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Article

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Matrix Solid-Phase Dispersion as a Sample Pretreatment for the Speciation of Arsenic in Seafood Products

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Matrix solid-phase dispersion (MSPD) has been applied to extract arsenical species (arsenite, As(III); arsenate, As(V); monomethylarsonic acid; dimethylarsinic acid, DMA; arsenobetaine, AsB; and arsenocholine) from seafood products. High-performance liquid chromatography coupled to inductively coupled plasma-mass spectrometry was used to separate and detect all arsenic species. Variables affecting MSPD, such as the solid support material (dispersing agent), solid support mass/sample mass ratio, elution solvent composition, and elution solvent volume, have been fully evaluated. Quantitative recoveries for inorganic and organic arsenic species have been obtained when using diatomaceous earth or octadecyl-functionalized silica gel (C18) as a solid support material, with a solid support mass/sample mass ratio of 7.0. Elution of arsenical compounds has been assessed using 10 mL of 50/50 methanol/ultrapure water as an elution solvent. The MSPD method has been found precise, with RSDs of ~9% for As(III), DMA, and As(V) and 3% for AsB. The developed procedure has been tested by analyzing different certified reference materials of marine origin such as DORM-2 and BCR 627, which offer certified contents for some arsenic species. The method has been also applied to assess arsenic speciation in different mollusks, cold water fishes, and white fishes.

It has been well established that the different chemical forms, oxidation states, or both, in which toxic elements occur often determine their toxicological and biological properties and also their bioaccumulation, distribution, and bioavailability.[1] Therefore, elemental speciation has become a very important field in analytical chemistry. In general, organometallic compounds (mainly methylated species) are more toxic than their corresponding inorganic species except in the case of arsenic. For this element, the inorganic species, arsenite (As(III)) and arsenate (As(V)), have been considered as more toxic than organic compounds, although tetramethylarsonium ion is an exception to this rule.[2] However, recent studies have shown that the oxidation state in organic arsenic forms varies toxicity. Therefore, trivalent methylated forms could be even more toxic than previously thought.[3,4] Arsenobetaine (AsB) is the major species in fish and seafood,and arsenocholine (AsC) has been suggested as a precursor of AsB, which is the end product of marine arsenic metabolism. Other arsenic species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide are also present in marine aquatic organisms, as well as arsenosugars (major arsenic compounds in marine algae and seaweed) or arsenic-containing lipids, although the metabolism and toxicology of these last species is still not clear yet.[2,5]

Since arsenic contamination is a worldwide problem, continuous efforts have been devoted to assess arsenic species in environmental and clinical materials. Most of them have led to improved arsenic species separation, mainly by high-performance liquid chromatography (HPLC), and detection, mainly by inductively coupled plasma-mass spectrometry (ICPMS) and hydride generation-fluorescence atomic spectrometry. Critical reviews such as that by Francesconi and Kuehnelt[6] and more recent reviews[2,7−10] are mainly focused on the challenges and pitfalls of the different hyphenated techniques, such as simultaneous separations of anionic and cationic species in a single chromatographic run, the establishment of new separations for new arsenic species, the use of reaction/collision cell technology to reduce interferences, and the design and use of new nebulizers to increase

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sensitivity. However, aspects concerning sample pretreatments, although partially commented on in most of the cited reviews,[2,6−10] have received less attention. In general, sample pretreatment for either liquid or solid samples is a critical stage of the analysis because of the complex nature of matrixes and the low detection levels required by current regulations. This is especially important when performing organometallic speciation because the integrity of the different chemical forms during the sample pretreatment must be guaranteed. In addition, other aspects, such as quantitative species release/isolation and sample pretreatment time, as well as environmental considerations such as low toxicity of reagents used for sample treatment and low wastes generated, are nowadays leading researchers to introduce new sample pretreatments and also to improve classic methods in order to obtain faster and more reliable and environmentally friendly methods. Classic sample pretreatments, such as Soxhlet extraction, ultrasound-assisted extraction methods, mainly ultrasound probe sonication, microwave-assisted extraction, and conventional enzymatic hydrolysis (and more recently ultrasound-assisted enzymatic hydrolysis methods)[11] have been widely described in literature as useful procedures for isolating arsenic species.[12] However, these sample treatments are usually multistep procedures and the subsequent removal of coextracted materials by cleanup steps prior to instrumental analysis is required. Modern extraction techniques, such as pressurized liquid extraction (PLE),[13,14] together with supercritical fluid extraction (SFE),[13,14] subcritical water extraction, pressurized hot water extraction (PHWE),[15] and matrix solid-phase dispersion (MSPD),[16,17] have demonstrated higher capabilities for organic analyte extraction from complex matrixes because extraction and cleanup stages can be performed simultaneously. Some applications by SFE,[1] PLE,[12,18] and PHWE[15] can be found in the literature for some organometallic species extraction. However, the feasibility of MSPD for metal speciation has not been tested yet.

Since the introduction of MSPD in 1989 by Barker and Long,[19] the use of this sample treatment for isolating/purifying organic compounds has increased as shown in recent reviews.[16,17] Detailed theoretical aspects concerning MSPD can be found in the literature;[16,17,20] but qualitatively, MSPD consists of sample architecture disruption by mechanical blending with a solid support bonded phase. After blending, a new sample matrix-solid support phase is formed and analytes tend to be more weakly bonded to it. Therefore, analyte extraction can easily be performed by using less-toxic reagents/solvents at atmospheric pressure and room temperature. Under these mild operating conditions, integrity of the different chemical species can be expected. This fact together with the possibility of performing a cleanup step simultaneously or just before extraction are the most appealing aspects that MSPD can offer to modern organometallic speciation studies.

Therefore, the aim of the current work has been the novel application of MSPD to extract arsenic species from seafood products. Different factors affecting MSPD, such as the nature of the solid support, the solid support mass to sample mass ratio, and volume and nature of the eluting solvent, have been exaustively studied. Arsenic species, such as As(III), As(V), MMA, DMA, AsB, and AsC, have been separated and determined by an optimized fast HPLC−ICPMS method based on a single chromatographic run.[21]

EXPERIMENTAL SECTION

Apparatus. HPLC−ICPMS analysis was performed with a Dionex HPLC UltiMateO 3000 LC (Dionex, Sunnyvale, CA), equipped with a GP50 gradient pump (Dionex), an AS50 thermal compartment (Dionex), and an AS50 autosampler (Dionex). Arsenic separation was carried out with an IonPac AS7 (250 mm × 4 mm i.d.) anion-exchange column (Dionex) and a guard column IonPac AG7 (Dionex). The chromatographic system was coupled with an ICPMS Thermo Finnigan X Series (Thermo Fisher Scientific Inc., Waltham, MA). Total arsenic content was measured using an 820-MS inductively coupled plasma quadrupole mass spectrometer (Varian, Mulgrave, Australia) equipped with SPS3 autosampler (Varian) and a MIcroMist nebulizer type (Varian). Rotavapor Büchi R-210 was equipped with a heating bath Büchi B-491 and a vacuum pump Büchi V-740 (Büchi Laboryechnik AG, Flawil, Switzerland). Vibrating ball mill, Retsch (Haan, Germany), was equipped with zircon cups (15 mL in size) and zircon balls (7-mm diameter). Other equipment were as follows: Laboratory Blender Stomacher 400 (Seward Med. Ltd., London, UK) with Stomacher closure bags 6041/CLR (Seward); a LYPH-LOCK 6-L freeze-dry system, model 77530 from Labconco Corp. (Kansas City, MO); an Ethos Plus microwave laboratory station (Milestone, Sorisole, Italy) with 100-mL closed Teflon vessels with Teflon covers; an HTC adapter plate and HTC safety springs (Milestone); 20-mL Omnifix plastic syringes from Braun (Melsungen, Germany); 20-mL polyethylene frits from Supelco (Bellefonte, PA). Powder funnels (65 mm) were from Barloworld Scientific (Stone, Staffs, UK) and 0.45-μm nitrocellulose membrane filters were from Millipore Co. (Bedford, MA).

Reagents. Ultrapure water of resistance 18 MΩ cm^{-1} obtained from a Milli-Q purification device (Millipore Co.). Methanol (gradient grade) was from Merck (Poole, U.K.). Arsenite and arsenate stock standard solutions, 1.000 mg L^{-1}, were from Panreac (Barcelona, Spain). Standard solutions of MMA, DMA, AsB, and AsC (1.000 g L^{-1}) were prepared by dissolving the appropriate amounts of MMA ($CH_3AsO(ONa_2)\cdot 6H_2O$) purchased from Carlo Erba (Milan, Italy), DMA ($C_2H_6AsNaO_2\cdot 3H_2O$) purchased from Merck, and AsB ($AsC_5H_{11}O_2$) and AsC ($AsC_5H_{14}O$) both purchased from Tri Chemical Laboratory Inc.

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(Yamanashi, Japan). The standard solutions for organic arsenic were stored in amber glass bottles and were kept at 4 °C. Low concentration standards were prepared daily from stock solution. Diatomaceous earth, 95% SiO_2, octadecyl-functionalized silica gel, and active magnesium silicate (Florisil), 60−100 mesh, were from Aldrich Chemical Co. (Milwaukee, WI). Sea sand (washed) QP, SiO_2 was from Panreac. Alumina (aluminum oxide 90 active neutral, 70−230 mesh) was from Merck. AnalaR nitric acid 69%, hydrochloric acid 37%, and hydrogen peroxide 33%(m/v) were from Panreac. DORM-2 (dog fish muscle) CRM was from National Research Council of Canada (Ottawa, Canada). BCR 627 (forms of As in tuna fish tissue) was from Community Bureau of Reference, Commission of the European Communities (Brussels, Belgium).

To avoid metal contamination, all glassware and plastic ware were washed, kept for 48 h in 10% (v/v) nitric acid, and then rinsed several times with ultrapure water before use.

Seafood Samples. Mollusks (mussel, *Mytilus galloprovincialis*; edible cockle, *Cerastoderma edule*; oyster, *Ostrea edulis*; scallop, *Pecten maximus*), white fishes (hake, *Merluccius merluccius*; sole, *Solea vulgaris*), and cold water fishes (sardine, *Sardina pilchardus*; Atlantic mackerel, *Scomber scombrus*) were obtained from a local supermarket. The byssus, shell, or both, were removed from mollusks (∼1 kg) and a composite sample from the soft tissues was prepared by pooling together all specimens. Then, the soft tissues were homogenized by mechanical blending and freeze-dried. Similarly, the bone and entrails were removed from fishes (∼1 kg) and the flesh was homogenized and freeze-dried. Finally, the dry seafood samples were pulverized by using a vibrating ball mill and were kept in polyethylene amber bottles with hermetic seals.

Microwave-Assisted Acid Digestion. Microwave-assisted acid digestion was carried out adding 2 mL of ultrapure water, 4 mL of nitric acid 69%, and 2 mL of hydrogen peroxide 33% (m/v) to 0.5 g of pulverized seafood sample. The reactors were then subjected to the microwave program listed in Table S1 (Supporting Information). After acid digestion was completed, the acid digests were made up to 25 mL with ultrapure water. The mussel sample used to optimize the MSPD procedure was acid digested seven times in order to assess the total arsenic concentration (reference value for optimization). Otherwise, seafood samples were subjected to the microwave-assisted acid digestion procedure in triplicate and at least two different blanks were performed for each set of microwave conditions.

Matrix Solid-Phase Dispersion Procedure. Around 0.25 g of sample was weighted and then blended thoroughly with 1.75 g of diatomaceous earth (DE) in a glass mortar (50-mL capacity) for 5 min using a glass pestle to obtain a homogeneous mixture. This mixture was quantitatively transferred by using a powder funnel to a 20-mL syringe containing 2.0 g of C18 between two polyethylene frits. Finally, a third polyethylene frit was placed at the top of the syringe and was slightly compressed with a syringe plunger to remove air and avoid preferential channels. Arsenic species were eluted from the syringes by gravity with 10 mL of 50/50 methanol/ultrapure water (MeOH/W). The eluated extracts were concentrated by rotary evaporation (water bath at 25 °C, vacuum pressure of 10 mmHg) to ∼2 mL (methanol removal), and then they were made up to 5 mL with ultrapure water. Three replicates were performed for each set of experiments (optimization procedure) and for seafood samples analysis, while seven replicates were performed when preparing CRMs. At least two different blanks were performed for each set of MSPD conditions.

Table 1. Anion-Exchange HPLC−ICPMS Conditions

ICPMS	radio frequency power/W	1400
	peristaltic pump speed/rpm	2.5
	nebulizer type	beat impact (cooled spray chamber)
gas flows/L min^{-1}	plasma	14.0
	auxiliary	0.8
	nebulizer	0.85
torch alignment/mm	horizontal	117
	vertical	317
	sampling death	210
ion optics/V	extraction	−102
	lens 1	−1150
	lens 2	−62
	focus	−7.8
	D1	−55.7
	D2	−140
	pole bias	−20
	hexapole bias	−17
CCT/mL min^{-1}	H_2/He, 5.85	
mass-to-ratio	As	75
	Ge (internal standard), postcolumn addition at 5 $\mu g\ L^{-1}$	72
HPLC	IonPac AS7 (250 mm × 4 mm i.d.) anion-exchange column	
	injection volume/μL	50
	column temperature/°C	25
	mobile phases flow rate /mL min^{-1}	1.35
mobile phase A	1.0 mM nitric acid (pH 2.9), 1% (v/v) methanol	
mobile phase B	80 mM nitric acid (pH 1.3), 1% (v/v) methanol	
	gradient program	100% A, 3.5 min 10% A, 5.0 min 100% A, 1.0 min

ICPMS Measurements. Total arsenic in acid digests from seafood samples was measured by ICPMS under operating conditions listed in Table S2 (Supporting Information). Determinations were performed by using an aqueous standard in 2.0 M nitric acid covering arsenic concentrations from 0 to 1000 $\mu g\ L^{-1}$.

HPLC−ICPMS Measurements. Anion-exchange HPLC conditions were optimized in order to obtain the separation of six arsenic species (As(III), As(V), MMA, DMA, AsB, and AsC) in a single chromatographic run. Based on the work by Dufailly et al.,[21] a chromatographic separation with a gradient elution by using nitric acid solutions as mobile phases, Figure S1 (Supporting Information) shows a chromatogram obtained by using the optimized anion-exchange HPLC conditions as well as ICPMS settings summarized in Table 1.

RESULTS AND DISCUSSION

Optimization of Arsenic Species MSPD Extraction. *Selection of the Solid Support.* A screening experiment was carried out to determine the proper supporting agent (dispersing material). Silica-based solid supports are by far the most popular sorbents for animal tissues because the lipophilic character of the reversed-phase material is believed to facilitate disruption, the retention of lipids, and the production of clean extracts.[16,19] Since arsenic species show polar properties, different silica-based solid supports, such as octyl-bonded silica, C18; DE, and sea sand, were tested. For comparison purposes, Florisil, a synthethic magnesia-silica gel commonly used to retain polar compounds, was also studied.

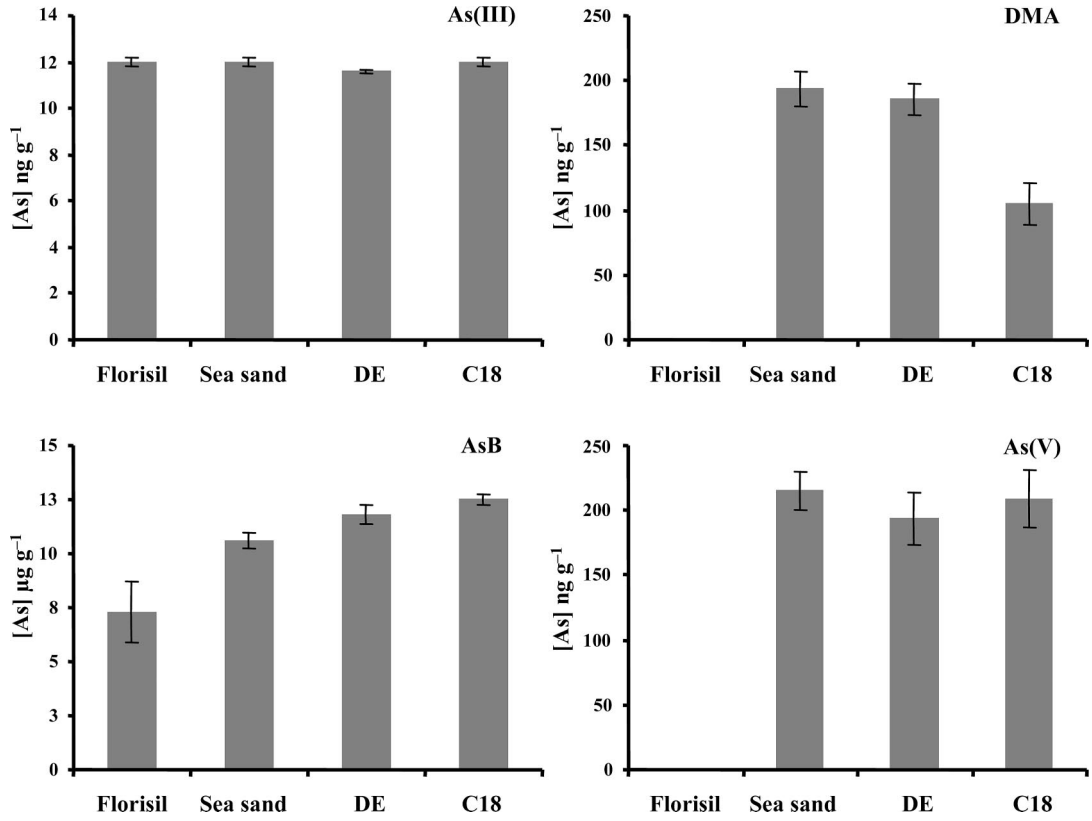

Figure 1. Effect of the nature of the solid support for the extraction of As(III), DMA, AsB, and As(V) from a mussel sample ($N = 3$).

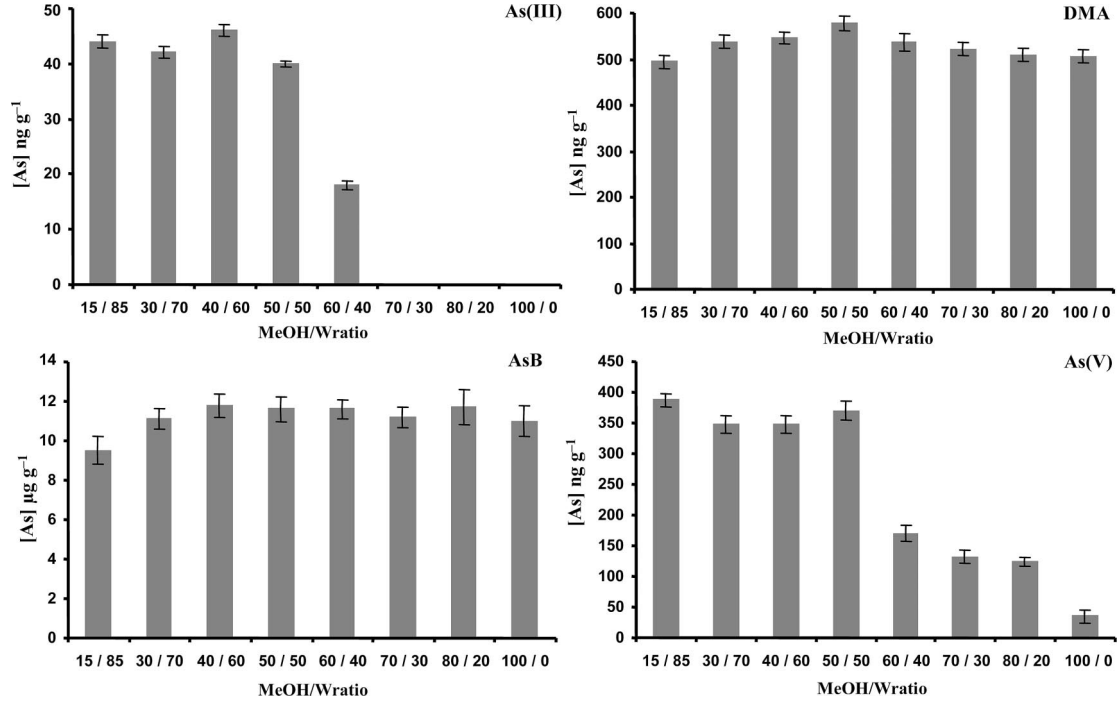

Figure 2. Effect of the MeOH/W composition of the eluting solvent for the extraction of As(III), DMA, AsB, and As(V) from a mussel sample ($N = 3$).

For all cases, MSPD was performed with 0.25 g of a dry mussel sample (mean particle diameter ~50 μm) and 1.0 g of supporting agent, (dispersing mass/sample mass ratio of 4) and eluting with 15 mL of 60/40 MeOH/W. Three different extractions were performed with each dispersing agent, and a blank was also prepared for each one. Results are plotted in Figure 1 for AsB, DMA, As(III), and As(V), arsenic species that are present in the mussel sample used for optimizing MSPD conditions. It can be seen that the use of silica-based solid supports offer similar AsB concentrations, around 13.0 μg g^{-1} for DE and C18 and close to 10.0 μg g^{-1} for the use of sea sand as a dispersing agent. However, a lower AsB concentration has been obtained when using Florisil

Table 2. Effect of the Volume of the Eluting Solution on the Extraction Efficiency of Arsenic Species

volume of 50/50 MeOH/W/mL	arsenic species concentration/ng g^{-1}[a]			
	As(III)	DMA	AsB[b]	As(V)
5.0	36.0 ± 2.45	691 ± 15.4	11.6 ± 0.374	342 ± 10.5
10.0	40.1 ± 3.28	765 ± 21.2	12.8 ± 0.523	332 ± 25.0
15.0	42.4 ± 2.77	689 ± 20.6	12.5 ± 0.311	344 ± 23.7
20.0	40.0 ± 3.43	709 ± 18.7	12.1 ± 0.466	290 ± 22.2

[a] $N = 3$. [b] Concentration expressed as $\mu g\ g^{-1}$.

Table 3. Mean Slopes for Calibration and Limits of Detection and Quantification

	mean calibration slope ± SD[a]	LOD[b]/ng g^{-1}	LOQ[b]/ng g^{-1}
As(III)	3778 ± 530	6.4	21.3
MMA	5062 ± 733	17.4	58.1
DMA	4827 ± 694	13.5	45.2
AsB	4656 ± 504	12.9	43.2
As(V)	3780 ± 435	23.3	77.8
AsC	1685 ± 160	9.7	32.5

[a] $n = 7$. [b] $n = 11$.

(close to 7.0 $\mu g\ g^{-1}$). As(III) is efficiently extracted from mussel tissue for all dispersing agents used, showing As(III) concentrations of ~12 ng g^{-1}. However, there were no chromatographic signals for As(V) and DMA for extracts prepared with Florisil, which means that both species are highly retained in this supporting material. As(V) concentration has been found to be ~200 ng g^{-1} when dispersing with C18, DE, and sea sand; while DMA concentrations were ~190 ng g^{-1} for the use of DE and sea sand and ~100 ng g^{-1} for the use of C18.

After total arsenic determination by microwave-assisted acid digestion (Table S1, Supporting Information) and ICPMS determination (Table S2, Supporting Information), arsenic recoveries of 49 ± 5, 85 ± 7, 94 ± 6, and 96 ± 7% were obtained for Florisil, sea sand, DE, and C18, respectively. Therefore, the use of DE and C18 has given the highest extraction efficiencies, with recoveries around 100%. As reported, the nature of the solid support is responsible for the affinity of certain analytes. It is well-known that the underivatized silanols on the surface of silica-based solid supports (C18, DE, and sea sand) interact with certain functional groups capable of hydrogen bonding,[20,22] such as the carboxylic acid group present in AsB and the arsinic acid group in DMA. The lower recoveries with sea sand (85 ± 7) than with the other silica-based solid materials can be attributed to the pore size and the particle size of the solid support, which allows higher flow rates than those obtained when using C18 and DE. In addition, the low recoveries found for the use of Florisil are to be expected because this material is commonly used to retain polar compounds.[23] Finally, since the DMA concentration found after dispersing with C18 (105 ± 16.2 ng g^{-1}) was lower than those obtained for the use of DE (186 ± 12.0 ng g^{-1}), DE was chosen as solid support for further experiments.

On-Column Cleanup Procedure. One of the outstanding advantages of MSPD is that extraction and cleanup procedures are carried out in a single step. Although a highly selective detector (ICPMS) was used, and there were no analytical signals from unknown coeluting substances, and although all extracts were filtered and the column was protected by using a precolumn, it was observed that certain polar substances reach the analytical column. The evidence is that the retention times of some species, such as DMA and AsB, tend to decrease after the analysis of real extracts, specially for extracts from fatty fishes. Thus, after two successive injections, AsB and DMA coelute without a cleanup step. Therefore, an on-column cleanup step was considered. Sorbents such as C18 and alumina, widely used for cleanup of polar compounds,[16] were tested. In addition, DE, found as an adequate solid support for dispersion in the current work, was also considered for cleanup purposes. Experiments have consisted of weighing ~2.0 g of C18, DE, or activated alumina (overnight heated) and placing it at the bottom of a column between two frits, with the blended DE/sample mixture (DE/sample ratio of 4) directly transferred to the top of the cleanup sorbents. After elution with 15 mL of 60/40 MeOH/W, it was proved that the successive injections of the extracts from fatty fishes did not change the retention times of DMA and, mainly, of AsB for the use of all sorbents used for cleanup. However, it should be mentioned that As(III) and DMA are partially retained by alumina, and the analytical signals for these species are lower than those obtained with the use of C18 and DE. When using either C18 or DE for cleanup purposes, after seven successive injections of extracts from fatty fishes, DMA and AsB do not coelute and the relative standard deviation for the retention time of these two species is similar to those obtained when injectioning aqueous standards (between 2 and 4% for DMA, and between 2 and 7% for AsB). However, it should be mentioned that higher blanks for inorganic arsenic, mainly As(V), have been obtained with the use of DE than for the use of C18. Therefore, C18 was been finally chosen for cleanup purposes.

Finally, the amount of C18 was studied for masses ranging from 2 to 4 g. No improvements were observed when using high amounts of C18, and thus, an amount of 2 g was adopted for further experiments.

DE Mass/Sample Mass Ratio. Another factor to consider in performing a MSPD extraction is the solid support mass to sample mass ratio. The most often applied ratio is 1 to 4, but it has varied from application to application.[15] Different ratios, 2, 4, 5, 6, 7, and 8, which for a mussel sample mass of 0.25 g imply DE masses of 0.5, 1.0, 1.25, 1.5, 1.75, and 2.0 g, respectively, were tested. Elution was performed with 15 mL of 60/40 MeOH/W. Results (Table S3, Supporting Information) have shown that the extraction of AsB, DMA, and As(V) is not affected by varying the DE mass/sample mass ratio. However, the concentration of As(III) is gradually increased when the DE mass/sample mass ratio is higher, and the highest As(III) concentration is reached for DE mass/sample mass ratios of 6−8. According to Barker,[17] the solid support mass/sample mass ratio is dependent on the application (analyte and sample) and ratios higher than 4 can lead to successful MSPD extractions. Therefore, a DE mass/sample mass ratio of 7 (0.25 g of sample and 1.75 g of DE) was finally chosen.

Eluting Solvent Composition. Owing to the high to moderate high polarity of arsenical compounds, a polar solvent such as methanol has been used extensively as a solvent, specially for

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Table 4. Results for Arsenic Species in CRMs

	AsB/μg g^{-1}		DMA/μg g^{-1}		As(III)/μg g^{-1}		total As/μg g^{-1}	
	certified value	found value	certified value	found value	certified value	found value	certified value	found value
BCR-627	3.9 ± 0.2	3.7 ± 0.28	0.15 ± 0.023	0.15 ± 0.0069	0.076[b]	0.080 ± 0.0092	4.8 ± 0.3	3.9 ± 0.4[d]
DORM-2	16.4 ± 0.5	16.2 ± 0.39	0.280 − 0.340[a]	0.309 ± 0.0268	0.075 − 0.110[c]	0.079 ± 0.0078	17.2 ± 0.4	16.6 ± 0.4[d]

[a] Not certified. DMA concentrations range reported by several authors.[21,25−30] [b] Not certified. As(III) concentration reported.[21] [c] Not certified. As(III) concentrations range reported by several authors.[21,27−29,31] [d] Total As concentrations as sum of As(III), AsB and DMA concentrations.

Table 5. Results (in μg g^{-1}; $N = 6$) for Arsenic Species and Total Arsenic in Different Seafood Samples and t_{tab} and t_{exp} (95% Confidence Level) for Statistical Evaluation of Extracted Arsenic and Total Arsenic Concentrations

	mussel	oyster	cockle	scallop	hake	sole	sardine	mackerel
As(III)	0.040 ± 0.0035	0.051 ± 0.012	<0.021	<0.021	<0.021	<0.021	<0.021	<0.021
As(V)	0.371 ± 0.0166	<0.078	0.158 ± 0.0287	<0.078	<0.078	<0.078	0.092 ± 0.00655	<0.078
DMA	0.565 ± 0.0323	0.388 ± 0.0225	0.394 ± 0.0277	0.932 ± 0.0853	0.138 ± 0.00121	0.207 ± 0.0188	0.919 ± 0.0561	0.491 ± 0.0113
AsB	11.8 ± 0.621	6.66 ± 0.352	9.56 ± 0.410	12.2 ± 0.553	13.1 ± 0.255	6.33 ± 0.496	14.2 ± 0.403	3.85 ± 0.185
total As[a]	12.8 ± 0.622	7.10 ± 0.353	10.1 ± 0.412	13.1 ± 0.562	13.2 ± 0.259	6.54 ± 0.496	15.2 ± 0.407	4.34 ± 0.192
total As[b]	12.9 ± 0.466	7.15 ± 0.796	10.6 ± 0.733	13.1 ± 0.450	13.4 ± 0.320	6.89 ± 0.651	15.7 ± 0.639	4.87 ± 0.125
t_{cal}[c]	0.189	0.0500	0.496	0.000	0.100	0.348	0.453	0.146

[a] Sum of concentration for As(III), As(V), DMA and AsB. [b] Total As concentration after microwave-assisted acid digestion. [c] t_{tab} (5 degrees of freedom, 95% confidence range) = 2.57.

AsB, the major arsenic compound in biological matrixes.[2] Preliminary experiments using only methanol as an eluting solvent led to the extraction of AsB, DMA, and As(V), but there was no signal for As(III). Therefore, the polarity of the eluting solvent was increased by mixing methanol and Milli-Q water at different MeOH/W ratios, from 15/85 to 100/0. Results are plotted in Figure 2, where a decrease in organic arsenic species concentration can be seen when the ratio of water is increased. Thus, AsB concentration decreases from 11.2−11.7 to 9.5 μg g^{-1} when using a methanol proportion lower than 30. In addition, the DMA concentration is also lower when a high ratio of water or methanol is used, and the highest concentration was reached for a MeOH/W of 50/50. Concerning inorganic arsenic species, water is necessary to increase the polarity of the eluting solution and As(V) can be easily leached for solvents with a water proportion higher than 50. This effect is more notorious for As(III) (Figure 2). Therefore, a compromise composition of the eluting solvent was needed to efficiently extract organic and inorganic arsenic species, and a composition of MeOH/W of 50/50 was finally chosen. Under these conditions, a total arsenic recovery of 98 ± 3% was achieved.

Volume of the Eluting Solvent. To ensure an efficient leaching of all arsenical compounds from the dispersed DE−sample mixture, different volumes of 50/50 MeOH/W eluting solution were tested. It can be seen in Table 2 that a less efficient extraction of all arsenic species was found when using 5 mL of the eluting solution (total arsenic recovery of 91 ± 3%), while the extraction is quantitative for volumes of 10, 15, and 20 mL (total arsenic recoveries of 100 ± 4, 97 ± 3, and 95 ± 3%, respectively). Therefore, further elution was performed with 10 mL of the eluting solvent.

Analytical Performances. Different aqueous calibration curves using germanium (5 μg L^{-1}) as an internal standard were obtained by covering As(III), As(V), MMA, DMA, and AsC concentrations of 0, 5, 10, 25, 50, 100, and 200 μg L^{-1}, expressed as As; and AsB concentrations of 0, 125, 250, 500, 750, 1000, and 2000 μg L^{-1}, also expressed as As. Table 3 lists the mean and standard deviation of the slopes of calibration graphs for each analyte. A good repeatability of the calibration curves can be seen over 6 different days, with RSD around 10% for all cases.

The limit of detection (LOD) and the limit of quantification (LOQ), based on the 3σ and 10σ criterion (σ, the standard deviation of background signal), were calculated. Keeping in mind the sample weight, the LODs and LOQs, expressed as ng g^{-1}, are also listed in Table 3, where it can be seen that the values are low enough to perform arsenic speciation in seafood samples.

The repeatability of the overall procedure was obtained by subjecting a mussel sample 11 times to the optimized MSPD procedure and analyzing each extract once. RSD values of 9% were obtained for As(III), As(V), and DMA determinations, while a 3% could be assessed for AsB.

Accuracy of the proposed method was assessed by analyzing different CRMs offering certified concentrations for some arsenic species, such as DORM-2 (certified AsB concentration) and BCR 627 (certified AsB and DMA concentrations). Each CRM was prepared seven times following the optimized MSPD-cleanup procedure, and each extract was analyzed twice by the optimized HPLC−ICPMS method. After subtracting reagent blanks, concentrations found in each CRM are listed in Table 4. A good agreement can be seen between the found AsB concentrations and the certified AsB contents in BCR 627 and DORM-2. This fact has been confirmed after applying a t test (95% confidence interval), showing t_{cal} of 1.97 and 1.92 for AsB in BCR 627 and DORM-2, respectively, being lower than the t_{tab} ($t_{tab} = 2.15$). In addition, DMA concentration in BCR 267 (0.15 ± 0.0069 μg g^{-1}) agrees with the certified DMA content (0.15 ± 0.023 μg g^{-1}), showing a t_{cal} of 0.000, lower than the $t_{tab} = 2.15$. For this species, a concentration of 0.309 ± 0.0268 μg g^{-1} was found in DORM-2, which is similar to most of those DMA concentrations found in this CRM by other authors (from 0.280−0.340 μg g^{-1}).[21,24−29]

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However, it must be mentioned that low (0.160 and 0.250 μg g^{-1})[30−32] and high (0.490 and 0.660 μg g^{-1})[33,34] values have also been reported for DMA in this material by other researchers. Similarly, As(III) content is not certified in either BCR 627 or DORM-2, but some reported concentrations can be found in the literature. As listed in Table 5, the As(III) concentration found in BCR 627 (0.080 ± 0.0092 μg g^{-1}) is quite similar to those reported by Dufailly et al.,[21] ~0.076 μg g^{-1}. In addition, As(III) levels found in DORM-2 (0.079 ± 0.0078 μg g^{-1}) agree with reported As(III) concentrations for this material (around 0.075 and 0.110 μg g^{-1}).[21,26−28,30]

Applications. The optimized MSPD-cleanup method was applied to four mollusk samples (mussel, edible cockle, oyster, and scallop), two white fishes (hake and sole) and two cold water fishes (sardine and mackerel). The samples were also microwave acid digested to obtain the total arsenic concentration. Each seafood sample was subjected to the MSPD and microwave-assisted acid digestion procedures three times and each extract or acid digest was measured twice by HPLC−ICPMS or ICPMS, respectively. Table 5 lists the concentrations for the different arsenic species as well as for total arsenic content. It can be seen that organic species (AsB and DMA) were present in all analyzed samples, withbeing AsB the major arsenic species. As(III) was found in mussel and oyster samples, while As(V) was found in mussel, cockle, and sardine samples. It must also be mentioned that the sum of the concentrations for the different arsenic species agrees with the total arsenic concentration found in the samples after microwave-assisted acid digestion. This fact has been confirmed by statistical analysis (Table 5).

CONCLUSIONS

The feasibility of MSPD, as a sample pretreatment, together with a fast anionic and cationic species HPLC separation, is an appealing method for modern speciation studies. MSPD has been demonstrated to be a successful methodology for extracting arsenic species from seafood samples. The procedure developed is fast because the on-column cleanup step is performed immediately after the extraction. DE and C18 have been found as adequate dispersing (solid support) materials to carry out the sample dispersion. In addition, C18 was found to be a successful material for an on column cleanup procedure. A compromise MeOH/W composition of 50/50 can be used to elute inorganic (As(III) and As(V)) and organic (DMA and AsB) arsenic species, with 10 mL enough volume to leach all species from the dispersed/blended sample. The mild conditions inherent to MSPD maintain the integrity of all arsenic species. This has been proved after analyzing different CRMs offering certified concentrations for some arsenic species, such as AsB or DMA. In addition, As(III) and DMA concentrations found in such CRMs agree with those concentrations reported for such species by other researchers. Therefore, the current work is a new trend for MSPD procedures, and speciation analysis based on this sample pretreatment must be tested to assess other organometallic species from biological or environmental materials.

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