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ORIGINAL PAPER

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Extraction and detection of arsenicals in seaweed via accelerated solvent extraction with ion chromatographic separation and ICP-MS detection

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Abstract An accelerated solvent extraction (ASE) device was evaluated as a semi-automated means of extracting arsenicals from ribbon kelp. The effect of the experimentally controllable ASE parameters (pressure, temperature, static time, and solvent composition) on the extraction efficiencies of arsenicals from seaweed was investigated. The extraction efficiencies for ribbon kelp (approximately 72.6%) using the ASE were fairly independent (< 7%) of pressure, static time and particle size after 3 ASE extraction cycles. The optimum extraction conditions for the ribbon kelp were obtained by using a 3 mL ASE cell, 30/70 (w/w) MeOH/H_2O, 500 psi (1 psi = 7 KPa), ambient temperature, 1 min heat step, 1 min static step, 90% vol. flush, and a 120 s purge. Using these conditions, two other seaweed products produced extraction efficiencies of 25.6% and 50.5%. The inorganic species present in the extract represented 62.5% and 27.8% of the extracted arsenic. The speciation results indicated that both seaweed products contained 4 different arsenosugars, DMA (dimethylarsinic acid), and As(V). One seaweed product also contained As(III). Both of these seaweed products contained an arsenosugar whose molecular weight was determined to be 408 and its structure was tentatively identified using ion chromatography-electrospray ionization-mass spectrometry/mass spectrometry (IC-ESI-MS/MS).

Introduction

Dietary intake is one of the major pathways for arsenic exposure. Dietary sources contain a variety of arsenicals which differ dramatically in their respective toxicities. Based on a "total" or acid digested arsenic analysis, seafood is one of the major sources of dietary arsenic [1, 2]. The majority of arsenicals associated with seafood are generally highly derivatized, non-toxic arsenicals (arsenobetaine, etc) while toxic inorganic arsenic [As(III) and As(V)] is usually a minor component (Table 1). One of the analytical needs required to assess risk is a quantitative or nearly quantitative extraction of the arsenicals with a major emphasis on preserving the original species specific information throughout the sample preparation procedure. The quantitative extraction aspect is also essential because without it, the extraction may be selectively removing the non-toxic species while leaving the toxic species unextracted/undetected within the solid matrix.

Sonication in combination with MeOH/water solvent mixtures has been used in an attempt to quantitatively extract arsenicals from seafoods [3–14]. Similar extraction procedures have been applied to seaweed products which are typically ingested by the Asian Pacific subpopulations [15–23]. Quantitative extraction has in some cases been achievable on standard reference materials (SRMs) while it has been somewhat more difficult to achieve a thorough extraction on actual seaweed samples. This has led to attempts to utilize enzymatic assisted extractions of seafoods [24]. Accelerated Solvent Extraction (ASE) is a relatively new extraction technique that has the capability of optimizing solvent mixtures, applied pressure, temperature and static time in order to obtain a more quantitative extraction [5, 18, 25]. Once the arsenicals are extracted, a variety of separation techniques have been used in combination with elemental [7, 10, 11, 13, 21, 25–27] and structural [9, 15, 16, 18, 28–30] based detection schemes. The degree to which a complete separation is needed is somewhat application dependent, but as the list of potential extractable arsenicals grows, the probability of misidentification also increases.

This paper addresses the optimization of the ASE parameters such as static time, pressure, solvent, and temperature for the extraction of arsenicals from seaweed. The ASE optimization includes an evaluation of the effect each parameter had on the determined distribution of arsenicals in ribbon kelp. Ribbon kelp was chosen for the optimization because it contains 3 arsenosugars [18]. Fi-

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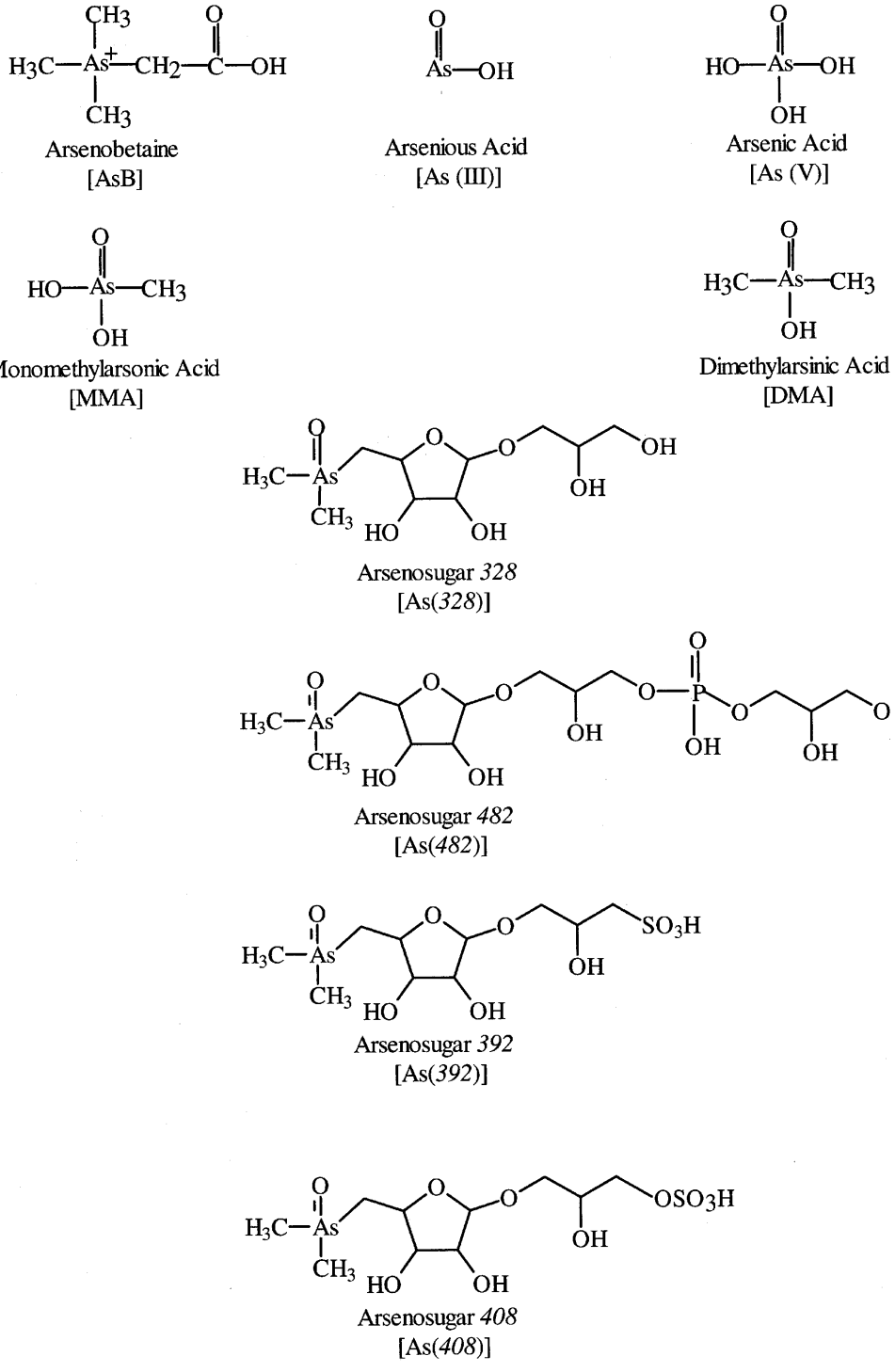
Table 1 Chemical structures of arsenicals



nally, the complex mixture of arsenicals extracted from these natural products are discussed with regard to chromatographic resolution and detector selectivity.

Experimental

Reagents

The ribbon kelp (Algaria Marginata) and Sargassum Muticum were received from Puget Sound, WA. The Seaweed A was purchased from a local specialty market. The HPLC grade methanol (MeOH), the certified A.C.S. hydrogen peroxide 30% (H_2O_2), and trace-metal-grade ammonium hydroxide (NH_4OH), were purchased from Fisher Scientific (Pittsburgh, PA). The A.C.S. reagent grade ammonium carbonate ($(NH_4)_2CO_3$) and the ultrapure reagent grade nitric acid (HNO_3) were purchased from Aldrich (Milwaukee, WI) and J. T. Baker (Phillipsburg, NJ), respectively. The 18 MΩ water was from a Milli-Q Water System by Millipore (Bedford, MA). The germanium and yttrium used to prepare the internal standard were SPEX Plasma Standards purchased from SPEX Industries, Inc. (Edison, NJ). The arsenite [As(III)] and arsenate [As(V)] were purchased from SPEX CertiPrep (Metuchen, NJ). Dimethylarsinic

acid [DMA] and disodium methyl arsenate [MMA] were obtained from Chem Services (West Chester, PA). Arsenobetaine [AsB] was obtained from the University of British Columbia, Department of Chemistry (Vancouver, Canada). All standard materials were prepared based on total arsenic and standardized against NIST 1643c obtained from the U.S. Department of Commerce, National Institute of Standards and Technology (Gaithersburg, MD).

Sample preparation and total arsenic digestion

The seaweed samples were freeze dried with a VirTis lyophilizer (Gardiner, NY), followed by homogenization in an Osterizer blender (Milwaukee, WI). The digestion procedure used to determine the $As_{Total\ Digest}$ (Fig. 1) concentration was a modification of US EPA method 200.3 [31] in which 0.5 g of seaweed was digested with conc. HNO_3 and H_2O_2 on a hot plate. Ge and Y were used as internal standards and the interference correction equation in US EPA Method 200.8 [32] was used to correct the reported arsenic concentrations. Samples were analyzed in triplicate.

ASE investigation

The freeze-dried, homogenized seaweed samples were extracted using an ASE 200 system (Dionex, Sunnyvale, CA). A specialized 3 mL ASE cell was utilized for this study so that the amount of dispersion media employed could be minimized. The specialized 3 mL ASE cell has a smaller inside diameter than the typical 11 mL ASE cell but the outside dimensions are the same. The seaweed was mixed with the dispersion media (Empore Filter Aid 400 High Density glass beads from Varian (Harbor City, CA)) to assure good sample/solvent contact and prevent the ASE cell from clogging. Clogging can result from the seaweed's tendency to expand upon hydration. The ribbon kelp sample was used to determine the optimum extraction conditions for the ASE.

Figure 1 outlines a complete extraction cycle on the ASE which consists of the following six steps: 1) the initial filling of the ASE cell with solvent; 2) the application of pressure (500–3000 psi [1 psi = 7 KPa]); 3) a cell heating step (ambient to 200 °C, time minimum is 1 min); 4) a static time (1–99 min); 5) a solvent flush step; and 6) a nitrogen purge to displace residual solvent (20–300 s). If multiple cycles are part of the extraction, then steps 4–5 are repeated. One additional term which is necessary to define when using the ASE is "flush %". "Flush %" defines the amount of solvent to be used by the ASE for extracting a sample. The cell size is necessary in the calculation of the "flush %". All calculations are based on an 11 mL ASE cell size because the instrument cannot differentiate between the specialized 3 mL ASE cell and the standard 11 mL ASE cell. The "flush %" is related to the volume flushed through the ASE cell and can be set from 1 to 150%. The volume flushed per cycle is determined by taking the "flush %" times the size of the ASE cell divided by the number of cycles. For example, if using the 11 mL ASE cell with a 90% vol. flush and 1 cycle, a 9.9 mL flush per cycle is performed, but if using 3 cycles, only a 3.3 mL flush per cycle is used. (This does not include the initial fill volume used in the first cycle).

Three different analytical arsenic determinations were made, $As_{Total\ Digest}$, $As_{Extraction\ Total}$, and $As_{Speciation}$, and are procedurally defined in Fig. 1. The sample extracts from the ASE were evaporated to dryness on a Zymark TurboVap LV evaporator at 50 °C with a nitrogen purge. The residue from the dry extract was then redissolved in 20 g of water, sonicated, vortexed and aliquots were taken for both a total arsenic measurement ($As_{Extraction\ Total}$, Fig. 1) and a speciation based analysis ($As_{Speciation}$, Fig. 1).

Arsenic determinations after ASE extraction

An aliquot of the redissolved extract from the ASE was prepared for $As_{Extraction\ Total}$, (Fig. 1) analysis by diluting with 2% HNO_3 and adding Ge and Y as internal standards. (The $As_{Extraction\ Total}$ samples were not digested.) For arsenic speciation analysis ($As_{Speciation}$, Fig. 1), a redissolved sample was treated with a maxi clean C_{18} cartridge (900 mg) from Alltech Associates, Inc. (Deerfield, IL) and was further diluted with water. The arsenic speciation by ion chromatography (IC) was completed utilizing a Dionex Gradient pump (Model GPM2) which utilizes a pre- and post-column six-way-valve injector. The post-column injection introduces the marker peak standard and the pre-column injection is used for chromato-

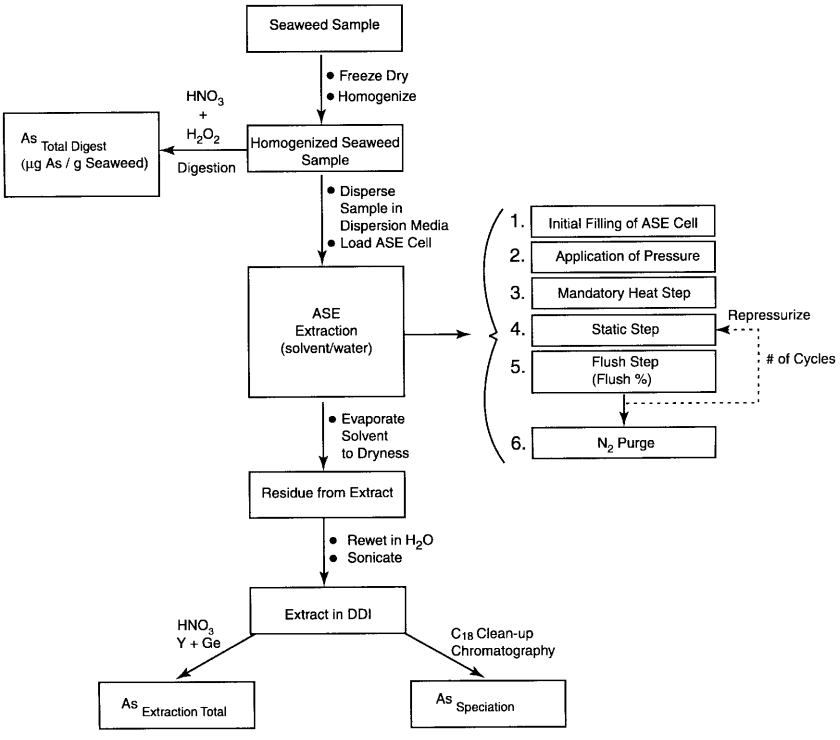


Fig. 1 Outline of sample handling and ASE extraction

graphic separation. The isocratic chromatographic separation conditions consisted of a PRP-X100 column from Hamilton (Reno, NV) and a 20 mM $(NH_4)_2CO_3$, pH 9.0 mobile phase. The flow rate was 1 mL/min with a 100 µL injection loop.

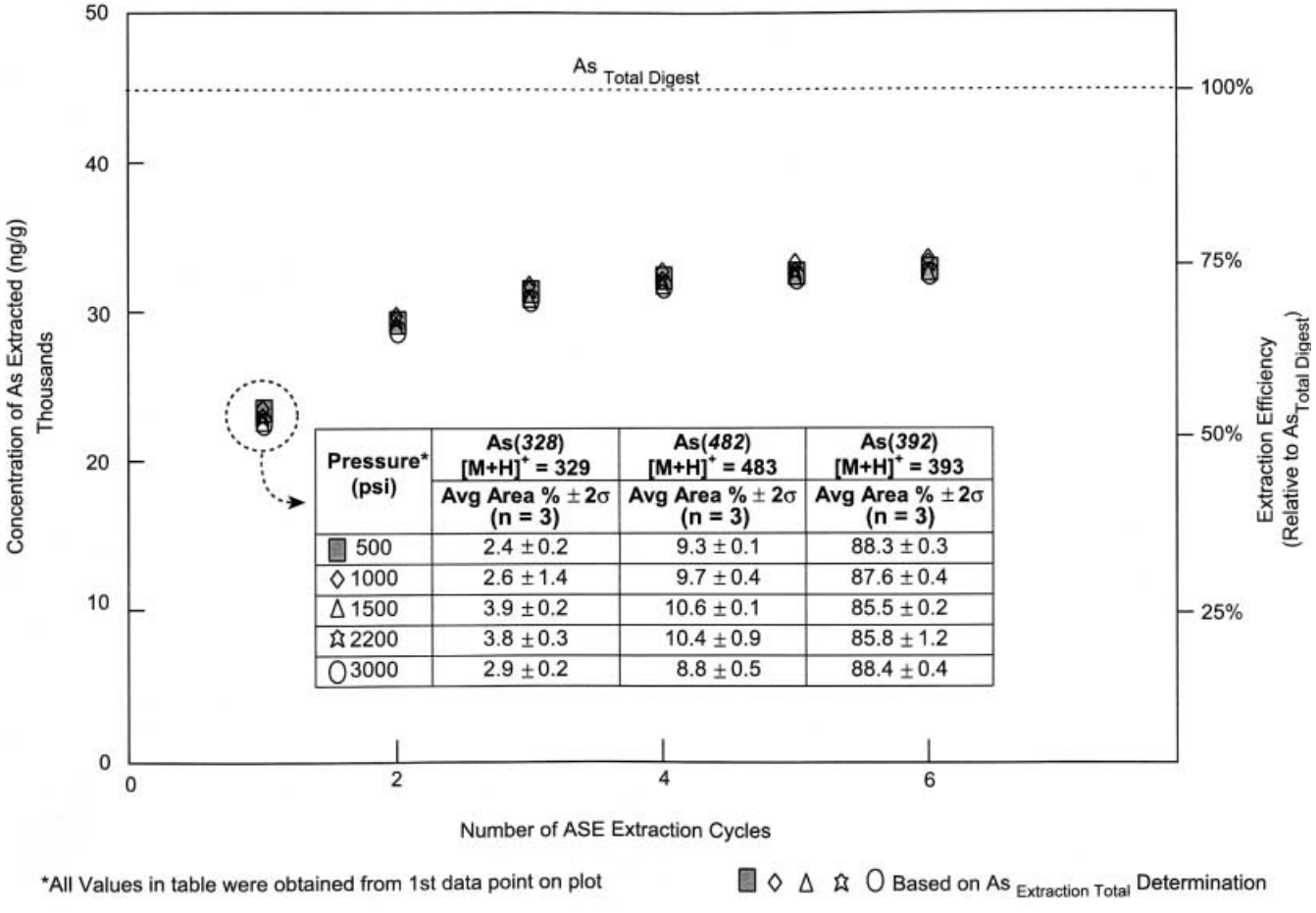
The inductively coupled plasma-mass spectrometry (ICP-MS) used was a Plasma Quad 3 from VG Elemental (Franklin, MA) with the Gilson Sample Changer (Middleton, WI). The flow rates for the plasma, auxiliary, and nebulizer were 13.0 L/min, 0.80 L/min, and 0.68 L/min, respectively, with a forward power of 1350 W. Selected ion monitoring (peak jump) was used for data collection.

Results and discussion

Optimization of ASE method for ribbon kelp

Previously, the analytical capability to extract arsenicals from solid matrices has predominantly depended on methanol/water mixtures in combination with sonication [3–23]. This procedure is tedious and labor intensive. However, the ASE approach lends itself to a semi-automated extraction of arsenicals in solid matrices. Figure 1 provides a procedural summary of the analysis with an emphasis on the sample handling by the ASE. Figures 2–5 demonstrate the effect of pressure, static time, temperature, and solvent composition on the extraction efficiency of As*(328)*, As*(482)*, and As*(392)* (Table 1) from the ribbon kelp using the ASE. Each parameter was optimized by changing one parameter at a time while utilizing standard conditions for the other parameters. The standard conditions initially used for the ASE method were generated using the specialized 3 mL ASE cell, a 5 min static time, 1 cycle, a pressure of 1500 psi, ambient temperature, 30% vol. flush, and a 50/50 (w/w) MeOH/H_2O solvent mixture. Five additional cycles (6 cycles total) were collected using each set of parameters during the optimization process. In order to determine the change in extraction efficiency after each ASE cycle, it was necessary to collect the solvent from each cycle in individual collection vials. These six individual extract collections were monitored so that the extraction efficiency (y axis Figs. 2–5) could be plotted against the number of ASE extraction cycles (x-axis Figs. 2–5) performed on the sample. The extraction efficiency was determined as a percent recovery of the total extracted arsenicals relative to the total arsenic determined via an acid digest ($[As_{Extraction\ Total}/As_{Total\ Digest}] \times 100$; Fig. 1). The extraction efficiency was then plotted as a function of ASE extraction cycles by summing the total

Fig. 2 Extraction efficiency and chromatographic distribution of arsenicals in ribbon kelp as a function of pressure. ■ 500 psi, ◇ 1000 psi, △ 1500 psi, ☆ 2200 psi, ○ 3000 psi (1 psi = 7 KPa). ASE parameters: 3 mL ASE cell, ambient temperature, 1 min heat step, 5 min static step, 1 cycle, 30% vol. flush, 120 s purge, 50/50 (w/w) MeOH/H_2O



Pressure* (psi)	As(*328*) $[M+H]^+$ = 329 Avg Area % ±2σ (n = 3)	As(*482*) $[M+H]^+$ = 483 Avg Area % ±2σ (n = 3)	As(*392*) $[M+H]^+$ = 393 Avg Area % ±2σ (n = 3)
■ 500	2.4 ±0.2	9.3 ±0.1	88.3 ±0.3
◇ 1000	2.6 ±1.4	9.7 ±0.4	87.6 ±0.4
△ 1500	3.9 ±0.2	10.6 ±0.1	85.5 ±0.2
☆ 2200	3.8 ±0.3	10.4 ±0.9	85.8 ±1.2
○ 3000	2.9 ±0.2	8.8 ±0.5	88.4 ±0.4

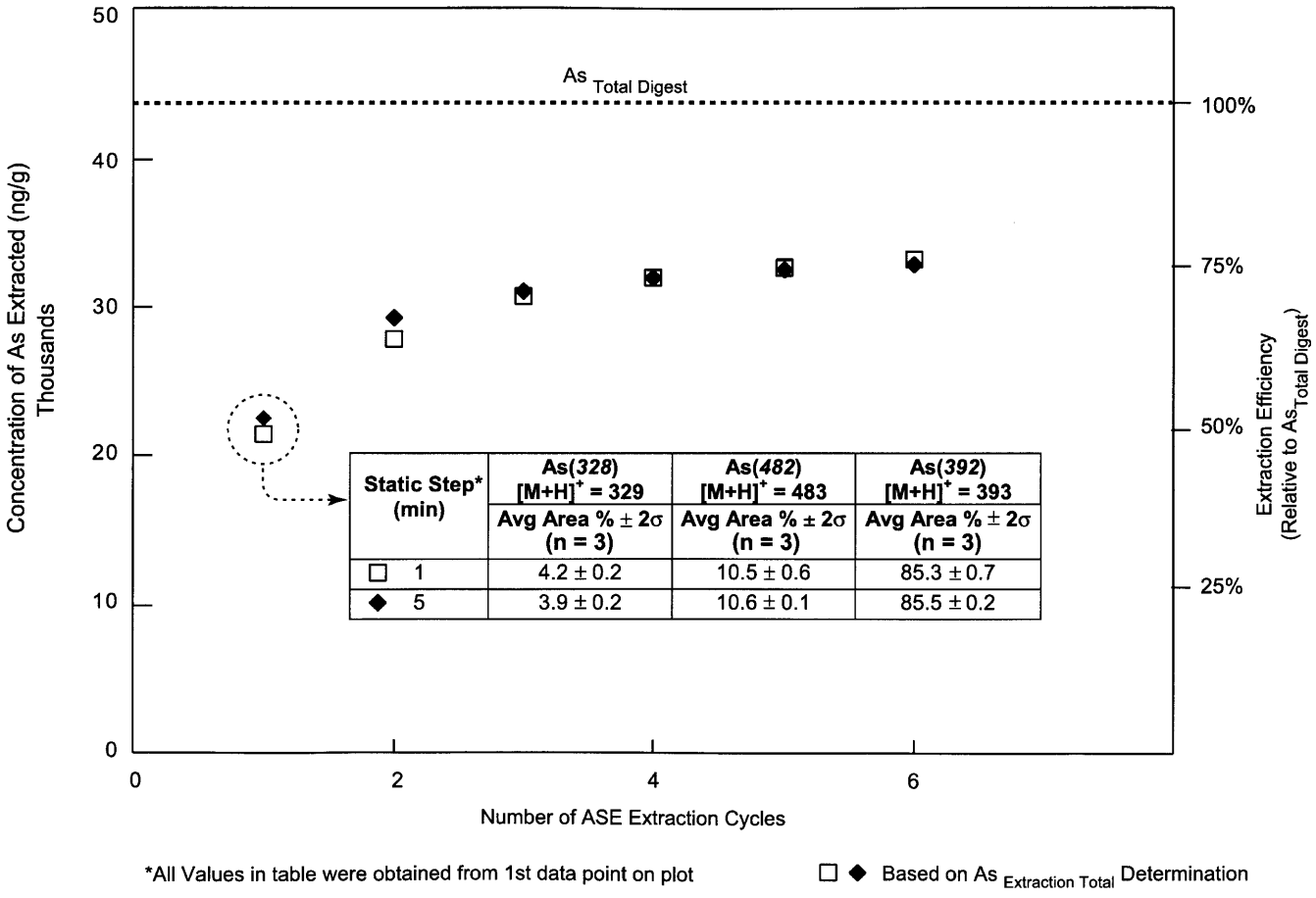
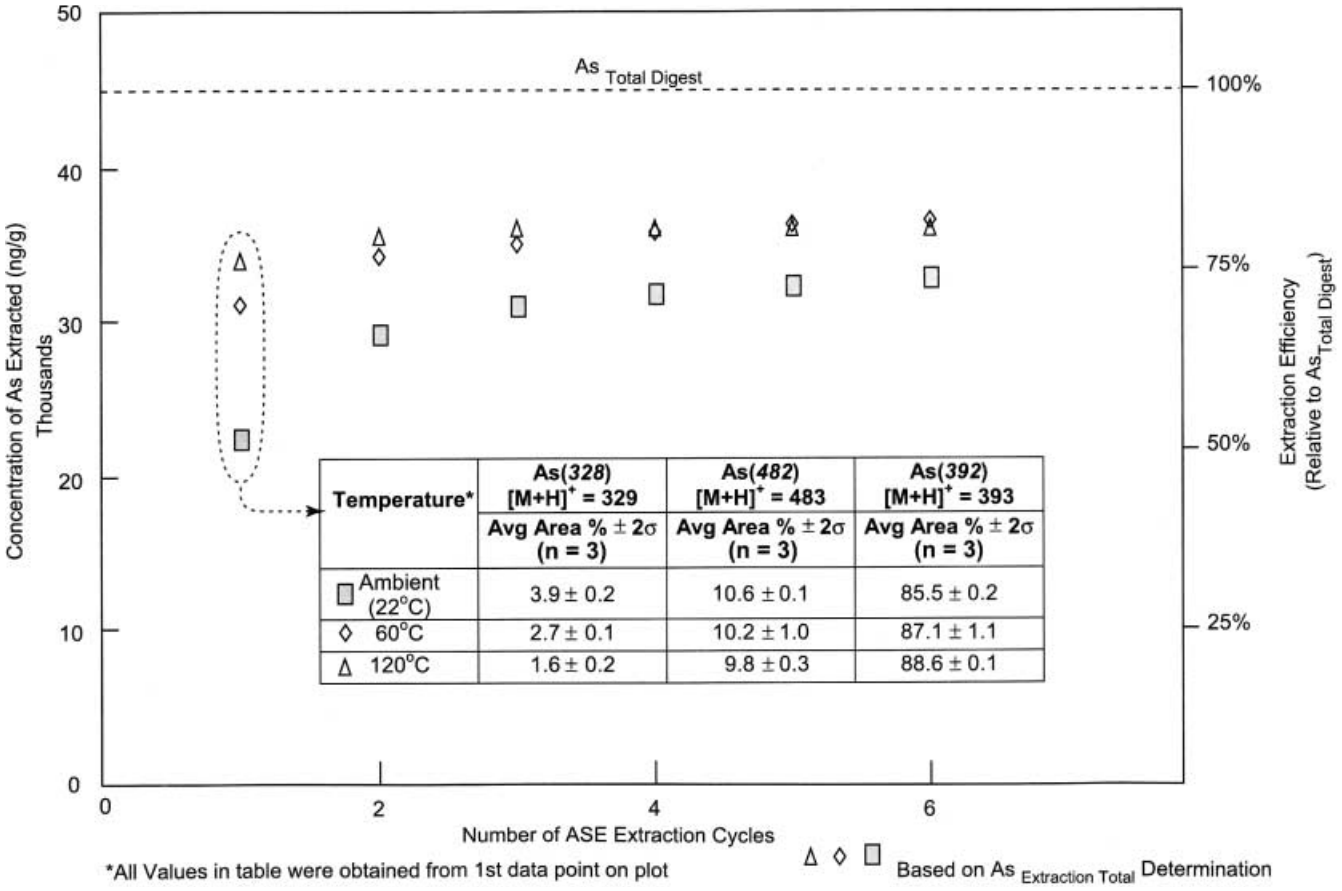


Fig. 3 Extraction efficiency and chromatographic distribution of arsenicals in ribbon kelp as a function of static step. □ 1 min static step, ◆ 5 min static step. ASE parameters: 3 mL ASE cell, 1500 psi, ambient temperature, 1 min heat step, 1 cycle, 30% vol. flush, 120 s purge, 50/50 (w/w) MeOH/H_2O

extracted arsenicals determined in all preceding vials. The area distributions for the three arsenosugars reported in each figure were collected using the isocratic chromatographic conditions outlined in the experimental section.

Figure 2 evaluates the effect of pressure (500–3000 psi) on extraction efficiency of ribbon kelp using a static step of 5 min and a 50/50 (w/w) MeOH/H_2O solvent mixture. The extraction efficiencies for all pressures evaluated in Fig. 2 show a plateau between the 3rd and the 4th ASE extraction cycle. The final 2 ASE extraction cycles extract very little (< 3%) additional arsenic from the ribbon kelp matrix. The extraction efficiency indicates that 25.9% (after 6 cycles) of the $As_{Total\ Digest}$ (Fig. 1) still remains in the extracted ribbon kelp matrix bonded to or sequestered in such a way that pressure alone is ineffective in enhancing its extraction. The table inserted in Fig. 2 summarizes the chromatographic relative area percent of each arsenical for the 1st data point. The table insert indicates that the distribution of arsenicals remains relatively constant for all pressures. Therefore, additional extraction pressure (500–3000 psi) does not assist in solubilizing any additional arsenic species or influences the existing distribution. Figure 2 also indicates that pressure has little effect on the recovery of arsenicals from the ribbon kelp sample. Thus, the use of elevated ASE pressures does not aid in penetrating the matrix and extracting the arsenicals from the ribbon kelp matrix. However, one sample parameter which may influence how pressure affects the extraction efficiency is sample particle size (see discussion below). Other important parameters may include the solvent diffusivity and sample density.

The next ASE parameter which was evaluated is the static step. The static step is the time in which the ASE cell is held at a set temperature and pressure. The minimum value for the static step is 1 min/cycle and a practical upper limit is 5 min/cycle. Figure 3 evaluates the static step as a function of ASE cycles using a 1500 psi pressure and a 50/50 (w/w) MeOH/H_2O solvent mixture. The five minute static step produces slightly (3%) higher extraction efficiencies for the first 2 ASE extraction cycles, but this small difference is eliminated by the 3rd ASE extraction cycle. From an analysis time perspective, it is better to use two 1 min ASE extraction (61.0% extraction efficiency) cycles than a single 5 min ASE extraction (50.1% extraction efficiency) cycle. The table insert in Fig. 3 summaries the chromatographic distribution associated with a 1 and 5 min static step for the 1st ASE extraction cycle. This distribution data indicates that the 5 min static step for the 1st ASE extraction cycle is extracting the same arsenicals as the 1 min static step.



Temperature*	As(*328*) $[M+H]^+$ = 329	As(*482*) $[M+H]^+$ = 483	As(*392*) $[M+H]^+$ = 393
	Avg Area % ± 2σ (n = 3)	Avg Area % ± 2σ (n = 3)	Avg Area % ± 2σ (n = 3)
□ Ambient (22°C)	3.9 ± 0.2	10.6 ± 0.1	85.5 ± 0.2
◇ 60°C	2.7 ± 0.1	10.2 ± 1.0	87.1 ± 1.1
△ 120°C	1.6 ± 0.2	9.8 ± 0.3	88.6 ± 0.1

Fig. 4 Extraction efficiency and chromatographic distribution of arsenicals in ribbon kelp as a function of temperature. ■ Ambient temperature, ◇ 60 °C △ 120 °C. ASE parameters: 3 mL ASE cell, 1500 psi, 1 min heat step, 5 min static step, 1 cycle, 30% vol. flush, 120 s purge, 50/50 (w/w) MeOH/H_2O

Figure 4 evaluates the effect of solvent temperature (ambient to 120 °C) on extraction efficiencies using a 50/50 (w/w) MeOH/H_2O solvent composition, and 1500 psi pressure. Figure 4 indicates that solvent temperature does have a pronounced effect on extraction efficiencies for the arsenosugars in the ribbon kelp sample using the ASE. A solvent temperature change from ambient to 60 °C produces a 19.7% change in the extraction efficiencies for the 1st ASE extraction cycle. Therefore, 1 ASE extraction cycle at 60 °C produces extraction efficiencies which would require 3 or more ASE extraction cycles to achieve the same results at ambient temperature regardless of pressure (Fig. 2) or static time (Fig. 3). This 19.7% difference in extraction efficiency (ambient vs 60 °C) is reduced to 8.9% after 3 ASE extraction cycles and this 8.9% difference remains constant up to 6 ASE extraction cycles. The difference in extraction efficiencies for 60 °C and 120 °C is less than 6.3% for the 1st ASE extraction cycle and by the 3rd ASE extraction cycle the extraction efficiencies associated with 60 °C and 120 °C are within 1.8%. The table insert in Fig. 4 provides the chromatographic distribution of each arsenical for the 1st ASE extraction cycle at ambient, 60 °C and 120 °C. This data indicates that the extraction efficiency of As*(328)* ($[M+H]^+$ = 329) has decreased from 3.9% to 1.6% for an increase in temperature of approximately 100 °C. This distribution change coupled with the unknown thermal stability of the species made an ambient extraction condition the most logical choice. In addition, the 120 °C extracts were much darker in color and produced a dark ring in the C_{18} cartridges used for sample clean up prior to speciation. This dark ring indicated a much higher co-extractant concentration. These co-extractants are generally not problematic when IC-ICP-MS is used as a detector, but these co-extractants do produce chromatograms with much higher total ion current (TIC) near the void volume and can cause detection problems via IC-electrospray ionization-mass spectrometry (ESI-MS).

In an attempt to improve the extraction efficiencies, solvent composition was evaluated as a function of ASE extraction cycles in Fig. 5. The solvent composition, similar to temperature, had a dramatic effect on extraction efficiencies within the 1st ASE extraction cycle. A 90/10 (w/w) MeOH/H_2O mixture extracts 9.7% of the arsenicals while 100% H_2O extracts almost 69.7% of the arsenicals within the 1st ASE extraction cycle. The differences in extraction efficiencies for the other solvent compositions (50/50, 30/70 and 0/100 (w/w) MeOH/H_2O) indicate that after 3 ASE extraction cycles the extraction efficiencies are within 4.9% of each other. In fact, after 2 ASE extraction cycles the 30/70 and 0/100 (w/w) MeOH/H_2O solvent compositions are within 1%. Therefore, the 100% H_2O solvent initially extracts more arsenicals, but the extrac-

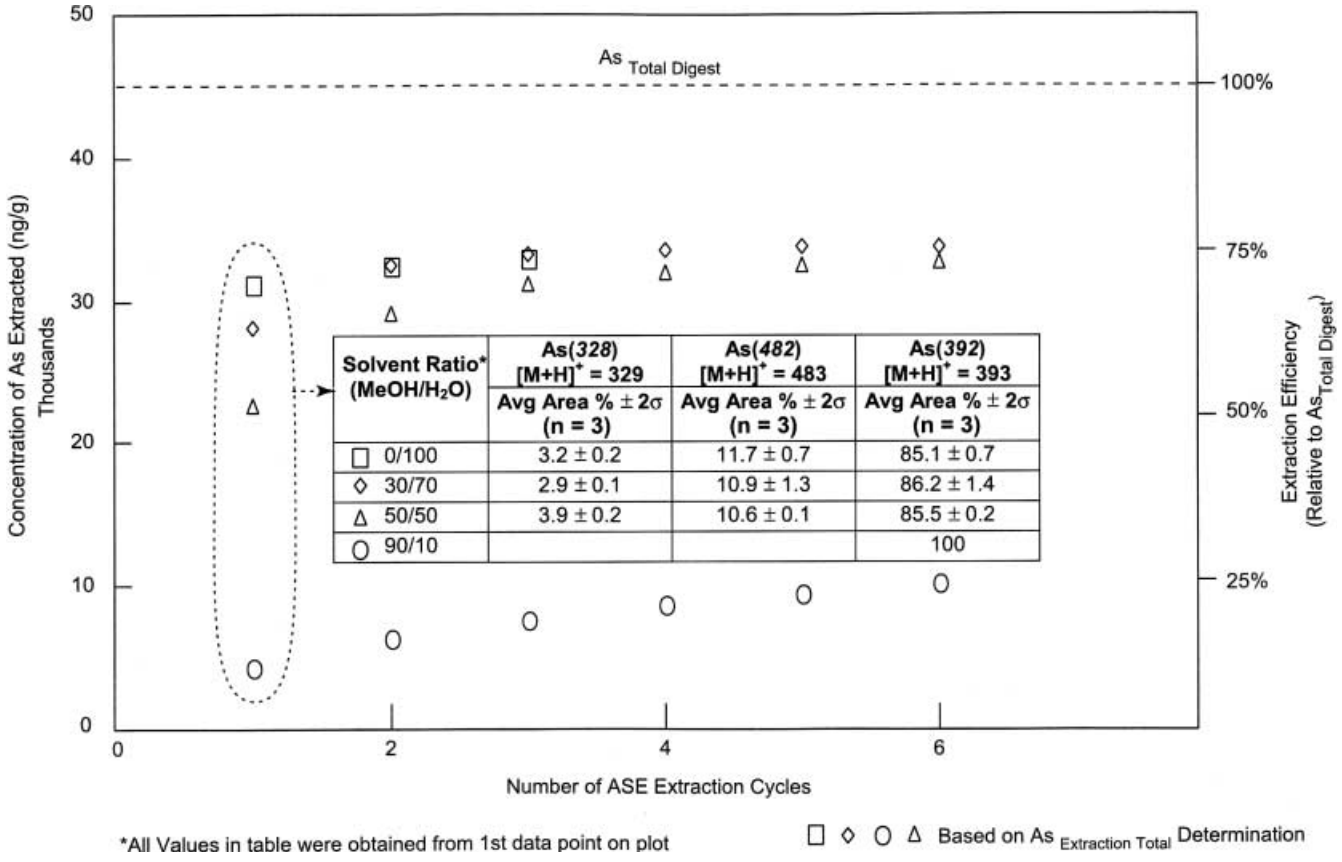


Fig. 5 Extraction efficiency and chromatographic distribution of arsenicals in ribbon kelp as a function of solvent. □ 100% H_2O, ◇ 30/70 (w/w) MeOH/H_2O. △ 50/50 (w/w) MeOH/H_2O, ○ 90/10 (w/w) MeOH/H_2O. ASE parameters: 3 mL ASE cell, 1500 psi, ambient temperature, 1 min heat step, 5 min static step, 1 cycle, 30% vol. flush, 120 s purge

tion efficiency profile generated by additional ASE extraction cycles is relatively flat. A problem which is not apparent in Fig. 5 is that the ribbon kelp has a tendency to swell upon hydration and in doing so produces a blockage within the ASE cell when 100% H_2O is used as the extraction solvent. Due to the blockage problem only three cycles could be completed in 100% H_2O and it limits the amount of ribbon kelp which can be loaded into the ASE cell for extraction. Therefore, a 30/70 (w/w) MeOH/H_2O solvent composition was used and resulted in very little loss as long as 2 or more ASE extraction cycles were collected.

Given that the static time and pressure were optimized with a 50/50 (w/w) MeOH/H_2O extraction solvent, an abbreviated re-evaluation using 30/70 (w/w) MeOH/H_2O was performed using the pressure and static time limits on the ASE. The minimum limits (500 psi, 1 min static step) produced an extraction efficiency of 73.4% after 3 ASE extraction cycles while the maximum limit (3000 psi, 5 min static step) produced an extraction efficiency of 68.7% after 3 ASE extraction cycles. Thus, static time and pressure have little effect on the extraction efficiency using the new 30/70 (w/w) MeOH/H_2O solvent mixture.

Effect of ASE cell size

The 3 mL ASE cell was compared to the 11 mL ASE cell in terms of extraction efficiency to see if sample dispersion within the ASE cell would improve sample/solvent contact. The two cells were compared using a 30/70 (w/w) MeOH/H_2O solvent, 500 psi, ambient temperature, 1 min heat step, 1 min static step, 1 cycle, 30% vol. flush (3 mL cell) and 100% vol. flush (11 mL cell). The arsenical extraction efficiency after the accumulation of 6 ASE extraction cycles was 74.9% for the 3 mL ASE cell and 75.9% for the 11 mL ASE cell. Therefore, the smaller ASE cell was not a hindrance to the extraction efficiency.

Effect of sample particle size

The ASE extraction procedure is a system in which the solvent/sample contact can be controlled by static time, sample dispersion within the cell, sample surface area, and indirectly pressure. The only parameter not evaluated above was surface area/particle size. All the samples above were homogenized by an Osterizer blender which produces a variety of particle sizes. For instance, 75–85% of the ribbon kelp sample (by weight) on average would pass through a 0.500 mm mesh screen while only 50–59% of the ribbon kelp sample (by weight) would pass through a 0.250 mm mesh screen. When the ribbon kelp sample was subjected to a cryogenic grinding process, a much smaller particle size was produced and in this case 100% of the

sample passed through a 0.250 mm mesh screen. Six individual ASE extraction cycles were collected for this study. Improved extraction efficiency was found in the first ASE extraction cycle collected, 59.0% for non-cryogenically ground and 72.4% for the cryogenically ground. However, by the 3rd ASE extraction cycle, the difference in extraction efficiency produced by particle size was reduced to < 2% . The arsenical extraction efficiency (after accumulation of 6 ASE extraction cycles) utilizing the optimum conditions was 77.2% for the seaweed ground in the Osterizer blender and 77.4% for the cryogenically ground seaweed. In addition, the cryogenically ground sample did not alleviate the clogging of the ASE cell and therefore, cryogenic grinding does not improve the overall extraction efficiency of arsenicals from the ribbon kelp.

Combining 3 ASE extraction cycles into 1 collection

Thus far, separate collection vials were utilized for each ASE cycle completed during an ASE extraction. In this configuration, the ASE extraction was repeated six times for each sample in order to monitor the extraction efficiencies produced by the different parameters. For each parameter studied, the majority of arsenicals were extracted in the first three extracts collected. In order to streamline the extraction process, a comparison of an ASE extraction with three cycles (all collected individually) was compared to the accumulation of three ASE extraction cycles (without the N_2 purge between solvent flushes) and combined into one collection vial. The flush volume was increased to a 90% vol. flush when the ASE extraction using three cycles was performed; therefore, each flush step in this ASE extraction was equivalent to the flush step in the ASE extraction with one cycle. The arsenical extraction efficiency utilizing the optimum conditions was 67.9% for the ASE extraction which combined the three cycles and 73.3% for the individual N_2 purge collection mode. The minimal loss due to the ASE extraction using three cycles is most likely due to the fact that the ASE cell was not purged of all solvent between each cycle. Since minimal extraction efficiency was lost due to the combined collection, the ASE extraction using three cycles was used for convenience and automation. Therefore, the final optimum ASE conditions for ribbon kelp using a 3 mL ASE cell were 30/70 (w/w) MeOH/H_2O, 500 psi, ambient temperature, 1 min heat step, 1 min static step, 3 cycles, 90% vol. flush, and a 120 s purge.

Analysis of other varieties of seaweed under the optimized conditions

Two other seaweeds were extracted using the above ASE conditions. Figure 6 contains the IC-ICP-MS chromatograms for these two seaweeds. Figure 6A is an IC-ICP-MS chromatogram of the ASE extract from seaweed A (a seaweed purchased from a local specialty grocery). The extraction efficiency for seaweed A was 25.6% ± 2.8 ($x \pm 2\sigma$, $n = 3$) using the optimized ASE conditions as for ribbon kelp. The arsenicals detected in seaweed A are As*(328)*, As(III), DMA, As*(482)*, As*(392)*, As*(408)*, and As(V) (see Table 1 for structural assignments). The relative area percent for each arsenical is reported in the legend in Fig. 6. Seaweed A has been found to contain 62.5% inorganic arsenic, which correlates with reports from Edmonds et al. [33]. Even with the relatively poor extraction efficiency, the ingestion of 1 g of this seaweed would be equivalent to drinking 350 mL of water at the current interim As MCL (maximum contaminate level) of 0.05 mg/L. Figure 6B is an IC-ICP-MS chromatogram of the ASE extract from a Sargassum Muticum seaweed product harvested from Puget Sound, WA. The extraction efficiency for the Sargassum Muticum was 50.5% ± 1.1 ($x \pm 2\sigma$, $n = 3$) using the optimized ASE conditions as for ribbon kelp. The arsenicals detected in Sargassum Muticum are As*(328)*, DMA, As*(482)*, As*(392)*, As*(408)*, and As(V) (see Table 1 for structural assignments). The inorganic fraction represents 27.8% of the extracted arsenicals and the ingestion of 1 g would be equivalent to the ingestion of 120 mL of water at the 0.05 mg/L As MCL. Whyte et al. found 38% of the arsenic present as inorganic for the same seaweed [34]. The figure insert in Fig. 6B is the ESI-MS/MS spectrum for the As*(408)* peak using the same experimental conditions reported in an earlier paper [18]. The only change in the experimental conditions is the use of a 25 eV collision energy instead of a 30 eV. The MS/MS daughter ions are in good agreement with those reported by Corr et al. [9]. Therefore, the fifth peak has been tentatively identified as As*(408)*, an arsenosugar, using the ESI-MS/MS spectrum. Given the relative area percent for each arsenical, shown in the legend of Fig. 6A and 6B, seaweed can be a source of arsenosugars, DMA, and inorganic arsenic. Recent reports [27, 35, 36] have demonstrated that the ingestion of arsenosugars can produce elevated DMA in urine. Therefore, the arsenosugars undergo a transformation after ingestion which may strongly influence the exposure assessment.

The poor extraction efficiencies of the other varieties of seaweed could be due to the differences in the arsenicals found in the seaweeds which were investigated. Ribbon kelp only contained 3 arsenosugars whereas the other two seaweeds investigated had at least 4 arsenosugars, DMA and As(V). The number of arsenicals present in the Sargassum Muticum and seaweed A samples indicates a potential for misidentification based on retention time alone. This potential problem is only exacerbated by utilizing shorter chromatographic analysis times. The need for detection systems which provide structural information is essential for methods development for an arsenic exposure assessment in a seaweed matrix. Alternatively, the sensitivity and selectivity of ICP-MS detection can be increased via the use of on-line hydride generation [18]. The most toxic forms of arsenic (As(III), As(V), MMA, DMA) are hydride active while highly substituted forms such as the arsenosugars (which complicate the chromatography) are not hydride active. The arsenosugars that were identified in the Sargassum Muticum and Sea-

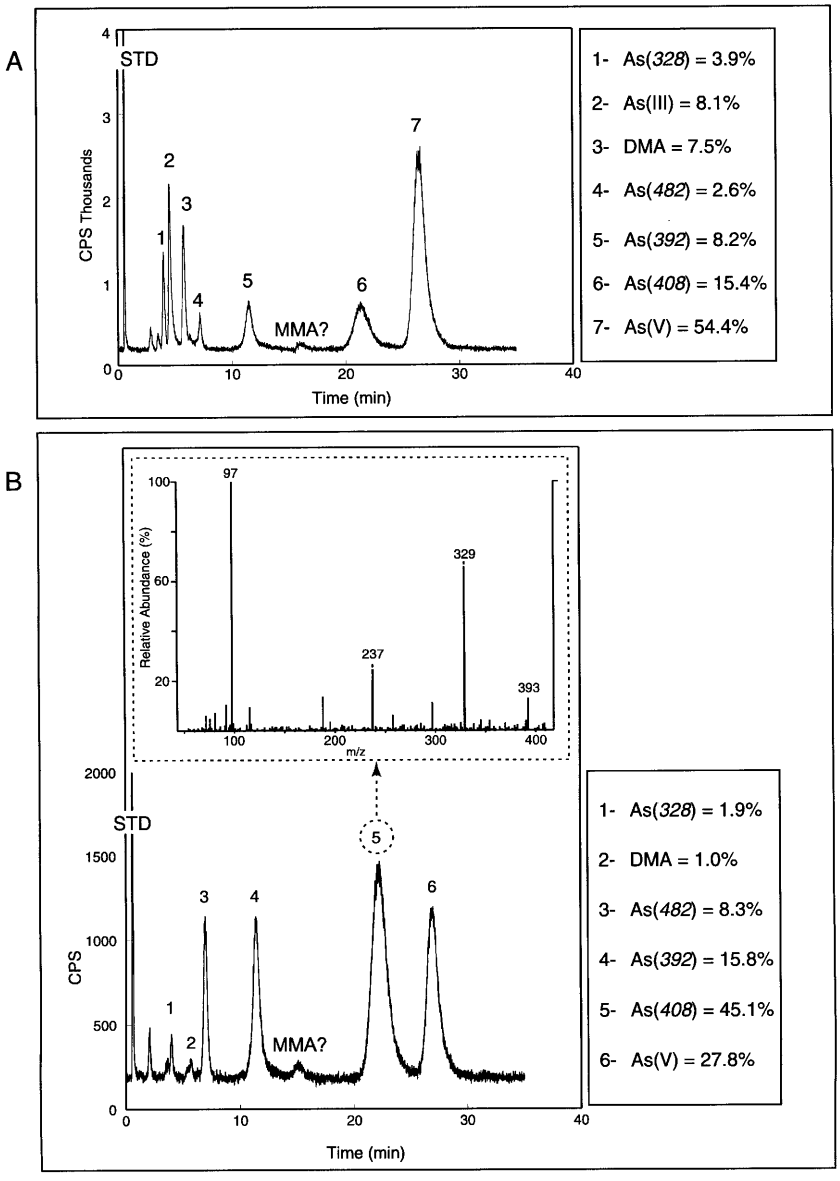


Fig. 6 *(A)* Chromatographic separation of seaweed A. *(B)* Chromatographic separation of Sargassum Muticum from Puget Sound, WA. Chromatographic conditions: PRP-X100 column, isocratic, 20 mM $(NH_4)_2CO_3$, pH 9.0, 1 mL/min. Numbers in parentheses [i.e. As*(328)*] refer to molecular weights established by ESI-MS. The relative area percent for each arsenical is reported in the legend

weed A sample have been shown to be hydride inactive via IC-hydride generation-ICP-MS [18]. Therefore, hydride generation in combination with ICP-MS detection can provide a degree of selectivity which minimizes the required chromatographic resolution in a seaweed matrix.

Conclusion

The ASE parameters which produce the most dramatic increase in extraction efficiency are solvent composition, and solvent temperature. Changes in these parameters resulted in dramatic improvements in extraction efficiencies when the 1st ASE extraction cycle was compared, but by the 3rd ASE extraction cycle these differences were significantly smaller. The pressure, which was initially thought to be an important parameter in ASE, was shown to be the least important parameter for extracting arsenicals from seaweed products. Overall, the ASE provides a semi-automated means of extracting arsenicals from seaweeds, but this initial evaluation indicates that the extraction efficiency asymptotically reaches a maximum at which point the quantitative nature of the extraction is matrix dependent. The authors plan to continue research into possible chemical/enzymatic methods to breakdown the cellulose backbone of the seaweed in the hope of obtaining a more quantitative extraction for all of the seaweed matrices.

Seaweed products can contain significant quantities of inorganic arsenic (~ 17 μg/g, seaweed A) and arsenosugars (~ 8 μg/g, seaweed A). The potential for misidentifying the arsenosugars as inorganic arsenic was minimized by using a 30 min separation and ICP-MS and ESI-MS/MS detection. From this data, seaweed products, although

directly ingested by a relatively small population, can result in an exposure to inorganic arsenic which exceeds the exposure reported for most dietary ingestions of fish. In addition, the presence of arsenosugars which can degrade into DMA after ingestion, also may add to the toxicity of this type of exposure.

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References

1. Gunderson EL (1995) J AOAC Int 78:910–921
2. Gunderson EL (1995) J AOAC Int 78:1353–1363
3. Francesconi K, Gailer J, Edmonds JS, Goessler W, Irgolic KJ (1999) Comparative Biochem Physiol Part C 122:131–137
4. Lagarde F, Amran MB, Leroy MJF, Demesmay C, Olle M, Lamotte A, Muntau H, Michel P, Thomas P, Caroli S, Larsen E, Bonner P, Rauret G, Foulkes M, Howard A, Griepink B, Maier EA (1999) Fresenius J Anal Chem 363:18–22
5. McKiernan JW, Creed JT, Brockhoff CA, Caruso, JA, Lorenzana RM (1999) J Anal At Spectrom 14:607–613
6. Francesconi KA, Goessler W, Panutrakul S, Irgolic KJ (1998) Sci Total Environment 221:139–148
7. Le XC, Li XF, Lai V, Ma M, Yalcin S, Feldmann J (1998) Spectrochim Acta Part B 53: 899–909
8. Larsen EH, Pedersen GA, McLaren JW (1997) J Anal At Spectrom 12:963–968
9. Corr JJ, Larsen EH (1996) J Anal At Spectrom 11:1215–1224
10. Larsen EH (1995) Fresenius J Anal Chem 352:582–588
11. Larsen EH, Pritzi G, Hansen SH (1993) J Anal At Spectrom 8:1075–1084
12. Shibata Y, Morita M (1992) Appl Organometall Chem 6:343–349
13. Beauchemin D, Siu KWM, McLaren JW, Berman SS (1989) J Anal At Spectrom 4:285–289
14. Shibata Y, Morita M (1989) Anal Chem 61:2116–2118
15. McSheehy S, Marcinek M, Chassaigne H, Szpunar J (2000) Anal Chim Acta 410 : 71–84
16. McSheehy S, Szpunar J (2000) J Anal At Spectrom 15:79–87
17. Koch I, Wang L, Ollson CA, Cullen WR, Reimer KJ (2000) Environ Sci Technol 34:22–26
18. Gallagher PA, Wei X, Shoemaker JA, Brockhoff CA, Creed JT (1999) J Anal At Spectrom 14:1829–1834
19. Koch I, Feldman J,Wang L, Andrewes P, Reimer KJ, Cullen WR (1999) Sci Total Environment 236:101–117
20. Lai VWM, Cullen WR, Harrington CF, Reimer KJ (1998) Appl Organometall Chem 12:243–251
21. Lai VWM, Cullen WR, Harrington CF, Reimer KJ (1997) Appl Organometall Chem 11:797–803
22. Le SXC, Cullen WR, Reimer KJ (1994) Environ Sci Technol 28:1598–1604
23. Shibata Y, Jin K, Morita M (1990) Appl Organometall Chem 4:255–260
24. Branch S, Ebdon L, O'Neill P (1994) J Anal At Spectrom 9: 33–37
25. Buldini PL, Cavalli S, Trifro A (1997) J Chromatogr A 789: 529–548
26. Londesborough S, Mattusch J, Wennrich R (1999) Fresenius J Anal Chem 363:577–581
27. Ma M, Le XC (1998) Clin Chem 44:539–550
28. Pergantis SA, Francesconi KA, Goessler W, Thomas-Oates JE (1997) Anal Chem 69:4931–4937
29. Lau BPY, Michalik P, Porter CJ, Krolik S (1987) Biomed Environ Mass Spectrom 14:723–732
30. Lawrence JF, Michalik P, Tam G, Conacher HBS (1986) J Agric Food Chem 34:315–319
31. McDaniel W (1991) Method for the Determination of Metals in Environmental Samples EPA/600/4–91–010:23–29
32. U.S. Environmental Protection Agency, Methods for the Determination of Metals in Environmental Samples EPA/600/R-94/111:200.8–2–200.8–58
33. Edmonds JS, Morita M, Shibata Y (1987) J Chem Soc Perkin Trans I :577–580
34. Whyte JNC, Englar JR (1983) Botanica Marina 26:159–164
35. Le XC, Ma M, Wong NA (1996) Anal Chem 68:4501–4506
36. Wei X, Shoemaker JA, Gallagher PA, Brockhoff CA, Creed JT (1999) "International Ion Chromatography Symposium" San Jose, CA