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Arsenic species extraction of biological marine samples (Periwinkles, *Littorina littorea*) from a highly contaminated site

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ABSTRACT

Arsenic is ubiquitous in the tissues of marine organisms and in uncontaminated environments it is dominantly present as the highly soluble and easily extractable non-toxic arsenical, arsenobetaine. However in contaminated environments, higher proportions of inorganic arsenic, which is much less soluble, are accumulated into the tissues of marine organisms, resulting in lower extraction efficiencies (defined as the percent extracted arsenic of the total arsenic). This study carried out a comparative analysis between three different two-step arsenic extraction methods based on Foster et al. [27] from highly contaminated tissue of the marine periwinkle, *Littorina littorea*. The first extraction step used 100% water, 1:1 methanol–water, or a 9:1 methanol–water as the extraction solvent and the second step consisted of a gently heated dilute nitric acid extraction. The optimized two step extraction method was 1:1 methanol–water extraction followed by a 2% HNO_3 extraction, based on maximum amounts of extracted species, including organoarsenic species.

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1. Introduction

Arsenic is prevalent in the marine environment as a result of natural processes but can become elevated through anthropogenic actions such as mining processes. This may have significant toxicological implications with regard to human and marine ecosystem health since marine organisms readily bioaccumulate arsenic into their tissue from their surrounding environment and human consumption of these organisms is common [1]. The toxic effects of arsenic have been recognized to be dependent on the form of arsenic that an organism is exposed to [2], retains and potentially biotransforms within their tissue. Thus, effective prediction and mitigation of negative impacts is largely dependent on the differentiation and identification of arsenic species present within an organism's tissue and the surrounding abiotic and biotic environment.

HPLC–ICPMS (high performance liquid chromatography linked to inductively coupled plasma mass spectrometry) is a highly effective analytical technique commonly used in arsenic speciation analysis. Chromatographic separation of different arsenic species allows researchers to define the arsenic distribution of a sample. However, this technique requires the arsenic species to be usually in aqueous solution [3]. Thus, extraction of arsenic species from a solid matrix (e.g. biological tissue) must occur prior to HPLC–ICPMS analysis. The success of this analytical technique is highly dependent on the effectiveness of the chosen extraction method (extractant and procedure). Ideally, the chosen method will remove a proportion of arsenic species that is sufficiently high to represent the sample and do so without changing the chemical properties of the arsenicals present (i.e. species transformation should be avoided).

Marine organisms have been examined extensively for arsenic species during recent decades [4] most likely because they naturally retain relatively high concentrations of arsenic and are commonly found in the human diet. In uncontaminated environments, the arsenic found within the tissues of marine animals is predominantly present as the non-toxic arsenical, arsenobetaine (AB) and in minor concentrations as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), arsenocholine (AC), tetramethylarsonium ion (Tetra) and the arsenosugars glycerol arsenosugar (Sugar 1), phosphate arsenosugar (Sugar 2), sulfonate arsenosugar (Sugar 3) and sulfate arsenosugar (Sugar 4). AB is a polar molecule and therefore easily soluble in aqueous solutions of methanol. This property of AB allows it to be easily extracted from biological tissue. As a result, many studies have observed and reported relatively good extraction efficiencies (EE's), such as those ranging from 60 to 85% [5] from uncontaminated marine biological samples. Studies that extracted arsenic (mostly present as AB) from the certified reference material DORM-2

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(National Research Council of Canada) (which is dried and homogenized marine dogfish tissue) have reported consistently high EE's of 85–109% [6–9]. Different solvent mixtures for the extraction of arsenic species from marine biological tissue reported in the literature are 100% water [10–12] and different ratios of methanol and water: 1:1 MeOH:H_2O [5,6,13–18] 3:1 MeOH:H_2O [19] and 9:1 MeOH:H_2O [20,21]. Justification for the choice of solvent mixture is rarely reported with the exception of a few studies [22–24]. An example of one of the exceptions is when three solvent mixtures (MeOH, 1:1 MeOH:chloroform and 1:1 MeOH:H_2O) were tested and 1:1 MeOH:H_2O was concluded to be the most appropriate extraction solvent for the marine tissues studied because it recovered the highest amount of arsenic from freeze-dried marine tissues [22]. Results of another recent study [24] which optimized the solvent extract composition for marine macroalgae supports the notion that different MeOH:H_2O solvent ratios extract varying amounts of arsenic from different sample matrices. More studies of this type are needed within the literature.

Recent studies have reported that marine samples from sites contaminated with arsenic contain a much smaller proportion of AB and significantly higher proportions of inorganic arsenic [23], which was a trend also seen in marine samples with arsenic concentrations exceeding 3 mg kg^{-1} wet weight [25]. This has significant implications with respect to the extraction efficiency because inorganic arsenicals (arsenous acid, $As(OH)_3$, or As(III); and arsenate, $H_2AsO_4^-$, or As(V)) are much less easily extracted than AB by aqueous extractants [26]. Currently, no study has reported an extraction method that allows for high extraction efficiencies while also maintaining the integrity of the arsenic species distribution specifically for a contaminated marine biological sample. However, a recent study by Foster et al. [27] reported an efficient method of a heat-assisted sequential extraction of plant samples where an initial extraction with 1:1 MeOH:H_2O was followed by a second extraction step with dilute nitric acid. Foster et al. [27] investigated the extraction efficiencies of each extraction step, as well as the stability of arsenic species at various temperatures and concentrations of dilute nitric acid. Overall, the study found that in biological samples first extracted with 1:1 MeOH:H_2O and then further extracted 2% HNO_3, the integrity of the arsenic species distribution was preserved and arsenic recoveries were increased when compared with traditional one-step methods. Mir et al. [28] carried out a similar study instead using dilute hydrochloric acid as the second extraction solvent and this yielded similar results.

The purpose of this study was to optimize the extraction of arsenic species from marine samples that originate from arsenic-contaminated sites, and that contain arsenic concentrations significantly above normal arsenic concentrations. Our approach was to combine the efficiency of aqueous methanol extraction for organoarsenic species with the apparent efficiency of remaining (probably inorganic) arsenic species by dilute acid extraction. Since increasing methanol content may increase the extraction of organoarsenicals [29,30], three mixtures of aqueous methanol were tested prior to the second acidic extraction step.

2. Materials and methods

2.1. Chemicals and reagents

Distilled deionized water (DDW) was prepared in-house to a minimum resistance of 18 MΩ cm (E-pure Barnstead). Trace metal grade nitric acid (~70%, Fisher Scientific) and HPLC grade methanol (Fisher Scientific) were used. Total arsenic matrix spikes and calibration curves were prepared from stock solutions with reported concentrations of 995 ± 3 mg L^{-1} and $10{,}006 \pm 25$ mg L^{-1} total arsenic (Inorganic Ventures).

If possible, different source of standards was used for calibration curves than for quality control calibration check solutions and matrix spikes; this was the case for TMAO trimethylarsine oxide, C_3H_9AsO, (MW = 136.02, Wako and Argus Chemicals) and AB, arsenobetaine, $C_5H_{11}AsO_2$, (MW = 178.06, Wako and Argus Chemicals) for cation exchange chromatography, and for AB (Wako and Argus Chemicals); DMA (V), cacodylic acid, (>99% purity from Fluka and 99% purity from City Chemical); As(III) (1000 ppm, 99.0%, Fluka and As_2O_3 with 99.995% purity from Aldrich); and As(V) (9775 ppm from Aldrich and 1000 ppm from Inorganic Ventures) for anion exchange chromatography. Only one source was available for AC, arsenocholine bromide, $C_5H_{14}AsBrO$, (MW = 244.99, Argus Chemicals); and Tetra, tetramethylarsonium iodide, $C_4H_{12}AsI$, (MW = 261.96, Wako) standards for cation exchange chromatography; and for the MMA(V) standard, monosodium acid methane arsonate sesquihydrate, (99.0%, Chemservice) for anion exchange chromatography.

The cation exchange chromatography mobile phase was prepared with pyridine (>99%, Aldrich), DDW and formic acid (98%, Fluka) using indium (10,000 mg L^{-1}) ICP-MS standard solution (PlasmaCAL) as the internal standard. The anion exchange mobile phases were prepared with H_3PO_4 (orthophosphoric acid) (85%, Fluka), DDW, NH_4OH and ammonium hydroxide solution (Fluka) using rhodium (in 5% weight HCl) AAS standard solution (Aldrich) as the internal standard. A certified reference material (CRM) Dogfish Muscle (DORM-2) was used and obtained from the National Research Council of Canada (NRCC).

2.2. Sample collection and preparation

Periwinkles (*Littorina littorea*) were collected in August of 2007 from Seal Harbour, Nova Scotia. Samples were frozen and shipped to Ontario with dry ice and stored at −20 °C until analysis. The frozen periwinkle tissue was obtained from the shell by cracking the shell and removing the tissue with tweezers. Three composite samples were created from 337 individual periwinkles. Tissue samples were placed in sterile, pre-weighed 50 ml Fisherbrand® disposable polypropylene centrifuge tubes and weighed to obtain wet weights. The samples were then freeze-dried for at least 24 h and weighed again to obtain dry weights. The freeze-dried samples were homogenized by grinding with a mortar and pestle.

2.3. Periwinkle total arsenic digestions

Analysis of total arsenic was carried out in duplicate. A quantity of 0.5 g of the freeze-dried, ground sample was added to a pre-weighed glass tube with a boiling chip. Samples were digested with 10 ml of 70% HNO_3 by heating to 120 °C on a heating block until the sample was reduced to 1–2 ml (approximately 12 h). Samples were vortexed and 10 ml of DDW was added. Finally samples were weighed, placed in syringes and filtered through disposable syringes outfitted with disposable 0.45 μm filters (Millipore® polypropylene 25 mm diameter hydrophilic PVDF durapore membrane) into 15 ml Fisherbrand® disposable polypropylene centrifuge tubes.

2.4. Periwinkle arsenic sequential extraction: step one

A quantity of 0.5 g of the dry sample was weighed out into 15 ml Fisherbrand® disposable polypropylene centrifuge tubes, with three replicates carried out for each extraction experiment. To each sample, 10 ml of either 9:1 MeOH/H_2O, 1:1 MeOH/H_2O or 100% water solution were added. These solutions were vortexed and left overnight at room temperature. Samples were placed in an

ultrasonic bath for 30 min and centrifuged for 30 min at 3000 rpm. The ultrasonication and centrifugation steps were repeated two more times for the aqueous methanol extractions.

The 9:1 and 1:1 supernatants were decanted into clean, labeled Syncore® tubes. Rinsings of their sample residues through a 0.20 μm filter (47 mm diameter Millipore® white nylon hydrophilic membrane) were combined with the corresponding supernatant. Methanol was then evaporated (Buchi Syncore® Analyst) at 60 °C, and pressures of 200 Mbar (start) to 100 Mbar (end). The extract (approximately 1–2 ml) was then pipetted into pre-weighed disposable syringes outfitted with 0.45 μm syringe filters (Millipore® polypropylene 25 mm diameter hydrophilic PVDF durapore membrane), filtered into 15 ml Fisherbrand® disposable polypropylene centrifuge tubes, diluted to a total volume of 10 ml and frozen before subsequent dilutions and arsenic speciation analysis.

For the 100% H_2O extraction procedure, supernatants were decanted from a single extraction directly into pre-weighed 15 ml Fisherbrand® disposable polypropylene centrifuge tubes. The washing step was not carried out for samples that had been extracted with 100% H_2O because of difficulty in passing the wash solution through the filter paper which became clogged with the periwinkle tissue residue.

2.5. Periwinkle arsenic sequential extraction: step two

Residues from the 100% H_2O extraction and filter paper + residues for the 9:1 and 1:1 extractions were placed in pre-weighed glass tubes and 10 ml of 2% HNO_3 was added to each sample. Samples were heated to 70 °C for 2 h in an oven, vortexed and centrifuged at 3000 rpm for 30 min. The extract was carefully poured into pre-weighed disposable syringes outfitted with disposable 0.45 μm filters (Millipore® polypropylene 25 mm diameter hydrophilic PVDF durapore membrane) and filtered into 15 ml Fisherbrand® disposable polypropylene centrifuge tubes, diluted to a total volume of 10 ml and frozen before subsequent dilutions and arsenic speciation analysis.

2.6. Final periwinkle residue digestion

Final residues were digested with 10 ml of 70% HNO_3. Samples were heated to 120 °C until the sample was reduced to 1–2 ml. Samples were vortexed and 10 ml of distilled deionized water (DDW) was added. Finally samples were weighed, placed in syringes and filtered through a 0.45 μm disposable syringes (Millipore® polypropylene 25 mm diameter hydrophilic PVDF durapore membrane) into 15 ml Fisherbrand® disposable polypropylene centrifuge tubes.

2.7. Arsenic species stability in dilute nitric acid

The stability of arsenic species in step 1 of extraction methodology over short time periods has been confirmed by previous studies [31] and therefore was not deemed necessary to repeat in the present study. However, similar to the method employed by Foster et al. [27], the examination of the stability of arsenic species during step 2 of the extraction methodology was carried out. A 10 ml quantity of 2% nitric acid was spiked with known concentrations of cation and anion standards, heated to 70 °C for 2 h, filtered and analyzed immediately to examine whether arsenic species degradation or interconversion occurred.

2.8. Total arsenic analysis

All samples were analyzed for total arsenic concentrations by either an inductively coupled plasma-mass spectrometer (ICP-MS) (Perkin Elmer DRC II) or an ICP-optical emission spectrometer (ICP-OES) (Optima 5300V Perkin Elmer). The operating conditions of the ICP-MS are shown in Table 1. The detection limit of the ICP-MS was 1 $\mu g\,L^{-1}$ and was based on the lowest concentration arsenic standard used within the calibration curve. Mass interferences from chloride were monitored by measuring m/z 77($ArCl^+$). Plasma conditions of the ICP-OES are listed in Table 1. The detection limit was established to be 49 $\mu g\,L^{-1}$ calculated from three times the standard deviation of the average arsenic concentration recorded from

Table 1
Instrumental operating conditions of ICP-MS, ICP-OES and HPLC–ICPMS.

Parameter	ICP-MS (PerkinElmer DRC II)	ICP-OES (PerkinElmer Optima)
Rf power	1400 W	1400 W
Nebulizer gas flow rate	0.99 L min^{-1}	0.65 L min^{-1}
Auxiliary gas flow rate	1.21 L min^{-1}	0.4 L min^{-1}
Coolant gas flow rate	15 L min^{-1}	–
Lens voltage	9.7	–
Nebulizer type	Concentric	Gemcone™
Analysis mode	Standard	–
Vacuum pressure	6.7×10^{-6} psi	–
Calibration curve solutions	0.1, 1, 10, 50, 100, 500, 1000 ppb	0.2, 1, 10, 200 ppm
QC solutions	Low QC: 5 ppb High QC: 50 ppb	0, 0.5, 10, 150 ppm
Internal standards	Sc, Y, Rh, In, Tb, Ho, B	Sc, Y

As species analysis	Parameter	HPLC–ICPMS
Cation (AB, TMAO, AC, Tetra)	Mobile phase	20 mM pyridine (C_5H_5N)/L DDW, pH = 2.7 (level achieved with formic acid)
	Column	PRP-X200
	Flow rate	1.5 ml min^{-1}
	Internal standard	Rh
	Detection limits	0.5 $\mu g\,L^{-1}$ = 0.25 mg kg^{-1} dw sample
Anion (AB, As(III), DMA, MMA, As(V))	Isocratic mobile phase	0.02 mM phosphoric acid (H_3PO_4)/L DDW, pH = 6.0 (level achieved with ammonium hydroxide)
	Gradient mobile phase	4 mM of ammonium nitrate (NH_4NO_3) as mobile A and 60 mM NH_4NO_3 as mobile B, pH = 8.65
	Column	PRP-X100
	Flow rate	1 ml min^{-1}
	Internal standard	Rh
	Detection limits	0.5 $\mu g\,L^{-1}$ = 0.25 mg kg^{-1} dw sample
	ICPMS	Same conditions as listed above

Table 2
Stability of cation and anion arsenic species in 2-h heated (70 °C) 2% nitric acid.

	Cation arsenic species				Anion arsenic species			
	AB	TMAO	AC	Tetra	As(III)	DMA	MMA	As(V)
N	4	4	4	4	4	4	4	4
Percent recovery (%)	98 ± 2	93 ± 6	83 ± 20	75 ± 13	100 ± 8	90 ± 6	104 ± 4	94 ± 2

17 aqua regia blank digestions that were run on the OES. Instrumental tests included instrumental blanks, and QC calibration checks run once every ten samples on the ICP-MS and OES; results were accepted when blanks were below detection limits and QC calibration check recoveries were between 80 and 120%.

2.9. Arsenic species analysis

All sample extracts were analyzed at a 10,000 times dilution to reduce potential matrix effects and ensure concentrations were within the calibration curve concentrations of the instrument. All samples were also run at a 500 times dilution to ensure that smaller proportions of organoarsenicals could be detected. Table 1 lists the instrumental parameters and mobile phase solutions used in the arsenic speciation analysis with HPLC–ICPMS. The instrumental software used for the HPLC–ICPMS was Chromera® Chromatography Data System. All chromatographic speciation data was then analyzed with Peak Fit© Version 4.12. Instrumental QC tests included blanks and QC calibration checks run once every ten samples and results were accepted when blanks were below detection limits and QC calibration check recoveries were between 80 and 120% (Supplementary Material, Table S1).

2.10. Statistical analysis

Statistical analysis was carried out using the statistical software SYSAT 12® Version 12.02. All data sets appeared to be approaching normality and thus no normalization was required. All statistical tests (ANOVA and Bonferroni pairwise comparisons) were carried out at 95% confidence levels.

2.11. Quality assurance/quality control (QA/QC)

Results are summarized in Supplementary Material, Table S1. Blanks were not detectable when analyzed for total arsenic on the ICP-OES, and duplicate results indicated acceptable precision, as they were within 15% of each other. One certified reference material DORM-2 sample was analyzed with the total digestion analysis of the sample, and three replicates were taken through the extraction and residue digestion process. The latter three results ($17 \pm 4\ mg\ kg^{-1}$) were within 23% of the certified value ($18.0 \pm 1.1\ mg\ kg^{-1}$) and therefore considered acceptable. The DORM-2 result accompanying the bulk sample was only 60% of the certified value, but the bulk sample results for the periwinkle tissue were accepted nevertheless because the results (mean ± standard error was $510 \pm 20\ mg\ kg^{-1}$, $n = 5$) were statistically indistinguishable ($p = 0.167$) from the residue + extract total arsenic results for the periwinkle (550 ± 10, $n = 27$). All blanks run with HPLC–ICPMS contained no detectable arsenic (<0.25 mg L^{-1}). Matrix spikes were prepared by adding a known amount of a mixture of arsenic standards to samples prior to analysis on both the anion and cation columns at a frequency of one spike every 10 samples and recoveries were generally between 70 and 130%, which was considered acceptable for this analysis. The AB recovery of three DORM-2 samples taken through the extraction methods was 104 ± 3.1% of the certified value. Column recovery (sum of species/total extracted arsenic) averages ranged from 99 to 113% (Table 2) which was considered acceptable.

3. Results and discussion

3.1. Arsenic species stability in heated dilute nitric acid

The chromatographic results showed that all the tested anion and cation standards (As(III), DMA(V), MMA(V), As(V), AB, TMAO, AC and Tetra) remained stable when placed in 10 ml of dilute nitric acid and heated to 70 °C for 2 h. Percent recoveries of the various anion and cation standards were all within acceptable ranges (Table 2). The average percent recoveries of AC and Tetra were lower than other tested arsenic compounds (indicating an average loss of approximately 20 and 25%). However, these values should be considered within the context of error in speciation measurements which was also considerably higher than other arsenic species measurements (Table 2). Therefore, the second extraction method carried out on the samples was assumed to not result in either significant degradation or interconversion of major arsenic species found in marine biological samples. The stability of arsenosugars in the heated dilute nitric acid was not tested in this study but Foster et al. [27] reported that under these conditions arsenosugars degraded to a product which eluted at the same retention time as glycerol arsenoribose. Thus, identification of arsenosugars in the heated nitric acid extracts should be done cautiously.

3.2. Comparison between sequential extraction methods

All extractions were carried out using composite samples of freeze-dried periwinkle tissue which had an average arsenic concentration of $510 \pm 20\ mg\ kg^{-1}$ (dry weight). These concentrations are well above reported background concentrations in marine shellfish (1–20 mg kg^{-1} dry weight) [1] and the sample is thus considered to be a contaminated marine sample. The extraction efficiency (EE) of the three different methods differed significantly in both Step 1 and Step 2 between the three methods. For Step 1, water gave a statistically higher average EE ($52 \pm 1\%$) then either 1:1 or 9:1 MeOH:H_2O ($21 \pm 0.9\%$ and $27 \pm 7\%$ respectively) (ANOVA, Posthoc Bonferroni, $p \leq 0.001$) (Fig. 1), but no significant difference was seen between the 1:1 and 9:1 MeOH:H_2O extraction solutions ($p = 0.96$). Thus, water extracted the most total arsenic from the contaminated marine tissue in the first extraction step. However, when results from Step 2 of the sequential extraction (extraction of the residue with dilute nitric acid) were summed with Step 1 results, the total EE of the H_2O method and 1:1 MeOH:H_2O are $90 \pm 1\%$ and $85 \pm 2\%$. These values were found not to be significantly different from each other ($p = 0.284$) and both were higher than the total EE of the 9:1 MeOH:H_2O method of $80 \pm 2\%$ (Fig. 1) ($p < 0.005$). Thus, the water method and the 1:1 method both provide high extraction efficiencies of the highly contaminated marine tissue. However, while EE's are a valuable measure to aid in determining an appropriate extraction method for any given sample, the arsenic species extracted from the matrix should also be considered. Thus, anion and cation exchange chromotographic analysis of the extracts were carried out to determine which of

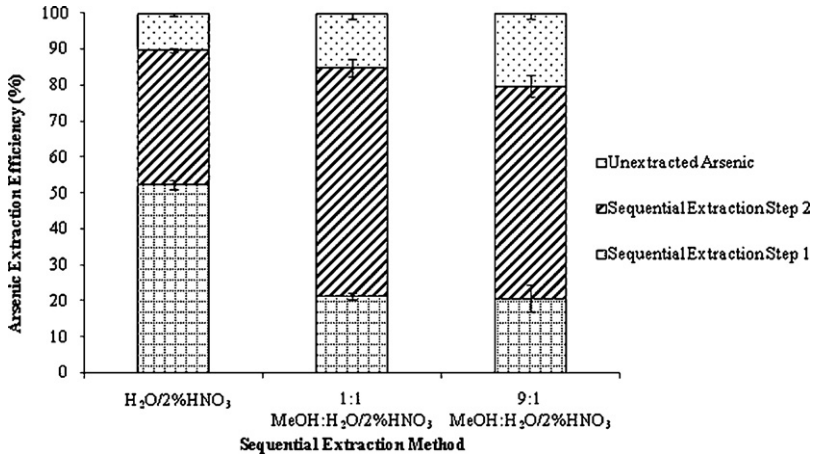

Fig. 1. Arsenic extraction efficiencies of three sequential extraction methods (H_2O/2% HNO_3, 1:1 MeOH:H_2O/2% HNO_3 and 9:1 MeOH:H_2O/2% HNO_3) from contaminated marine biological tissue. Error bars represent the standard error around the arithmetic mean of replicate (N = 7–11) extractions.

these sequential extractions methods was the most appropriate for a highly arsenic contaminated marine tissue matrix.

The anion and cation exhange chromatograhic analysis allowed for the identification of twelve arsenic species commonly found in biological samples as shown in Supplementary Material, Fig. S1: arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AB), trimethylarsine oxide (TMAO), arsenocholine (AC), tetramethylarsonium ion (Tetra), glycerol arsenosugar (Sugar 1), phosphate arsenosugar (Sugar 2), sulfonate arsenosugar (Sugar 3) and sulfate arsenosugar (Sugar 4). The results of the speciation analysis are depicted in Fig. 2 which shows that in the first step of the sequential extraction, similar amounts of As(III), AB and Sugar 1 were extracted from all three methods but water was able to extract more As(V) and MMA then the 1:1 and 9:1 MeOH:H_2O solutions. However, analysis of the second step of the sequential extractions (2% HNO_3 solution) indicate that the As(V) and the MMA left behind by the 1:1 and 9:1 MeOH:H_2O was extracted by the second step. It should be emphasized that the only arsenosugar identified was found in the "first-step" extracts (water, 1:1 MeOH:H_2O and 9:1 MeOH:H_2O) and therefore the instability of arsenosugars in heated dilute nitric acid which Foster et al. [27] reported did not impact the results of this study.

A difference was seen in the ability of the three extraction methods in extracting Tetra from the marine tissue ($p < 0.001$). Tetra was only present in the two methanol-containing extraction solutions and the general trend in the Tetra extraction effectiveness was water < 1:1 MeOH:H_2O < 9:1 MeOH:H_2O (Table 3; Fig. 2). In other words, Tetra was not identified in the water extracts at all, even when the more concentrated extract (500× dilution) was analyzed. We hypothesize that Tetra was more effectively extracted when more methanol was present; this was a trend also seen for polar species [30].

If the methods in the present study were assessed according to the maximum total arsenic extracted in a single step, as is typical [22,26,29] we may have concluded that water was the best extractant. However, the speciation analysis showed that water was not as effective as 1:1 and 9:1 MeOH:H_2O in extracting organoarsenicals such as Tetra; the use of water in such cases would erroneously fail to identify all species present. Additionally, high proportions of inorganic arsenic could potentially chromatographically overlap with organoarsenical peaks present in much smaller proportions (although this was not observed in the present study). Thus, the use of an extraction method that allows one step to optimally extract organoarsenicals, and another step to optimally extract inorganic arsenic (as seen with the 1:1 and 9: 1 MeOH + 2% HNO_3), allows minor amounts of organoarsenicals also present in the sample to be quantified. In other words, carrying out sequential extractions on our samples was more effective in attaining high extraction efficiencies and a representative characterization

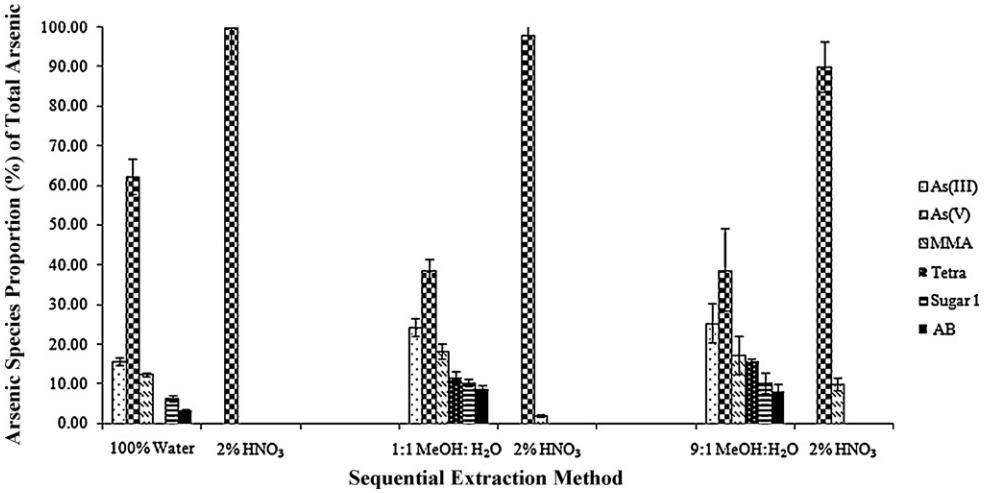

Fig. 2. Comparison of three sequential extraction methods (H_2O/2% HNO_3, MeOH:H_2O/2% HNO_3 and 9:1 MeOH:H_2O/2% HNO_3) of arsenic species (As(III), As(V), MMA, Tetra, Sugar 1 and AB) from highly contaminated marine snail tissue. Error bars represent the standard error around the arithmetic mean of replicate extractions (N = 7–11).

Table 3
Arsenic species concentrations (mg kg^{-1}) determined from extractions, total arsenic concentration in residues (mg kg^{-1}) of freeze-dried contaminated marine tissue of the edible periwinkle (*Littorina littorea*) and column recovery (%) (*N* = number of replicate extractions).

	N	As(III)	AB	Sugar 1	MMA	As(V)	Tetra	Residue	Column recovery (%)
100% water	7	52 ± 3	11 ± 1	21 ± 3	41 ± 2	208 ± 15	–	59 ± 6	99 ± 7
2% HNO_3		–	–	–	–	173 ± 15	–		
1:1 MeOH:H_2O	11	37 ± 3	13 ± 1	16 ± 2	28 ± 3	59 ± 5	10 ± 2	90 ± 2	99 ± 11
2% HNO_3		–	–	–	3 ± 0.5	169 ± 19	–		
9:1 MeOH:H_2O	9	7 ± 7	11 ± 3	14 ± 4	24 ± 7	54 ± 14	18 ± 8	114 ± 7	113 ± 8
2% HNO_3		–	–	–	28 ± 5	254 ± 18	–		

of the arsenic species distribution, compared with a single extraction.

4. Conclusion

This study found that the optimized extraction method for contaminated marine snails used an initial extraction with 1:1 MeOH:H_2O to ensure extraction of the organoarsenicals present, followed by a second extraction with 2% HNO_3 to extract a large proportion of the inorganic arsenic (i.e. As(V)). We anticipate that this extraction method is applicable to other marine tissues and possibly other biological samples with significantly higher arsenic concentrations. While slightly more time consuming, future work that involves the extraction of arsenical species from biological matrices, especially those from contaminated sites, should consider the use of sequential extraction as a method that is more suitable than traditional procedures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.10.030.

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