Establishment of a Method for Determination of Arsenic Species in Seafood by LC-ICP-MS

Ariane V. Zmozinski\textsuperscript{a}, Toni Llorente-Mirandes\textsuperscript{b}, José F. López-Sánchez\textsuperscript{b*}, Mária M. da Silva\textsuperscript{a}

\textsuperscript{a} Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

\textsuperscript{b} Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E-08028, Barcelona, Spain

*Corresponding author: Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, Barcelona E-08028, Spain. E-mail address: fermin.lopez@ub.edu

Abstract

An analytical method for determination of arsenic species (inorganic arsenic (iAs), methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenobetaine (AB), trimethylarsine oxide (TMAO) and arsenochoine (AC)) in Brazilian and Spanish seafood samples is reported. This study was focused on extraction and quantification of inorganic arsenic (iAs), the most toxic form. Arsenic speciation was carried out via LC with both anionic and cationic exchange with ICP-MS detection (LC-ICP-MS). The detection limits (LODs), quantification limits (LOQs), precision and accuracy for each arsenic species were established. The proposed method was evaluated using eight reference materials (RMs). Arsenobetaine was the main species found in all samples. The total and iAs concentration...
in 22 seafood samples and RMs ranged between 0.27–35.2 and 0.02–0.71 mg As kg\(^{-1}\), respectively. Recoveries of between 100% and 106% for iAs, based on spikes, were achieved. The present results provide reliable iAs data for future risk assessment analysis.

**Keywords:** arsenic speciation; seafood; inorganic arsenic; certified reference materials (CRMs); liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS).

1. Introduction

The rapid expansion in trade of seafood products makes this an important market worldwide (De Silva & Bjondal, 2013). The increase in global consumption of seafood is associated with several benefits such as a reduction in risk of several diseases (Innis, 2007; Zmozinski, Passos, Damin, Espirito Santo, Vale, & Silva, 2013). On the other hand, concerns about human health have arisen since several arsenic species have been detected in seafood (Leufroy, Noël, Dufailly, Beauchemin, & Guérin, 2011). The toxicity of As is dependent on its chemical species, with inorganic species (iAs) such as arsenite (As(III)) and arsenate (As(V)) being the most toxic (Geng, Komine, Ohta, Nakajima, Takanashi, & Ohki, 2009). Other arsenic species such as monomethylarsonic acid (MA) and dimethylarsenic acid (DMA) are less toxic to humans, with asenobetaine (AB) being considered non-toxic (Feldmann & Krupp, 2011; Geng et al., 2009).

Seafood contains intrinsically more total arsenic than terrestrial foods, and more than 50 species of arsenic were identified in seafood (Francesconi, 2010). Inorganic As
species in seafood are commonly present as low percentages of the total amount of As (Borak & Hosgood, 2007). However, high concentrations have been reported in some types of seafood, e.g. in bivalve mussels, where concentrations of up to 5 mg As kg\(^{-1}\) were found (Sloth & Julshamn, 2008). The different toxicities of the As species reinforce the importance of its chemical speciation, as the total amount of As does not provide enough information about the toxicity of the analysed sample.

The analysis of arsenic species usually involves many steps, including extraction, separation and detection. Several methods have been employed to perform As speciation analysis: high-performance liquid chromatography (HPLC) and detection by inductively coupled plasma-optical emission spectrometry (ICP–OES), inductively coupled plasma–mass spectrometry (ICP–MS), hydride generation–atomic absorption spectrometry (HG–AAS) and hydride generation–atomic fluorescence spectrometry (HG–AFS) (Francesconi & Kuehnelt, 2004).

Countries such as New Zealand and Australia have legislation for the maximum levels of inorganic arsenic (iAs) in seafood and established a maximum level of inorganic arsenic of 2 mg kg\(^{-1}\) for crustaceans and fish, and 1 mg kg\(^{-1}\) for molluscs and seaweed (Australia New Zealand Food Authority, 2013). The Republic of China establishes a maximum level of inorganic arsenic of 0.1 mg kg\(^{-1}\) for fish and 1.0 mg kg\(^{-1}\) for shells, shrimps and crabs (dry weight), respectively (MHC, 2005). On the other hand, the Brazilian government through the Ministry of Agriculture, Livestock and Food Supply (MAPA) establishes a reference value of 1 mg kg\(^{-1}\) for total As in fish (National Program for Residue and Contaminant Control, 2012). However, the European Union has not established a limit for total or inorganic As in fish and seafood in its legislation (Commission regulation, 2006).
Aware of this situation, the EFSA (European Food Safety Authority) published in 2009 and 2014, two reports about the dietary exposure to arsenic in the European population (European Food Safety Authority, 2009 and 2014). Both reported the urgent need for further data on arsenic species, particularly iAs data, in particular in fish and seafood, and in food groups that provide a significant contribution to the dietary exposure to iAs (e.g. rice and wheat-based products) to reduce the uncertainty of the exposure assessments to iAs. Thus, the need to introduce specific legislation is becoming evident (European Food Safety Authority, 2009; Feldmann & Krupp, 2011). Furthermore, the need to create certified reference materials for seafood and to develop arsenic speciation methods for a large range of food samples and arsenic species was also emphasized (European Food Safety Authority, 2009). The increased focus on inorganic arsenic in food has led to several initiatives towards development of methods for selective determination of inorganic arsenic in seafood. For this purpose, the Institute for Reference Materials and Measurements (IRMM) organised two proficiency tests (PT) in 2010 for measuring iAs, and trace metals in seafood (IMEP-109 and IMEP-30). The determination of iAs in seafood test material presented serious analytical problems. The expert laboratories were not able to agree on a value for the iAs within a reasonable degree of uncertainty (Baer, Baxter, Devesa, Vélez, Raber, Rubio, et al., 2011). It was concluded that more research in extraction and chromatographic procedures was required to quantify the iAs in seafood (Baer et al., 2011). The complexity of the seafood matrix requires accurate and robust procedures. However, the analytical procedures used to date do not comply with these requirements (Feldmann & Krupp, 2011).

Some authors reported inorganic arsenic values in several seafood CRM collected from previously published studies (Leufroy et al. 2011; Pétursdóttir, Gunnlaugsdóttir,
Jörundsdóttir, Mestrot, Krupp, & Feldmann, 2012a; Pétursdóttir, Gunnlaugsdóttir, Jörundsdóttir, Raab, Krupp, & Feldmann, 2012b; Pétursdóttir, Gunnlaugsdóttir, Krupp, & Feldmann 2014). The results of iAs varied widely according to the extraction and detection method. This emphasizes the need for the development of reliable methods for the determination of iAs in seafood and a certified value of inorganic As in a seafood-based reference material.

The goal of this work was to determine total As and As species in seafood samples comprising fish, crustaceans and bivalves. Due to the increasing focus on inorganic arsenic in food, the study was focused on the extraction, identification, separation and accurate quantification of inorganic arsenic (iAs), the most toxic form, which was selectively separated and determined using anion exchange LC-ICP-MS. Finally, due to the lack of CRMs for iAs in seafood samples, previously published values were compared with results obtained in the present study.

2. MATERIALS AND METHODS

2.1 Instruments

For total As, all measurements were carried out using an Agilent 7500ce ICP-MS (Agilent, Germany) with a BURGENER Ari Mist HP type nebulizer. For As speciation, LC-ICP-MS was used with an Agilent 1200 LC quaternary pump, equipped with an auto sampler. The analytical columns Hamilton PRP-X100 (250 x 4.1 mm, 10 µm, Hamilton, USA) and Zorbax-SCX300 (250 x 4.6 mm, 5 µm, Agilent, Germany) were protected by guard columns filled with the corresponding stationary phases. The outlet of the LC column
was connected via PEEK capillary tubing to the nebulizer of the ICP-MS system. A microwave (Milestone Ethos Touch Control) was used for digesting and extracting the samples. The fish samples supplied by MAPA (Brazil) were lyophilized in a Modulyon Freeze Dryer lyophilizer (Thermo Electron Corporation, USA) and milled in an A 11 Basic micro-mill (IKA – Werke, Germany).

2.2. Reagents and standards

Analytical grade reagents were used exclusively. Deionized water with a specific resistivity of 18 MΩ cm$^{-1}$ from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for the preparation of all solutions. Formic acid (98%) (Panreac, p.a., Barcelona, Spain), ammonium dihydrogen phosphate (Panreac, p.a., Barcelona, Spain), aqueous ammonia solution (25%) (Panreac, p.a., Barcelona, Spain), and pyridine (Scharlau, p.a., Barcelona, Spain) were used for the preparation of mobile phases. The following reagents were used for sample digestion and extraction: 31% H$_2$O$_2$ (Merck, Selectipur, Darmstadt, Germany) and 69% HNO$_3$ (Panreac, Hiperpur, Barcelona, Spain). External calibration standards for total As were prepared daily by dilution of a standard stock solution traceable to the National Institute of Standards and Technology (Gaithersburg, USA) with a certified concentration of 1001 ± 5 mg As L$^{-1}$ (Inorganic Ventures Standards, Christiansburg, USA). A solution of $^9$Be, $^{103}$Rh and $^{205}$Tl was used as the internal standard in ICP-MS measurements. An arsenate standard solution of 1000 ± 5 mg As L$^{-1}$ (Merck, Darmstadt, Germany) was used for external quality control in total arsenic and arsenic speciation measurements. Stock standard solutions (1000 mg As L$^{-1}$) for arsenic speciation were prepared as follows: As(III), from As$_2$O$_3$ (NIST, Gaithersburg, USA, Oxidimetric
Primary Standard 83d, 99.99%) dissolved in 4 g L\(^{-1}\) NaOH (Merck, Suprapure, Darmstadt, Germany); As(V), from Na\(_2\)HASO\(_4\).7H\(_2\)O (Carlo Erba, Milano, Italy) dissolved in water; MA, prepared from (CH\(_3\))AsO(ONA)\(_2\).6H\(_2\)O (Carlo Erba, Milano, Italy) dissolved in water; DMA, prepared from (CH\(_3\))\(_2\)AsNaO\(_2\).3H\(_2\)O (Fluka, Buchs, Switzerland) dissolved in water. Arsenocholine (AC) from (CH\(_3\))\(_3\)As\(^+\)(CH\(_2\))\(_2\)CH\(_2\)OHBr\(^-\) was supplied by the “Service Central d’Analyse” (CNRS Vernaison, Solaize, France) and trimethylarsine oxide (TMAO) was prepared from (CH\(_3\))\(_3\)AsO (Argus Chemicals, Vernio, Italy) dissolved in water. The certified reference material of arsenobetaine (AB) from (CH\(_3\))\(_3\)As\(^+\)CH\(_2\)COO\(^-\) was supplied by NMIJ (Tsukuba, Japan) as a standard solution, NMIJ CRM 7901-a. For our internal quality control, the As concentration in in-house prepared As speciation standards was determined by ICPMS. For this, As(V), As(III), DMA, MA, AC, TMAO and AB were standardized against two arsenic certified standard solutions (Merck, Darmstadt, Germany and Inorganic Ventures, Christiansburg, USA) as well as against As\(_2\)O\(_3\) solution. All stock solutions were kept at 4 °C, and further diluted solutions for the analysis were prepared daily.

2.3. Reference materials and samples

The following certified reference materials (CRM) were used for method development: DOLT-4 (Dogfish), TORT-2 (Lobster Hepatopancreas) (both from the National Research Council, Canada); NIST SRM 2976 (Mussel Tissue) and NIST SRM 1566b (Oyster Tissue) (National Institute of Standards and Technology, Gaithersburg, MD, USA); BCR-627 (Tuna fish), ERM-BC211 (Rice) and ERM-CE278 (Mussel Tissue) (Institute for Reference Materials and Measurements of the European Commission’s Joint Research Centre, Geel, Belgium). The reference material (RM) 9th PT on fish from the
Community Reference Laboratory-Istituto Superiore di Sanità (CRL-ISS, Rome, Italy) was also analysed.

Four fresh fish muscle samples were provided by the Laboratory of Trace Metals and Contaminants (LANAGRO/RS) of the Ministry of Agriculture, Livestock and Supply (MAPA/Brazil). The total amount of these four samples were initially washed with Milli-Q water, cut and then lyophilized for a period of 5 hours. They were then ground in a vibratory mill and sieved through polyester mesh of 85 µm to improve the particle size distribution.

Ten fish samples and a clam sample were supplied by the Laboratory of the Public Health Agency of Barcelona (ASPB, Barcelona, Spain). Three crustacean samples and four bivalve samples were purchased from local supermarkets in Barcelona, Spain, during 2013. All these samples were analyzed in a raw state (wet weight) without lyophilization or other pretreatments. Only edible parts of each fish and seafood were used for the analysis. Samples were washed with Milli-Q water, cut, and homogenized using a blender (non-contaminating kitchen mixer; Multiquick 5 Hand Processor, Braun, Barcelona, Spain). After homogenization, samples were stored in the refrigerator at 4–10 °C until analysis (before 2 days).

2.4. Procedures

2.4.1. Moisture determination

The moisture of fresh samples was determined in triplicate by drying 0.5 g aliquots in an oven at 102 ± 3°C until constant weight. Moisture ranged from 45% to 94%, and all results are expressed as dry mass.
2.4.2. Total arsenic analysis

The total arsenic content in seafood and CRM samples was determined by ICP-MS following microwave digestion. Initially, 0.5 g and 2 g aliquots of lyophilized and fresh samples, respectively, were weighed in digestion vessels, after which 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. The microwave digestion procedure was carried out according to the following programme: 10 min from room temperature to 90 °C, maintained for 5 min at 90 °C, 10 min from 90 °C to 120 °C, 10 min from 120 °C to 190 °C and 10 min maintained at 190 °C. After cooling to room temperature, the digested samples were diluted in water up to 25 mL. Helium gas was used in the collision cell to avoid interference in the ICP-MS measurements. A solution of $^9$Be, $^{103}$Rh and $^{205}$Tl was used as the internal standard. The samples were quantified by means of an external calibration curve from As(V) standards. Triplicate analyses were performed for each sample. For quality control purposes, the standards of the calibration curve were run before and after each sample series. The corresponding digestion blanks (one for each sample digestion series) were also measured. Quality control standard solutions at two concentrations were measured after constructing the calibration curve. To assess the accuracy of the ICP-MS method, seven CRMs (DOLT-4, TORT-2, SRM 2976, SRM 1566b, BCR-627, ERM-BC211 and ERM-CE278) and one RM (9th PT) were analysed.

2.4.3 Arsenic speciation analysis

The extraction of As species was based on our previous study (Llorente-Mirandes, Calderón, Centrich, Rubio, & López-Sánchez, 2014). For this, 0.2 g and 1.0 g aliquots of
lyophilized and fresh samples, respectively, were weighed in digestion vessels and 10 mL of a solution containing 0.2% (w/v) of nitric acid and 1% (w/v) of hydrogen peroxide were added to perform a microwave assisted extraction (MAE) at temperature of 95 °C. Samples were cooled to room temperature and centrifuged at 3500 rpm for 25 min. The supernatant was filtered through PET filters (Chromafil, Macherey–Nagel, pore size 0.45 µm). Triplicate analyses were performed for each sample. This extraction method completely oxidizes As(III) into As(V), without conversion of the other organoarsenic species into inorganic arsenic (iAs). The iAs was identified and quantified as As(V) in the extracts by comparing the chromatographic peak for the samples with the peak of As(V) standard solution. Total arsenic in the extracts was determined by ICP-MS (as described previously). Arsenic speciation was carried out in the extracts by LC-ICP-MS. Two chromatographic separation methods were used for separation of the arsenic species. As(III), As(V), DMA and MA were analysed by anion exchange chromatography. AB, AC and TMAO were analysed by cation-exchange chromatography. The performance characteristics of anion-exchange chromatographic system are previously described (Llorente-Mirandes, Calderón, Centrich, Rubio, & López-Sánchez, 2014). The main chromatographic conditions of cation-exchange chromatography were: mobile phase of 20 mM pyridine, pH = 2.6, flow rate at 1.5 mL min⁻¹, and injection volume of 50 µL. Arsenic species in extracts were identified by comparison of retention times with standards. External calibration curves were used to quantify MA, DMA, As(III), As(V), AB, TMAO and AC according to the corresponding standards. Extraction blanks were also analysed by LC-ICP-MS in each work session. The ion intensity at m/z 75 (⁷⁵As) was monitored using time-resolved analysis software. Additionally, the ion intensities at m/z 77 (⁴⁰Ar³⁷Cl) and m/z 35 (³⁵Cl) were monitored to detect possible argon chloride (⁴⁰Ar³⁵Cl) interference at m/z 75. In each speciation run, an
As(V) certified standard solution (Merck, Darmstadt, Germany) and a certified reference material solution were measured every ten samples and at the end of the sequence to ensure stable instrument sensitivity.

3. RESULTS AND DISCUSSION

3.1 Quality control

3.1.1 Analysis of the total As concentration

To evaluate the accuracy of the applied procedure, several CRMs were analysed. Seafood CRMs (TORT-2, DOLT-4, SRM 2976, SRM 1566b, BCR-627, ERM-BC211 and ERM-CE278) and one material reference (9Th) were analysed during the study. The concurrent analyses of the CRMs listed above were used to measure the accuracy of the determination of total As (Table 1). For quality control of acid digestion, a CRM was analysed in every batch of samples measurements (total As concentration). The comparison between each obtained value of total As with its corresponding certified value (Table 1) showed no significant difference at a 95% confidence level when Student’s t-test was applied. The repeatability (six times within a day, n=6) was assessed for the results obtained by analysis of different replicates of CRMs (Table 1). The RSD (%) values were: 4.9% for TORT-2 and 1.2% for DOLT-4. The detection (LOD) and quantification limits (LOQ) were calculated as three times the standard deviation (3σ) and ten times the standard deviation signal (10σ) of ten digestion blanks, respectively (Llorente-Mirandes et al., 2014). The results obtained were as follows: 0.006 mg As kg\(^{-1}\) dry weight basis for method detection limit and 0.021 mg As kg\(^{-1}\) dry weight basis for method quantification limit.
3.1.2 Analysis of As species

**Extraction efficiencies**

The extraction efficiency was evaluated by calculating the ratio between total arsenic present in the samples, given by the acid digestion, and the total arsenic present in the extracts. The extraction efficiencies are presented in Table 1 for the CRMs and Table 2 for the real samples. The efficiency obtained in this work varied between 73% and 104% with an average of 89%, which is consistent with the literature (Amayo, Petursdottir, Newcombe, Gunlaugsdottir, Raab, Krupp, et al., 2011; Pétursdóttir et al., 2014; Zheng & Hintelmann, 2004). Thus, the solution containing 0.2% (w/v) of HNO$_3$ and 1% (w/v) of H$_2$O$_2$ proved to be an effective solvent in the extraction of As species in seafood. A recent study compared nine extraction methods for determination of iAs in seafood, including the HNO$_3$/H$_2$O$_2$ (Pétursdóttir et al., 2014). The highest extraction efficiency for all samples was achieved by HNO$_3$/H$_2$O$_2$ method, which corroborate with this work. An average extraction efficiency of 93% was obtained for most samples, with the exception of DOLT-4, ERM CE278 and salmon-2, for which the average was 75%. According to Pétursdóttir et al. (2012b) and Amayo et al. (2011) this difference in extraction efficiencies can be attributed to the different amount of lipids in the samples. Salmon has a high lipid content and possibly contained arsenolipids that could not be extracted by the present extractant. Zheng & Hintelmann (2004) attributed the remaining arsenic (lower efficiencies in the extraction procedures) to the arsenolipids, which is not soluble in the methanol/water solvent. For DOLT-4 extraction efficiency, the value of 77% found in this work is similar to (78%) reported by Pétursdóttir et al. (2014) that used the same extraction method. On the other hand, whitefish and swordfish, which have low lipid content, had high extraction efficiencies of 97% and 95%, respectively.
Column recovery

Column recovery is expressed as the ratio of total As (sum of all arsenic species) eluted from the chromatographic column to the total As in the extract injected into the chromatographic column. Measurement of column recovery is essential to provide a control of chromatographic separation and to evaluate the quantification of the As species. The column recovery values ranged from 58% to 99% for CRMs (Table 1) and 70% to 104% for all samples (Table 2). These values are in agreement with those reported by Zheng & Hintelmann (2004), which found values from 85 to 110% using HPLC-ICP-SFMS and methanol/water as extracting agent.

Recovery of inorganic arsenic

Standards of As(III) and As(V) were spiked in solid samples of red porgy, tuna-1, clam-1, mussel and CRM TORT-2 and then homogenized. Samples were taken for extraction 30 minutes after spiking. Quantitative oxidation of As(III) to As(V) was achieved since only As(V) was found as iAs in the spiked samples. Thus, anion LC-ICP-MS was used to quantify the As(V) as iAs in the samples. The recoveries found for red porgy, tuna-1, clam-1, mussel and TORT-2 were 102 ± 2, 100 ± 5, 100 ± 4, 101 ± 2 and 106 ± 2 (mean % ± standard deviation, n=3), respectively. These recovery values were calculated according to the literature (Llorente-Mirandes et al., 2014) and show good recovery of iAs. As an example, Figure 1 and Figure 2 show the chromatograms of clam-1 and red porgy extracts, respectively. The clam-1 was fortified with 0.200 mg As kg\(^{-1}\) of As(III) and As(V); the red porgy with 0.250 mg As kg\(^{-1}\) of As(III) and As(V). As can be seen, iAs was recovered successfully as As(V) from the two samples.
**Accuracy**

In order to verify the accuracy of the proposed speciation method, two CRMs were analysed and evaluated: BCR-627 (Tuna fish) and ERM-BC211 (Rice). The CRM BCR-627 has a certified value of $3.9 \pm 0.22 \text{ mg As kg}^{-1}$ for AB and $0.15 \pm 0.02 \text{ mg As kg}^{-1}$ for DMA. To assess the accuracy of the inorganic arsenic results, the ERM-BC211 rice material was analysed because there is no CRM for measurement of inorganic arsenic in seafood. The ERM-BC211 has a certified value of $0.124 \pm 0.011 \text{ mg As kg}^{-1}$ for iAs and $0.119 \pm 0.013 \text{ mg As kg}^{-1}$ for DMA. The values found for the ERM-BC211 and CRM BCR-627 are shown in Table 1 and did not differ significantly from certified values at a 95% confidence level.

**Limits of detection and quantification**

Limits of detection (LOD) and quantification (LOQ) were estimated for each As species. To calculate these parameters, the standard deviation of the base line and the chromatographic peak base of each analyte multiplied by 3 or 10 (LOD and LOQ respectively) were interpolated in the slope of the height calibration curve. The instrumental limits were converted to sample limits by multiplying by the extraction dilution factor. The LODs for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0010, 0.0014, 0.0017, 0.0024, 0.0010, 0.0028 and 0.0018 mg As kg$^{-1}$ dry weight basis, respectively. The LOQs for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0033, 0.0047, 0.0056, 0.0080, 0.0033, 0.0093, 0.0060 mg As kg$^{-1}$ dry weight basis, respectively.

3.2 Comparison of inorganic arsenic in seafood Reference Materials
The concentrations of iAs in TORT-2, DOLT-4, BCR-627 and SRM 1566b CRMs found in the literature since 2005 are given in Table 3. These concentrations vary widely according to the extraction and detection method. According to Table 3, the concentrations of iAs ranged from 0.09-1.233 mg kg\(^{-1}\) for TORT-2, 0.010-0.152 mg kg\(^{-1}\) for DOLT-4, 0.004-1.161 mg kg\(^{-1}\) for SRM 1566b and 0.015-0.192 mg kg\(^{-1}\) for BCR-627. No iAs concentrations were found in the literature for NIST SRM 2976, ERM-CE278 and 9\(^{th}\) PT RMs, however the concentrations found in this work are given in Table 1.

The international measurement evaluation programme (IMEP) and the EU-RL-HM performed two proficiency tests in 2010 for the determination of trace metals, methylmercury and iAs, in seafood. In these proficiency tests, CRM DOLT-4 was used as the test material and the iAs values reported by expert laboratories using different extraction methods and techniques (Baer et al., 2011) ranged between 0.040 and 0.152 mg kg\(^{-1}\) (Table 3), highlighting strong discrepancies among the reported results. In other words, it was not possible to establish an assigned value for iAs, which was clearly more difficult to analyse in the seafood matrix than other matrices (Baer et al., 2011). Due to these problems, Pétursdóttir et al. have been published several works about determination of iAs concentration in CRMs using different extraction and detection methods (Pétursdóttir, 2012a and 2012b; Pétursdóttir et al., 2014). In the most recent study, nine different extraction methods were used to extract DOLT-4 and TORT-2 (Pétursdóttir et al., 2014). The reported values ranged between 0.010–0.036 mg kg\(^{-1}\) and 0.315–0.823 mg kg\(^{-1}\) for DOLT-4 and TORT-2, respectively (Table 3). This fact illustrates that solvent plays a role in the extraction of iAs, and therefore, a difficulty in obtaining a consistent value of iAs in DOLT-4 and TORT-2. The concentrations of iAs found in the present study for DOLT-4
(0.020 ± 0.003 mg kg$^{-1}$) and TORT-2 (0.71 ± 0.04 mg kg$^{-1}$) are concordant with Pétursdóttir et al. (2014) work (0.017 ± 0.003 mg kg$^{-1}$ and 0.714 ± 0.092 mg kg$^{-1}$ for DOLT-4 and TORT-2, respectively), which used a similar extraction method (MAE, 2% HNO$_3$ in 3% H$_2$O$_2$). On the other hand, Leufroy et al. (2011) used two MAE methods (water and methanol/water) and found a mean concentration of 1.183 mg kg$^{-1}$ iAs for TORT-2 that is higher than found in HNO$_3$/H$_2$O$_2$ extraction method. For CRM BCR-627, the concentration found in this study was 0.02 ± 0.002 iAs. Leufroy et al. (2011) found 0.074 ± 0.014 mg kg$^{-1}$ iAs with water and 0.192 ± 0.071 mg kg$^{-1}$ iAs with methanol/water. Santos, Nunes, Barbosa, Santos, Peso-Aguiar, Korn, et al. (2013) using MAE (methanol/water) method found 0.325 mg kg$^{-1}$ iAs. Sloth & Julshamn (2008) using MAE (ethanol/NaOH) method found 0.015 mg kg$^{-1}$ iAs. The latter concentration was the most similar to that found in this work. In relation to SRM 1566b, the concentration of iAs found was 0.05 ± 0.001 mg kg$^{-1}$, different from that reported by Santos (1.161 mg kg$^{-1}$) and Sloth (0.004 mg kg$^{-1}$) (Santos et al., 2013; Sloth & Julshamn, 2008).

In summary, the concentrations of iAs found in this work (Table 1) are within the range reported by several authors (Table 3), which show that proposed method give comparable results. However, the large variability of iAs concentration illustrates that it is difficult to obtain a consistent value for iAs in these CRMs. Therefore, the lack of a CRM for iAs in seafood limits the comparison and validation of values found by different authors. The development of seafood CRMs would help in the validation of speciation data and in the creation of legislation that could establish the maximum amount of iAs (Pétursdóttir et al., 2012b).

3.3 Total arsenic in samples
Total As was determined in 22 seafood samples, four of which were Brazilian fish samples and the remainder Spanish seafood samples. The samples were classified as fish (n=14), crustaceans (n=3) and bivalves (n=5) and the values found for total As in seafood samples are reported in Table 4. The concentration of total As ranged from 1.2–35.2 mg kg$^{-1}$ dry mass. Crustaceans and bivalves contained more total As than fish (with the exception of three fish samples). A mean of 10.2 mg kg$^{-1}$ dry mass (dm) was found in fish, while in bivalves and crustaceans the mean were 15.0 and 2.2 mg kg$^{-1}$, respectively. These results are consistent with the literature (Baeyens, Gao, De Galan, Bilau, Van Larebeke, & Leermakers, 2009; Fontcuberta, Calderon, Villalbí, Centrich, Portaña, Espelt, et al., 2011; Leufroy et al., 2011; Moreda-Piñeiro, Peña-Vázquez, Hermelo-Herbello, Bermejo-Barrera, Moreda-Piñeiro, Alonso-Rodríguez, et al., 2008; Sirot, Guérin, Volatier, & Leblanc, 2009). The 2004 EU SCOOP report (European Commission, 2004) and Sirot et al. (2009) highlighted the importance of geographical, seasonal and environmental factors in the large variation in arsenic levels in seafoods. Two Brazilian fish samples (whitefish and red porgy) and one Spanish fish sample (forkbeard) showed the highest levels of total As: 35.2 ± 1.14 mg kg$^{-1}$, 35.0 ± 0.16 mg kg$^{-1}$ and 31.8 ± 1.27 mg kg$^{-1}$ respectively. The levels of total As in oyster and mussel samples were 24.6 ± 0.30 mg kg$^{-1}$ and 12.9 ± 0.74 mg kg$^{-1}$, respectively. Leufroy et al. (2011) found similar values in five different oyster samples (average of 20.4 mg kg$^{-1}$ for total As) and ten different mussel samples (average of 11.3 mg kg$^{-1}$ for total As). The Brazilian government, through the Ministry of Agriculture, Livestock and Food Supply (MAPA), established a reference value of 1 mg kg$^{-1}$ for total As in fish (National Program for Residue and Contaminant Control, 2012). The values found in this work are above the values recommended by the Brazilian government. Although the seafood samples had high levels of total As, the dominant species was AB (approximately
66\% for oyster and mussel, and 95\% for fish, Table 2), which is considered non-toxic. In contrast, Zheng & Hintelmann (2004) found lower levels of AB in samples collected from the Moira Lake (less than 16\% of total arsenic). Those data demonstrate the need to carry out speciation in seafood samples as the total amount of As does not provide enough information about the toxicity of the analysed sample.

3.4 Arsenic species in samples

A selection of 22 seafood samples including crustaceans, bivalves and fish, were analysed for their content of As species. The results are reported in Table 2. AB was found the main arsenic species in all analysed samples as expected (Leufroy et al., 2011; Sirot et al., 2009) ranging from 48 to 95\% of the total arsenic. DMA was also detected as minority compounds in mussels, clams and prawns, as reported in the literature (Cao, Hao, Wang, Yang, Chen, & Wang, 2009; Cava-Montesinos, Nilles, Cervera, & Guardia, 2005; Leufroy et al., 2011; Moreda-Piñeiro et al., 2008; Sirot et al., 2009; Súñer, Devesa, Clemente, Vélez, Montoro, Urieta, et al., 2002). DMA was found in 73\% of samples, and MA appeared in 36\% of samples (prawns, shrimp, cockles and oysters). DMA was found at higher levels than MA in fish samples which is in agreement with other published studies (Cava-Montesinos et al., 2005; Leufroy et al., 2011; Sirot et al., 2009; Súñer et al., 2002). TMAO and AC were found in 50\% and 18\% of all samples respectively. As mentioned before, an interesting study was carried out by Zheng & Hintemann (2004), which reported an unusual distribution of As species in fresh water fish samples. In this study, high concentration of DMA was found in a predatory fish sample and a high TETRA content was observed in the muscle tissue of pumpkinseed (34.9\%) and largemouth bass (24.4\%).
An unknown compound with a retention time of 279 s was found using the cationic column (UC-A, ranged from 0.6% to 27% of total arsenic) (Figure 1), along with a second unknown compound (UC-B, ranged from 0.3% to 6% of the total arsenic) with a retention time of 360 s. These unknown cation species could be attributed to trimethylarsoniopropionate (TMAP) and tetramethylarsonium ion (TETRA), respectively, according to Kirby, Maher, Ellwood, & Krikowa (2004). However, it was not possible to check this attribution due to the lack of appropriate standards.

In terms of anionic species, two unknown compounds, UA-A and UA-B, with a retention time of 148 and 251 s respectively, were found as minor species in crustacean and bivalve samples (Figure 1). These unknown peaks ranged from 0.4% to 0.9% and from 0.2% to 15% of the total arsenic, for UA-A and UA-B, respectively. These peaks could correspond to arsenosugar compound such as dimethylarsinoylsugarglycol and dimethylarsinoylsugarphosphate, which were identified in fish and molluscs (Nischwitz & Pergantis, 2005). Due to the lack of appropriate standards, this attribution was not checked.

The inorganic arsenic was extracted, identified and quantified as As(V), and selectively separated from other arsenic compounds. It was found in 36% of all samples being always below 3.3% of the total arsenic. For fish samples, the inorganic arsenic content is in all cases below the limit of detection. (n=14). This is illustrated in Figure 2a, which shows that inorganic arsenic was not detected in red porgy extracts (continuous line), and also shows that the all the spiked iAs was successfully recovered as As(V) (dotted line). The extraction method not converted the other organoarsenic species into inorganic arsenic (iAs). Figure 2b shows that the major arsenic compound in red porgy extracts was arsenobetaine. Low concentrations for iAs (<0.037 mg kg\(^{-1}\)) in fish have been reported in other studies which are in agreement with the results found in the present study.
(Fontcuberta et al., 2011; Larsen, Engman, Sloth, Hansen, & Jorhem, 2005; Leufroy et al., 2011). However, iAs was found in bivalves and crustaceans at concentrations of up to 0.35 mg kg\(^{-1}\). In all samples analysed in this work, iAs accounted for less than 3.3% of the total arsenic and was below the limits allowed by Australia/New Zealand (Australia New Zealand Food Authority, 2013) and China (MHC, 2005). The highest concentration of iAs (0.35 ± 0.009 mg kg\(^{-1}\)) was found in the clam-1 sample, followed by cockle (0.27 ± 0.008 mg kg\(^{-1}\)). Chromatograms of the clam-1 extract from anion exchange (a) and cation exchange (b) are shown in Figure 1. Inorganic arsenic was found in the clam-1 sample (Fig. 1a, continuous line), which was fortified with As(III) and As(V), and as can be seen, iAs was recovered successfully as As(V) (Fig. 1a, dotted line). The lowest concentration of iAs (0.033 ± 0.003 mg kg\(^{-1}\)) was found in shrimp, as previously observed (Baeyens et al., 2009; Leufroy et al., 2011; Sirot et al., 2009; Sloth, Larsen & Julshamn, 2005).

The present results showed a wide variability in the arsenic species found in seafood samples, highlighting the need to carry out speciation to discern the toxic from the non-toxic species.

### 4. CONCLUSIONS

The differences found in the literature among the concentrations of iAs in several CRMs reinforce the need to develop reliable methodology to its determination. Therefore, a method for the determination of inorganic arsenic as well as for AB, DMA, MA, AC and TMAO species in seafood was proposed. Regarding the advantages of the proposed method, the conversion of As(III) to As(V) which allows the quantification of iAs as As(V) is the most notable factor. As(III) elutes near the void volume in the anion-exchange
column and it could co-elute with other cationic species usually found in seafood (specially AB). Therefore, the oxidation of As(III) to As(V) allows the determination of iAs as As(V) which is well separated from other As species. Also it is remarkable that is not necessary to quantify two peaks to determine iAs, so errors are minimized. Thus, the present method allows an accurate quantification of iAs and could be a valuable tool for food control laboratories which assessing the iAs in seafood samples.

To assess the applicability of the method, total arsenic and arsenic species in different seafood samples, including fish, crustaceans and bivalves, were determined. AB was the predominant arsenic species in all samples. Inorganic arsenic content was below the detection limit in all fish samples, whereas it was found in all bivalves and crustacean samples ranged from 0.02 to 0.71 mg As kg\(^{-1}\) of iAs.

For an accurate assessment of food safety more efforts will be needed such as validation and interlaboratory comparison exercise for iAs determination in seafood that, up to date, have shown unsatisfactory performances. Despite the lack of Brazilian and European legislation regulating the maximum levels of iAs in seafood, the present results have increased the availability of reliable results on inorganic arsenic in seafood and could be useful for EFSA in future dietary exposure to iAs and in further Directives on iAs in food commodities.

ACKNOWLEDGMENTS

The authors are grateful to CAPES (process n° BEX 12866121) and CNPQ for scholarships awarded to A.V.Z. and M.M.S. The authors thank the DGICYT (Project No. CTQ2010-15377) and the Grup de Recerca Consolidat (Project No. SGR2009-1188) for financial help.
received in support of this study. T. Llorente-Mirandes acknowledges the Ajuts Predoctorals de Formació en Docència i Recerca (ADR) of the University of Barcelona for a pre-doctoral grant. The authors also thank Dr. A. Padró (Centres Científics i Tecnològics de la Universitat de Barcelona, CCiTUB) for his valuable support with LC-ICP-MS measurements. The authors are grateful to Josep Calderon from the Laboratory of the Public Health Agency of Barcelona (ASPB), Maria Aparecida B. Espírito Santo and Isabel C. F. Damin from LANAGRO (RS, Brazil) for the kind donation of the seafood samples.

5. REFERENCES

Amayo, K. O., Petursdottir, A. H., Newcombe, C., Gunnlaugsdottir, H., Raab, A., Krupp, E. M., & Feldmann, J. r. (2011). Identification and Quantification of Arsenolipids Using Reversed-Phase HPLC Coupled Simultaneously to High-Resolution ICPMS and High-Resolution Electrospray MS without Species-Specific Standards. *Analytical Chemistry, 83*(9), 3589-3595.

Australia New Zealand Food Standards Code (2013). Standard 1.4.1 - Contaminants and Natural Toxicants. Federal Register of Legislative Instruments F2013C00140 Issue: 139. URL: http://www.comlaw.gov.au/Details/F2013C00140. Accessed on 14-05-2014.

Baer, I., Baxter, M., Devesa, V., Vélez, D., Raber, G., Rubio, R., Llorente-Mirandes, T., Sloth, J. J., Robouch, P., & de la Calle, B. (2011). Performance of laboratories in
speciation analysis in seafood – Case of methylmercury and inorganic arsenic. *Food Control*, 22(12), 1928-1934.

Baeyens, W., Gao, Y., De Galan, S., Bilau, M., Van Larebeke, N., & Leermakers, M. (2009). Dietary exposure to total and toxic arsenic in Belgium: Importance of arsenic speciation in North Sea fish. *Molecular Nutrition & Food Research*, 53(5), 558-565.

Borak, J., & Hosgood, H. D. (2007). Seafood arsenic: Implications for human risk assessment. *Regulatory Toxicology and Pharmacology*, 47(2), 204-212.

Cao, X., Hao, C., Wang, G., Yang, H., Chen, D., & Wang, X. (2009). Sequential extraction combined with HPLC-ICP-MS for As speciation in dry seafood products. *Food Chemistry*, 113(2), 720-726.

Cava-Montesinos, P., Nilles, K., Cervera, M. L., & Guardia, M. d. l. (2005). Non-chromatographic speciation of toxic arsenic in fish. *Talanta*, 66(4), 895-901.

Commission regulation (2006). Number 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Commun L364:5–24.

De Silva, D., & Bjondal, T. (2013). An open innovation and its role in global fish and seafood value chains: beyond the conventional wisdom. *Journal of Agricultural Sciences*, 8(3), 161-173.
Dufailly, V., Noel, L., Fremy, J.-M., Beauchemin, D., & Guerin, T. (2007). Optimisation by experimental design of an IEC/ICP-MS speciation method for arsenic in seafood following microwave assisted extraction. *Journal of Analytical Atomic Spectrometry, 22*(9), 1168-1173.

European Commission (2004). Report of experts participating in Task 3.2.11 “Assessment of the dietary exposure to arsenic, cadmium, lead, and mercury of the population of the EU Member States”

European Food Safety Authority (2009). Scientific opinion on arsenic in food. Panel on Contaminants in the Food Chain (CONTAM). *EFSA Journal 2009; 7*(10), 1351-1549.

European Food Safety Authority (2014). Dietary exposure to inorganic arsenic in the European population. *EFSA Journal 2014; 12*(3), 3597-3665.

Feldmann, J., & Krupp, E. M. (2011). Critical review or scientific opinion paper: Arsenosugars—a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? *Analytical and Bioanalytical Chemistry, 399*(5), 1735-1741.

Fontcuberta, M., Calderon, J., Villalbí, J. R., Centrich, F., Portaña, S., Espelt, A., Duran, J., & Nebot, M. (2011). Total and Inorganic Arsenic in Marketed Food and Associated Health Risks for the Catalan (Spain) Population. *Journal of Agricultural and Food Chemistry, 59*(18), 10013-10022.
Foster, S., Maher, W., Krikowa, F., & Apte, S. (2007). A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues. *Talanta, 71*(2), 537-549.

Francesconi, K. A. (2010). Arsenic species in seafood: Origin and human health implications. *Pure and Applied Chemistry, 82*(2), 373-381.

Francesconi, K. A., & Kuehnelt, D. (2004). Determination of arsenic species: A critical review of methods and applications, 2000-2003. *Analyst, 129*(5), 373-395.

Geng, W., Komine, R., Ohta, T., Nakajima, T., Takanashi, H., & Ohki, A. (2009). Arsenic speciation in marine product samples: Comparison of extraction–HPLC method and digestion–cryogenic trap method. *Talanta, 79*(2), 369-375.

Hirata, S., Toshimitsu, H., & Aihara, M. (2006). Determination of Arsenic Species in Marine Samples by HPLC-ICP-MS. *Analytical Sciences, 22*(1), 39-43.

Innis, S. M. (2007). Dietary (n-3) fatty acids and brain development. *The Journal of nutrition, 137*(4), 855-859.

Kirby, J., Maher, W., Ellwood, M., & Krikowa, F. (2004). Arsenic Species Determination in Biological Tissues by HPLC–ICP-MS and HPLC–HG–ICP-MS. *Australian Journal of Chemistry, 57*(10), 957-966.

Larsen, E., Engman, J., Sloth, J., Hansen, M., & Jorhem, L. (2005). Determination of inorganic arsenic in white fish using microwave-assisted alkaline alcoholic sample
dissolution and HPLC-ICP-MS. *Analytical and Bioanalytical Chemistry, 381*(2), 339-346.

Leufroy, A., Noël, L., Dufailly, V., Beauchemin, D., & Guérin, T. (2011). 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. *Talanta, 83*(3), 770-779.

Llorente-Mirandes, T., Calderón, J., Centrich, F., Rubio, R., & López-Sánchez, J. F. (2014). A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by IC–ICPMS. *Food Chemistry, 147*(0), 377-385.

MHC (2005). Maximum levels of contaminants in foods, GB2762-2005. Beijing: Ministry of Health of China.

Moreda-Piñeiro, A., Peña-Vázquez, E., Hermelo-Herbello, P., Bermejo-Barrera, P., Moreda-Piñeiro, J., Alonso-Rodríguez, E., Muniategui-Lorenzo, S., López-Mahía, P. n., & Prada-Rodríguez, D. o. (2008). Matrix Solid-Phase Dispersion as a Sample Pretreatment for the Speciation of Arsenic in Seafood Products. *Analytical Chemistry, 80*(23), 9272-9278.

National Program for Residue and Contaminant Control (2012). Normative Instruction number 11, Brasilia, section 1, Pages 4-8.
Nischwitz, V., & Pergantis, S. A. (2005). Liquid Chromatography Online with Selected Reaction Monitoring Electrospray Mass Spectrometry for the Determination of Organoarsenic Species in Crude Extracts of Marine Reference Materials. *Analytical Chemistry, 77*(17), 5551-5563.

Pétursdóttir, Á., Gunnlaugsdóttir, H., Jörundsdóttir, H., Mestrot, A., Krupp, E., & Feldmann, J. (2012a). HPLC-HG-ICP-MS: a sensitive and selective method for inorganic arsenic in seafood. *Analytical and Bioanalytical Chemistry, 404*(8), 2185-2191.

Pétursdóttir, Á. H., Gunnlaugsdóttir, H., Jörundsdóttir, H., Raab, A., Krupp, E. M., & Feldmann, J. (2012b). Determination of inorganic arsenic in seafood: emphasizing the need for certified reference materials. *Pure and Applied Chemistry, 84*(2), 191-202.

Pétursdóttir, A. H., Gunnlaugsdóttir, H., Krupp, E. M., & Feldmann, J. (2014). Inorganic arsenic in seafood: Does the extraction method matter? *Food Chemistry, 150*(0), 353-359.

Reyes, L. H., Mar, J. L. G., Rahman, G. M. M., Seybert, B., Fahrenholz, T., & Kingston, H. M. S. (2009). Simultaneous determination of arsenic and selenium species in fish tissues using microwave-assisted enzymatic extraction and ion chromatography–inductively coupled plasma mass spectrometry. *Talanta, 78*(3), 983-990.
Santos, C. M. M., Nunes, M. A. G., Barbosa, I. S., Santos, G. L., Peso-Aguiar, M. C., Korn, M. G. A., Flores, E. M. M., & Dressler, V. L. (2013). Evaluation of microwave and ultrasound extraction procedures for arsenic speciation in bivalve mollusks by liquid chromatography–inductively coupled plasma-mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy, 86*(0), 108-114.

Sirot, V., Guérin, T., Volatier, J. L., & Leblanc, J. C. (2009). Dietary exposure and biomarkers of arsenic in consumers of fish and shellfish from France. *Science of The Total Environment, 407*(6), 1875-1885.

Sloth, J. J., Larsen, E. H., & Julshamn, K. (2005). Survey of Inorganic Arsenic in Marine Animals and Marine Certified Reference Materials by Anion Exchange High-Performance Liquid Chromatography–Inductively Coupled Plasma Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 53*(15), 6011-6018.

Sloth, J. J., & Julshamn, K. (2008). Survey of Total and Inorganic Arsenic Content in Blue Mussels (Mytilus edulis L.) from Norwegian Fiords: Revelation of Unusual High Levels of Inorganic Arsenic. *Journal of Agricultural and Food Chemistry, 56*(4), 1269-1273.

Súñer, M. A., Devesa, V., Clemente, M. J., Vélez, D., Montoro, R., Urieta, I., Jalón, M., & Macho, M. L. (2002). Organoarsenical Species Contents in Fresh and Processed Seafood Products. *Journal of Agricultural and Food Chemistry, 50*(4), 924-932.
Zheng, J., & Hintelmann, H. (2004). Hyphenation of high performance liquid chromatography with sector field inductively coupled plasma mass spectrometry for the determination of ultra-trace level anionic and cationic arsenic compounds in freshwater fish. *Journal of Analytical Atomic Spectrometry, 19*(1), 191-195.

Zmozinski, A. V., Passos, L. D., Damin, I. C. F., Espirito Santo, M. A. B., Vale, M. G. R., & Silva, M. M. (2013). Determination of cadmium and lead in fresh fish samples by direct sampling electrothermal atomic absorption spectrometry. *Analytical Methods, 5*(22), 6416-6424.
Table 1. Total arsenic and arsenic species in reference materials; concentrations are expressed as mg As kg\(^{-1}\) dry mass (mean ± SD, n = 3 and *n=6).

| Reference Materials | Total As | Total extracted As | Arsenic species | Sum of As species | Extraction Efficiency (%) | Column Recovery (%) |
|---------------------|----------|--------------------|-----------------|-------------------|---------------------------|---------------------|
|                     |          |                    | DMA            | MA                | UA-B \(^c\)             | iAs                  | AB                 | TMAO  | AC        | UC-A \(^d\) | UC-B \(^e\) |                     |
| TORT-2\(^x\)        | 22.4 ± 1.1 | 21.9 ± 1.7         | 1.57 ± 0.05    | 0.20 ± 0.01       | 0.12 ± 0.02             | 0.71 ± 0.04           | 13.1 ± 0.45         | 0.19 ± 0.02 | 0.05 ± 0.004 | 0.94 ± 0.05 | 0.08 ± 0.02 | 17.0 ± 0.64 | 98 | 78 |
| Certified value \(^a\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| DOLT-4\(^x\)        | 9.64 ± 0.11 | 7.39 ± 0.39        | 0.45 ± 0.07    | 0.10 ± 0.02       | 0.07 ± 0.01             | 0.02 ± 0.003          | 5.17 ± 0.51         | 0.32 ± 0.01 | <LOD       | 0.10 ± 0.01 | <LOD       | 6.24 ± 0.63 | 77 | 84 |
| Certified value \(^a\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| ERM-CE278           | 6.09 ± 0.21 | 4.46 ± 0.23        | 0.62 ± 0.04    | 0.10 ± 0.02       | 0.03 ± 0.007            | 0.07 ± 0.003          | 2.27 ± 0.17         | <LOD       | <LOD       | 0.09 ± 0.005 | 0.17 ± 0.012 | 3.36 ± 0.26 | 73 | 75 |
| Certified value \(^a\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| NIST 1566           | 7.67 ± 0.13 | 6.85 ± 0.19        | 0.84 ± 0.06    | <LOD              | 0.45 ± 0.02             | 0.05 ± 0.001          | 2.63 ± 0.07         | <LOD       | <LOD       | <LOD        | <LOD        | 3.97 ± 0.15 | 89 | 58 |
| Certified value \(^a\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| NIST 2976           | 13.7 ± 0.25 | 13.3 ± 0.52        | 0.41 ± 0.05    | 0.12 ± 0.002      | 0.30 ± 0.04             | 0.11 ± 0.013          | 10.3 ± 0.20         | <LOD       | <LOD       | 0.14 ± 0.02 | 0.13 ± 0.012 | 11.5 ± 0.33 | 97 | 86 |
| Certified value \(^a\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| 9th PT (CRL-ISS)    | 7.00 ± 0.32 | 6.89 ± 0.06        | 0.5 ± 0.06     | 0.05 ± 0.01       | 0.25 ± 0.04             | 0.24 ± 0.02           | 4.3 ± 0.19          | <LOD       | 0.16 ± 0.03 | <LOD        | 5.73 ± 0.36 | 98 | 83 |
| Assigned value \(^b\) |          |                    |                |                   |                          |                      |                    |                   |                   |                      |                   |                   |
| BCR-627             | 4.84 ± 0.13 | 4.75 ± 0.08        | 0.13 ± 0.02    | 0.02 ± 0.004      | 0.03 ± 0.006            | 0.02 ± 0.002          | 3.8 ± 0.07          | <LOD       | 0.05 ± 0.008 | 0.05 ± 0.003 | 0.06 ± 0.006 | 4.16 ± 0.11 | 98 | 88 |
|                | Value   | Certified value | Assigned value | Unknown anion arsenic species (UA-B) with a retention time of 251 s. | Unknown cation arsenic species (UC-A) with a retention time of 279 s. | Unknown cation arsenic species (UC-B) with a retention time of 360 s. |
|----------------|---------|----------------|----------------|---------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
|                | Mean    | 4.80 ± 0.3     | 0.15 ± 0.02    | 3.9 ± 0.2                                                            |                                                                     |                                                                     |
| ERM-BC211      | 0.263 ± 0.011 | 0.265 ± 0.010 | 0.128 ± 0.006  | 0.016 ± 0.004 <LOD                                                  | 0.119 ± 0.005 <LOD                                                 | 0.124 ± 0.011 <LOD                                                  |
| Certified value | 0.260 ± 0.013 | 0.119 ± 0.013  | 0.124 ± 0.011  | <LOD                                                             | <LOD                                                             | <LOD                                                             |

\(^a\) Certified value: mean ± uncertainty.

\(^b\) Assigned value: mean ± uncertainty.

\(^c\) Unknown anion arsenic species (UA-B) with a retention time of 251 s.

\(^d\) Unknown cation arsenic species (UC-A) with a retention time of 279 s.

\(^e\) Unknown cation arsenic species (UC-B) with a retention time of 360 s.
Table 2. Arsenic speciation analysis of selected seafood samples; concentrations are expressed as mg As kg\(^{-1}\) dry mass (mean ± SD, n = 3).

| Sample       | Total extracted As | Arsenic species | Sum of As species | Extract Efficiency (%) | Column Recovery (%) |
|--------------|--------------------|-----------------|-------------------|------------------------|---------------------|
|              |                    | DMA  | MA  | UA-A\(^a\) | UA-B\(^b\) | iAs  | AB  | TMAO | AC | UC-A\(^c\) | UC-B\(^d\) |                |
| Fish         |                    |      |     |            |            |      |     |      |    |            |            |                |
| White fish   | 34.3 ± 0.89        | <LOD | <LOD| <LOD       | <LOD       | 33.5| 0.04| <LOD | <LOD| 0.1 ± 0.004| 33.6 ± 2.96| 97             | 98             |
| Red porgy    | 33.8 ± 1.84        | <LOD | <LOD| <LOD       | <LOD       | 33.2| 0.04| <LOD | <LOD| 0.94 ± 0.062| 34.1 ± 2.77| 97             | 101            |
| Hake-1       | 6.70 ± 0.16        | <LOD | <LOD| <LOD       | <LOD       | 6.58| 0.03| <LOD | <LOD| 0.04 ± 0.002| 6.65 ± 0.39| 94             | 99             |
| Hake-2       | 3.80 ± 0.03        | 0.13 | 0.012| <LOD       | <LOD       | 3.2 | 0.07| <LOD | <LOD| 0.07 ± 0.026| 3.41 ± 0.25| 90             | 89             |
| Forkbeard    | 27.6 ± 1.22        | 0.24 | 0.015| <LOD       | <LOD       | 20.3| 0.07| <LOD | <LOD| 4.53 ± 0.29 | 25.0 ± 1.43| 86             | 89             |
| Sardine      | 6.88 ± 0.27        | 0.16 | 0.02 | <LOD       | <LOD       | 5.27| 0.03| <LOD | <LOD| 0.04 ± 0.003| 6.0 ± 0.14 | 93             | 87             |
| Salmon-1     | 1.45 ± 0.04        | 0.012| 0.010| <LOD       | <LOD       | 1.18| 0.024| <LOD | <LOD| 1.21 ± 0.056| 1.21 ± 0.056| 86             | 85             |
| Salmon-2     | 1.38 ± 0.08        | 0.03 | 0.006| <LOD       | <LOD       | 0.86| 0.03| <LOD | <LOD| 0.93 ± 0.009| 0.93 ± 0.009| 76             | 70             |
| Tuna-1       | 1.41 ± 0.09        | 0.05 | 0.008| <LOD       | <LOD       | 0.90| 0.08| <LOD | <LOD| 1.08 ± 0.054| 1.08 ± 0.054| 98             | 77             |
| Tuna-2       | 1.71 ± 0.06        | 0.02 | 0.006| <LOD       | <LOD       | 1.43| 0.01| <LOD | <LOD| 1.46 ± 0.10 | 1.46 ± 0.10 | 94             | 86             |
| Louvar       | 4.65 ± 0.07        | 0.04 | 0.008| <LOD       | <LOD       | 4.15| 0.09| <LOD | <LOD| 0.09 ± 0.007| 4.3 ± 0.32 | 104            | 93             |
| Swordfish-1  | 5.20 ± 0.08        | 0.16 | 0.008| <LOD       | <LOD       | 4.20| 0.08| <LOD | <LOD| 0.35 ± 0.05 | 4.73 ± 0.22| 102            | 91             |
| Species       | Value (± Error)  | LOD 1 | LOD 2 | LOD 3 | LOD 4 | LOD 5 | LOD 6 | LOD 7 |
|---------------|-----------------|-------|-------|-------|-------|-------|-------|-------|
| Swordfish-2   | 3.00 ± 0.11     | <LOD  | <LOD  | <LOD  | <LOD  | 1.73 ± 0.02 | 0.01 ± 0.002 | <LOD  |
| Swordfish-3   | 2.58 ± 0.05     | <LOD  | <LOD  | <LOD  | <LOD  | 1.96 ± 0.05 | <LOD  | <LOD  |
| **Crustaceans** |                 |       |       |       |       |       |       |       |
| Prawn-1       | 2.0 ± 0.07      | 0.06 ± 0.008 | 0.08 ± 0.009 | <LOD  | <LOD  | 0.06 ± 0.08  | 1.44 ± 0.023 | <LOD  |
| Prawn-2       | 2.9 ± 0.05      | <LOD  | 0.012 ± 0.002 | <LOD  | 0.007 ± 0.007  | <LOD  | 0.016 ± 0.003  | 2.21 ± 0.039 |
| Shrimp        | 1.0 ± 0.09      | <LOD  | 0.016 ± 0.001 | <LOD  | 0.033 ± 0.003  | <LOD  | 0.005 ± 0.001  | 0.61 ± 0.017 |
| **Bivalves**  |                 |       |       |       |       |       |       |       |
| Clam-1        | 16.8 ± 0.94     | 0.25 ± 0.006 | 0.18 ± 0.02 | 2.07 ± 0.08 | 0.35 ± 0.009  | 11.7 ± 0.73 | <LOD  | 0.29 ± 0.03  |
| Clam-2        | 10.5 ± 0.06     | 0.14 ± 0.002 | <LOD  | 1.86 ± 0.44 | 0.20 ± 0.005  | 7.93 ± 0.27 | <LOD  | 0.02 ± 0.009  |
| Mussel        | 10.3 ± 0.08     | 0.07 ± 0.007 | <LOD  | 0.04 ± 0.005 | 0.65 ± 0.10 | 8.79 ± 0.07 | <LOD  | 0.08 ± 0.006  |
| Cockle        | 7.5 ± 0.45      | <LOD  | 0.13 ± 0.009 | <LOD  | 0.16 ± 0.008 | 0.27 ± 0.008 | 4.01 ± 0.193 | <LOD  | 0.38 ± 0.011 |
| Oyster        | 21.7 ± 0.28     | 0.10 ± 0.009 | 0.08 ± 0.006 | <LOD  | 0.29 ± 0.021 | 0.10 ± 0.009 | 15.9 ± 0.75 | 0.06 ± 0.007 |

*a* Unknown anion arsenic species (UA-A) with a retention time of 148 s.

*b* Unknown anion arsenic species (UA-B) with a retention time of 251 s.

*c* Unknown cation arsenic species (UC-A) with a retention time of 279 s.

*d* Unknown cation arsenic species (UC-B) with a retention time of 360 s.
Table 3. Inorganic arsenic (iAs) concentrations in TORT-2, DOLT-4, BCR 627 and SRM 1566b CRMs found in literature since 2005.

| CRMs | Techniques | Extractions | iAs (mg kg⁻¹) | References |
|------|------------|-------------|---------------|------------|
|      | HPLC-ICP-MS| MAE/(HCl/H₂O₂) | 0.648         | Pétursdóttir et al., 2012 |
| HPLC-ICP-MS | MAE/(HNO₃) | 0.663        |               |            |
| HPLC-ICP-MS | MAE/(NaOH/EtOH) | 0.417 |               |            |
| HPLC–HG-ICP-MS | MAE/(HCl/H₂O₂) | 0.614 |               |            |
| HPLC–HG-ICP-MS | MAE/(HNO₃) | NM³ |               |            |
| HPLC–HG-ICP-MS | MAE/(NaOH/EtOH) | 0.453 |               |            |
| IEC/ICP-MS | MAE/(H₂O) | 1.133 | Leufroy et al., 2011 |
| IEC/ICP-MS | MAE/(MeOH/H₂O) | 1.233 |               |            |
| HPLC–ICP-MS | MAE/(MeOH/H₂O) | 0.320 | Foster et al., 2007 |
| HPLC–ICP-MS | MAE/(HNO₃) | 0.780 |               |            |
| HPLC–ICP-MS | MAE/(H₂O) | 0.100 | Hirata et al., 2006 |
| HPLC–ICP-MS | MAE/(EtOH/NaOH) | 0.190 | Sloth et al., 2005 |
| HPLC–ICP-MS | SON/(Acetone/MeOH/HCl) | 0.09 | Cao et al., 2009 |
| HPLC–ICP-MS | MAE/(EtOH/NaOH) | 0.340 | Pétursdóttir et al., 2012 |
| HPLC–HG-ICP-MS | MAE/(EtOH/NaOH) | 0.470 |               |            |
| HPLC–HG-AFS | MAE/(EtOH/NaOH) | 0.369 |               |            |
| HPLC–HG-AFS | MAE/(EtOH/NaOH) | 0.188 | Larsen et al., 2005 |
| HPLC–HG-AFS | Mineralization/(HCl/KI/Ascorbic acid) | 0.320 | Baeyens et al., 2009 |
| HPLC–HG-AFS | Shaking/(H₃PO₄) | 0.450 | Geng et al., 2009 |
| CT-HG AAS | Alkaline digestion/(NaOH) | ND³ |               |            |
|      | MAE/(HCl/H₂O₂) | 0.614 |               |            |
| HPLC–HG-ICP-MS | MAE/(H₂O/MeOH) | 0.676 | Pétursdóttir et al., 2014 |
| HPLC–HG-ICP-MS | SON and MAE/(TFA /H₂O₂) | 0.315 |               |            |
|      | Described in reference | 0.331 |               |            |
| Method                  | MAE/(HNO₃) | MAE/(HNO₃/H₂O₂) | MAE/(H₂O) | SON/(H₂O) | MAE/(NaOH/EtOH) |
|------------------------|------------|-----------------|-----------|-----------|-----------------|
| DOLT-4                 |            |                 |           |           |                 |
| HPLC-ICP-MS            | MAE/(HCl/H₂O₂) 0.039 | MAE/(HNO₃) 0.028 | MAE/(NaOH/EtOH) 0.027 |           |                 |
|                        | MAE/(HCl/H₂O₂) 0.011 | MAE/(HNO₃) 0.011 | MAE/(NaOH/EtOH) 0.010 |           |                 |
|                        | MAE/(HCl/H₂O₂) <0.040 | MAE/(MeOH/H₂O) ND | SON/(Trifluoracetic acid/H₂O₂) 0.047 |           |                 |
|                        | MAE/(HCl/H₂O₂) 0.011 |             |           |           |                 |
| FI-HG-AAS              | Shaking/(H₂O/HCl/HBr/Hydrazine sulphate) 0.075 |             |           |           |                 |
| HR-ICP-MS              | Shaking/(H₂O/HCl/HBr/Hydrazine sulphate) 0.152 |             |           |           |                 |
|                        | MAE/(HCl/H₂O₂) 0.011 |             |           |           |                 |
|                        | MAE/(H₂O/MeOH) 0.012 |             |           |           |                 |
|                        | SON and MAE/(Trifluoracetic acid/H₂O₂) 0.011 |             |           |           |                 |
|                        | Described in reference 0.036 |             |           |           |                 |
|                        | MAE/(HNO₃) 0.011 |             |           |           |                 |
|                        | MAE/(HNO₃/H₂O₂) 0.017 |             |           |           |                 |
|                        | MAE/(H₂O) 0.011 |             |           |           |                 |
|                        | SON/(H₂O) 0.010 |             |           |           |                 |
|                        | MAE/(NaOH/EtOH) 0.010 |             |           |           |                 |
| Method                  | Extraction Type                                      | Recovery | Reference                        |
|------------------------|------------------------------------------------------|----------|----------------------------------|
| BCR 627                |                                                      |          |                                  |
| IEC/ICP-MS             | MAE/(H₂O)                                           | 0.074    | Leufroy et al., 2011             |
|                        | MAE/(MeOH/H₂O)                                      | 0.192    |                                  |
| IEC/ICP-MS             | MAE/(MeOH)                                          | 0.100    | Dufailly et al., 2007            |
| HG–AFS                 | SON/(HNO₃/Triton X-100)                             | 0.070    | Cava-montesinos et al., 2005     |
| HPLC–ICP-MS            | MAE/(EtOH NaOH)                                     | 0.015    | Sloth et al., 2005               |
| HPLC–ICP-MS            | Matrix solid phase extraction/(MeOH/H₂O)            | 0.080    | Moreda-Piñeiro et al., 2008      |
| IC–ICP-MS              | MAE-enzymatic/(pronase/lipase)                      | NDᵇ      | Reyes et al., 2009               |
| LC–ICP-MS              | MAE/(MeOH/H₂O)                                      | 0.325    | Santos et al., 2013              |
| HPLC-HG-AFS            | Shaking/(H₃PO₄)                                     | NDᵇ      | Geng et al., 2009                |
| CT-HG AAS              | Alkaline digestion/(NaOH)                           |          |                                  |
| SRM 1566b              |                                                      |          |                                  |
| HPLC–ICP-MS            | MAE/(EtOH/NaOH)                                     | 0.004    | Sloth et al., 2005               |
| HPLC-ES-SRM            | Shaking/(H₂O)                                       | NDᵇ      | Nischwitz & Pergantis, 2005      |
| LC–ICP-MS              | MAE/(MeOH/H₂O)                                      | 1.161    | Santos et al., 2013              |

*NM not measured  *ND not detected

MAE Microwave Assisted Extraction  SON Sonication
**Table 4.** Total arsenic in seafood samples, concentrations are expressed as mg As kg$^{-1}$ dry mass (mean ± SD, n = 3).

| Samples | Species                  | Trade name | Origin | Total As   |
|---------|--------------------------|------------|--------|------------|
| Fish    | *Urophycis cirrata*      | White fish | Brazil | 35.2 ± 1.14|
|         | *Pagrus pagrus*          | Red porgy  | Brazil | 35.0 ± 0.16|
|         | *Merluccius hubbsi*      | Hake-1     | Brazil | 7.10 ± 0.04|
|         | *Merluccius gayi*        | Hake-2     | Brazil | 4.20 ± 0.11|
|         | *Phycis blennoides*      | Forkbeard  | Spain  | 31.8 ± 1.27|
|         | *Sardina pilchardus*     | Sardine    | Spain  | 7.42 ± 0.08|
|         | *Salmo sp.*              | Salmon-1   | Spain  | 1.70 ± 0.09|
|         | *Salmo sp.*              | Salmon-2   | Spain  | 1.77 ± 0.10|
|         | *Thunnus sp.*            | Tuna-1     | Spain  | 1.44 ± 0.09|
|         | *Thunnus sp.*            | Tuna-2     | Spain  | 1.71 ± 0.12|
|         | *Luvarus imperialis*     | Louvar     | Spain  | 4.46 ± 0.08|
|         | *Xiphias gladius*        | Swordfish-1| Spain | 5.10 ± 0.08|
|         | *Xiphias gladius*        | Swordfish-2| Spain | 3.30 ± 0.21|
|         | *Xiphias gladius*        | Swordfish-3| Spain | 2.90 ± 0.04|
| Crustaceans | *Aristeus antennatus* | Prawn-1   | Spain  | 2.3 ± 0.07 |
|          | *Aristaeopsis edwardsiana* | Prawn-2   | Spain  | 3.1 ± 0.08 |
|          | *Crangon crangon*        | Shrimp     | Spain  | 1.2 ± 0.05 |
| Bivalves | *Tapes pullastra*        | Clams-1    | Spain  | 17.0 ± 1.40|
|          | *Tapes Decussatus*       | Clams-2    | Spain  | 12.2 ± 0.16|
|          | *Mytilus edulis*         | Mussel     | Spain  | 12.9 ± 0.74|
|          | *Cerastoderma edule*     | Cockle     | Spain  | 8.3 ± 0.02 |
|          | *Ostrea sp.*             | Oyster     | Spain  | 24.6 ± 0.30|
Figure captions

**Figure 1.** Chromatograms of clam-1 extract from anion exchange (a) (continuous line: non-spiked sample and dotted line: sample spiked with iAs) and cation exchange (b) by LC–ICP-MS.

**Figure 2.** Chromatograms of red porgy extract from anion exchange (a) (continuous line: non-spiked sample and dotted line: sample spiked with iAs) and cation exchange (b) by LC–ICP-MS.
