JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Survey of Inorganic Arsenic in Marine Animals and Marine Certified Reference Materials by Anion Exchange High-Performance Liquid Chromatography−Inductively Coupled Plasma Mass Spectrometry

JENS J. SLOTH,*,†,‡ ERIK H. LARSEN,‡ AND KÅRE JULSHAMN†

National Institute of Nutrition and Seafood Research (NIFES), Postboks 2029 Nordnes, N-5817 Bergen, Norway, and Danish Institute for Food and Veterinary Research (DFVF), Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

A method for the determination of inorganic arsenic in seafood samples using high-performance liquid chromatography−inductively coupled plasma mass spectrometry is described. The principle of the method relied on microwave-assisted alkaline dissolution of the sample, which at the same time oxidized arsenite [As(III)] to arsenate [As(V)], whereby inorganic arsenic could be determined as the single species As(V). Anion exchange chromatography using isocratic elution with aqueous ammonium carbonate as the mobile phase was used for the separation of As(V) from other coextracted organoarsenic compounds, including arsenobetaine. The stability of organoarsenic compounds during the sample pretreatment was investigated, and no degradation/conversion to inorganic arsenic was detected. The method was employed for the determination of inorganic arsenic in a variety of seafood samples including fish, crustaceans, bivalves, and marine mammals as well as a range of marine certified reference materials, and the results were compared to values published in the literature. For fish and marine mammals, the results were in most cases below the limit of detection. For other sample types, inorganic arsenic concentrations up to 0.060 mg kg^{-1} were found. In all samples, the inorganic arsenic content constituted less than 1% of the total arsenic content.

KEYWORDS: Inorganic arsenic; seafood samples; HPLC−ICPMS; alkaline dissolution; certified reference materials

INTRODUCTION

Typical concentrations of total arsenic in marine organisms are in the range of 1−100 mg kg^{-1} (fresh weight) (*1*), whereas samples of terrestrial origin including freshwater fish usually contain lower concentrations than their marine counterparts (*2*). A variety of arsenic species found in marine samples are listed in **Table 1**. Arsenobetaine (AB) is usually the predominant species in fish, bivalves, and crustaceans, whereas arsenosugars are the major species in algae (*3*−*5*). Other arsenic species, including the inorganic species As(III) and As(V), usually only contribute with a few percent (*6*−*8*). In the context of human health risk assessment, inorganic arsenic may lead to serious adverse effects, including cancer. This applies to acute toxicity as well as to long-term effects resulting from low dose exposure (*9*). As illustrated by the acute toxicity LD_{50} values in **Table 2**, the inorganic arsenic species, As(III) and As(V), are the most toxic species followed by tetramethylarsonium-ion (TETRA), methyl arsonate (MA), and sodium dimethylarsinate (DMA), whereas the trimethylated forms [e.g., AB and arsenocholine (AC)] are virtually nontoxic. Inorganic arsenic has been proposed to bind to thiol groups of biologically active proteins, thus inhibiting the function of various metabolic enzymes (*10*). Seafood is the main source of arsenic in the diet (*11*−*13*), and consequently, accurate data for inorganic arsenic in these sample types are important to make a correct dietary risk assessment analysis. In 1988, the World Health Organization established a provisional tolerable weekly intake (PTWI) for longer term exposure to inorganic arsenic, being a recognized human carcinogen, at 15 μg kg^{-1} body weight (*14*). The vast majority of the consumers are not in danger of exceeding the PTWI value from consumption of seafood (*13*). Well-known exceptions are cases of high concentrations of inorganic arsenic in the drinking water, which is the case in certain areas throughout the world (*15*, *16*).

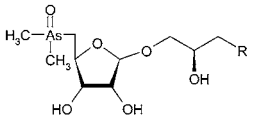
A number of sample preparation approaches have been applied for the analysis of inorganic arsenic. Commonly reported is solvent extraction using water or methanol or mixtures thereof, followed by high-performance liquid chromatography (HPLC) separation of inorganic arsenic from other arsenic species and determination by, e.g., inductively coupled plasma mass spectrometry (ICPMS) (*17*−*20*). Although these solvent extraction

* To whom correspondence should be addressed. E-mail: jjs@dfvf.dk.
† National Institute of Nutrition and Seafood Research (NIFES).
‡ Danish Institute for Food and Veterinary Research (DFVF).

10.1021/jf047950e CCC: $30.25
Published on Web 06/30/2005

Table 1. Examples of Arsenic Compounds Occurring in the Marine Environment[a]

Acronym	Arsenic species	Formula
As(V)	Arsenite	$O=As(O^-)_3$
As(III)	Arsenate	$As(O^-)_3$
MA	Methylarsonate	$CH_3AsO(O^-)_2$
DMA	Dimethylarsinate	$(CH_3)_2AsO(O^-)$
AB	Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
TMAO	Trimethylarsine oxide	$(CH_3)_3AsO$
AC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2OH$
TETRA	Tetramethylarsonium-ion	$(CH_3)_4As^+$
DMAA	Dimethylarsinoylacetate	$(CH_3)_2As(O)CH_2COO^-$
TMAP	Trimethylarsoniopropionate	$(CH_3)_3As^+CH_2CH_2COO^-$



Arsenosugar **1** (glycerol sugar)	R = OH
Arsenosugar **2** (phosphate sugar)	$R = OP(O)(O^-)OCH_2CH(OH)CH_2OH$
Arsenosugar **3** (sulphonate sugar)	$R = SO_3^-$
Arsenosugar **4** (sulphate sugar)	$R = OSO_3^-$

[a] For simplicity, the compounds are depicted in their fully deprotonated form. Nomenclature is as proposed in a recent review by Francesconi and Kuehnelt (*37*).

Table 2. LD_{50} Values in Mice and Rats for Some Arsenic Species (*7*, *38*, *39*)

	LD_{50} values (mg/kg)
As(III)	14–42
As(V)	20–800
MA	700–1800
DMA	700–2600
TETRA	900
AC	6500
AB	>10000
TMAO	10600

systems have been reported to extract arsenic from fish tissue quantitatively, the procedure is often time-consuming, and the extraction of arsenic species from certain sample types (e.g., bivalves) may be less efficient (*21*). Incomplete extraction of As(III) from seafood matrices using methanol was reported by Munoz et al. (*6*), who found that 85% of As(III) added to a cockle sample was retained in the residue after extraction with methanol and when using methanol/chloroform−water only 31% of added As(III) was recovered in the extract. These results suggest that the thiol groups in the proteins with an affinity to As(III) remain active during the extraction procedures investigated; therefore, quantitative extraction of As(III) was not achievable. More efficient reagents are necessary to dissociate and dissolve As(III) in a solvent.

Several approaches involving hydrolysis with a strong hydrochloric acid solution for the release of inorganic arsenic have been reported. By this procedure, $AsCl_3$ is produced, which subsequently may be extracted (*6*, *22*−*24*) with organic solvents or collected by distillation (*25*, *26*) followed by determination of inorganic arsenic content as total arsenic by, e.g., HG-AAS. However, a potential error associated with these procedures is the coextraction of MA, DMA, and trimethylarsine oxide (TMAO) by chloroform and, consequently, overestimation of the inorganic arsenic (*6*).

Recently, Larsen et al. (*27*) reported the use of microwave-assisted alkaline sample dissolution and gradient elution anion exchange HPLC-ICPMS for the detection of inorganic arsenic in samples of salmon, plaice, and mackerel. Spike experiments with As(III) gave quantitative recovery as As(V) in plaice and salmon; however, in mackerel, the recovery of As(III) was incomplete and this was attributed to the high content of lipid in mackerel. The methodology was thus reported to be restricted to lean white fish.

Table 3. Instrumental Settings Used in This Work

ICPMS settings	
RF power (W)	1600
carrier gas flow (L min^{-1})	1.15–1.25
plasma gas flow (L min^{-1})	15
auxiliary gas flow (L min^{-1})	1
nebulizer	Meinhard concentric
spray chamber	water-cooled double pass
spray chamber temp (°C)	2
interface cones	platinum
lens voltage (V)	2–3
mass resolution (u)	0.8
integration time (ms)	1000
HPLC settings	
injection volume (μL)	25
operating pressure (bar)	45–50
mobile phase concn [mmol L^{-1} $(NH_4)_2CO_3$]	50
mobile phase pH	10.3
mobile phase flow (mL min^{-1})	1

In the present study, the same sample preparation approach was applied. The aim was to further simplify the HPLC methodology for faster analyses and additionally to document the stability of naturally occurring organoarsenic compounds when subjected to this sample preparation procedure. Furthermore, the scope was to report data for inorganic arsenic in a range of different seafood samples including fish, crustaceans, bivalves, and marine mammals. Finally, because no certified reference materials (CRMs) exist for inorganic arsenic, the paper aims at discussing the existing literature values in comparison with results produced in the present study.

MATERIALS AND METHODS

Instrumentation. For the analysis of the total arsenic content, the lyophilized seafood samples were digested using an Ethos Pro microwave system (Milestone, Holger Teknologi, Oslo, Norway). An Agilent quadrupole ICPMS 7500c instrument (Yokogawa Analytical Systems Inc., Tokyo, Japan), which was used as an arsenic specific detector, was run in the standard mode. The sample introduction system consisted of an ASX-500 Auto sampler (CETAC Technologies, Omaha, NE), a peristaltic pump, a concentric nebulizer (CPI International, Amsterdam, The Netherlands), and a water-cooled (2 °C) spray chamber.

For the arsenic speciation analysis, the ICPMS instrument was coupled to an Agilent 1100 series quaternary HPLC pump, degasser, and autosampler (Agilent Technologies, Waldbronn, Germany). Polypropylene autosampler vials were used, as it was found that glass vials could contaminate with arsenate. The outlet of the HPLC column was connected to the nebulizer of the ICPMS instrument by a short length of polyethylene tubing. For anion exchange chromatography, a polymer-based strong anion exchange HPLC column, ICSep ION-120 (4.6 mm × 120 mm; 10 μm particles) equipped with a guard column (Transgenomics, San Jose, CA), was used. In **Table 3**, the ICPMS settings and the HPLC settings are given. Data were collected and processed using the Agilent Chemstation ICPMS chromatographic software.

Standard Substances and Chemicals. For the microwave-assisted digestion of seafood samples prior to analysis of their total arsenic content, concentrated HNO_3 and 30% w/w H_2O_2 (both Merck, Darmstadt, Germany) were used. External calibration standards were prepared daily by dilution of a 1000 mg L^{-1} certified arsenic stock solution (Spectrascan, TeknoLab, Drøbak, Norway) in 5% (v/v) HNO_3 (Merck). A diluted solution (0.5 mg L^{-1}) of a 1000 mg L^{-1} rhodium stock

Table 4. Results from Recovery Experiments with Added As(III) or As(V) (both 50 ng as As) to Selected Samples

sample	recovery (ng)		recovery (%)	
	As(III)	As(V)	As(III)	As(V)
TORT-2 (lobster hepatopancreas)	48	51	96	102
DORM-2 (dogfish muscle)	46	46	91	92
BCR CRM278R (mussel tissue)	46	50	91	100
crab meat (*Cancer pagurus*)	56	53	112	107
Norway lobster (*Nephrops norvegicus*)	47	54	94	108
cod (*Gadus morhua*)	51	50	102	100
herring (*Clupea harengus*)	45	55	90	110
mackerel (*Scomber scombrus*)	48	52	95	104
mean $\pm 2\sigma$	48 ± 7	51 ± 6	97 ± 15	103 ± 12

solution (Spectrascan, TeknoLab) was added on-line and served as an internal standard.

For the microwave-assisted sample dissolution for inorganic arsenic analysis, a solution of 0.9 mol L^{-1} sodium hydroxide (Merck) in 50% ethanol (Arcus, Oslo, Norway) was used. Standard stock solutions of the following chemicals were prepared in water: arsenite standard with a concentration of 1000 ± 3 mg L^{-1} (Fluka, Buchs, Switzerland), arsenate standard with a concentration of 1000 ± 3 mg L^{-1} (Spectrascan, TeknoLab), MA hexahydrate (Chem Service, West Chester, PA), DMA trihydrate (Merck, Hohenbrunn, Germany), TMAO, AC bromide, and TETRA iodide (Hot Chemicals, Tokyo, Japan), respectively. A certified standard solution was used for AB (BCR CRM626, 1031 ± 6 mg kg^{-1} as AB [Institute for Reference Materials and Measurements (IRMM), Geel, Belgium]. Dimethylarsinoylacetic acid (DMAA), trimethylarsoniumpropionic acid (TMAP), and four arsenosugar standards (see **Table 1**) were kindly donated by Professor K. A. Francesconi (Karl-Franzens University, Graz, Austria). The stock solutions were stored in the dark at 4 °C and were checked for impurities of other arsenic species using the HPLC-ICPMS system (no impurities found). The mobile phase solutions were prepared by dissolving an appropriate amount of ammonium carbonate (J. T. Baker, Phillipsburg, NJ) in an aqueous 3% (v/v) methanol solution (Merck) followed by adjustment of the pH to 10.3 with 25% (v/v) aqueous ammonia (Merck) and filtration through a 0.45 μm polyvinyldifluoride filter (Pall Gelman Sciences, Ann Arbor, MI) prior to use. The high pH of the mobile phase ensured complete ionization of the arsenic species. Deionized water (resistance > 17 MΩ cm^{-1}, Nanopure-system, Nanopure, Barnstead, United Kingdom) was used throughout the work. All chemicals were of pro analysi quality or better.

Samples and Sample Preparation. The following CRMs have been used in this work: DORM-2 (dogfish muscle), DOLT-2 (dogfish liver), and TORT-2 (lobster hepatopancreas) (National Research Council of Canada, Ontario, Canada); BCR CRM627 (tuna fish tissue), BCR CRM422 (cod muscle), and BCR CRM278R (blue mussel) (IRMM) and NIST1566b (oyster tissue) (National Institute for Science and Technology (NIST), Gaithersburg, MD). Furthermore, seafood samples from the Norwegian surveillance program on contaminants in marine animals were analyzed (see list of samples in **Table 5**). The CRMs were used as received, and all other samples were freeze-dried and thoroughly homogenized using a laboratory mill (Retsch, Haan, Germany) prior to the microwave treatment.

For the analysis of their total arsenic content, subsamples (approximately 0.2 g) were submitted to microwave-assisted wet digestion using 2.0 mL of HNO_3 and 0.5 mL of H_2O_2. Prior to the ICPMS determination, the sample digests were diluted to a final volume of 25 mL with water. For the analysis of inorganic arsenic, subsamples of approximately 0.2 g (dry weight) were mixed with (A) 10 mL of a solution of 0.9 M NaOH in 50% (v/v) ethanol or (B) 10 mL of a solution of 50% (v/v) methanol and dissolved using microwave-assisted heating (90 °C for 20 min). Subsequently, the samples were centrifuged (4400 rpm, 10 min), and the supernatant was removed and filtered through a 0.45 μm single use syringe filter (Sartorius, Göttingen, Germany) prior to analysis. The microwave treatment with alkaline ethanol efficiently dissolved the sample matrix, and only a small amount of undissolved remained after centrifugation and filtration. To maintain satisfactory performance for the ION-120 column, frequent regeneration was performed after approximately every 30−40 injections under conditions reported in ref *27*.

Table 5. Results from the Analysis of Inorganic Arsenic in Various Seafood Samples[a]

sample identification	inorganic arsenic	total arsenic
salmon (*Salmo salar*)	<0.0006	1.9 ± 0.2
cod (*Gadus morhua*)	<0.0006	17 ± 2
cod (*G. morhua*)	<0.0006	15 ± 2
wolffish (*Anarhichas lupus*)	<0.0006	4.1 ± 0.5
wolffish (*A. lupus*)	<0.0006	31 ± 4
anglerfish (*Lophius piscatorius*)	<0.0006	15 ± 2
anglerfish (*L. piscatorius*)	<0.0006	44 ± 6
Atlantic halibut (*Hippoglossus hippoglossus*)	<0.0006	12 ± 1
mackerel (*Scomber scombrus*)	<0.0006	1.7 ± 0.2
mackerel (*S. scombrus*)	<0.0006	2.8 ± 0.4
herring (*Clupea harengus*)	<0.0006	1.5 ± 0.2
herring (*C. harengus*)	<0.0006	1.7 ± 0.2
herring (*C. harengus*)	<0.0006	1.7 ± 0.2
tuna fish (*Thunnus alalunga*)	0.008 ± 0.001	0.9 ± 0.1
lobster, tail meat (*Homarus gammarus*)	<0.0006	14 ± 2
lobster, head and thorax meat (*H. gammarus*)	0.037 ± 0.005	22 ± 3
crab, white meat (*Cancer pagurus*)	0.016 ± 0.002	32 ± 4
crab, head and thorax meat (*C. pagurus*)	0.060 ± 0.009	26 ± 3
king crab, white meat (*Paralithodes camschaticus*)	0.005 ± 0.001	26 ± 3
Norway lobster (*N. norvegicus*)	0.020 ± 0.003	21 ± 3
shrimp (*Pandalus borealis*)	<0.0006	3.8 ± 0.5
shrimp (*P. borealis*)	<0.0006	60 ± 8
shrimp (*P. borealis*)	<0.0006	67 ± 8
horse mussel (*Modilous modiolus*)	0.0012 ± 0.002	3.4 ± 0.4
scallop muscle (*Pecten maximus*)	0.008 ± 0.001	3.1 ± 0.3
oyster (*Ostrea edulis*)	0.014 ± 0.002	1.8 ± 0.2
mink whale (*Balaenoptera acutorostrata*)	<0.0006	0.61 ± 0.08
harp seal (*Pagophilus groenlandicus*)	<0.0006	0.9 ± 0.1
hooded seal (*Cystophora cristata*)	<0.0006	0.22 ± 0.03

[a] All results in mg kg^{-1} as As $\pm 2\sigma$ on a wet weight basis.

RESULTS AND DISCUSSION

Method Development. Previously, gradient elution had been used for the analysis of inorganic arsenic in samples of fish following alkaline sample dissolution (*27*). To shorten the time of analysis, an isocratic elution approach was pursued in this work. A variety of ammonium carbonate concentrations were tested as isocratic mobile phase aiming at a short time of analysis without compromising the separation of the target species As(V) from the organoarsenic compounds. A mobile phase concentration of 50 mM was optimum and resulted in a relative short time of analysis of approximately 6 min per run, and in contrast to gradient elution, no column reequilibration between runs was needed. Additionally, by using this procedure, chloride was chromatographically resolved from As(V), and consequently, the potential interference from $^{40}Ar^{35}Cl^+$ did not pose any problems. In **Figure 1**, a chromatogram of a standard mixture solution, which contained eight arsenic species, illustrates that As(V) is baseline separated from the other arsenic species. Another convenient feature is that no blank peak from As(V) impurities in the mobile phase reagents was observed (*27*, *28*). However, a small peak, corresponding to a concentration of 0.08 μg L^{-1}, originating from As(V) impurities in NaOH, which was used in the sample preparation solution, was detected. The quantitative conversion of As(III) to As(V) by the micro-

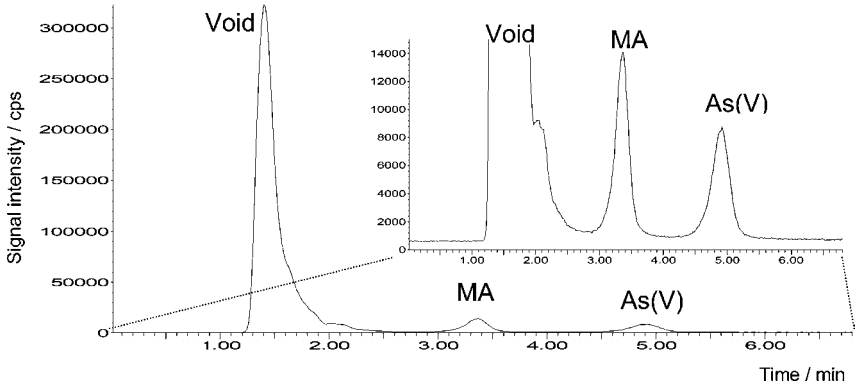


Figure 1. Anion exchange chromatogram of a mixed standards solution (enlargement inserted) containing As(V), 10 μg L^{-1}; MA, 10 μg L^{-1}; DMA, 10 μg L^{-1}; AB, 95 μg L^{-1}; TMAO, 10 μg L^{-1}; AC, 10 μg L^{-1}; TETRA, 2.6 μg L^{-1}; and TMAP, 10 μg L^{-1}. See **Table 2** for information on instrumental settings. For peak identification, see **Table 1**.

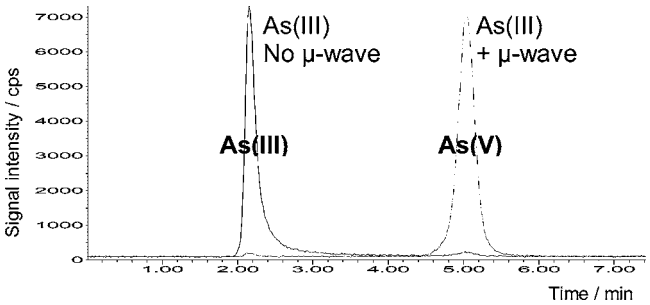


Figure 2. Overlaid chromatograms of an As(III) standard solution (10 μg L^{-1}) before and after microwave treatment, respectively.

wave treatment in alkaline ethanol is illustrated in **Figure 2**, which shows overlaid chromatograms of an As(III) standard solution without and with microwave treatment. Analysis of total arsenic in a range of CRMs after microwave treatment with alkaline ethanol gave a recovery of 98 ± 6% [mean ± relative standard deviation (RSD)] of the certified concentrations. This clearly indicated that arsenic present in the samples was completely solubilized by this procedure.

Stability of Organoarsenic Compounds during the Sample Preparation Step. Any conversion of the predominant organoarsenic species in seafood to inorganic species during the sample pretreatment procedure would impede the analytical accuracy of the inorganic arsenic analysis. In contrast, conversion of organoarsenic compounds to other organoarsenic compounds would not affect its determination.

To illustrate the stability of the species, the authentic arsenic standards listed in **Table 1** were prepared at concentrations in the range of 2−190 μg As L^{-1} in water and alkaline ethanol, respectively. Speciation analysis of the aqueous standard solutions was performed in order to ensure that no conversion of the arsenical in question had occurred in the stock solutions. The alkaline ethanol solutions were subjected to microwave treatment followed by speciation analysis using HPLC-ICPMS. For all arsenic compounds investigated, no conversion of the parent species to inorganic arsenic was observed. Some indications of interconversions among some of the organoarsenic compounds were found, but because this did not influence the inorganic arsenic content, this was not pursued further in this study. Gamble et al. (*29*) investigated the chemical stability of the four main arsenosugars (see **Table 1**) in basic environment at 60 °C and found degradation of arsenosugars **2** and **4** to arsenosugar **1** and DMA, but no presence of inorganic arsenic was reported, supporting the findings in the present study.

Comparison with Methanol/Water Extraction. To compare the extraction efficiency of the sample preparation suggested in this work (A), with the efficiency of a commonly used extractant such as methanol/water (1 + 1) (B), two samples (TORT-2 and crab meat) were selected and prepared in five replicates by each of the methods. For TORT-2, the inorganic arsenic concentration (mean ± 2σ) was (A) 0.176 ± 0.007 and (B) 0.168 ± 0.003 mg kg^{-1} and for crab meat (A) 0.229 ± 0.008 and (B) 0.220 ± 0.005 mg kg^{-1}, respectively. In either case, no significant difference between the two different sample preparation procedures was found. These results may at a first glance seem contradictory to the hypothesis that methanol/water is insufficient to quantitatively release As(III) from a proteinaceous matrix as discussed in the Introduction. However, the results possibly reflect that the inorganic arsenic in the two sample types is present predominantly as As(V) whereas

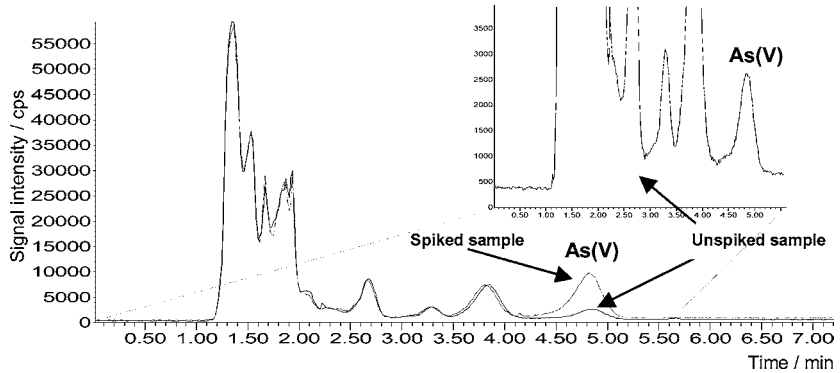


Figure 3. Overlaid chromatograms of anion exchange analysis of a BCR CRM278R mussel tissue sample unspiked and spiked with 50 ng of As(III). The spiked amount of As(III) is quantitative recovered as As(V). The other (not assigned) coeluting peaks in the chromatogram are organoarsenic species. The insert shows an enlargement of the chromatogram for the unspiked sample.

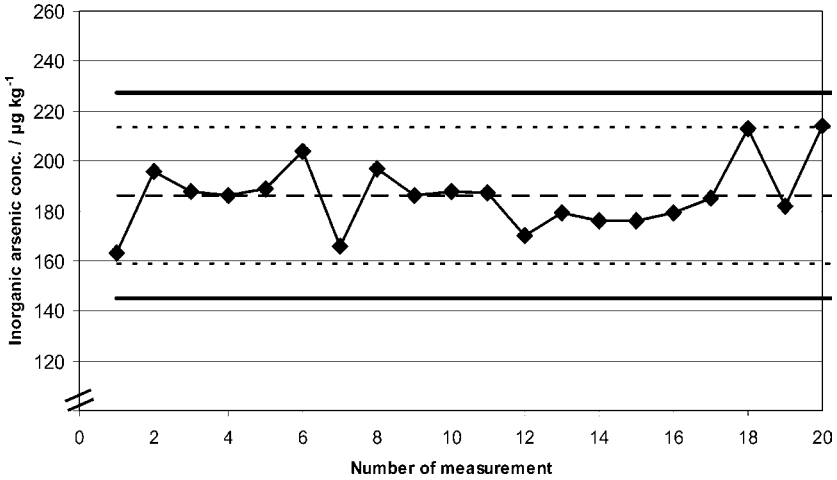


Figure 4. Control chart for inorganic arsenic in TORT-2 from 20 measurements. A mean value of 0.186 mg kg^{-1} with a CV of 7.3% RSD was found. Upper and lower action limits (mean ± 3 s) are given in full lines; upper and lower warning limits (mean ± 2 s) are given in dotted lines.

Table 6. Results from the Analysis of Inorganic Arsenic in Various Marine CRMs[a]

	As(III)	As(V)	As(III) + As(V)	sample preparation	detection	ref
DORM-2			<0.002	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
(dogfish muscle)	0.05 ± 0.01	0.05 ± 0.02		dispersion unit/[methanol/water(1 + 1)]	HPLC-ICPMS	*17*
	ND	0.0063 ± 0.0015		sonication/water	HPLC-ICPMS	*40*
	ND	0.36 ± 0.09		microwave/water	HPLC-ICPMS	*32*
	0.11 ± 0.04	ND		microwave/[methanol/water(1 + 1)]	IC-ICPMS	*33*
	ND	0.05 ± 0.01		sonication/(chloroform/methanol/water)	HPLC-ICPMS	*41*
	0.1	0.4		mechanical agitation/water	HPLC-ICPMS	*42*
	<0.03	<0.03		mechanical agitation/(methanol/water(9+1))	HPLC-ICPMS	*43*
	0.08	0.48		aqueous extract	IC-ICPMS	*44*
			0.102 ± 0.029[b]	microwave assisted distillation/HCl	HG-AAS	*25*
			0.145 ± 0.011[c]	dissolution (HCl)/extraction (chloroform)	HG-AAS	*6*
			0.15 ± 0.01	distillation/HCl	FI-HG-AAS	*26*
TORT-2 (*Lobster*			0.19 ± 0.03	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
hepatopancreas)	ND	0.47 ± 0.13		microwave/water	HPLC-ICPMS	*32*
	ND	0.0928 ± 0.0371		sonication/water	HPLC-ICPMS	*40*
	ND	0.684 ± 0.405		various techniques[d]/water	HPLC-ICPMS	*45*
	ND	0.41 ± 0.03		microwave/[methanol/water(1 + 1)]	HPLC-ICPMS	*46*
			0.506 ± 0.031[b]	microwave assisted distillation/HCl	HG-AAS	*25*
			0.581 ± 0.055[c]	dissolution (HCl)/extraction (chloroform)	HG-AAS	*6*
			0.46 ± 0.07	distillation/HCl	FI-HG-AAS	*26*
DOLT-2			<0.002	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
(dogfish liver)	ND	0.0071 ± 0.0015		sonication/water	HPLC-ICPMS	*40*
	ND	0.004		dispersion/(methanol/NaOH)	IC-ICPMS	*47*
BCR CRM 627			0.015 ± 0.003	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
(tuna fish tissue)	ND	ND		Soxhlet/[methanol/water(1 + 1)]	HPLC-HG-ICPMS	*48*
	0.0018 ± 0.0001	0.0045 ± 0.0002		microwave/water	HPLC-HG-ICPMS	*49*
BCR CRM 422 (cod muscle)			<0.002	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
BCR CRM 278R (mussel tissue)			0.025 ± 0.002	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
NIST 1566b			0.004 ± 0.001	microwave/(ethanol/NaOH)	HPLC-ICPMS	this work
(oyster tissue)			0.647 ± 0.027[c]	dissolution (HCl)/extraction (chloroform)	HG-AAS	*6*

[a] All results in mg kg^{-1} as As on a dry weight basis. Blank cells were not analyzed; ND, not detected. [b] As(III) + As(V) + MA + DMA (11%) + TMAO (0.2%). [c] As(III) + As(V) + MA + TMAO (3–10%). [d] Various procedures were employed including Soxhlet, sonication, room temperature mixing, and microwave-assisted and supercritical extraction.

As(III) is only present in negligible amounts. A similar comparative analysis using a sample with a significant content of As(III) would provide data for a better evaluation of the two sample preparation procedures. At present, no such sample has been found.

Figures of Merit. None of the CRMs available are certified for inorganic arsenic content. Alternatively, the trueness of the method was evaluated by recovery experiments of As(V) and As(III); see **Table 4**. For both species, 50 ng as As was added to the sample prior to the microwave treatment. Eight different samples representing different sample categories were selected and recoveries of (mean ± 2σ) 103 ± 12 and 97 ± 15% were found for As(V) and As(III), respectively. The experiments with As(III) spiked to the samples additionally confirmed the fundamental feature of the method, namely, the complete conversion of As(III) to As(V) in the sample preparation step. **Figure 3** illustrates an experiment with As(III) spiked to the CRM BCR278R mussel tissue sample. The spiked amount of As(III) is fully recovered as As(V), which is chromatographically separated from other organoarsenic species. The precision of the method was estimated from the analysis of the CRM TORT-2, which was included in each batch of samples analyzed.

The mean value found was 0.19 mg kg^{-1} dry matter with a RSD of 7.3% ($N = 20$). **Figure 4** shows a control chart of the obtained results for inorganic arsenic in TORT-2. The limit of detection (3σ) was based on the measurement of peak area for 12 blank samples and was estimated to be 0.045 μg L^{-1} in sample solutions. This is equivalent to 0.002 mg kg^{-1} dry matter or 0.0006 mg kg^{-1} wet weight in the samples (i.e., 250 g dry matter kg^{-1} and sample mass = 0.2 g dry matter) and is among the lowest reported so far for inorganic arsenic.

Analysis of Inorganic Arsenic in Seafood Samples and CRMs. A selection of 29 seafood samples representing different sample types such as fish, crustaceans, bivalves, and marine mammals were analyzed for their content of inorganic arsenic and total arsenic. Quantification was based on a peak area evaluation using a three point external calibration (0−10 μg L^{-1}) with matrix-matched standard solutions. All samples were analyzed in duplicate, and the results are given in **Table 5**. For fish, lobster, shrimps, and marine mammals, the inorganic arsenic content is in all cases below the limit of detection, except for tuna fish, where a concentration of 0.008 mg kg^{-1} was found. Low concentrations for inorganic arsenic (<0.037 mg kg^{-1}) in fish have been reported in other studies substantiating the results found in the present study (*27*, *30*, *31*). In the bivalves, crab, and lobster samples, inorganic arsenic concentrations up to 0.06 mg kg^{-1} were found. In all samples investigated in the present study, the inorganic arsenic content constituted less than 1% of the total arsenic content. On the basis of the data presented here, even extreme seafood eaters (365 g fish day^{-1}) (*30*) would not be in danger of exceeding the PTWI value of 15 μg kg^{-1} body weight (i.e., 900 μg inorganic arsenic for 60 kg person per week). Other sources of inorganic arsenic in the diet and in the drinking water should of course also be taken into consideration in order to make a correct intake calculation, although seafood is by far the primary source of arsenic in the diet (*13*).

Table 6 shows the results from the analysis of marine CRMs and also includes published results for inorganic arsenic in these samples and the method of analysis used. A large variability exists between the reported values; hence, no consensus value can be established in either of the CRMs. Further work toward development of accurate method(s) for the determination of inorganic arsenic in seafood is therefore needed. The production of reference materials certified for inorganic arsenic would provide a useful and mandatory tool in this process, and the commercialization of such CRMs is therefore called upon.

The values for inorganic arsenic reported in the present study are generally among the lowest reported for all CRMs with the exception of BCR CRM627 (tuna fish tissue). It is noteworthy that the two works by Muñoz et al. (*6*, *25*) report the possibility of coextraction and determination of MA, DMA, and TMAO with their method, thus leading to an overestimation of the inorganic arsenic content. It is also remarkable that the two reports by Karthikeyan et al. (*32*, *33*) for As(III) and As(V) in DORM-2 present very different values without commenting on this discrepancy in any of the two reports.

Critical Evaluation of the Proposed Method for Routine Analysis. In several publications, the need for reliable analytical methods for the analysis of inorganic arsenic has been emphasized, e.g., in the recently published Codex Alimentarius Position Paper on Arsenic as a contaminant (*13*). The European Committee for Standardization (CEN) has currently an ongoing activity regarding the standardization of methods for the analysis of total arsenic content in foodstuffs (*34*−*36*). However, to date, no methods for the speciation analysis of arsenic have been standardized by CEN or any other standardization organization. Considering the possibility that the method of analysis characterized in this report might be considered for future international standardization, the advantages and drawbacks are as follows. Advantages: (i) simple and rapid sample preparation procedure resulting in an almost total dissolution of the sample; (ii) short chromatographic procedure facilitating a high sample throughput; and (iii) low limits of detection making analysis of inorganic arsenic at biological and toxicological important levels possible. Drawbacks: (i) HPLC-ICPMS equipment is a costly investment but benefits from its flexibility; and (ii) frequent cleaning of the analytical column is required to maintain acceptable performance.

In conclusion, a method for the determination of inorganic arsenic in seafood has been described. The method was employed on wide range of seafood samples and marine CRMs. Low concentrations were found in all sample types implying that there is no risk of exceeding present recommendations for intake of inorganic arsenic associated with the consumption of seafood. A comparison of existing literature values, including the present study, of inorganic arsenic in marine CRMs showed a large variation between the results. This emphasizes the need for further research toward the development of reliable methods for the determination of inorganic arsenic in biological samples and CRMs with certified values for inorganic arsenic.

LITERATURE CITED

(1) Edmonds, J. S.; Francesconi, K. A. Organoarsenic compounds in the marine environment. In *Organometallic Compounds in the Environment*; Craig, P. J., Ed.; John Wiley and Sons Ltd.: Chichester, United Kingdom, 2003; pp 195−222.

(2) Kuehnelt, D.; Goessler, W. Organoarsenic compounds in the terrestrial environment. In *Organometallic Compounds in the Environment*; Craig, P. J., Ed.; John Wiley and Sons Ltd.: Chichester, United Kingdom, 2003; pp 223−275.

(3) Van Hulle, M.; Zhang, C.; Zhang, X.; Cornelis, R. Arsenic speciation in Chinese seaweeds using HPLC-ICP-MS and HPLC-ES-MS. *Analyst* **2002**, *127*, 634−640.

(4) Raber, G.; Francesconi, K. A.; Irgolic, K. J.; Goessler, W. Determination of "arsenosugars" in algae with anion-exchange chromatography and an inductively coupled plasma mass spectrometer as element-specific detector. *Fresenius' J. Anal. Chem.* **2000**, *367*, 181−188.

(5) Tukai, R.; Maher, W. A.; McNaught, I. J.; Ellwood, M. J.; Coleman, M. Occurrence and chemical form of arsenic in marine macroalgae from the east coast of Australia. *Mar. Freshwater Res.* **2002**, *53*, 971−980.

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

(7) Donohue, J.; Abernathy, C. Exposure to Inorganic Arsenic from Fish and Shellfish. In *Arsenic Exposure and Health Effects*; Chappell, W., Abernathy, C., Calderon, R., Eds.; Elsevier Science Ltd.: Oxford, United Kingdom, 1999; pp 89−98.

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

(9) Hughes, M. F. Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett.* **2002**, *133*, 1−16.

(10) Liebl, B.; Muckter, H.; Nguyen, P. T.; Doklea, E.; Islambouli, S.; Fichtl, B.; Forth, W. Differential effects of various trivalent and pentavalent organic and inorganic arsenic-species on glucose-metabolism in isolated kidney-cells. *Appl. Organomet. Chem.* **1995**, *9*, 531−540.

(11) Tao, S. S.-H.; Bolger, P. M. Dietary arsenic intakes in the United States: FDA Total Diet Study, September 1991-December 1996. *Food Addit. Contam.* **1998**, *16*, 465−472.

(12) Llobett, J. M.; Falco, G.; Casas, C.; Teixido, A.; Domingo, J. L. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J. Agric. Food Chem.* **2003**, *51*, 838−842.

(13) Larsen, E. H.; Berg, T. Trace element speciation and international food legislation—A codex alimentarius position paper on arsenic as a contaminant. In *Trace Element Speciation for Environment, Food and Health*; Ebdon, L., Pitts, L., Cornelis, R., Crews, H., Donard, O. F. X., Quevauviller, P., Eds.; RSC: Cambridge, United Kingdom, 2001; pp 251−260.

(14) Food and Agriculture Organization, World Health Organization. *WHO Food Additives Series No. 24*; WHO: Geneva, 1988.

(15) Rahman, M. M.; Chowdhury, U. K.; Mukherjee, S. C.; Mandal, B. K.; Paul, K.; Lodh, D.; Biswas, B. K.; Chanda, C. R.; Basu, G. K.; Saha, K. C.; Roy, S.; Das, R.; Palit, S. K.; Quamruzzaman, Q.; Chakraborti, D. Chronic arsenic toxicity in Bangladesh and West Bengal, India—A review and commentary. *J. Toxicol. Clin. Toxicol.* **2001**, *39*, 683−700.

(16) Smedley, P. L.; Kinniburgh, D. G. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* **2002**, *17*, 517−568.

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

(18) Li, W. H.; Wei, C.; Zhang, C.; Van Hulle, M.; Cornelis, R.; Zhang, X. R. A survey of arsenic species in Chinese seafood. *Food Chem. Toxicol.* **2003**, *41*, 1103−1110.

(19) McKiernan, J. W.; Creed, J. T.; Brockhoff, C. A.; Caruso, J. A.; Lorenzana, R. M. A comparison of automated and traditional methods for the extraction of arsenicals from fish. *J. Anal. At. Spectrom.* **1999**, *14*, 607−613.

(20) Lai, V. W.-M.; Cullen, W. R.; Ray, S. Arsenic speciation in sea scallop gonads. *Appl. Organomet. Chem.* **2001**, *15*, 533−538.

(21) Amran, M.; Lagarde, F.; Leroy, M. Determination of arsenic species in marine organisms by HPLC-ICP-OES and HPLC-HG-QFAAS. *Microchim. Acta* **1997**, *127*, 195−202.

(22) Munz, H.; Lorenzen, W. Selective determination of inorganic and organic arsenic in foods by atomic-absorption spectroscopy. *Fresenius Z. Anal. Chem.* **1984**, *319*, 395−398.

(23) Beard, H. C.; Lyerly, L. A. Separation of arsenic from antimony and bismuth by solvent extraction. *Anal. Chem.* **1961**, *33*, 1781−1782.

(24) Yasui, A.; Tsutsumi, C.; Toda, S. Selective determination of inorganic arsenic (III), (V) and organic arsenic in biological-materials by solvent extraction atomic absorption spectrophotometry. *Agric. Biol. Chem.* **1978**, *42*, 2139−2145.

(25) Munoz, O.; Velez, D.; Cervera, M.; Montoro, R. Rapid and quantitative release, separation and determination of inorganic arsenic [As(III) plus As(V)] in seafood products by microwave-assisted distillation and hydride generation atomic absorption spectrometry. *J. Anal. At. Spectrom.* **1999**, *14*, 1607−1613.

(26) Øygard, J. K.; Lundebye, A. K.; Julshamn, K. Determination of inorganic arsenic in marine food samples by hydrochloric acid distillation and flow-injection hydride-generation atomic absorption spectrometry. *J. AOAC* **1999**, *82*, 1217−1223.

(27) Larsen, E. H.; Sloth, J. J.; Hansen, M.; Jorhem, L.; Engman, J. Determination of inorganic arsenic in white fish using microwave-assisted alkaline-alcoholic sample dissolution and HPLC-ICP-MS. *Anal. Bioanal. Chem.* **2005**, *381*, 339−346.

(28) Sloth, J. J.; Larsen, E. H.; Julshamn, K. Selective arsenic speciation analysis of human urine reference materials using gradient elution ion-exchange HPLC-ICP-MS. *J. Anal. At. Spectrom.* **2004**, *19*, 973−978.

(29) Gamble, B. M.; Gallagher, P. A.; Shoemaker, J. A.; Parks, A. N.; Freeman, D. M.; Schwegel, C. A.; Creed, J. T. An investigation of the chemical stability of arsenosugars in basic environments using IC-ICP-MS and IC-ESI-MS/MS. *Analyst* **2003**, *128*, 1458−1461.

(30) Urieta, I.; Jalón, M.; Macho, M. L. Arsenic intake in the Basque Country (Spain): A real need for speciation. In *Trace Element Speciation for Environment, Food and Health*; Ebdon, L., Pitts, L., Cornelis, R., Crews, H., Donard, O. F. X., Quevauviller, P., Eds.; RSC: Cambridge, United Kingdom, 2001; pp 241−250.

(31) Munoz, O.; Devesa, V.; Suner, M.; Velez, D.; Montoro, R.; Urieta, I.; Macho, M.; Jalon, M. Total and inorganic arsenic in fresh and processed fish products. *J. Agric. Food Chem.* **2000**, *48*, 4369−4376.

(32) Karthikeyan, S.; Hirata, S.; Iyer, C. S. P. Determination of arsenic species by microwave-assisted extraction followed by ion-pair chromatography-ICPMS: Analysis of reference materials and fish tissues. *Int. J. Environ. Anal.* **2004**, *84*, 573−582.

(33) Karthikeyan, S.; Hirata, S. Ion chromatography-inductively coupled plasma mass spectrometry determination of arsenic species in marine samples. *Appl. Organomet. Chem.* **2004**, *18*, 323−330.

(34) European Committee for Standardization (CEN). *European Standard EN 14332 Determination of Arsenic in Seafood by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) after Microwave Digestion*; CEN: Brussels, 2004.

(35) European Committee for Standardization (CEN). *Provisional European Standard prEN 14546 Determination of Total Arsenic in Seafood by HGAAS after Dry Ashing*; CEN: Brussels, 2004.

(36) European Committee for Standardization (CEN). *Provisional European Standard prEN 14627 Determination of Total Arsenic and Selenium by HGAAS after Pressure Digestion*; CEN: Brussels, 2004.

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

(38) Shiomi, K. Arsenic in marine organisms: Chemical forms and toxicological aspects. In *Arsenic in the Environment. Part II: Human Health and Ecosystem Effects*; Nriagu, J., Ed.; John Wiley and Sons: New York, 1994; pp 261−282.

(39) Kaise, T.; Fukui, S. The chemical form and acute toxicity of arsenic compounds in marine organisms. *Appl. Organomet. Chem.* **1992**, *6*, 155−160.

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

(41) Wrobel, K.; Wrobel, K.; Parker, B.; Kannamkumarath, S. S.; Caruso, J. A. Determination of As(III), As(V), monomethylarsonic acid, dimethylarsinic acid and arsenobetaine by HPLC-ICP-MS. Analysis of reference materials, fish tissues and urine. *Talanta* **2002**, *58*, 899−907.

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

(43) Goessler, W.; Kuehnelt, D.; Schlagenhaufen, C.; Slejkovec, Z.; Irgolic, K. J. Arsenobetaine and other arsenic compounds in the National Research Council of Canada Certified Reference Materials DORM 1 and DORM 2. *J. Anal. At. Spectrom.* **1998**, *13*, 183−187.

(44) Mattusch, J.; Wennrich, R. Determination of anionic, neutral, and cationic species of arsenic by ion chromatography with ICPMS detection in environmental samples. *Anal. Chem.* **1998**, *70*, 3649−3655.

(45) Brisbin, J. A.; B'Hymer, C.; Caruso, J. A. A gradient anion exchange chromatographic method for the speciation of arsenic in lobster tissue extracts. *Talanta* **2002**, *58*, 133−45.

(46) Kirby, J.; Maher, W. Measurement of water-soluble arsenic species in freeze-dried marine animal tissues by microwave-assisted extraction and HPLC-ICPMS. *J. Anal. At. Spectrom.* **2002**, *17*, 838−843.

(47) Zbinden, P.; Andrey, D.; Blake, C. A routine ion chromatography ICP-MS method for the analysis of arsenic species applicable in the food industry. *At. Spectrosc.* **2000**, *21*, 205−215.

(48) Gómez Ariza, J. L.; Sanchez Rodas, D.; Giraldez, I.; Morales, E. Comparison of biota sample pretreatments for arsenic speciation with coupled HPLC-HG-ICP-MS. *Analyst* **2000**, *125*, 401−407.

(49) Nakazato, T.; Taniguchi, T.; Tao, H.; Tominaga, M.; Miyazaki, A. Ion-exclusion chromatography combined with ICP-MS and hydride generation-ICP-MS for the determination of arsenic species in biological matrixes. *J. Anal. At. Spectrom.* **2000**, *15*, 1546−1552.

Received for review December 6, 2004. Revised manuscript received May 31, 2005. Accepted June 2, 2005. This work was funded by the Norwegian Research Council (Project 142468/140).

JF047950E