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Improved Arsenic Speciation Analysis for Extracts of Commercially Available Edible Marine Algae Using HPLC-ES-MS/MS

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Their increasing commercial availability and their rather high total arsenic contents necessitate a more detailed arsenic speciation analysis of marine algal products. Compared to current HPLC-ICPMS methods, HPLC on-line with electrospray tandem mass spectrometry in the selected reaction monitoring mode (HPLC-ES-SRM) offers much higher detection selectivity with similarly high sensitivity for most known organoarsenic species. This study demonstrates the advantages of HPLC-ES-SRM for the detection of the main as well as trace arsenic species in extracts of 12 commercially available marine algal powders. The main focus was the unambiguous identification of the detected arsenic species. Four quality control tools were applied for this purpose, including matching chromatographic retention times, comparing SRM transition ratios, recording product ion spectra, and determining accurate masses. As a result, evidence was obtained for the presence of 19 organoarsenic species in the analyzed algal extracts. The method of standard addition was used for quantification. Estimated matrix effects on the analyte signal were similar for most of the investigated arsenic species in extracts of different types of brown algae. This allowed the comparison of the contents of the arsenic species present in the 12 algal extracts on the basis of normalized peak areas. A partial correlation of the arsenic speciation pattern with the algal family or algal order, respectively, was found.

KEYWORDS: Liquid chromatography; electrospray mass spectrometry; selected reaction monitoring; arsenic speciation; marine algae

INTRODUCTION

Marine macroalgae, commonly known as seaweed or kelp, are advertised by several companies, especially in North America, as food supplements due to their high contents of iodine, minerals, and vitamins. However, the algae also accumulate arsenic from the seawater, which results in relatively high total arsenic contents, typically in the range from 7 to 50 mg/kg of dry weight (*1*). Therefore, regular consumption of marine algal supplements, especially in combination with various other kinds of seafood, results in a rather high daily intake of arsenic (*2*).

It is known that arsenic, which is mainly taken up in the form of arsenate from seawater, is metabolized by the algae to a variety of organoarsenic species, especially arsenosugars (*3*). Detailed toxicological data for all of these arsenic compounds are currently not available, but it is generally accepted that the inorganic forms of arsenic have far greater acute toxicity than the organoarsenic species. However, not a great deal is known about the chronic toxicity resulting from these species. It is therefore apparent that identification and quantification of all the individual arsenic species present in the sample are required as a basis for accurate toxicological assessments. Currently, it cannot be excluded that arsenic species present at even low concentrations (<0.5 μg/g) may be of toxicological relevance. The necessity of a more detailed investigation of the arsenic speciation in edible marine algae is further emphasized by recent results which suggest that algal arsenosugars containing pentavalent arsenic may be reduced in vivo to arsenosugars containing trivalent arsenic. The latter show higher toxicity in vitro than their pentavalent forms (*2*).

During the past 25 years of arsenic speciation in marine algae, several methods have been developed and applied. The pioneering work, which involved preparative isolation by using repeated liquid chromatography and subsequent characterization by nuclear magnetic resonance (NMR) spectroscopy, resulted in the identification and characterization of the first dimethylated arsenosugars (*4*). Currently, the predominantly applied technique for arsenic speciation is high-performance liquid chromatography (HPLC) on-line with inductively coupled plasma mass spectrometry (ICPMS) for element selective detection (*5*−*7*). In addition, HPLC on-line with atomic fluorescence spectrometry was recently applied for the characterization of arsenic species in edible seaweed (*8*). The main limitations of these techniques are the requirement for complete chromatographic separation of all arsenic species present in the sample and the

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Table 1. Literature Review on Arsenic Species in Marine Algae[a]

alga	reported arsenic species[b]	ref[c]
Hizikia fusiforme	arsenite, arsenate, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA)	Tagawa (*15*)
Ecklonia radiata	DMAsSugarGlycol, DMAsSugarSulfonate	Edmonds (*4*)
Ecklonia radiata	DMAsSugarPhosphate	Edmonds (*16*)
Hizikia fusiforme	DMAsSugarSulfate, DMAsSugarAminoSulfonate	Edmonds (*17*)
Sargassum thunbergii	**TMAsSugarSulfate**	Shibata (*18*)
Sargassum lacerifolium	**DMAsSugarMethoxy, DMAsSugarMannitol**, DMArsonioSugarSulfate derivate	Francesconi (*19*)
Laminaria	**TMAsSugarAminoSulfonate**	McSheehy (*12*)
Fucus vesiculosus, *Ascophyllum nodosum*	**DMArsinoyl acetate (DMAA), DMArsinoylethanol (DMAE), DMArsinoyl propionate**	Sloth (*6*)
Ascophyllum nodosum, *Laminaria digitata*, *Fucus vesiculosus*, *Ulva lact.*, *Padina pav.*	**arsenobetaine (AsB)**	Nischwitz (*14*)
kelp powder	DMThioAsSugarGlycol, DMThioAsSugarPhosphate, DMThioAsSugarSulfonate, DMThioAsSugarSulfate	Nischwitz (*20*)

[a] Only the first reports of new compounds in marine algae are considered. Those arsenic species that have never again been reported to be present in marine algae are given in bold type. [b] The abbreviations for the arsenosugars are composed of the following four parts: 1, number of methyl groups bound to the arsenic, DM for dimethyl, TM for trimethyl; 2, only for the new group of thioarsenosugars, Thio; 3, the common core for all arsenoribofuranoside derivatives, AsSugar; 4, the characteristic functional group of the aglycone, e.g., glycol, phosphate, etc. (*13*). [c] Only the first author is mentioned here.

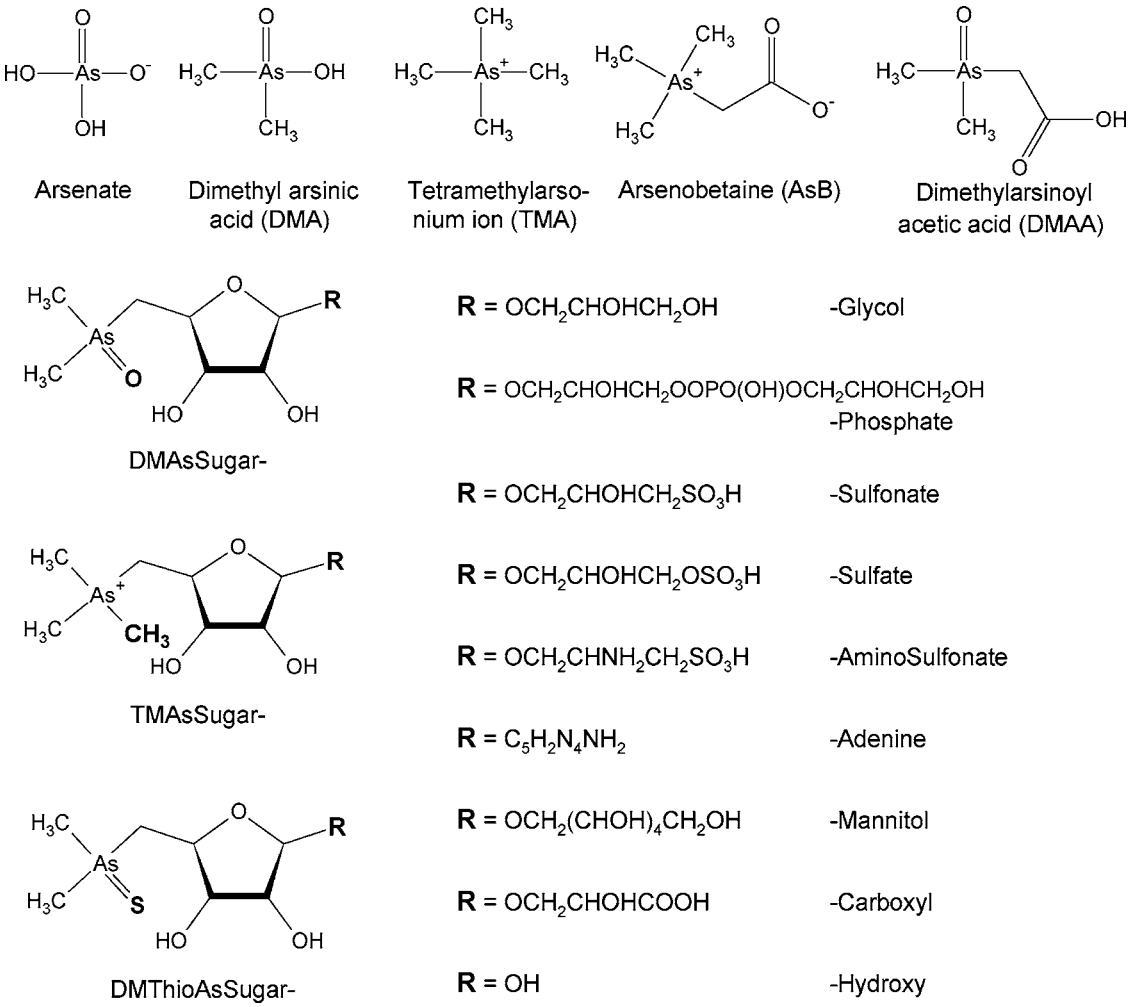

Figure 1. Structures and abbreviations of the arsenic species detected in this study in extracts of marine algae.

need for standards of all detected arsenic species for peak identification. In complex matrices, such as extracts of marine organisms, there is a high risk for peak misidentification due to the possibility of different arsenic species coeluting during HPLC-ICPMS. A recent review on an interlaboratory trial for the determination of arsenic species in algal extracts using HPLC-ICPMS highlights these difficulties and concludes that "there is a wide variation between the results showing that quantitative species determination is still in its beginnings" (*9*). Considering the extensive research efforts on arsenic speciation and the fact that certified reference materials for some arsenic species are currently available, this statement is quite disappointing.

All of this clearly demonstrates that there is a need for a more selective detection method with similarly high sensitivity

Table 2. Type, Origin, Total Arsenic Content, and Extraction Efficiency of Arsenic for the Commercial Algal Samples Analyzed in This Study ($n = 2$)

sample	alga type	origin	total As (mg/kg)	extraction efficiency for As (%)
KE1	"kelp"	Canada?	25.2 ± 1.4	63.2 ± 2.8
KE2	*Ascophyllum nodosum*	United States	29.2 ± 2.0	61.6 ± 3.3
KE3	*Ascophyllum nodosum*	United States	29.4 ± 0.1	61.6 ± 5.3
KE4	"kelp"	Canada?	50.9 ± 7.3	66.3 ± 6.7
KE5	*Ascophyllum nodosum*	United States	32.0 ± 3.5	59.8 ± 4.8
KE6	*Ascophyllum nodosum*	Iceland	30.4 ± 3.7	59.1 ± 5.3
KE7	*Laminaria digitata*	Iceland	58.2 ± 3.3	69.7 ± 2.9
KE8	*Laminaria* spp.	?	62.5 ± 0.5	69.1 ± 3.4
KE9	*Laminaria digitata*	Iceland	78.6 ± 2.4	66.4 ± 5.4
KE10	"granulated kelp"	?	83.0 ± 2.9	70.8 ± 1.9
KE11	*Fucus vesiculosus*	Iceland	28.5 ± 0.3	63.3 ± 2.5
KE12	*Fucus vesiculosus*	United States	31.5 ± 0.8	74 ± 18

compared to ICPMS. Molecule selective detection using electrospray ionization tandem mass spectrometry (ES-MS/MS) allows for the unambiguous identification of organoarsenic species in algal extracts. However, most studies employed scanning tandem mass spectrometric techniques (product ion scan), which exhibit high selectivity (*10*−*12*) but insufficient sensitivity for the detection of trace arsenic species.

In contrast, HPLC-ES-MS/MS in the selected reaction monitoring (SRM) mode combines high selectivity with high sensitivity for the detection of organoarsenic species. Recently, we have developed an anion exchange method and a combined cation−anion exchange method using this detection technique for the identification and quantification of up to 28 arsenic species in a single run (*13*). Extracts of fish and bivalve reference materials were analyzed for method validation. An example of the advantages of this approach compared to HPLC-ICPMS is the detection of arsenobetaine in extracts of commercial and fresh marine algae (*14*). The identification of this well-known arsenic species in marine algae has not been reported in previous studies.

A short review on published arsenic speciation results for marine algae is given in **Table 1**, showing only the first reports of new arsenic compounds in this matrix (most of the corresponding structures are given in **Figure 1** or in ref *13*). The abbreviations used for the arsenosugars are explained in the footnote of **Table 1**. A few arsenic species such as DMAsSugarGlycol, DMAsSugarPhosphate, DMAsSugarSulfonate, DMAsSugarSulfate, and DMA are present at relatively high concentrations in the extracts of most marine algae and therefore have been detected in many studies. However, for several arsenic species present at trace levels the first report remains the only one up to now (see species in bold type in **Table 1**). The reasons for the lack of subsequent identification of some species may be either their resulting as artifacts in the first study or insufficient sensitivity or selectivity of the methods applied in later investigations. The latter may be the case for arsenic species such as TMAsSugarSulfate and DMAsSugarMannitol, which have been identified by preparative isolation and NMR, but not in studies using HPLC-ICPMS. On the other hand, TMAsSugarAminoSulfate may be an example of an artifact of the first report which applied multidimensional liquid chromatography with parallel ICPMS and electrospray MS/MS detection (product ion scan). In this case identification was based on the detection of a molecular ion at *m*/*z* 407 in the positive ion mode and the product ions *m*/*z* 164, 178, 213, and 236 (*12*). The molecular mass of TMAsSugarAminoSulfate is correctly given as 406 in the report and in analogy to the dimethylated arsenosugars a $[M + H]^+$ with *m*/*z* 407. However, the trimethylated arsenosugars have already a permanent positive charge at the arsenic atom, which means that the molecular ion of TMAsSugarAminoSulfate would be expected at $[M]^+$ *m*/*z* 406, and not at *m*/*z* 407.

Currently, there is no method available that is capable of monitoring all of the arsenic species given in **Table 1**. In the present study, we applied the HPLC-ES-SRM approach to extracts of 12 commercially available marine algae to investigate and demonstrate the advantages of this technique compared to HPLC-ICPMS. Our aim was to include far more of the species from **Table 1** in one method than in any previous study on arsenic speciation in marine algae. The main focus was on the unambiguous identification of the detected arsenic species, but also quantification was performed in cases when standards of the analytes were available in sufficient purity and concentration. Several quality control measures were used to check for artifacts.

MATERIALS AND METHODS

Chemicals. Ammonium bicarbonate (puriss.) was obtained from Riedel-de Haen, Seelze, Germany. Ammonium hydroxide solution (puriss., p.a.) and nitric acid (65%, puriss, p.a.) were purchased from Fluka, Buchs, Switzerland. Methanol (gradient grade for HPLC) and acetic acid (100%, GR for analysis) were supplied from Merck, Darmstadt, Germany.

Standards of Arsenic Species. Cacodylic acid (DMA) (puriss., >99%) and sodium arsenate heptahydrate were purchased from Fluka. Monosodium acid methane arsonate (MMA) (sesquihydrate) was obtained from Chem Service, West Chester, PA. A certified reference solution of arsenobetaine (AsB) (BCR-626) was obtained from the Institute for Reference Materials and Measurements, Geel, Belgium.

The majority of the arsenic species were derived from earlier studies in which they were either isolated from marine organisms (DMAsSugarAminoSulfonate, -Carboxyl, -Carbamate, -Glycol, -Methoxy, and -Phosphate) (*16*, *19*) or synthesized [TMAsSugar-Glycol, -Methoxy, -Phosphate, -Sulfonate, and -Sulfate (*21*), DMAsSugarAdenine (*22*), trimethylarsoniopropionate (AsB-2), trimethylarsoniobutyrate (AsB-3) (*23*), arsenocholine (AsC), tetramethylarsonium ion (TMA), and trimethylarsine oxide (TMAO) (*24*)]. DMAsSugarHydroxy and TMAsSugarHydroxy were prepared by acidic hydrolysis of DMAsSugarMethoxy and TMAsSugarPhosphate. Algal extracts were used as source for DMAsSugar-Mannitol, -Sulfonate, -Sulfate, and dimethylarsinoylacetic acid (DMAA). DMThioAsSugars were prepared by reaction of the DMAsSugars with hydrogen sulfide. Details were previously published (*13*).

Algal Samples. Twelve samples of kelp powder (KE1−KE12) were obtained from various suppliers in the United States and Canada. Three algal types, *Ascophyllum*, *Laminaria*, and *Fucus*, were considered from locations in North America and Europe. The algal type and origin of samples KE1, KE4, and KE10 are not known, nor is the origin of KE8. The available information is summarized in **Table 2**.

Instrumentation and Conditions for HPLC-ES-MS/MS. A Surveyor HPLC system with a quarternary gradient pump (including solvent degassing facilities) and an autosampler was coupled to a TSQ Quantum enhanced resolution triple-quadrupole mass spectrometer

(Thermo Finnigan, San Jose, CA) equipped with an electrospray (ES) source. Measurements were performed in the positive ion mode using an ES voltage of 4.1 kV, a sheath gas pressure of 45 arbitrary units, an auxiliary gas pressure of 25 arbitrary units, a capillary temperature of 300 °C, and no source collision-induced dissociation (CID) voltage.

Two previously developed HPLC methods were applied (*13*): Anion exchange chromatography was performed on a PRP-X100 column (250 × 4.1 mm) using two PRP-X800 cation exchange precolumns (Hamilton, Reno, NV). Gradient elution was done with 20 mM NH_4HCO_3, pH 10 (eluent A), and 20 mM NH_4HCO_3, 40% methanol, pH 10 (eluent B) (*13*). For combined cation−anion exchange chromatography the second precolumn of the anion exchange method was substituted by an IonPac CS10 cation exchange column (250 × 4.0 mm) (Dionex, Camberley, Surrey, U.K.). Gradient elution was employed using 10 mM ammonium acetate at pH 3 (adjusted with acetic acid) (A), 20 mM NH_4HCO_3, 10% methanol, pH 10 (B), and deionized water (C) (*13*). In both cases the HPLC flow rate was 1 mL/min, and a postcolumn split was used that reduced the sample flow rate to the ES source to 240 μL/min. The injection volume was 20 μL.

Accurate mass measurements were performed with the anion exchange HPLC method in the selected ion monitoring mode (SIM) using signals of ammoniated polyethyleneglycol (PEG) as lock masses (two replicate analyses for each species). The PEG solution was supplied with a syringe pump via the sheath flow channel at 2.5 or 5 μL/min. The accurate mass of the molecular ions was calculated as the average of at least 30 scans around the peak maximum. In the case of DMAsSugarGlycol and DMAsSugarMannitol a fraction of these species was collected from the separation of an algal extract (KE10) with the combined method, preconcentrated by lyophilization, and redissolved in deionized water prior to injection on the anion exchange system.

Instrumentation and Conditions for HPLC-ICPMS. Anion exchange chromatography was coupled on-line with ICPMS using a Marathon pump (Rigas Labs, Thessaloniki, Greece) with manual injection and an X-Series ICPMS (Thermo Electron Elemental Analysis, Winsford, U.K.) equipped with a concentric nebulizer and an impact bead spray chamber. The PRP-X100 column (250 × 4.1 mm) was operated isocratically with 20 mM NH_4HCO_3 in 3% aqueous methanol, pH 10, at 1 mL/min. The injection volume was 20 μL. Postcolumn addition of 5% HNO_3 containing 10 μg/L In as an internal standard was realized via a T-piece. Two replicate extracts of each sample were analyzed on different days. External calibration was performed with mixed standard solutions of DMAsSugarGlycol, DMA, and DMAsSugarAdenine for quantification of the detected arsenic species. For the second replicate additional calibration with arsenate was done. Mean values were calculated from the results of the three or four calibrations for those species that were not available as standards. Uncertainties of the mean contents were derived by propagation of error from the confidence intervals of the individual results obtained from the linear regression. If the confidence intervals of the replicate results did not overlap, the standard deviation was calculated instead because this is larger in these cases.

Extraction of Algal Samples. Algal powder (0.25 g) was suspended in 5 mL of deionized water and shaken for 30 min. After centrifugation (10 min, 100% speed, Universal II, Hettich, Germany), the supernatant was pipetted into a plastic tube of known weight. The residue was re-extracted once in the same way. The supernatants were combined, and the total extract volume was determined gravimetrically. The extracts were analyzed on the same day using the anion exchange HPLC-ES-MS/MS method and then stored frozen until analysis with the combined HPLC-ES-MS/MS method and HPLC-ICPMS on the following days.

Determination of Total Arsenic Contents with ICPMS. Algal powders (150−200 mg) were digested using 5 mL of concentrated nitric acid in a closed vessel microwave system (MWS-2, Berghof, Germany). After digestion, the samples were diluted with deionized water to a final volume of 50 mL. Arsenic concentrations in the digestion solutions and in the extracts were determined with ICPMS using external calibration with In as the internal standard. The extraction efficiency for arsenic was calculated as percentage ratio of the extracted arsenic content and the total arsenic content (**Table 2**). The Tuna CRM 627 was digested and analyzed in the same way for quality control. The obtained arsenic content of 5.14 ± 0.12 mg/kg ($n = 3$) agrees well with the certified content of 4.8 ± 0.3 mg/kg.

RESULTS AND DISCUSSION

Total Arsenic Contents and Extraction Efficiency. The total arsenic contents determined after microwave-assisted acid digestion of the kelp powder ranged from 25 to 83 mg of As/kg (**Table 2**), which is in agreement with previously published data (*1*, *8*). The applied mild extraction procedure for the determination of the arsenic species using only deionized water as extractant, without sonication, resulted in extraction efficiencies between 59 and 74%. The main focus of this study was the unambiguous identification of the arsenic species in the algal extracts, so preservation of species stability was considered to be more important than maximization of the extraction efficiency. However, the achieved extraction efficiency is satisfactory considering a recent study on arsenic speciation in commercially available algae, which reported a similar extraction efficiency of 63% for a Canadian kelp powder even though 50% aqueous methanol solutions were used in three extraction cycles (*8*). Lai et al. performed five extraction cycles with 50% methanol and 10 min sonication each and calculated an extraction efficiency of 85% for a Canadian kelp powder (*1*).

Conventional Approach: HPLC-ICPMS. A typical anion exchange chromatographic method coupled with ICPMS was used for screening the main arsenic species in the algal extracts. A chromatogram obtained from an extract of a granulated kelp sample (KE10; probably *Laminaria* type) is shown in **Figure 2**. On the basis of the analysis of standards of DMAsSugarGlycol, DMA, and arsenate with HPLC-ICPMS as well as the elution order and major detected species obtained from anion exchange HPLC-ES-MS/MS, the peaks were identified as shown in **Figure 2**. The slopes of the calibration curves obtained for DMAsSugarGlycol, DMA, DMAsSugarAdenine, and arsenate were all in good agreement. These calibration curves were used for the quantification of the arsenic species for which standards were not available. The use of four species for calibration and the similarity of the structures of the calibrants and the analytes justified this approach, which assumes that the ICPMS response for arsenic is independent of the species and that the chromatographic recoveries of calibrants and analytes are the same. The summary of the quantitative results ($n = 2$) is given in **Table 3**.

The obtained results indicate a partial correlation of the arsenic speciation pattern with the algal type. The samples KE1−KE6, which are attributed to the *Ascophyllum* type (family Fucaceae), and KE11 and KE12, which are *Fucus vesiculosus* (family Fucaceae), contain higher amounts of DMAsSugarSulfate than the samples KE7−KE10, which are assigned to the *Laminaria* type (family Laminariaceae). On the contrary, the latter contain more DMAsSugarSulfonate and DMAsSugarPhosphate. Peaks with the same retention time as arsenate were detected only in the *Laminaria* samples. The percentage amount of the quantified arsenic species from the total extracted arsenic content ranged from 76 to 94%.

In previous studies detection of arsenate was reported for algae from the Sargassaceae family [*Hizikia fusiforme* (*25*, *26*) and *Sargassum lacerifolium/piluliferum* (*19*, *27*)] and from the family Laminariaceae [*Laminaria* (*12*, *25*)] but not for species from the Fucaceae family [*Fucus serratus* (*28*)]. These findings agree with our results and indicate that significant differences in the arsenate contents and the arsenate metabolism may occur at the level of the algal family but not on the level of the algal order, because Sargassaceae and Fucaceae both belong to the

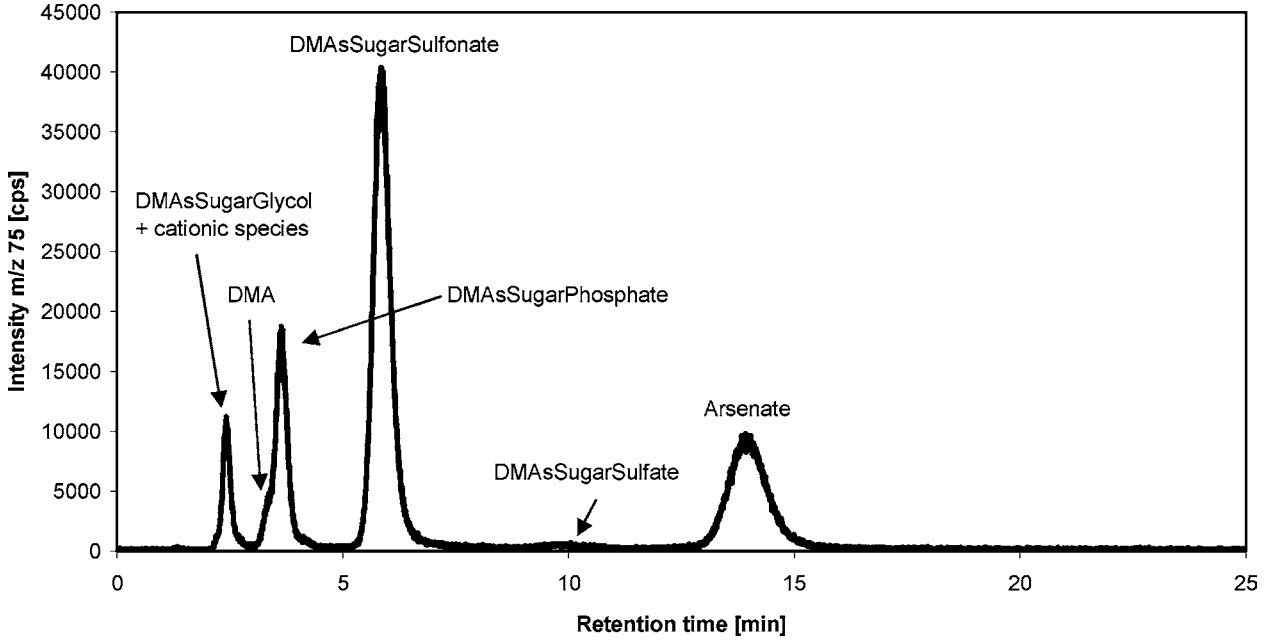

Figure 2. Anion exchange HPLC-ICP-MS chromatogram of an extract of a granulated kelp sample (KE10).

Table 3. Quantitative Results Obtained by Using HPLC-ICPMS for Arsenic Species in Extracts of 12 Marine Algal Samples ($n = 2$)[a]

sample	DMAsSugar-Glycol	DMAsSugar-Phosphate	DMA	DMAsSugar-Sulfonate	DMAsSugar-Sulfate	arsenate	sum of species in % of total extracted As
KE1	4.0 ± 0.5	0.8 ± 0.1	0.4 ± 0.2	1.4 ± 0.1	5.8 ± 0.6	nd[b]	77.3 ± 9.2
KE2	3.9 ± 0.2	0.9 ± 0.1	0.3 ± 0.2	2.3 ± 0.2	7.8 ± 0.6	nd	84.7 ± 7.1
KE3	3.8 ± 0.2	0.9 ± 0.1	0.4 ± 0.2	2.0 ± 0.1	8.4 ± 0.7	nd	85.3 ± 6.1
KE4	5.0 ± 0.2	1.4 ± 0.1	0.6 ± 0.2	12.7 ± 1.0	11.3 ± 0.9	nd	91.9 ± 4.2
KE5	4.3 ± 0.2	0.9 ± 0.1	0.3 ± 0.2	2.3 ± 0.2	8.3 ± 0.7	nd	83.3 ± 4.1
KE6	2.4 ± 0.2	1.4 ± 0.1	0.2 ± 0.2	2.8 ± 0.2	7.8 ± 0.6	nd	80.5 ± 4.3
KE7	3.5 ± 1.2	4.7 ± 0.4	0.5 ± 0.2	20.9 ± 2.1	0.8 ± 0.4	7.1 ± 0.9	92.4 ± 9.4
KE8	2.8 ± 0.4	5.4 ± 0.7	0.3 ± 0.2	18.8 ± 2.3	0.9 ± 0.7	12.0 ± 1.2	93.2 ± 4.2
KE9	3.3 ± 0.2	6.8 ± 1.5	0.3 ± 0.2	21.6 ± 1.8	0.6 ± 0.1	14.9 ± 1.2	91.1 ± 4.3
KE10	3.8 ± 0.2	7.3 ± 0.6	0.6 ± 0.4	27.5 ± 2.1	1.0 ± 0.4	15.2 ± 1.2	94.2 ± 4.4
KE11	2.4 ± 0.3	1.3 ± 0.2	0.2 ± 0.2	2.5 ± 0.4	7.2 ± 0.9	nd	76 ± 15
KE12	4.8 ± 0.2	0.9 ± 0.1	0.3 ± 0.2	3.1 ± 0.2	9.0 ± 0.8	nd	79 ± 15

[a] All contents are given as μg of As/g referring to an extraction with deionized water. [b] Not detected.

order Fucales. Due to the high acute toxicity of arsenate, this is a very important aspect for the use of marine algae for the production of food.

Improved Approach for the Identification of Arsenic Species: HPLC-ES-SRM. Altogether 30 organoarsenic species were targeted in the algal extracts using HPLC-ES-SRM. CID curves and optimized SRM conditions for 28 arsenic species were reported earlier (*13*). In the present study dimethylarsinoyl acetic acid (DMAA) and DMAsSugarMannitol were additionally included. All species were analyzed both with an anion exchange (AE) method and with a combined cation−anion (CM) HPLC method, except for the four DMThioAsSugars, which were considered using only the anion exchange system. Results for only those arsenic species that were either detected in the algal extracts using our analytical method or listed in **Table 1** are discussed in this section. The SRM transitions used for detecting the arsenic species are summarized in **Table 4**. The summed peak areas of both SRM transitions were used with the exception of the calculation of the SRM transition ratios.

The application of the two HPLC-ES-SRM methods for the characterization of organoarsenic species in the algal extracts resulted in peaks for several additional arsenic species other than those identified by using HPLC-ICPMS. As a typical example, the chromatograms obtained with the anion exchange and the combined HPLC-ES-SRM method for a granulated kelp powder extract (KE10; the same sample as in **Figure 2**) are presented in **Figure 3**. However, in complex matrices, such as extracts of biological samples, there is the possibility of artifact peaks, which are generated by matrix compounds having at least one SRM transition in common with an analyte. Because arsenic is monoisotopic, the observed isotopic pattern cannot be used to check if the signal originates from an arsenic-containing compound. In spite of this limitation, the HPLC-ES-MS/MS technique offers several alternative quality control tools, which were exploited here to ensure unambiguous peak identification. For two representative commercial marine algae, KE2 (*Ascophyllum nodosum*) and KE10 (probably *Laminaria*), the application of four quality control instruments for peak identification is demonstrated in the following sections.

The first quality control, in analogy to HPLC-ICPMS, involves matching chromatographic retention times of the analyte peaks obtained from the extracts to those from standard solutions. As shown in **Table 5**, good agreement of the retention times was observed for most species both with the anion exchange and with the combined HPLC-ES-SRM method. Of course, this tool is limited to those compounds that are available

Table 4. Optimized SRM Transitions [Parent Ion → Product Ion (Collision Energy [eV])] for Targeted Determination of Organoarsenic Species

species	SRM 1	SRM 2
TMA	135 → 120 (22)	135 → 105 (22)
AsB	179 → 120 (21)	179 → 105 (30)
AsB-2	193 → 105 (28)	193 → 120 (28)
AsB-3	207 → 87 (20)	207 → 121 (20)
AsC	165 → 121 (23)	165 → 105 (23)
TMAO	137 → 107 (26)	137 → 122 (20)
TMAsSugarGlycol	327 → 193 (34)	327 → 120 (34)
TMAsSugarHydroxy	253 → 193 (25)	253 → 163 (25)
TMAsSugarMethoxy	267 → 75 (26)	267 → 120 (38)
TMAsSugarPhosphate	481 → 327 (30)	481 → 389 (30)
TMAsSugarSulfonate	391 → 235 (26)	391 → 293 (26)
TMAsSugarSulfate	407 → 327 (20)	407 → 193 (40)
DMA	139 → 91 (24)	139 → 109 (24)
DMAA	181 → 119 (15)	181 → 139 (15)
DMAsSugarAdenine	372 → 237 (20)	372 → 178 (28)
DMAsSugarAminoSulfonate	392 → 97 (30)	392 → 295 (25)
DMAsSugarCarboxyl	343 → 97 (28)	343 → 237 (16)
DMAsSugarCarbamate	356 → 237 (15)	356 → 97 (27)
DMAsSugarGlycol	329 → 97 (25)	329 → 237 (15)
DMAsSugarHydroxy	255 → 97 (24)	255 → 195 (24)
DMAsSugarMannitol	419 → 97 (38)	419 → 237 (20)
DMAsSugarMethoxy	269 → 97 (21)	269 → 105 (32)
DMAsSugarPhosphate	483 → 97 (40)	483 → 237 (25)
DMAsSugarSulfonate	393 → 97 (30)	393 → 237 (20)
DMAsSugarSulfate	409 → 329 (15)	409 → 97 (35)
DMThioAsSugarGlycol	345 → 97 (20)	345 → 253 (10)
DMThioAsSugarPhosphate	499 → 253 (15)	499 → 97 (27)
DMThioAsSugarSulfonate	409 → 97 (25)	409 → 253 (15)
DMThioAsSugarSulfate	425 → 97 (30)	425 → 253 (15)
MMA	141 → 91 (30)	

as well-characterized standards. The application of two different chromatographic systems in combination with the high selectivity of the SRM detection provides strong support for the fact that these arsenic species are present in the algal extracts.

The second quality control indicator involves the peak area ratio of the two SRM transitions, monitored for most of the arsenic species (**Table 4**). The ratios were calculated by dividing the peak area of the more abundant transition by the peak area of the transition with lower intensity. The presence of a peak in the chromatograms of both analyte SRM transitions, at the same retention time, with a peak area ratio similar to that obtained for standard solutions, further supports correct peak identification. From **Table 5** it is obvious that in many cases (11 of 29) the ratios obtained for extracts and standards match well (<20% difference, given in bold type), whereas in the remaining cases the mean values differ by 26−156%. The much larger differences of 606 and 1024% for DMAsSugarHydroxy are most likely caused by matrix interferences in the anion exchange system. However, only in a few cases (7 of 29) are the ratios significantly different. The general prerequisite for stable transition ratios are peaks of sufficient intensity for both transitions. This is especially critical for species present at low concentrations, such as TMA and DMAsSugarAdenine, because the peaks of their lower abundant SRM transition are close to the limit of detection and therefore influenced significantly by the background noise. This problem is intensified for species with high ratios; that is, the first transition is much more abundant than the second, such as TMAsSugarSulfate, but less relevant for species with SRM ratios close to 1, such as TMAsSugarSulfonate. For DMAsSugarAdenine it was found that the SRM ratio is significantly higher for standards with low peak areas, which agrees with the results obtained for the algal extracts (Figure B in the Supporting Information and **Table 5B**). The same effect was observed for TMAsSugarPhosphate, but not for TMAsSugarSulfonate, which had no significant difference in ratios at low peak intensities.

As a third quality control tool product ion spectra were recorded using the anion exchange HPLC-ES-MS/MS method. Due to the lower sensitivity of the scanning technique, compared to the SRM mode, mass spectra of the arsenic species were recorded for the extract with the higher content of the analyte, either KE2 or KE10. Spectra with good signal-to-noise ratios were obtained for most of the species (**Figure 4** and Supporting Information). The detection of additional product ions in the mass spectra that were not used in the SRM mode and the possibility of comparing the spectra with previously published data in the literature further confirm the identity of the detected species.

Finally, a fourth tool is available as a result of recent improvements in the ES triple-quadrupole instrumentation. Thus, it is possible to make accurate mass (AM) determinations with the enhanced resolution triple stage quadrupole (TSQ) Quantum AM mass spectrometer. High-accuracy mass determination, which is usually achieved by time-of-flight (TOF) or ion cyclotron resonance mass spectrometers, enables the determination of the elemental composition of the molecular ion (*29*, *30*). The measurements in this study were performed in SIM mode because lock mass signals are more easily accessible in this mode than in the SRM mode. However, the lower selectivity of SIM limits the use of this technique to arsenic species present at high concentrations (DMAsSugarGlycol, DMAsSugarPhosphate, DMAsSugarSulfonate, and DMAsSugarSulfate) or well-separated species such as DMAsSugarMannitol (analyzed after two-dimensional chromatography) and TMAsSugarSulfate. Relative standard deviations of the accurate masses determined from individual scans ($n = 32-60$) range from 0.0012 to 0.0026%. The relative accuracy $\Delta m/m$ of the mass measurements was determined from the difference of the experimental and the calculated mass divided by the calculated mass. Low deviations between 0.4 and 36.2 ppm were found for the experimentally determined masses (**Table 5**). A previous study on accurate mass measurements for organoarsenic species using ES-TOF-MS detection reported differences of 0.3−37.7 ppm for arsenosugar standards (the highest deviation of 55.5 ppm reported for DMAsSugarPhosphate seems to be due to a mistake in the calculated mass of this compound; it should be 15.1 ppm) (*31*). This indicates that the enhanced resolution quadrupole technique with the use of lock masses allows for comparable mass accuracy as TOF-MS without employing lock mass signals.

The use of four quality control tools with HPLC-ES-MS/MS for peak identification provided evidence for the presence of 19 organoarsenic species in the analyzed algal extracts. Depending on their publication history these species are classified into four groups, briefly discussed in the following paragraphs.

The first group comprises the most frequently reported arsenic species in extracts of marine algae: DMAsSugarGlycol, DMAsSugarPhosphate, DMAsSugarSulfonate, DMAsSugarSulfate, and DMA (*4*, *15*−*17*). Although purified standards were not available for some of these compounds, their identification is well supported by the recorded tandem mass spectra and the accurate mass measurements.

The second group covers DMThioAsSugarGlycol, DMThioAsSugarPhosphate, DMThioAsSugarSulfonate, DMThioAsSugarSulfate, and arsenobetaine. The presence of these species in marine algae was recently reported by us for the first time (*14*, *20*). Rather high contents of the four DMThioAsSugars were found in extracts of a Canadian kelp sample (KE4);

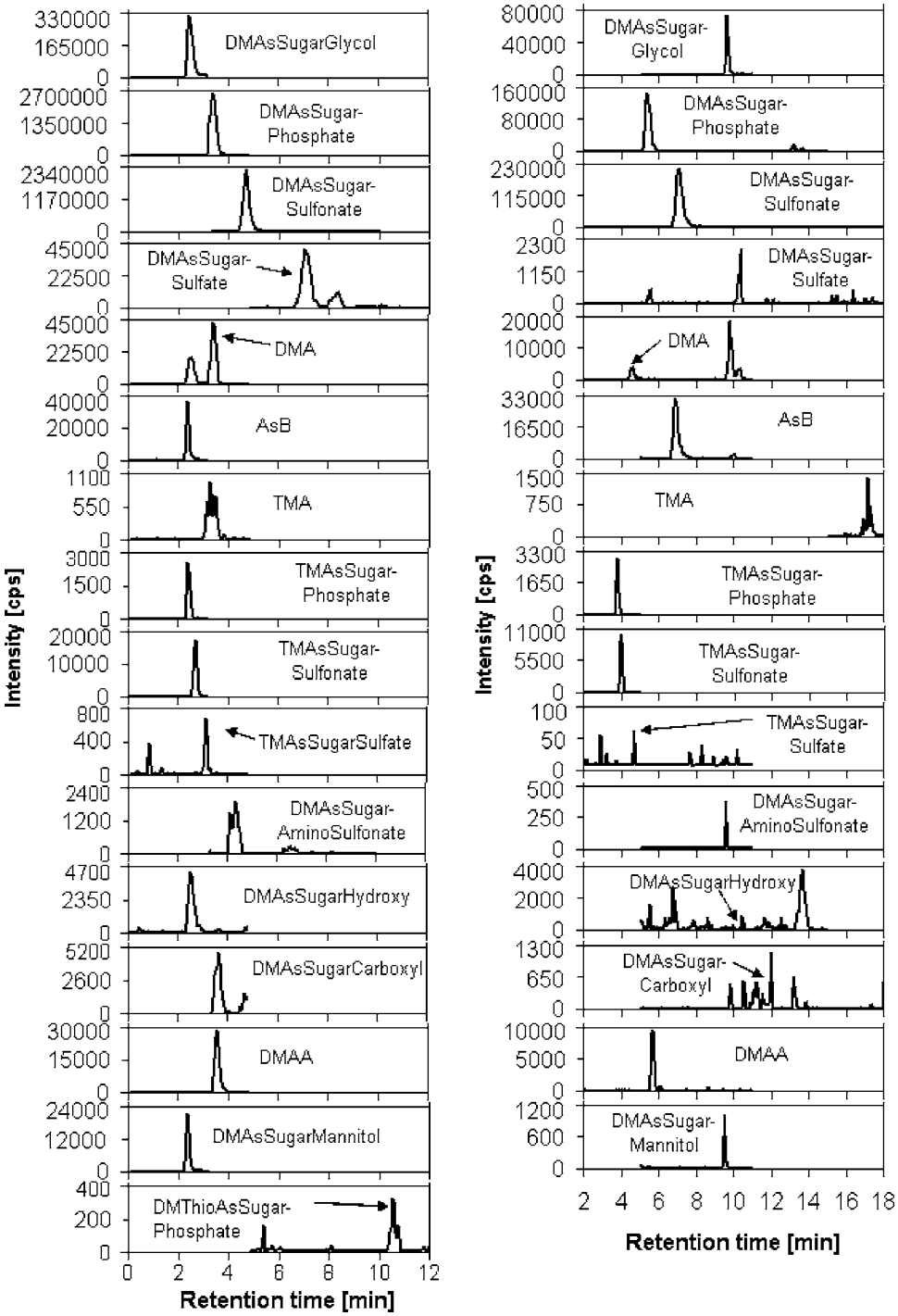

Figure 3. HPLC-ES-SRM chromatograms of an extract of a granulated kelp sample (KE10) obtained with the anion exchange method (left column) and the combined method (right column).

detailed data on the identification of these compounds in the extracts of this sample were already published (*20*). The detection of arsenobetaine has been reported in extracts of commercial and fresh marine algae (*14*).

The third group contains DMAsSugarAminoSulfonate, TMAsSugarSulfate, dimethylarsinoylacetic acid (DMAA), and DMAsSugarMannitol. The detection of these species in marine algae has been reported before (*6*, *17*−*19*), but apart from DMAsSugarAminoSulfonate there is only a single reference on these compounds (**Table 1**). DMAsSugarAminoSulfonate was found only at low intensity in the extracts of *Laminaria* type algae, but the availability of a standard of this compound provided sufficient evidence for its identification (compare **Table 5A** for retention times and SRM ratios). TMAsSugarSulfate was detected at relatively high intensity in the *A. nodosum* extract (KE2), and also in this case a standard was available for comparison of retention times. The product ion 327 has a far higher abundance than all other product ions, which results in less precise SRM ratios and no additional structural information from the product ion spectrum. However, the accurate mass determination further supports the identification of this species. Although standards of TMAsSugarSulfate are available, the detection of this species has not been reported in previous HPLC-ICPMS studies. Only recently has the presence of DMAA in extracts of marine algae been reported (*6*). Currently, we have no standard of this species, but the good quality of the product

Table 5. Identification of Arsenic Species in (**A**) Extracts of a Granulated Kelp Sample (KE10) and in (**B**) Extracts of *A. nodosum* (KE2) Using the Anion Exchange (AE) and the Combined Cation–Anion Exchange (CM) HPLC-ES-SRM Method[a]

	RT AE (min)		RT CM (min)		SRM ratio AE[b]		SRM ratio CM[b]			
species	extract	standard	extract	standard	extract	standard	extract	standard	MS/MS spectrum	accurate mass (g/mol) (Δm, ppm)[c]
(**A**) Extracts of Granulated Kelp Sample (KE10)										
DMAsSugarGlycol	2.61 ± 0.01	2.62 ± 0.02	9.70 ± 0.02	9.69 ± 0.02	**3.1 ± 0.2**	**3.1 ± 0.2**	3.4 ± 0.3	4.9 ± 1.7		329.0617 (11.0)
DMAsSugarPhosphate	3.40 ± 0.01	na[d]	5.44 ± 0.01	na	1.36 ± 0.03	na	1.35 ± 0.08	na	ESI_2[e]	483.0597 (3.3)
DMAsSugarSulfonate	4.60 ± 0.01	na	6.96 ± 0.03	na	2.67 ± 0.04	na	2.68 ± 0.07	na	ESI_3[e]	393.0204 (0.9)
DMAsSugarSulfate	6.74 ± 0.06	na	10.22 ± 0.04	na	3.9 ± 0.2	na	na	na		
DMA	3.41 ± 0.01	3.53 ± 0.01	4.58 ± 0.01	4.59 ± 0.02	1.9 ± 0.1	3.09 ± 0.06	3.8 ± 0.6	2.7 ± 0.2	ESI_5[e]	
AsB	2.51 ± 0.01	2.56 ± 0.02	6.61 ± 0.08	6.9 ± 0.1	**1.8 ± 0.2**	**1.76 ± 0.08**	**1.8 ± 0.2**	**1.79 ± 0.04**	ESI_6[e]	
TMA	3.57 ± 0.06	3.53 ± 0.05	16.79 ± 0.06	17.0 ± 0.1	**1.7 ± 0.7**	**2.1 ± 0.2**	3.0 ± 0.6	2.2 ± 0.1	ESI_7[e]	
TMAsSugarPhosphate	2.54 ± 0.09	2.59 ± 0.02	3.88 ± 0.03	3.78 ± 0.01	9.0 ± 5.5	3.5 ± 0.2	6.9 ± 3.2	4.1 ± 0.6	ESI_8[e]	
TMAsSugarSulfonate	2.87 ± 0.01	2.83 ± 0.04	4.04 ± 0.01	4.02 ± 0.02	**1.1 ± 0.1**	**0.99 ± 0.03**	**1.1 ± 0.1**	**1.1 ± 0.1**	ESI_9[e]	
DMAsSugarAminoSulfonate	4.31 ± 0.06	4.38 ± 0.04	9.72 ± 0.03	9.68 ± 0.02	**1.9 ± 0.7**	**2.1 ± 0.5**	7.0 ± 3.6	4.0 ± 4.2	ESI_12[e]	
DMAsSugarHydroxy	2.57 ± 0.04	2.66 ± 0.02	na	na	24.5 ± 4.1	3.5 ± 0.3	na	na		
DMAsSugarCarboxyl	3.61 ± 0.02	3.69 ± 0.02	11.7 ± 0.3	11.4 ± 0.3	5.6 ± 3.2	2.9 ± 0.1	4.1 ± 4.1	2.97 ± 0.05	ESI_13[e]	
DMAA	3.56 ± 0.04	na	5.50 ± 0.02	na	1.8 ± 0.1	na	1.9 ± 0.2	na	Figure 4a	
DMAsSugarMannitol	2.48 ± 0.01	na	9.59 ± 0.03	na	1.8 ± 0.3	na	7.6 ± 7.2	na	Figure 4b	419.0913 (3.6)
DMThioAsSugarPhosphate	10.2 ± 0.1	na	na	na	2.8 ± 1.9	na	na	na		
(**B**) Extracts of *A. nodosum* (KE2)										
DMAsSugarGlycol	2.65 ± 0.04	2.62 ± 0.02	9.67 ± 0.01	9.69 ± 0.02	**3.13 ± 0.09**	**3.1 ± 0.2**	3.6 ± 0.3	4.9 ± 1.7	ESI_1[e]	
DMAsSugarPhosphate	3.48 ± 0.01	na	5.46 ± 0.03	na	1.34 ± 0.03	na	1.47 ± 0.04	na		
DMAsSugarSulfonate	4.67 ± 0.02	na	7.10 ± 0.04	na	2.80 ± 0.09	na	3.4 ± 0.6	na		
DMAsSugarSulfate	6.88 ± 0.02	na	10.20 ± 0.05	na	3.77 ± 0.02	na	4.4 ± 0.7	na	ESI_4[e]	409.0152 (0.4)
DMA	3.49 ± 0.01	3.53 ± 0.01	4.60 ± 0.03	4.59 ± 0.02	**3.1 ± 0.2**	**3.09 ± 0.06**	3.5 ± 0.7	2.7 ± 0.2		
AsB	2.54 ± 0.02	2.56 ± 0.02	6.70 ± 0.1	6.9 ± 0.1	**1.5 ± 0.2**	**1.76 ± 0.08**	**1.9 ± 0.1**	**1.79 ± 0.04**		
TMAsSugarSulfate	3.22 ± 0.01	3.24 ± 0.03	4.59 ± 0.03	4.49 ± 0.02	87 ± 30	65.2 ± 1.2	228 ± 123	129 ± 47	ESI_10[e]	407.0504 (36.2)
DMAsSugarAdenine	7.03 ± 0.09	6.8 ± 0.2	14.68 ± 0.02	14.44 ± 0.08	7.3 ± 3.1	3.34 ± 0.09	8.1 ± 0.6	3.53 ± 0.08	ESI_11[e]	
DMAsSugarHydroxy	2.6 ± 0.2	2.66 ± 0.02	na	na	39 ± 33	3.5 ± 0.3	na	na		
DMAsSugarCarboxyl	3.69 ± 0.02	3.69 ± 0.02	11.4 ± 0.2	11.4 ± 0.3	5.2 ± 3.8	2.9 ± 0.1	na	2.97 ± 0.05		
DMAA	3.62 ± 0.03	na	5.68 ± 0.04	na	1.8 ± 0.2	na	1.7 ± 1.1	na		
DMAsSugarMannitol	2.53 ± 0.04	na	na	na	1.5 ± 0.4	na	na	na		
DMThioAsSugarGlycol	7.0 ± 0.2	na	na	na	1.5 ± 0.2	na	na	na		
DMThioAsSugarPhosphate	10.1 ± 0.1	na	na	na	2.3 ± 0.8	na	na	na		
DMThioAsSugarSulfate	23.50 ± 0.05	na	na	na	1.4 ± 0.2	na	na	na		

[a] Retention times (RT) and SRM ratios are mean values (± SD) of three replicate extracts. Only one extraction step; no re-extraction of the residue. The concentration of the arsenic species in the mixed standard solution was 25 μg of As/L each. [b] Extract and standard ratios are indicated in bold type if they differ by <20%. [c] Referring to the molecular ion in the positive ion mode ($n = 2$). [d] Not available (either no standard available or no peak detected for at least one of the SRM transitions). [e] Electronic Supporting Information.

ion spectrum (**Figure 4a**), which agrees well with those published by Sloth et al. and McSheehy et al., provided sufficient evidence for the identification of DMAA in the algal extracts (*6*, *31*). The DMAA content in the extracts of the granulated kelp sample could be used for the optimization of the SRM conditions via the compound's CID breakdown curve (Figure A in the Supporting Information). An interesting case is also DMAsSugarMannitol, which has been reported only in a single study 15 years ago. Identification had been performed on the basis of NMR data and the fact that mannitol could be detected following acidic hydrolysis (*19*). A pure standard of DMAsSugarMannitol was also not available in our study. The product ion spectrum of *m*/*z* 419 obtained for an extract of the granulated kelp sample using the anion exchange HPLC-ES-MS/MS method contains signals for *m*/*z* 97, 195, and 237, which are typical product ions of DMAsSugars (**Figure 4b**). The pattern of the CID breakdown curve with a maximum for *m*/*z* 237 at a collision energy of 20 eV and a broad maximum for *m*/*z* 97 at higher collision energies further confirms the presence of a dimethylated arsenosugar (**Figure 5**). Unfortunately, the tandem mass spectrum in the positive ion mode provided no information about the structure of the aglycone, and the sensitivity in the negative ion mode was too low for direct analysis of the algal extract using the anion exchange HPLC method. However, the accurate mass determination after a two-dimensional chromatographic separation agreed within 3.6 ppm with the calculated molecular mass and thus strongly supports the identification of DMAsSugarMannitol. On both HPLC systems DMAsSugarMannitol elutes close to DMAsSugarGlycol, which is expected due to the similarity of the molecular structures. Therefore, an HPLC system that is not optimized for the separation of these compounds in combination with element selective detection (ICPMS) will most likely fail to detect DMAsSugarMannitol due to the far higher content of DMAsSugarGlycol in the algal extracts.

Finally, the fourth group covers five arsenic species that have not been identified in extracts of marine algae before: TMAsSugarPhosphate, TMAsSugarSulfonate, TMA, DMAsSugarCarboxyl, and DMAsSugarAdenine. Standards of all these species were available, and the retention times of the peaks detected in the extracts matched well with those of the standards on both chromatographic systems. All five species were clearly detected in the SRM mode, but due to their low concentrations significant differences in the mean SRM ratios obtained for extracts and standards were observed in some cases. The reasons for this have already been discussed at the beginning of this section (see Figure B in the Supporting Information). The tandem MS spectra show at least the major product ions, which were previously recorded for the standard compounds (see Supporting Information of ref *13*). Considering all of the presented data, the identification of these compounds in the algal extracts is convincing.

Quantification with HPLC-ES-SRM: Standard Addition. In contrast to HPLC-ICPMS, the sensitivity in HPLC-ES-SRM

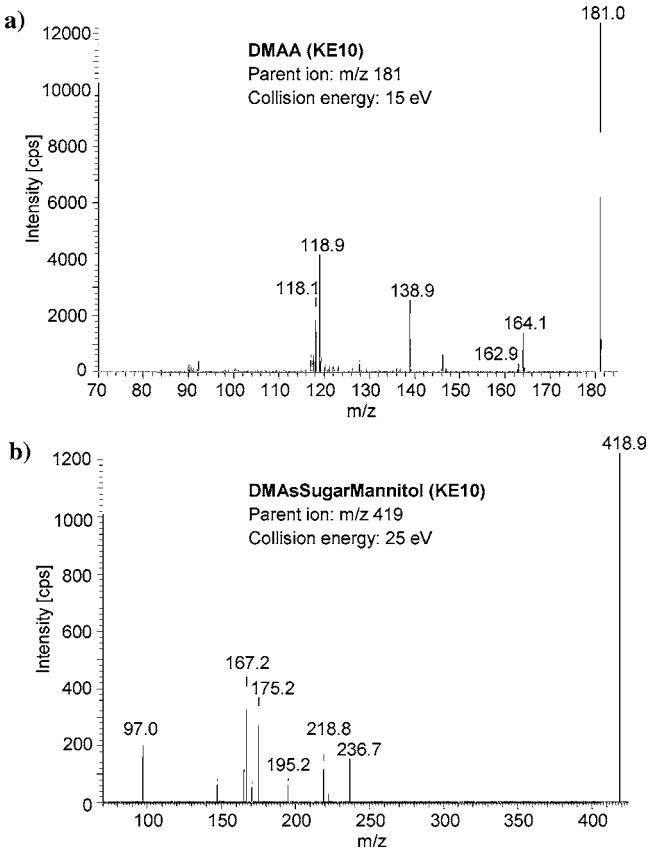

Figure 4. Product ion spectra of dimethylarsinoylacetic acid (DMAA) (**a**) and DMAsSugarMannitol (**b**) obtained with anion exchange HPLC-ES-MS/MS for an extract of a granulated kelp sample (KE10). Product ion spectra of additional species are available as Supporting Information.

is much more species dependent. As a major consequence, accurate quantification is possible only if standards of the analytes are available for calibration. Moreover, coeluting matrix compounds may have a significant influence on the ionization of the analyte in the electrospray process (*13*). Therefore, it is necessary to check for relevant matrix effects on the analyte signal at least for each new type of sample. For this purpose a calibration by standard addition was performed and compared to an external calibration. One sample of each algal type was selected for these investigations ($n = 2$): *Ascophyllum nodosum* (KE2), *Laminaria digitata* (KE7), and *Fucus vesiculosus* (KE11). For 18 selected organoarsenic species the percentage ratios of the slope of the standard addition linear regression and the slope of the external calibration were calculated for each extract. The results are displayed in **Figure 6** for the anion exchange method. Significant signal suppression occurs mainly for the early eluting species such as AsB and DMAsSugar-Glycol. However, for some trimethylated species such as TMAsSugarGlycol and TMA, an enhancement of the signal was observed. Most importantly, the slope ratios obtained from extracts of three different algal types match well for most of the arsenic species. This allows a direct comparison of the arsenic speciation pattern of the algal samples on the basis of peak areas (see next section).

The standard addition method also provided quantitative results for those arsenic species that were available as standards and present at concentrations above the limit of quantification (**Table 6**). On the basis of the HPLC-ICPMS data, the recoveries for both HPLC-ES-SRM methods range from 45 to 111% (mean = 76%, SD = 21%; $n = 6$) for DMAsSugarGlycol and from 35 to 103% (mean = 73%, SD = 30%; $n = 6$) for DMA. In addition to the HPLC-ICPMS results, AsB (*14*), TMAsSugar-Sulfate, TMAsSugarSulfonate, and DMAsSugarAminoSulfonate were also quantified at levels between 0.015 and 0.26 μg of As/g. DMAsSugarAdenine and TMA were found only at levels of $\leq$0.01 μg of As/g. On the basis of the percentage ratios of the contents from both HPLC-ES-SRM methods (smaller content divided by larger content for 15 cases where the species were quantified with both methods), the results match well in 6 cases (ratios between 75 and 100%). Considering the low contents of the species and the use of molecular mass spectrometric detection with high selectivity but matrix effects on the analyte signal, ratios between 50 and 75% (5 cases) are still in good agreement. The remaining 3 cases have ratios between 25 and 50%, and 1 case has a ratio below 25%. Limits of detection (LOD) for aqueous standard solutions were calculated from the standard deviation of blank measurements during the validation of both HPLC-ES-SRM methods (*13*). Because the background intensity in the SRM mode is usually low, the matrix

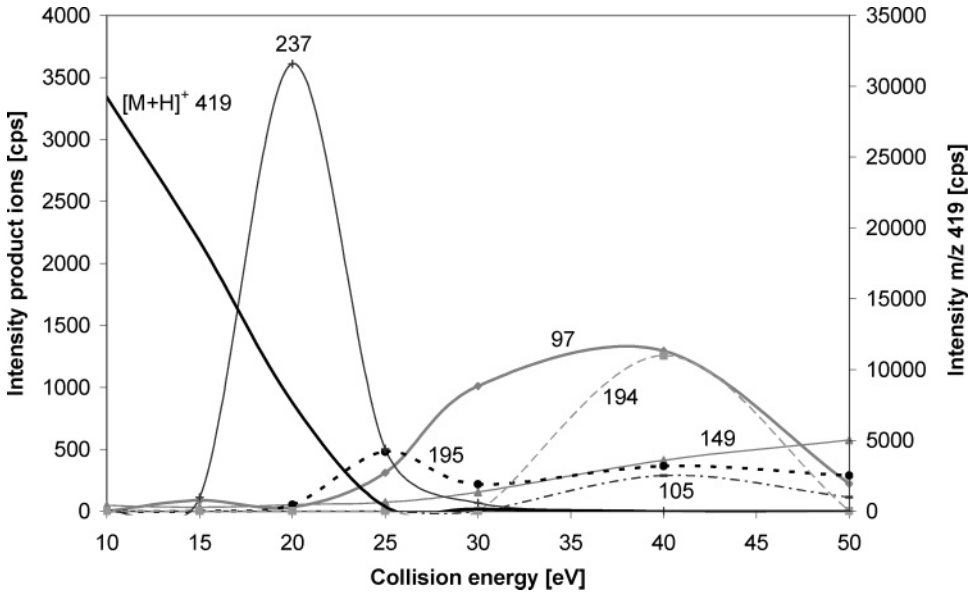

Figure 5. CID breakdown curve for DMAsSugarMannitol derived from product ion spectra at various collision energies recorded with anion exchange HPLC-ES-MS/MS.

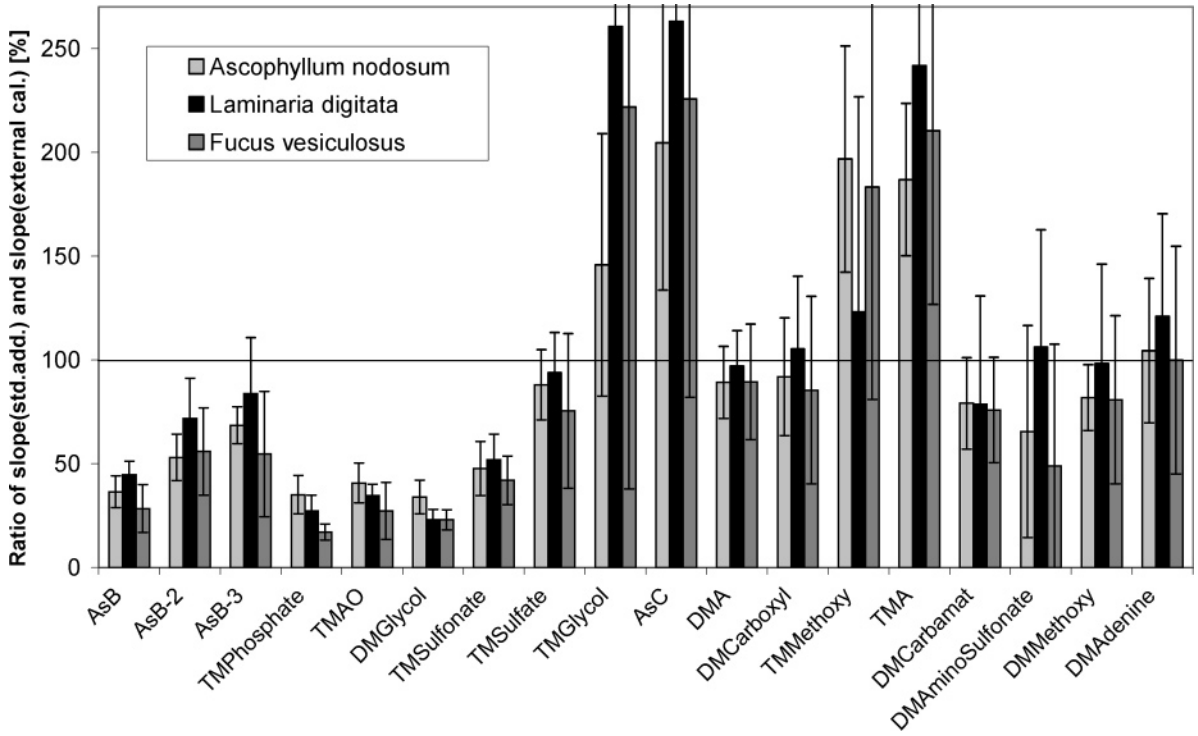

Figure 6. Percentage ratios of the slopes of standard addition linear regressions and the slopes of corresponding external calibration curves for extracts of three different types of brown algae: *A. nodosum* (KE2), *L. digitata* (KE7), and *F. vesiculosus* (KE11).

Table 6. Quantitative Results from both the Anion Exchange HPLC-ES-SRM Method and the Combined HPLC-ES-SRM Method Using Standard Addition for Quantification ($n = 2$)[a]

	A. nodosum (KE2)		*L. digitata* (KE7)		*F. vesiculosus* (KE11)	
species	anion	combined	anion	combined	anion	combined
DMAsSugarGlycol	2.8 ± 0.6	3.0 ± 1.7[b]	2.5 ± 0.7	1.6 ± 0.4[b]	1.9 ± 0.4	2.6 ± 1.3
DMA	0.18 ± 0.07	0.3 ± 0.2	0.24 ± 0.04	0.18 ± 0.07	0.17 ± 0.13	0.16 ± 0.12
AsB	0.03 ± 0.02	0.04 ± 0.02	0.16 ± 0.03	0.17 ± 0.05	0.26 ± 0.12	0.21 ± 0.04
TMAsSugarSulfate	0.05 ± 0.01	0.02 ± 0.05	nd[c]	nd	0.05 ± 0.05	0.002 ± 0.047[b]
TMAsSugarSulfonate	nd	nd	0.03 ± 0.02	0.015 ± 0.018	nd	nd
DMAsSugarAminoSulfonate	nd	nd	0.04 ± 0.02	nd	nd	nd
DMAsSugarAdenine	0.006 ± 0.002	0.002 ± 0.002	nd	nd	0.01 ± 0.01	0.004 ± 0.003
TMA	nd	nd	0.005 ± 0.004	0.009 ± 0.005	nd	nd

[a] All contents are given as μg of As/g referring to the commercial powder without further drying and an extraction with deionized water. [b] $n = 1$. [c] Not detected.

effects influence mainly the signal but not the noise. Therefore, the LODs for the analysis of the algal extracts were estimated by dividing the LOD for aqueous solution by the mean slope ratio (see **Figure 6**) for each arsenic species. The obtained LOD for the extract in micrograms of As per liter was then converted to micrograms of As per gram of algal powder according to the extraction conditions (**Table 7**). The LODs for those species that were available as standards in sufficient amounts were estimated in the range of 1−5 ng of As/g for the anion exchange method. Using the combined method, higher LODs were calculated for some species, mainly due to the lower compatibility of the mobile phase with optimum electrospray conditions.

HPLC-ES-SRM Results for 12 Commercial Algal Samples. As already mentioned, the similarity of the slope ratios (**Figure 6**) for the different algal types enables a comparison of the arsenic speciation pattern in the 12 commercial algal samples without the need for quantification by standard addition. For the five major arsenic species that were quantified by HPLC-ICPMS, the validity of this approach can be checked by linear regression of the peak areas from HPLC-ES-SRM versus the concentrations determined by HPLC-ICPMS. This was done separately for each of the two measuring days (first and second

Table 7. Estimated Limits of Detection (LOD) (Micrograms of As per Gram) for Selected Organoarsenic Species in Commercial Algal Samples Using Anion Exchange or Combined Cation–Anion Exchange HPLC-ES-SRM[a]

species	anion exchange	combined method
TMA	0.001	0.001
AsB	0.002	0.003
TMAsSugarGlycol	0.001	0.004
TMAsSugarPhosphate	0.001	0.001
TMAsSugarSulfonate	0.001	0.001
TMAsSugarSulfate	0.001	0.001
DMA	0.001	0.02
DMAsSugarAdenine	0.001	0.01
DMAsSugarAminoSulfonate	0.005	na[b]
DMAsSugarGlycol	0.003	0.006
DMAsSugarMethoxy	0.001	0.2
MMA	na	0.2

[a] Calculations are based on the standard deviation of blank measurements, conversion to concentration units with an aqueous calibration standard, and correction for matrix effects by the ratio of slopes from standard addition and external calibration. [b] Not available.

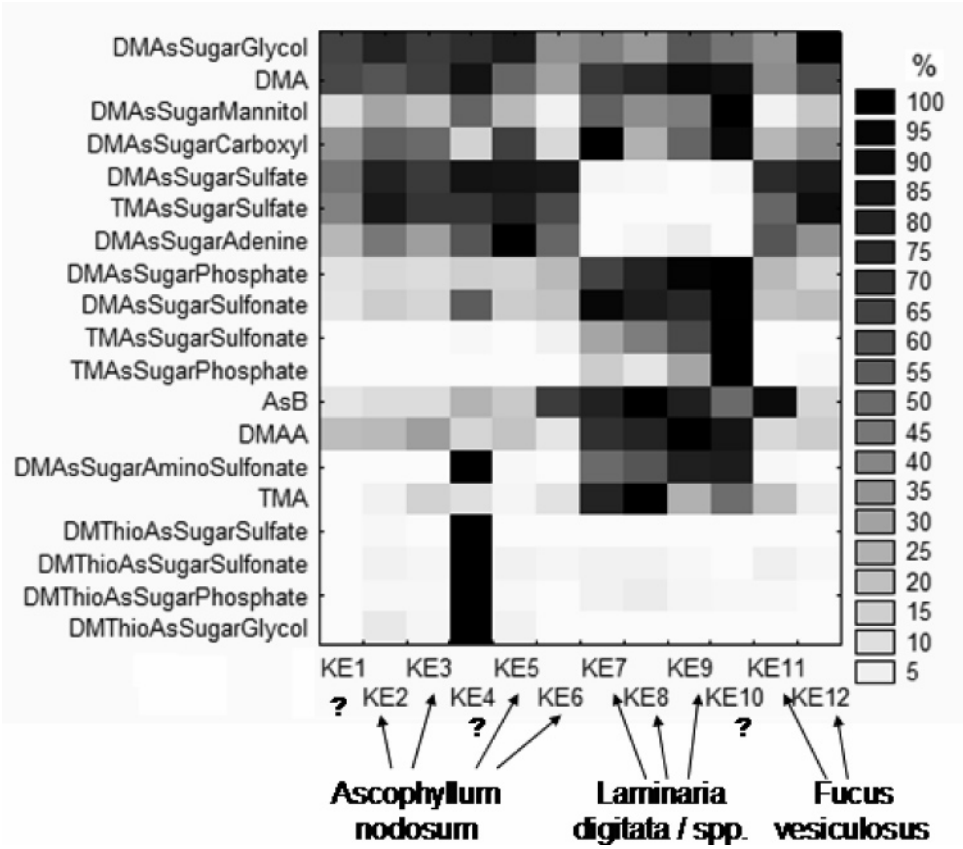

Figure 7. Overview on the relative intensities of the detected arsenic species in extracts of 12 commercial marine algal samples obtained from the anion exchange HPLC-ES-SRM analysis. Peak areas (counts) were first converted to contents (counts/g) according to the extraction conditions and then separately for each arsenic species normalized to the sample with the highest intensity. Mean values of the relative contents were calculated ($n = 2$) with the exception of DMAA and DMAsSugarMannitol ($n = 1$).

replicates) for the anion exchange method and the combined method. Quite good correlation coefficients were obtained for DMAsSugarPhosphate, DMAsSugarSulfonate, and DMAsSugarSulfate under all conditions ($0.883 < R^2 < 0.992$). Correlation coefficients for DMAsSugarGlycol ($0.628 < R^2 < 0.914$) and DMA ($0.457 < R^2 < 0.621$) were lower. This could be explained by the low retention of DMAsSugarGlycol on the anion exchange chromatography, which has a negative effect on both the HPLC-ICPMS and the HPLC-ES-SRM due to coelution with cationic species and matrix compounds. In the case of DMA the lower concentration of this compound and especially the insufficient chromatographic resolution from DMAsSugarPhosphate complicate its quantification by HPLC-ICPMS.

To provide an overview of the contents of the detected arsenic species in the extracts of the 12 analyzed commercial algal powders, a comparison on the basis of the peak areas obtained using the anion exchange HPLC-ES-SRM method was conducted. This approach enables the inclusion of those species that are not available as standards. However, the comparison is limited to the intensity of the same arsenic species in the different samples. A comparison of the contents of different arsenic species of the same sample is not possible with these data. For better visualization the peak areas of each arsenic species were normalized to the sample with the highest intensity, which was set to 100%. Mean values ($n = 2$) obtained from the anion exchange HPLC-ES-SRM method are shown in **Figure 7**. These results indicate that there is a partial correlation between the arsenic speciation pattern and the algal type. For example, DMAsSugarSulfate, TMAsSugarSulfate, and DMAsSugarAdenine were found in much higher intensity in the *Ascophyllum* and *Fucus* samples (both algal species belong to the order Fucales) than in the *Laminaria* samples (order Laminariales). On the other hand, DMAsSugarPhosphate, DMAsSugarSulfonate, and DMAA are present in higher amounts in the *Laminaria* extracts compared to the extracts of other algal types. On the basis of early results on arsenosugar contents in marine algae, Edmonds et al. have suggested a similar chemotaxonomy (*32*). In combination with detailed toxicological data these differences in arsenic speciation patterns may be relevant to the identification of those algal powders that have the lowest arsenic borne risk to human health.

Another aspect of the data in **Figure 7** arises from the comparison of the intensities (peak areas) of DMAsSugarSulfate and TMAsSugarSulfate. For the two data sets obtained from the analyses of the first and second replicates with the anion exchange method correlation coefficients $R^2 = 0.946$ and 0.916 were calculated from the linear regression. This suggests a metabolic link between both compounds. Probably, the trimethylated arsenosugar is synthesized by reduction and methylation of the dimethylated arsenosugar. In analogy, the highest intensities of TMAsSugarSulfonate and TMAsSugarPhosphate were detected in the extracts with the highest contents of the corresponding dimethylated arsenosugars (**Figure 7**). Due to the lower intensities of those TMAsSugars, the correlation is less apparent.

The identification of several arsenic species that have not been detected in marine algal extracts before may help to obtain more detailed knowledge on the metabolism of arsenic in marine algae, which are the first and most important link in the marine arsenic accumulation chain. For example, DMAsSugarAdenine has been proposed as a key intermediate of the biosynthesis of arsenosugars (*32*). The fact that the analyzed samples are

commercially available edible marine algae further illustrates the complexity of a toxicological risk assessment for these algal supplements and for seafood in general. During the production and storage of the commercial algal samples changes in the arsenic speciation pattern may have occurred and contamination of the samples, for example, by epifauna species cannot be excluded. Therefore, the analyses of fresh algal samples will be necessary to check if all of these arsenic species are natural products of arsenic metabolism in marine algae.

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Supporting Information Available: The CID curve of DMAA, a graphical display of the dependence of the SRM ratios on the peak area, and additional product ion spectra of the arsenic species detected in the algal extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

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