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Determination of seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled plasma-mass spectrometry following microwave assisted extraction: Method validation and occurrence data

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ABSTRACT

The determination of seven arsenic species in seafood was performed using ion exchange chromatography on an IonPac AS7 column with inductively coupled plasma mass spectrometry detection after microwave assisted extraction. The effect of five parameters on arsenic extraction recoveries was evaluated in certified reference materials. The recoveries of total arsenic and of arsenic species with the two best extraction media (100% H_2O and 80% aqueous MeOH) were generally similar in the five seafood certified reference materials considered. However, because MeOH co-elutes with arsenite, which would result in a positively biased arsenite concentration, the 100% H_2O extraction conditions were selected for validation of the method. Figures of merit (linearity, LOQs (0.019–0.075 mg As kg^{-1}), specificity, trueness (with recoveries between 82% (As(III)) and 104% (As(V) based on spikes or certified concentrations), repeatability (3–14%), and intermediate precision reproducibility (9–16%) of the proposed method were satisfactory for the determination of arsenite, monomethylarsonic acid, dimethylarsinic acid, arsenate, arsenobetaine and arsenocholine in fish and shellfish. The performance criteria for trimethylarsine oxide, however, were less satisfactory. The method was then applied to 65 different seafood samples. Arsenobetaine was the main species in all samples. The percentage of inorganic arsenic varied between 0.4–15.8% in shellfish and 0.5–1.9% at the utmost in fish. The main advantage of this method that uses only H_2O as an extractant and nitric acid as gradient eluent is its great compatibility with the long-term stability of both IEC separation and ICP-MS detection.

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1. Introduction

Arsenic (As) speciation in marine ecosystems has been the subject of much attention over the past 20 years. Seafood was identified as a source of major exposure to As through human consumption, and various As species have been detected in fish products [1–3]. Among these species, inorganic arsenite (As(III)) and arsenate (As(V)) are the most toxic forms and are carcinogenic [4] while the methylated forms monomethylarsonic acid (MA) and dimethylarsinic acid (DMA) are cancer promoters [5]. Arsenobetaine (AsB) the major species in fish and crustaceans, and arsenocholine (AsC), trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TMAs) are regarded as being non toxic [1]. Because of its species-dependent toxicity, traditional approaches involving the determination of total As concentration are not adequate to truly assess the health risk to consumers from As exposure and intake [6].

Many methods have been developed to perform As speciation analysis [7–9]. Separation of the species by high-performance liquid chromatography (HPLC) coupled with element-specific detection by inductively coupled plasma-mass spectrometry (ICP-MS) is a widely applied technique [10]. This hyphenation indeed combines a rapid, powerful and reproducible separation method with a very efficient detector that is known for its high sensitivity and large linear dynamic range [11].

The chemical nature of As compounds differs in charge and pK_a value, molecular size, and functional groups. Consequently, many chromatographic approaches have been applied, including anion exchange [12–15], cation exchange [10], reversed phase and size exclusion [9,10]. The Dionex Ion pac AS7 column has strong anion-exchange and hydrophobic properties. Based on the work of Londesborough et al. [14], chromatographic conditions (nitric

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acid gradient as eluent) have already been optimized [16]. They are most compatible with ICP-MS detection, as they avoid the clogging problems that are frequently encountered with phosphate- or carbonate-based eluents.

The sample extraction of arsenicals from solid samples is a critical step in the sequence of analytical operations due to possible loss of analyte, changes of the species or incomplete extraction, which may lead to poor or erroneous results. Extraction recoveries depend of the matrix, species present, type of solvents and extraction time and temperature. Traditional techniques such as Soxhlet and liquid–solid extractions as well as sonication are time-consuming and require large amounts of solvents. More recent approaches, including accelerated solvent extraction (ASE), pressurised liquid extraction (PLE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE), avoid some of these problems [17]. MAE has been successfully applied to various food samples such as fish products [18–21] or plants [13]. A low power is generally selected to keep the carbon–arsenic bonds intact [10]. Commonly reported is solvent extraction using a methanol/water (MeOH/H_2O) mixture [13,18–20,22–26], or only water [19,24,26–29]. Sometimes, other solvents were tested such as tetramethylammonium hydroxide (TMAH) [24,30,31], HNO_3 [13], alkaline alcohol for inorganic species [32,33] or a "Suc/2(*N-morphilino*)ethanesulphonic acid (MES)/ethylene diamine tetraacetic acid (EDTA)/ascorbate" mixture [30]. Foster et al. [13] showed that 2% HNO_3 (or extraction with a MeOH/H_2O mixture followed by 2% HNO_3) improved the extraction of arsenic from difficult-to-extract materials (plant and animal digestive tissue). However, the total As extraction recoveries reported with HNO_3 from DORM-2 and TORT-2 (102% in both cases) were similar to those observed with 50% MeOH (98 and 88%, respectively). Moreover, the low HNO_3 pH is likely to disturb the chromatographic separation of arsenic species in extracted samples. Ackley et al. [24] reported that TMAH allowed a satisfactory extraction of total As (95%) from DORM-2 [25]. Nevertheless, Quaghebeur et al. [30] showed that this reagent induced oxidation of As(III) to As(V) during the extraction process [25]. Brisbin and Caruso [26] studied a MAE method for various solvents (water, methanol/water mixture or nitric acid) and various extraction times (2–6 min) on a certified reference material (CRM) of lobster (TORT-1) [25]. They showed that MAE was the simplest, fastest and most reproducible extraction method, which resulted in better or similar extraction rates than those observed with other extraction methods. A second paper from this group confirmed that these conditions were suitable for the quantification of As species in lobster [25].

The three aims of this work were: firstly, to optimize conditions of the MAE procedure for the determination of total As and As species in seafood samples, secondly, to evaluate the figures of merit of the IEC/ICP-MS method that was previously developed [16]: linearity, limits of detection and quantification, specificity, trueness, repeatability and intermediate precision reproducibility in order to validate the method, and finally to analyze samples of the second French total diet study (TDS) to assess extraction recoveries on real samples and to provide As occurrence data on fish and shellfish [34].

2. Experimental

2.1. Instrumentation

IEC/ICP-MS analysis was performed with an Ultimate 3000 chromatographic system equipped with an injection valve and a 100-μL injection loop, an IonPac AG7 guard column and an IonPac AS7 ion exchange column (250 mm × 4 mm; 10-μm particles) (all Dionex, Voisins le Bretonneux, France). The chromatographic system was coupled to an X-Series^II instrument (Thermo Scientific, Courtaboeuf, France) equipped with a concentric nebulizer and impact bead spray chamber) via a 50-cm-long PEEK tubing (0.17-mm i.d.). The sample solutions for total As analysis, were fed by a peristaltic pump from tubes arranged on an ASX 500 autosampler model 510 (CETAC, Omaha, NE, USA). Torch position and ion lenses of the ICP-MS system were optimized daily by performing short-term stability tests with a 1 μg L^{-1} tuning solution (containing especially arsenic (As), barium (Ba) and indium (In)) to maximize As signal and stability while minimising oxide levels ($BaO^+/Ba^+ < 2\%$). Signals were monitored in the time resolved analysis (TRA) mode of the ICP software. Further details of instrument settings are given in Table 1. Other equipments were as follows: closed-vessel microwave digestion system (Anton-Paar, Courtaboeuf, France) equipped with 80-mL quartz vessels (80-bar operating pressure), Universal 32R centrifuge (Hettich, Tuttlingen, Germany).

Table 1
Instrumental operating conditions for IEC/ICP-MS system.

ICP-MS parameters	
Plasma gas flow	14.9 L min^{-1}
Auxiliary gas flow	0.8 L min^{-1}
Nebulizer gas flow	0.9 L min^{-1}
Plasma power	1450 W
Monitored signals	*m/z* 75 (^{75}As), *m/z* 77 ($^{40}Ar^{37}Cl$)
Dwell time	500 ms
IC parameters	
Guard column	IonPac AG7 (50 mm × 4 mm, 10-μm particles, Dionex)
Analytical column	IonPac AS7 (250 mm × 4 mm, 10-μm particles, Dionex)
Flow rate	1.35 mL min^{-1}
Mobile phase A	0.8 mM HNO_3, 1% MeOH, pH = 3.8
Mobile phase B	500 mM HNO_3, 1% MeOH, pH = 1.4
Gradient program	0–3 min: 99% A 3–5 min: 10% A 5–12 min: 80% A

2.2. Chemicals

All solutions were prepared with analytical reagent grade chemicals and ultra pure water (18 MΩ cm) generated by purifying distilled water with the Milli-Q^TM PLUS system combined to an Elix 5 pre-system (Millipore S.A., St Quentin en Yvelines, France). Methanol (HPLC gradient grade, Sigma Aldrich), and nitric acid (Suprapur, 67%, Merck) were used as eluents. Standard solutions of the individual As species with an As concentration of 0.5 or 1 g L^{-1} were prepared from the following reagents: sodium (meta) arsenite (≥99.0%), sodium arsenate dibasic heptahydrate (≥98.5%), disodium methylarsenate (≥98.4%), cacodylic acid (≥99.0%) (all Sigma Aldrich, Saint-Quentin Fallavier, France), methylarsonic acid, arsenocholine, trimethylarsine oxide (all Tri-Chemicals, Yamanashi, Japan), arsenobetaine calibrated solution (BCR 626, 1031 ± 6 mg kg^{-1} Community Bureau of Reference, Geel, Belgium) and tetramethylarsonium ($TMAs^+$) kindly provided by Prof. K.A. Francesconi (Institute of chemistry, University Graz, Austria). Each stock solution was further diluted to 1 mg L^{-1}. Stock solutions were stored in the dark at 4 °C to prevent decomposition or oxidation. The stability of these standards in terms of total As content and purity of the species was checked by ICP-MS using As stock solution from Analytika (Prague, Czech Republic). An internal standard solution was prepared with 1000 mg L^{-1} standard stock solutions of indium (In), purchased from Analytika (Prague, Czech Republic). Multi-species calibration standard solutions of 0–20 μg L^{-1} were prepared daily from these stock solutions by appropriate dilution. A multi-elemental standard solution (10 mg L^{-1}) (Perkin–Elmer, Courtaboeuf, France) was used to prepare tuning solutions in 6% (v/v) nitric acid.

Table 2
As species and total As concentrations (mg kg^{-1}) for both extraction conditions in 5 seafood CRMs ($n = 5$).

		As(III)	MA	DMA	As(V)	AB	TMAO + TMAP	AC	ΣAs species	Total As extracted	Total As certified
TORT-2	A	0.408 ± 0.095[a]	0.460 ± 0.060	1.27 ± 0.14	0.725 ± 0.017	13.0 ± 1.8	1.41 ± 0.11	<0.080[b]	17.3 ± 2.2	20.6 ± 1.2	21.6 ± 1.8
	B	0.588 ± 0.113	0.364 ± 0.150	0.711 ± 0.150	0.645 ± 0.099	12.2 ± 0.6	1.25 ± 0.18	<0.080	15.8 ± 1.3	22.2 ± 1.4	
DORM-2	A	0.031 ± 0.014	0.061 ± 0.008	0.359 ± 0.032	0.029 ± 0.018	16.7 ± 0.6	0.201 ± 0.016	<0.080	17.6 ± 0.7	17.9 ± 0.9	18.0 ± 1.1
	B	0.064 ± 0.011	0.033 ± 0.024	0.314 ± 0.029	0.026 ± 0.002	18.3 ± 0.7	0.200 ± 0.041	<0.080	19.2 ± 0.8	19.7 ± 0.4	
DOLT-3	A	0.074 ± 0.011	0.047 ± 0.008	0.480 ± 0.032	0.073 ± 0.007	8.11 ± 0.65	0.357 ± 0.093	<0.080	9.1 ± 0.8	10.0 ± 0.4	10.2 ± 0.5
	B	0.136 ± 0.004	<0.020	0.417 ± 0.024	<0.020	7.56 ± 0.20	0.452 ± 0.048	0.081 ± 0.022	8.6 ± 0.3	9.6 ± 1.1	
DORM-3	A	0.085 ± 0.014	0.091 ± 0.028	0.459 ± 0.007	0.243 ± 0.023	4.69 ± 0.16	0.221 ± 0.028	<0.080	5.8 ± 0.3	5.8 ± 0.4	6.9 ± 0.3
	B	0.129 ± 0.018	0.066 ± 0.024	0.495 ± 0.054	0.276 ± 0.036	5.24 ± 0.26	0.188 ± 0.064	<0.080	6.4 ± 0.5	7.1 ± 0.4	
BCR 627	A	0.054 ± 0.014	0.063 ± 0.010	0.154 ± 0.011	<0.020	4.86 ± 0.3	0.051 ± 0.010	<0.080	5.2 ± 0.3	5.2 ± 0.5	4.8 ± 0.3
	B	0.172 ± 0.071	<0.020	0.158 ± 0.019	<0.020	3.82 ± 0.4	0.099 ± 0.044	<0.080	4.2 ± 0.5	4.8 ± 0.3	

A: 100% H_2O, and B: 80% MeOH extraction.
[a] ±SD ($n \geq 3$).
[b] Limit of quantification (see Table 4).

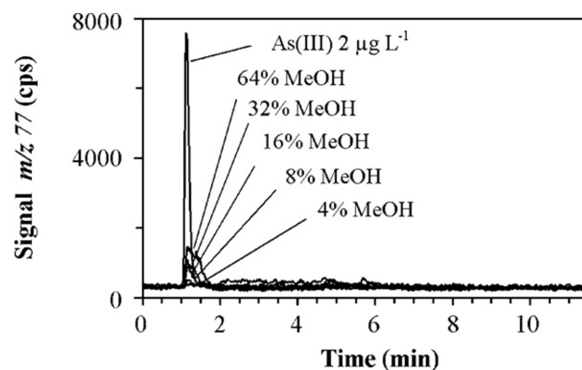

Fig. 1. Methanol effect on the measurement at m/z 75.

2.3. Reference materials

Certified reference materials DORM-2 (Dogfish muscle), DOLT-3 (Dogfish liver), DORM-3 (Fish protein), DOLT-4 (Dogfish liver), and TORT-2 (Lobster hepatopancreas) from the National Research Council of Canada (CNRC), and BCR 627 (Tuna fish tissue) from the Institute for Reference Materials and Measurements (IRMM), were all purchased from Promochem (Molsheim, France). All samples were used as provided without further grinding.

2.4. Seafood samples

All the seafood composite samples from the second French Total Diet Study (TDS) were analysed: fish (saithe, salmon, smoked salmon, tuna and canned tuna), mollusc (mussel, oyster, scallop) and crustacean (shrimp) products. Each of the samples was composed of up to 15 sub-samples of equal weight of the same food item and was prepared "as normally consumed". Only the edible part was used to prepare the sample, (i.e., inedible parts fish bones, fish skin, shells etc., were removed). Then the core foods were prepared as consumed, i.e., as prepared by the average consumer (salmon smoked or steamed, tuna oven cooked or canned in oil or brine, saithe cooked, oyster raw, shrimp and mussel boiled, scallop steamed). So, the TDS has considered the impact of home cooking on the possible decomposition of less stable chemicals and the formation of new ones [34].

2.5. Total arsenic determination

Aliquots of ~0.150–0.300 g sample were separately weighed in quartz vessels in duplicate and 3 mL nitric acid and 3 mL ultra pure water were added. The digestion program was as described previously [35]. After cooling, sample solutions were quantitatively transferred into 50-mL calibrated polyethylene flasks. Before final dilution with water to 50 mL, 1 mg L^{-1} internal standard solution (In,) was added to a final concentration of 2 µg L^{-1} to allow drift correction and to compensate for possible matrix effects. Total As concentration in extracted samples was determined by ICP-MS according to a validated and accredited "in-house" method [36]. Quantification was performed by external calibration using five aqueous As standard solution from 0 to 20 µg L^{-1}.

2.6. Arsenic speciation

Aliquots of ~0.150 g freeze-dried sample were separately weighed in the microwave digestion vessels and 10 mL of a MeOH/H_2O mixture or water was added. The vessels were closed and placed into the microwave system. The samples were heated and maintained at 80 °C for 6 min. After cooling, suspensions were transferred into 50-mL polyethylene flasks, filled to volume with

Table 3
As species concentrations (mg kg^{-1}) observed in literature for DORM-2, TORT-2 and BCR 627.

	As(III)	MA	DMA	As(V)	AsB	TMAO + TMAP	AC	TMA+	Total As	References	Extraction
DORM-2	*0.031*	*0.061*	*0.359*	*0.029*	*16.7*	*0.201*	*<0.08*[a]	*0.265*	*17.9*	This work	A
					16.4			**0.248**	**18.0**	**Certified**	
	0.075	nd	0.281	0.024	18.3	0.152	nd		18.8	[16]	A
	nd	0.070	0.320	0.020	17.1	0.160	nd	0.270	18.4	[13]	A
	nd	0.050	0.340	0.020	16.2	0.160	0.010	0.250	17.4	[13]	A
	nd	0.019	0.320	0.004	17.5				17.8	[23]	A
	0.200		0.410	0.650	13.2	0.010	nd	0.430	15.0	[22]	A
	nd		0.160	1.90	16.5	nd	nd	0.360	18.9	[49]	A
			0.250	0.360	16.0					[19]	A
	0.110	0.310	0.160	nd	16.9	0.340	nd	0.360	18.2	[18]	A
	nd	nd	0.280	nd	16.8	0.170	0.023	0.240	18.5	[20]	A
			0.390		15.0		0.460		15.9	[48]	B
			0.235		17.9	0.18	nd	0.173	16.84	[58]	B
			0.300		16.9	0.181	0.010	0.260	17.8	[37]	B
	0.050	0.140	0.490	0.050	16.1	0.300	nd	0.300	17.4	[39]	B
	nd	nd	0.290	0.050	16.1					[59]	B
		<0.003	0.300		15.9	<0.001	0.110	0.110	18.0	[60]	B
	0.100	nd	0.300	0.400	13.5	0.400	0.020	0.100	14.8	[14]	B
			0.204	nd	15.6				17.2	[17]	C
	nd	0.015	0.230	0.006	17.6	0.154	0.024	0.266	19.6	[61]	C
	nd	0.123	0.610	0.330	16.7				17.8	[54]	D
	0.079		0.309		16.2				16.6	[15]	E
TORT-2	*0.408*	*0.460*	*1.27*	*0.725*	*13.0*	*1.41*	*<0.08*		*20.6*	This work	*A*
									21.6	**Certified**	
	nd	nd	1.04	0.320	13.9	1.20	0.040	0.050	19.0	[13]	A
	nd	nd	1.70	0.780	14.4	1.40	0.060	0.050	22.0	[13]	A
	0.500		1.10	0.500	12.2	0.80	nd	nd	15.1	[22]	A
				0.190[b]						[33]	A
			1.06	0.470	12.8				19.1	[19]	A
		0.147	1.33	0.684	13.6		0.299		16.2	[25]	A
	nd	0.200	1.03	0.410	13.1	1.20	nd	0.055	19.9	[20]	A
			1.39		13.0	1.08	0.024	0.055		[37]	B
		0.030	0.97		13.8	0.150	<0.003	<0.003	23.0	[60]	B
	nd	0.093	0.84	0.093	14.3	0.84	0.043	0.044	19.7	[61]	C
	0.093	0.093	0.84	0.093	14.2		0.024		21.6	[12]	D
BCR 627	*0.068*	*0.045*	*0.148*	*0.041*	*3.6*	*0.082*	*<0.08*		*5.2*	This work	A
			0.150		**3.9**				**4.8**	**Certified**	
	0.076	<0.016	0.157	<0.024	4.1	0.037	< 0.042		4.4	[16]	A
	0.002	0.010	0.154	0.005	4.1	<0.002			4.2	[28]	A
			0.050		4.0	nd	0.008	0.029	4.1	[58]	B
				0.015[b]						[33]	B
			0.140		3.7	0.043	0.012	0.037	4.1	[37]	B
		<0.003	0.163		4.1	<0.001	<0.003	<0.003	4.9	[60]	B
			nd	nd	5.3		nd		5.8	[17]	C
	nd	nd	0.166	0.070	4.3				4.6	[54]	D
	nd	nd	0.140	0.010	3.6				3.8	[31]	D
	nd	nd	0.135	nd	3.8				4.1	[62]	D
	0.080		0.150		3.7				3.9	[15]	E
	nd	nd	0.180	nd	4.2				4.5	[3]	AF

A: MAE, B: liquid/solid, C: ASE, D: sonication, E: matrix solid phase extraction, F: enzymatic, G: soxhlet. nd: not detected.
[a] Limit of quantification (see Table 4).
[b] As(III) + As(V) values.

ultra-pure water and centrifuged at 3500 rpm for 5 min. Supernatants were filtered through a 0.45-μm Millipore syringe filter and 5-fold diluted before injection. Dilution of the extract reduced matrix effect, resulting in similar retention times for a given species in standard solutions and various seafood matrices. Although this approach degrades the limit of quantification (LOQ), it is common practice [37] because it greatly simplifies the analysis and increases sample throughput as it avoids the time-consuming method of standard addition. The separation was performed at a flow rate of 1.35 mL min^{-1}, using a nitric acid gradient between pH 3.4 and pH 1.8 [16]. For quantification using peak area, the chromatographic software (PlasmaLab) of the ICP-MS instrument was used. A five-point external calibration with the respective standard compounds was carried out to compensate for any difference in sensitivity between species as a result of gradient elution. The concentrations of unknown species were estimated using the calibration curve of the nearest eluting standard compound.

2.7. *Calculations and statistical methods*

The concentrations of arsenic species were always expressed in milligrams of As per kg (mg kg^{-1}) of dry mass. The average moisture of the 65 seafood was calculated to be 71%. All TDS samples were analyzed in triplicate. For several samples, concentrations were below the LOQs. For calculations, values below the LOQs were taken as equal to the LOQs.

3. Results and discussion

3.1. *Optimization of MAE procedure*

A preliminary study was made to determine factors that could have a significant effect on the total As extraction recovery from seafood samples. Five factors were selected for the study: (1) sample weight (0.050–0.200 g, in 0.050-g increments), (2) heating time

Table 4
Figures of merit of the speciation method.

	As(III)	MA	DMA	As(V)	AsB	TMAO + TMAP	AsC
Linearity							
Range ($\mu g\,L^{-1}$)	0–20	0–20	0–20	0–20	0–60	0–20	0–20
LOQ							
(mg As kg^{-1})	0.020	0.020	0.020	0.020	0.020	0.050	0.080
Specificity							
$t_{critical\ value}$	2.898	2.861	2.898	2.898	2.878	2.845	2.898
$t_{observed}$ (slope $\neq$ 1)	2.630	0.803	1.429	1.520	1.315	3.733	2.885
$t_{observed}$ (intercept $\neq$ 0)	1.204	0.111	0.439	1.454	0.254	0.190	0.824
Trueness							
Recovery (%)							
BCR 627	–	–	102	–	96	–	–
Spikes							
0.500 $\mu g\,L^{-1}$	–	98[a]	–	104[a]	–	78[a]	99[b]
2.00 $\mu g\,L^{-1}$	82[c]	96[a]	–	90[a]	–	–	97[c]
Repeatability							
CV_r (%) ($n \geq 5$)	5	4	10	4	3	20	14
Reproducibility							
CV_R (%) ($n \geq 10$)	16	10	16	9	11	36	15

[a] Spike on BCR 627.
[b] Spike on TORT-2.
[c] Spike on DORM-3.

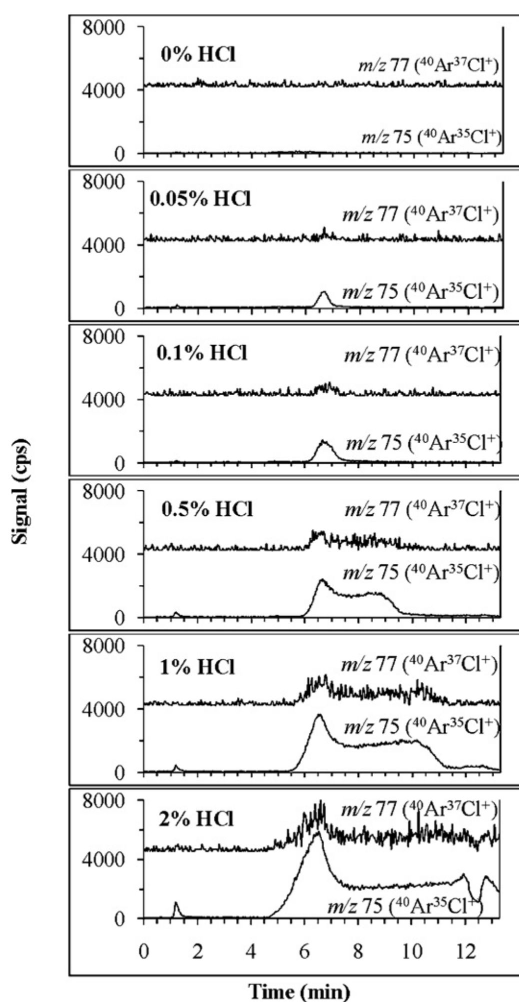

Fig. 2. ArCl interferences on the measurement at m/z 75 and m/z 77.

(2, 4, 6 min), (3) MeOH concentration (0–100%, in 20%-increments), (4) solvent volume (10–20 mL) and (5) heating temperature (80, 100, 120 °C). Initially, the total As extraction recovery (R) from each of DORM-2 and BCR 627 (certified in total As: 18.0 and 4.8 mg kg^{-1}, respectively) was selected as the response to optimise, which was calculated as $R = (X_f/X_c) \times 100$ (with X_f and X_c being, respectively, the found and certified total arsenic concentrations). Results of this preliminary study (data not shown) indicated that the MeOH concentration had the most effect on the 2 responses and that the best total As extraction recoveries were obtained with either 100% H_2O or 80% MeOH. These results agree with those of Brisbin and Caruso [26] (95–106%) for TORT-2, Ackley et al. [24] (79–98%) and Wang et al. [23] (99%) for DORM-2 who reported the best total As extraction recovery with 80% MeOH. Moreover, the extraction recovery observed in 100% H_2O with TORT-2 by Brisbin and Caruso [26] (89–93%) was also satisfactory, as well as results obtained by Narukawa et al. [29] in rice (97–106%). Even if Hirata et al. [22] reported slightly better total As extraction recovery in DORM-2 with 50% MeOH, only H_2O was used for the extraction of others seafood matrices. Finally, total As extraction recoveries observed by Karthikeyan et al. [19] in seafood samples after 100% H_2O extraction were in the range of 85–105%, whereas those reported by Karthikeyan and Hirata [18] using a 50% MeOH ranged from 84 to 105%.

The other parameters appeared to have no significant effect on extraction recoveries. Hence, a 0.150-g sample weight, with 6-min heating time at 80 °C in 10 mL of solvent was used to compare the effect of 100% H_2O and 80% MeOH on both total As and As species extraction recoveries from several seafood CRMs.

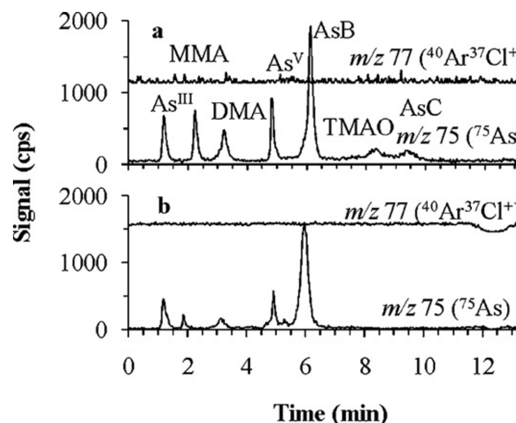

Fig. 3. Chromatographic separation of arsenic species (a) in standard solution 0.2 $\mu g\,L^{-1}$ and (b) in a mussel extract (5-fold diluted).

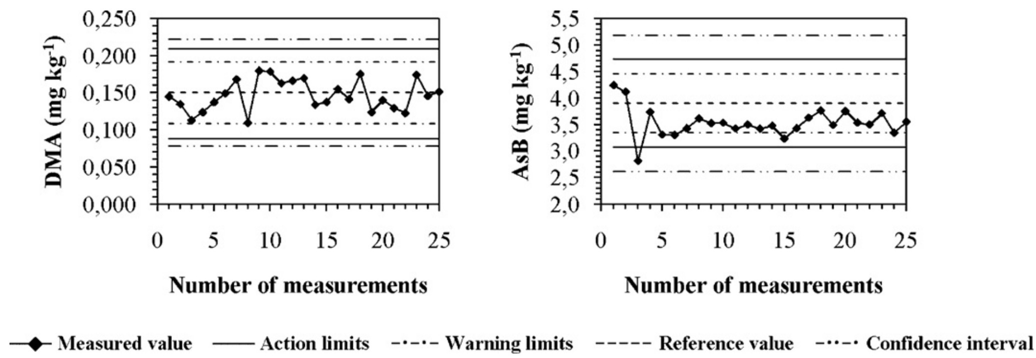

Fig. 4. Control charts for DMA and AsB in BCR 627 from 25 measurements. Means of 0.147 mg kg^{-1} and 3.5 mg kg^{-1} with *CV* of 14% and 8% RSD were found. Warning limits were calculated as $M \pm 2s$, action limits as $M \pm 3s$, confidence interval as $M \pm (k \times CV_R \times M)/100$ with *M* the certified value, *s* the standard deviation, $k = 3$ ($p = 99\%$) and CV_R, the intermediate precision coefficient of variation, set at 16% for DMA and 11% for AsB.

Table 5a
Concentration of As in shellfish (mg kg^{-1} dry mass).

	As(III)	MA	DMA	As(V)	AsB	TMAO + TMAP	AsC	Sum of As species	Total As	%Asi	Humidity (%)
Mussel	0.928	0.278	0.085	0.468	4.27	0.284	*0.080*	7.15	9.63	14	72
	0.220	0.472	0.192	0.362	4.82	0.116	0.121	6.38	11.4	5.1	74
	0.559	0.334	0.311	0.689	2.85	0.104	*0.080*	5.23	10.3	12	72
	0.409	0.109	0.222	0.677	3.67	0.198	*0.080*	5.41	12.3	8.8	74
	0.681	0.623	0.413	0.839	3.83	*0.050*	*0.080*	6.88	13.1	12	72
	0.296	0.502	0.287	0.390	4.40	*0.050*	*0.080*	6.23	13.5	5.1	72
	0.279	0.449	0.155	0.478	4.05	*0.050*	*0.080*	5.65	11.8	6.4	72
	0.303	0.150	0.180	0.685	3.86	*0.050*	*0.080*	5.34	10.0	9.9	75
	0.888	0.322	0.300	1.00	4.78	*0.050*	*0.080*	7.49	12.0	16	77
	0.460	0.171	0.316	0.464	3.06	*0.050*	*0.080*	4.65	8.92	10	72
Mean (n = 10)	0.502	0.341	0.246	0.606	3.96	0.100	0.084	6.04	11.3	9.8	73
Oyster	1.14	0.612	0.299	0.496	13.2	*0.050*	0.448	16.5	20.9	7.8	89
	0.800	1.19	0.376	0.482	11.6	*0.050*	*0.080*	15.1	20.7	6.2	91
	0.714	1.38	0.302	0.132	13.5	*0.050*	1.085	17.6	20.6	4.1	91
	1.13	0.512	0.975	0.754	1.08	0.378	*0.080*	8.41	24.2	7.8	91
	0.749	1.40	0.347	0.099	11.5	*0.050*	*0.080*	14.2	15.6	5.4	90
Mean (n = 5)	0.908	1.02	0.459	0.393	10.2	0.116	0.355	14.4	20.4	6.4	90
Scallop	0.173	*0.020*	*0.020*	0.217	0.676	*0.050*	*0.080*	1.24	10.4	3.7	74
	0.075	0.022	*0.020*	*0.020*	3.16	0.349	*0.080*	3.72	8.06	1.2	73
	0.127	0.025	*0.020*	*0.020*	5.40	0.380	*0.080*	6.06	12.4	1.2	73
	0.091	*0.020*	0.028	0.026	4.38	0.987	*0.080*	5.61	9.03	1.3	73
	0.102	*0.020*	*0.020*	0.030	4.90	0.677	*0.080*	5.83	8.19	1.6	73
Mean (n = 5)	0.114	0.021	0.022	0.063	3.70	0.488	0.080	4.49	9.61	1.8	73
Shrimp	*0.020*	*0.020*	0.054	0.103	8.22	*0.050*	*0.080*	8.55	8.21	1.5	74
	0.063	*0.020*	*0.020*	*0.020*	17.3	*0.050*	*0.080*	17.6	21.2	0.4	74
	0.123	*0.020*	*0.020*	0.036	3.54	*0.050*	*0.080*	3.87	4.75	3.4	74
	0.168	*0.020*	*0.020*	0.049	8.63	*0.050*	*0.080*	9.02	10.9	2.0	74
	0.086	*0.020*	*0.020*	0.036	5.68	*0.050*	*0.080*	5.97	7.52	1.6	74
	0.021	*0.020*	*0.020*	*0.020*	0.826	*0.050*	*0.080*	1.04	1.98	2.1	74
	0.094	*0.020*	*0.020*	*0.020*	12.3	*0.050*	*0.080*	12.6	15.9	0.7	74
	0.020	*0.020*	*0.020*	*0.020*	0.710	*0.050*	*0.080*	0.920	1.84	2.2	74
	0.096	*0.020*	*0.020*	*0.020*	4.66	0.072	*0.080*	4.97	12.7	0.9	74
	0.073	*0.020*	*0.020*	*0.020*	4.52	*0.050*	*0.080*	4.78	6.74	1.4	74
	0.088	*0.020*	*0.020*	*0.020*	3.75	*0.050*	*0.080*	4.03	7.21	1.5	74
	0.186	*0.020*	*0.020*	*0.020*	24.6	*0.050*	*0.080*	25.0	34.1	0.6	75
	0.245	*0.020*	0.367	0.052	8.31	*0.050*	*0.080*	9.13	17.7	1.7	74
	0.136	*0.020*	0.092	0.030	7.76	*0.050*	*0.080*	8.16	13.3	1.2	74
	0.039	*0.020*	*0.020*	*0.020*	3.04	*0.050*	*0.080*	3.26	5.98	1.0	74
Mean (n = 15)	0.097	0.020	0.050	0.032	7.59	0.051	0.080	7.92	11.3	1.5	74
Total mean (n = 35)	**0.331**	**0.255**	**0.161**	**0.252**	**6.37**	**0.137**	**0.120**	**7.82**	**12.4**	**4.6**	**76**
Median	0.173	0.020	0.054	0.052	4.52	0.050	0.080	6.06	11.4	2.2	74
P90	0.853	0.619	0.359	0.687	12.9	0.366	0.080	16.0	20.8	11.1	89
Minimum	0.020	0.020	0.020	0.020	0.676	0.050	0.080	0.920	1.84	0.4	72
Maximum	1.14	1.40	0.975	1.00	24.6	0.987	1.08	25.0	34.1	16	91
n < LOQ	2	18	16	11	0	25	32	–	–	–	–

*Values below the LOQ are indicated in italic.

Table 5b
Concentration of As in fish (mg kg^{-1} dry mass).

	As(III)	MA	DMA	As(V)	AsB	TMAO + TMAP	AsC	Sum of As species	Total As	Asi (%)	Humidity (%)
Saithe	0.036	*0.020*	0.033	0.025	4.47	0.069	*0.080*	4.73	7.23	0.8	76
	0.023	*0.020*	0.033	*0.020*	3.86	*0.050*	*0.080*	4.08	7.94	0.5	77
	0.045	*0.020*	*0.020*	*0.020*	6.40	*0.050*	*0.080*	6.64	7.96	0.8	76
	0.043	*0.020*	*0.020*	*0.020*	6.74	*0.050*	*0.080*	6.98	8.52	0.7	76
	0.035	*0.020*	*0.020*	*0.020*	8.00	*0.050*	*0.080*	8.23	10.7	0.5	76
	0.025	*0.020*	*0.020*	*0.020*	5.83	*0.050*	*0.080*	6.05	8.39	0.5	76
Mean ($n = 6$)	0.034	0.020	0.024	0.021	5.88	0.053	0.080	6.12	8.46	0.7	76
Salmon	*0.020*	*0.020*	*0.020*	*0.020*	2.11	*0.050*	*0.080*	2.32	2.92	1.4	61
	0.020	*0.020*	*0.020*	*0.020*	2.24	*0.050*	*0.080*	2.45	3.41	1.2	61
	0.020	*0.020*	0.027	0.025	1.88	*0.050*	*0.080*	2.10	2.43	1.9	61
	0.020	*0.020*	0.080	*0.020*	2.43	*0.050*	*0.080*	2.70	3.16	1.3	61
	0.026	*0.020*	0.071	*0.020*	2.63	*0.050*	*0.080*	2.90	3.24	1.4	63
	0.043	*0.020*	*0.020*	*0.020*	5.54	*0.050*	*0.080*	5.77	5.07	1.2	62
	0.020	*0.020*	0.049	*0.020*	1.42	*0.050*	*0.080*	1.66	2.36	1.7	63
	0.020	*0.020*	0.037	*0.020*	1.93	*0.050*	*0.080*	2.15	3.25	1.2	63
	0.020	*0.020*	0.045	0.024	2.09	*0.050*	*0.080*	2.32	3.72	1.2	59
	0.020	*0.020*	0.035	*0.020*	1.92	*0.050*	*0.080*	2.14	3.15	1.3	60
	0.020	*0.020*	*0.020*	*0.020*	2.41	*0.050*	*0.080*	2.62	4.06	1.0	63
	0.020	*0.020*	0.051	*0.020*	1.96	*0.050*	*0.080*	2.20	3.07	1.3	63
	0.020	*0.020*	0.059	*0.020*	2.57	*0.050*	*0.080*	2.82	3.63	1.1	63
	0.020	*0.020*	0.048	*0.020*	2.22	*0.050*	*0.080*	2.46	3.63	1.1	63
	0.020	*0.020*	*0.020*	*0.020*	2.37	*0.050*	*0.080*	2.58	3.40	1.2	60
	0.020	*0.020*	*0.020*	*0.020*	2.11	*0.050*	*0.080*	2.32	2.92	1.4	61
Mean ($n = 16$)	0.022	0.020	0.042	0.021	2.36	0.050	0.080	2.60	3.34	1.3	62
Smoked salmon	0.028	*0.020*	0.037	*0.020*	3.01	*0.050*	*0.080*	3.25	3.95	1.2	64
	0.020	*0.020*	0.070	*0.020*	3.03	*0.050*	*0.080*	3.29	3.79	1.1	64
Mean ($n = 2$)	0.024	0.020	0.054	0.020	3.02	0.050	0.080	3.27	3.87	1.1	64
Tuna	0.031	*0.020*	0.117	*0.020*	4.79	*0.050*	*0.080*	5.11	6.74	0.8	66
	0.020	*0.020*	0.053	*0.020*	5.53	*0.050*	*0.080*	5.77	7.32	0.5	68
Mean ($n = 2$)	0.025	0.020	0.085	0.020	5.16	0.050	0.080	5.44	7.03	0.7	67
Canned tuna	*0.020*	*0.020*	*0.020*	*0.020*	1.56	*0.050*	*0.080*	1.77	2.49	1.6	65
	0.020	*0.020*	0.030	*0.020*	1.44	*0.050*	*0.080*	1.66	2.22	1.8	65
	0.020	*0.020*	0.034	*0.020*	1.48	*0.050*	*0.080*	1.71	2.31	1.7	65
	0.020	*0.020*	*0.020*	*0.020*	1.72	*0.050*	*0.080*	1.93	2.45	1.6	65
Mean ($n = 2$)	0.020	0.020	0.026	0.020	1.55	0.050	0.080	1.77	2.37	1.7	65
Total mean ($n = 30$)	**0.024**	**0.020**	**0.040**	**0.020**	**3.19**	**0.051**	**0.080**	**3.42**	**4.51**	**1.2**	**66**
Median	0.020	0.020	0.033	0.020	2.39	0.050	0.080	2.60	3.52	1.2	63
P90	0.037	0.020	0.072	0.020	5.89	0.050	0.080	6.10	8.00	1.7	76
Minimum	0.020	0.020	0.020	0.020	1.42	0.050	0.080	1.66	2.22	0.5	59
Maximum	0.045	0.020	0.117	0.025	8.00	0.069	0.080	8.23	10.7	1.9	77
n < LOQ	20	30	11	27	0	29	30	–	–	–	–

*Values below the LOQ are indicated in italic.

3.2. Comparison of 100% H_2O and 80% MeOH extraction conditions

Table 2 shows the concentration of extracted As in five CRMs. For DORM-2 and BCR 627, it was within the allowable certified value's error (10%, $k = 2$) with both extractants. No significant difference was noticed between both extraction conditions. Because of the co-elution of TMAO and trimethylarsoniopropionate (TMAP) under the chromatographic conditions used [38], the sum of the two species are given as results.

Total As recoveries in TORT-2, DORM-2 and BCR 627 using both 100% H_2O (95%, 99% and 108%) and 80% MeOH (103%, 109% and 100%) were in substantial agreement with those reported in literature [3,13,18–24,26]. However, in TORT-2 and BCR 627, the quantitative recoveries obtained using H_2O were higher than those reported by Hirata et al. [22] and Nakazato et al. [28] (respectively, 76 and 88%). The concentrations for DMA, As(V), AsB, TMAO + TMAP and AsC after 100% H_2O extraction were generally close to those obtained with 80% MeOH for all CRMs. However, As(III) values were systematically higher with 80% MeOH. In fact, the overestimation of As(III) is likely due to the elution of MeOH in conjunction with As(III) (Fig. 1), as reported by Kohlmeyer et al. [39]. Consequently, in order to avoid the possible over-estimation of As(III), and also to simplify the extraction procedure, the 100% H_2O extraction conditions were selected for the remainder of this work. Moreover, these conditions are also the most compatible with the long-term stability of ICP-MS detection.

In TORT-2, the difference between the sum of As species concentrations and the total extracted As concentration may be partially explained by the presence of arsenosugars identified as OH-arsenoribose and PO_4-arsenoribose (0.35 and 0.56 mg kg^{-1}, respectively) by Foster et al. [13].

Concentrations of As species and total As for DORM-2, TORT-2 and BCR 627 are compared with those reported in the literature over the last decade in Table 3. To the best knowledge of the authors, no other study reported As species concentrations in DOLT-3 and

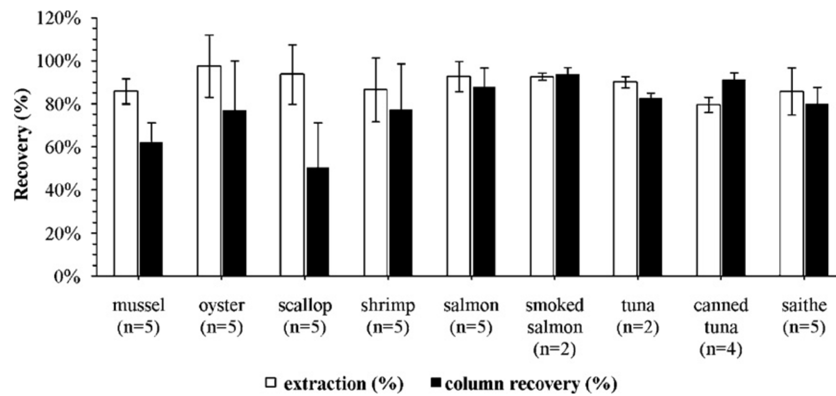

Fig. 5. Extraction recoveries of total arsenic and arsenic species after 100% H_2O MAE extraction. Error bars indicate the standard deviation of the results.

DORM-3. The values for DMA, AB, TMAO + TMAP, AC and $TMAs^+$ for the 3 CRMs were generally in good agreement with the majority of mean values reported previously.

As discussed by Sloth et al. [33], a large variability exists between the reported species concentrations obtained by different extraction and separation methods. Hence, no consensus value for inorganic arsenic could be established in any of these CRMs. This is particularly obvious in the case of As(V) in DORM-2 where six values are higher than 0.280 mg kg^{-1} (including one higher than 1 mg kg^{-1}) whereas 8 values are lower than 0.05 mg kg^{-1} (including 5 similar to the value in this work).

3.3. Validation parameters

The optimised extraction and separation conditions for arsenic speciation analysis of seafood samples were used to validate the method. Figures of merit including linearity, limits of quantification, specificity, trueness, repeatability and intermediate precision reproducibility are presented in Table 4.

3.3.1. Linearity and limits of quantification

A linearity study was performed with standard solutions (n = 20) of different concentration levels (0, 2, 5, 10 and 20 μg L^{-1}). Statistical tests based on application of analysis of variance to the least-squares regression (data not shown) indicated that the linear regression model was acceptable for the seven species in the defined range. The LOQs (in mg As kg^{-1}) were defined as six times the standard deviation of the average from the blank samples (n = 21) quantified on different days over several months, following correction for sample weight (0.150 g of dry material) and dilution (50 mL). The LOQs were experimentally verified, by measuring a spiked standard solution of each species corresponding to the target value of LOQ under repeatability conditions (n = 10) and by checking that the found value was acceptable under both trueness and precision conditions. These LOQ values, obtained and verified under robust conditions (Table 4), can be higher than those previously obtained over short-term conditions [16,18,23,31,37,39], but are sometimes in good agreement with those previously reported in marine samples [3,15,18,22,39–41] or better [42]. In general, the large variability existing between reported LOQs can be attributed to differences in methods of evaluation, such as extrapolation of the calibration curve, number of blanks analysed, LOQs estimated over short or long terms conditions.

3.3.2. Specificity (freedom from interferences)

Specificity involves the confirmation that interferences on the ICP-MS measurement process are not significant.

3.3.2.1. Spectroscopic interferences. In the speciation analysis of As, interference by chloride cannot be disregarded. The $^{40}Ar^{35}Cl^+$ polyatomic ion can interfere with the detection of As species at m/z 75 when analyzing seafood containing a substantial amount of chlorides. In order to evaluate the possible interference of chlorides on the chromatographic separation and ICP-MS detection, several HCl solutions (0, 0.05, 0.1, 0.5, 1, and 2%) were prepared and analyzed in the same conditions as for the samples. Under the selected conditions, two peaks due to $^{40}Ar^{35}Cl^+$ appeared on the chromatogram at the retention time of As(III) and soon after the retention time of AsB from 0.1% HCl (Fig. 2). Those peaks increase with the concentration of HCl and the second one results in a deformation of the baseline and an increase of the apparent AsB peak width. At 0.05% HCl, this interference is negligible and from 0.1% HCl the deformation of the baseline just after AsB and the increase of AsB peak width would indicate the presence of chloride in the solutions. It must be noticed that the peak quantified as As(III) at 0.1% HCl was lower than the LOQ (0.03 μg L^{-1}).

Moreover, the signal at m/z 77 (corresponding to $^{40}Ar^{37}Cl^+$) was monitored simultaneously during the As measurement and systematically checked. Chromatograms indicate that the presence of chlorides is evident from 0.1% HCl added. Therefore, the $^{40}Ar^{35}Cl^+$ interference on the measurement of As species does not appear to be significant when analysing real samples (Fig. 3).

3.3.2.2. Matrix effects. In order to check for possible matrix effects, recoveries of 0.5–20 μg L^{-1} (or 60 μg L^{-1} for AsB) spikes were measured in several real samples (oyster, mussel, scallop, shrimp, salmon and tuna). Spiking was done before extraction, each spiked sample then being processed in the same way as the unspiked sample. The regression line was tested against the line of unity (slope = 1, intercept = 0) by simultaneously testing the hypotheses of slope different from 1 and intercept different from 0, using Student's t-test. These results ($t_{observed} < t_{critical}$) show that specificity of the method is acceptable for all species except TMAO. In fact, a bad resolution of the TMAO peak was achieved in spite of the 5-fold dilution, which is likely due to the remaining salt concentration. Owing to the low concentration of TMAO in the samples, a 10-fold dilution was not possible. However, as this species is non toxic and thus its accurate determination is not critical for risk assessment purposes, the method was not further modified.

3.3.3. Trueness

Trueness was assessed either using available CRMs with certified species concentrations, such as DMA and AsB in BCR 627 [43,44] or by spiking BCR 627, TORT-2 and DORM-3 with 0.5 and 2.0 μg L^{-1} of each species. The mean of five analyses must lie within the confidence interval (CI) calculated from the certified value (M)

of the CRM or spike as: $CI = M \pm (k \times CV_R \times M)/(100 \times n^{1/2})$ where $k = 2$, n the number of samples, and CV_R the intermediate precision coefficient of variation ($p = 99\%$) defined as intermediate precision reproducibility for each of species. Furthermore, the coefficient of variation obtained from these results ($n = 5$) must be less than the defined CV_R.

The results presented in Table 4 demonstrate a satisfactory trueness for the seven species on the three CRMs. The obtained recoveries from the certified values of DMA and AsB in BCR 627 are 102 and 96%, respectively. In BCR 627, DORM-3 and TORT-2, the recoveries from spikes are in the range of 78–104%, with the lowest recovery obtained for TMAO.

3.3.3.1. Precision under repeatability and intermediate reproducibility conditions. Repeatability was evaluated by analyzing BCR 627 spiked with 2 μg L^{-1} of As(III), MA, As(V), TMAO and AsC. The calculated CV_r for the seven species are in the range of 3–20%, with the highest CV_R observed for TMAO and AsC.

The intermediate precision reproducibility was investigated by analyzing a homogeneous sample in duplicate ($n \geq 10$), on different days and by three different analysts. BCR 627 spiked with 2 μg L^{-1} of As(III), MA, As(V), TMAO and AsC was also used. Intermediate reproducibility variance () was calculated as: where S_L^2 is the variance due to sample variations and S_r^2 is the repeatability variance.

The calculated CV_R was in the range of 9–16% for all species, except for TMAO (36%) (Table 4).

Compared to the literature, CV_r seems higher but in these previous studies, repeatability was estimated on standard solutions and not on sample extracts [14,18,22]. In conclusion, the performance of this method in terms of precision is satisfactory.

3.4. Application

3.4.1. Quality assurance

Following the proposed IEC/ICP-MS procedure, sample solutions were analyzed in batches including internal quality control (IQC) such as the 5-point calibration standards to monitor linearity ($r^2 \geq 0.995$), a reagent blank to monitor possible cross-contamination or memory effects, a CRM to check trueness, and a standard solution every six samples and at the end of the sequence to monitor instrument drift. The mean values obtained for DMA and AsB were 0.147 and 3.5 mg kg^{-1} with a RSD of 14 and 8%, respectively ($n = 25$). Control charts indicate that the concentrations found were well within the confidence interval (CI) (Fig. 4).

3.4.2. Extraction recoveries for real samples

All real samples of fish and shellfish selected for the second French TDS were used to assess the effect of fat content on extraction recoveries. The average fat content, obtained from the French Data Centre on Food Quality unit of ANSES (CIQUAL), was 12.4% for fish for smoked salmon, 11.8% for steamed salmon, 8% for canned tuna, 5% for oven cooked tuna, 1.0% for saithe, and for shellfish 3.0% for boiled mussel, 1.4% for steamed scallop, 1.1% for boiled shrimp and 0.53% for raw oyster [45]. These samples correspond to the species most consumed by the general French population and were prepared as consumed [34]. Extraction recoveries were calculated as As_{TE}/As_T, with As_{TE} the total As extracted by H_2O and MAE and As_T the total As mineralized with HNO_3 and quantified by ICP-MS. To ensure and confirm the analytical accuracy of the As_T method, the laboratory regularly participated in proficiency test schemes (PT-Schemes) as an external quality control such as the Central Science Laboratory-Food Analysis Performance Assessment Scheme (CSL-FAPAS) and the Community Reference Laboratory for Heavy Metals (Instituto Superiore di Sanità – ISS). The results for total arsenic in fish and shellfish were considered satisfactory with Z-score in the range −2 and +2 [46].

Total extraction (extracts directly quantified by ICP-MS) and column recoveries (the sum of species quantified by IEC/ICP-MS) for various real seafood samples after MAE using 100% H_2O are shown in Fig. 5. Total extraction recoveries were in the range of 80–98%, with a minimum for canned tuna ($n = 4$) and a maximum for oyster ($n = 5$). In a fatty fish such as salmon, an extraction recovery rate of 93% was achieved ($n = 7$, including two smoked salmons).

A difference was observed between the sum of As species and the total As extracted in shellfish (50–77% against 86–98% on average, respectively), whereas in fish, both were in concordance (80–94% against 80–93% on average, respectively). A greater difference was found in scallop (44% difference on average) than in mussel and oyster (about 22% difference on average), as previously observed [22]. However, the results for a given sample type indicated a variation in the proportion of unknown arsenic species (13–35% in mussels, 7–37% in oysters and 33–68% in scallop), such as arsenosugars (not determined by this method), as already reported in the literature [9,27,47]. This hypothesis is further supported by the presence of unknown peaks in the mussel, oyster and scallop chromatograms. The identification of this type of compound is difficult because of the lack of commercially-available certified standards. Since arsenosugars are non toxic, further studies were not undertaken as the aim of this work was to validate a method for risk assessment of French seafood consumers.

3.5. Occurrence data

A selection of 65 seafood samples representing different types, such as shellfish and fish, were analyzed for their content of As species and total As. The results are reported in Table 5a for shellfish and in Table 5b for fish.

Shellfish samples contained more total As than fish. A mean level of 12.4 mg kg^{-1} dry mass (dm) was found in shellfish, against 4.51 mg kg^{-1} in fish. The highest level of total As was found in shrimp (34.1 mg kg^{-1}) where As levels were very variable. These results are in good agreement with those previously reported [3,15,48–51] but sometimes much higher than those from the previous French study, notably in shrimps and mussels [51]. Even if the contamination results are generally consistent with the literature, nevertheless, as it was underlined by the 2004 EU SCOOP report, the diversity of origins of seafood results in variability in contamination levels. For example, data from European countries show a mean As level range for fish, molluscs, crustaceans and echinoderms from less than 0.1 to 18 mg As kg^{-1} fresh mass [52]. Recent studies indicate a great As concentration range for different samples of a same species: for example [5.4 ± 0.6; 34.2 ± 6.4] mg kg^{-1} for shrimp [22]. This large range could be related to geographical, seasonal or environmental differences [51].

The non toxic AsB was quantified in 100% of samples as the prevalent species. In canned tuna, the low values compared with cooked tuna are probably due the migration of AsB from muscle to brine. This phenomenon can be explained by the weak electrostatic interaction of AsB and fish muscle and the greater osmotic pressure of the external medium [53]. Two samples were found to contain abnormally low level of AsB (an oyster and a scallop containing 1.08 mg kg^{-1} and 0.676 mg kg^{-1}, respectively). Since these samples had a dubious aspect (smell and colour), these differences might be due to AsB degradation occurring during cooking.

The less toxic MA and DMA were quantified in 26% and 58% of all the samples, respectively. MA was only quantified in mussel and oyster, whereas DMA was detected as a minor component in mussel, oyster and fish, as previously reported in the literature [12,15,51,53,54]. TMAO and AsC were below the LOQ for 83% and 95% of samples but were found in some mussels, oysters and scallops.

Inorganic arsenic (Asi) forms were quantified in 94% of the shellfish samples as As(III) and in 69% as As(V), whereas, in fish, As(III) was quantified in 33% of the samples and As(V) in only 10%. Oysters were found to be the most contaminated on average by Asi (1.30 mg kg^{-1}), followed by mussels (1.11 mg kg^{-1}) in shellfish, and to a lesser extent by salmon (0.063 mg kg^{-1}) and saithe (0.061 mg kg^{-1}) in fish. The concentrations of Asi in shrimp were very low for all samples analysed, as previously observed [32,33,51,55–57]. Asi represents on average 4.6% of the total As content in shellfish (min–max 0.4–15.8%) and 1.2% at the utmost in fish (min–max 0.5–1.9%). These results are generally in good agreement with those reported in literature [15,18,32,48,50,51,54,57] but sometimes higher [33,51].

4. Conclusions

In this work, figures of merit (linearity, LOQ, specificity, trueness, repeatability, and intermediate precision reproducibility) of the proposed IEC/ICP-MS procedure were satisfactory for the determination of As(III), MA, DMA, As(V), AsB and AsC in fish and shellfish. The procedure was successfully applied to various real samples, e.g., mussels, oysters, shrimps, and different types of fish containing more or less fat. Using only H_2O as extractant and a nitric acid gradient as eluent is most compatible with the long-term stability of both IEC separation and ICP-MS detection. Combined to a fast continuously leaching system [38], this method will allow the determination of bio-accessible As species in these samples.

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