K. A. Volatile analytes formed from arsenosugars: determination by HPLC-HG-ICPMS and implications for arsenic speciation analyses. *Anal. Chem.* **2004**, *76*, 418−423.

(34) Wickenheiser, E. B.; Michalke, K.; Drescher, C.; Hirner, A. V.; Hensel, R. Development and application of liquid and gas-chromatographic speciation techniques with element specific (ICP-MS) detection to the study of anaerobic arsenic metabolism. *Fresenius' J. Anal. Chem.* **1998**, *362*, 498−501.

(35) Grüter, U. M.; Kresimon, J.; Hirner, A. V. A new HG/LT-GC/ICP-MS multi-element speciation technique for real samples in different matrices. *Fresenius' J. Anal. Chem.* **2000**, *368*, 67−72.

(36) GALAB (http://www.galab.de/laboratories/services/applications.html).

(37) McSheehy, S.; Pohl, P.; Lobinski, R.; Szpunar, J. Investigation of arsenic speciation in oyster test reference material by multidimensional HPLC-ICP-MS and electrospray tandem mass spectrometry (ES-MS-MS). *Analyst* **2001**, *126*, 1055−1062.
(38) Phillips, D. J. H. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. *Environ. Pollut.* **1977**, *13*, 281−317.
(39) Scanes, P. R.; Roach, A. C. Determining natural "background" concentrations of trace metals in oysters from New South Wales, Australia. *Environ. Pollut.* **1999**, *105*, 437−446.
(40) Chen, C. Y.; Chen, M. H. Investigation of Zn, Cu, Cd and Hg concentrations in the oyster of Chi-ku, Tai-shi and Tapeng Bay, Southwestern Taiwan. *J. Food Drug Anal.* **2003**, *11*, 32−38.
(41) Lin, S.; Hsieh, I. J. Occurrences of green oyster and heavy metals contaminant levels in the Sien-San area, Taiwan. *Mar. Pollut. Bull.* **1999**, *38*, 960−965.
(42) CFSAN/USFDA. *Guidance Document for Arsenic in Shellfish*; Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration: Washington, DC, 1993; http://www.cfsan.fda.gov/∼frf/guid-as.html.
(43) Francesconi, K. A.; Edmonds, J. S. Arsenic species in marine samples. *Croat. Chem. Acta* **1998**, *71*, 343−359.
(44) FSANZ. *Food Standards Australia New Zealand Issue 53, Standard 1.4.1*; 2005; http://www.foodstandards.gov.au/_srcfiles/Standard141_Contaminants_v63.pdf.
(45) U.S. EPA. *EPA Region 3 Risk-Based Concentration Table, Technical Background Information, Development of Risk-Based Concentrations*; 2003; http://www.epa.gov/reg3hwmd/risk/human/info/tech.htm.
(46) Han, B. C.; Jeng, W. L.; Chen, R. Y.; Fang, G. T.; Hung, T. C.; Tseng, R. J. Estimation of target hazard quotients and potential health risks for metals by consumption of seafood in Taiwan. *Arch. Environ. Contam. Toxicol.* **1998**, *35*, 711−720.
(47) U.S. EPA. *Risk-Based Concentration Table, EPA Region III RBC Table 4/7/2005*; 2005; http://www.epa.gov/reg3hwmd/risk/human/rbc/rbc0405.pdf.
(48) Guo, H. R. Cancer risk assessment for arsenic exposure through oyster consumption. *Environ. Health Perspect.* **2002**, *110*, 123−124.
(49) OEHHA. *Chemicals in Fish: Consumption of Fish and Shellfish in California and the United States*; 2001; http://www.oehha.ca.gov/fish/pdf/Fishconsumptionrpt.pdf.
(50) U.S. EPA. *Estimated Per Capita Fish Consumption in the United States (EPA-821-C-02-003)*; 2002; http://www.epa.gov/waterscience/fish/consumption_report.pdf.
(51) NMFS. *Fisheries of the United States 2003*; 2003; http://www.st.nmfs.gov/st1/fus/fus03/2003_fus.pdf.

Received for review May 21, 2005. Revised manuscript received January 31, 2006. Accepted February 2, 2006. This study was supported by the National Science Council of the Republic of China under Grant NSC92-2611-M-019-014.

JF051181X