

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

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13

14 **Abstract**

15 An analytical method for determination of arsenic species (inorganic arsenic (iAs),
16 methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenobetaine (AB),
17 trimethylarsine oxide (TMAO) and arsenocholine (AC)) in Brazilian and Spanish seafood
18 samples is reported. This study was focused on extraction and quantification of inorganic
19 arsenic (iAs), the most toxic form. Arsenic speciation was carried out via LC with both
20 anionic and cationic exchange with ICP-MS detection (LC-ICP-MS). The detection limits
21 (LODs), quantification limits (LOQs), precision and accuracy for each arsenic species were
22 established. The proposed method was evaluated using eight reference materials (RMs).
23 Arsenobetaine was the main species found in all samples. The total and iAs concentration

24 in 22 seafood samples and RMs ranged between 0.27–35.2 and 0.02–0.71 mg As kg⁻¹,
25 respectively. Recoveries of between 100% and 106% for iAs, based on spikes, were
26 achieved. The present results provide reliable iAs data for future risk assessment analysis.

27

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

31

32 **1. Introduction**

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

44 Seafood contains intrinsically more total arsenic than terrestrial foods, and more
45 than 50 species of arsenic were identified in seafood (Francesconi, 2010). Inorganic As

46 species in seafood are commonly present as low percentages of the total amount of As
47 (Borak & Hosgood, 2007). However, high concentrations have been reported in some types
48 of seafood, e.g. in bivalve mussels, where concentrations of up to 5 mg As kg⁻¹ were found
49 (Sloth & Julshamn, 2008). The different toxicities of the As species reinforce the
50 importance of its chemical speciation, as the total amount of As does not provide enough
51 information about the toxicity of the analysed sample.

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

59 Countries such as New Zealand and Australia have legislation for the maximum
60 levels of inorganic arsenic (iAs) in seafood and established a maximum level of inorganic
61 arsenic of 2 mg kg⁻¹ for crustaceans and fish, and 1 mg kg⁻¹ for molluscs and seaweed
62 (Australia New Zealand Food Authority, 2013). The Republic of China establishes a
63 maximum level of inorganic arsenic of 0.1 mg kg⁻¹ for fish and 1.0 mg kg⁻¹ for shells,
64 shrimps and crabs (dry weight), respectively (MHC, 2005). On the other hand, the Brazilian
65 government through the Ministry of Agriculture, Livestock and Food Supply (MAPA)
66 establishes a reference value of 1 mg kg⁻¹ for total As in fish (National Program for Residue
67 and Contaminant Control, 2012). However, the European Union has not established a limit
68 for total or inorganic As in fish and seafood in its legislation (Commission regulation,
69 2006).

70 Aware of this situation, the EFSA (European Food Safety Authority) published in
71 2009 and 2014, two reports about the dietary exposure to arsenic in the European
72 population (European Food Safety Authority, 2009 and 2014). Both reported the urgent
73 need for further data on arsenic species, particularly iAs data, in particular in fish and
74 seafood, and in food groups that provide a significant contribution to the dietary exposure
75 to iAs (e.g. rice and wheat-based products) to reduce the uncertainty of the exposure
76 assessments to iAs. Thus, the need to introduce specific legislation is becoming evident
77 (European Food Safety Authority, 2009; Feldmann & Krupp, 2011). Furthermore, the need
78 to create certified reference materials for seafood and to develop arsenic speciation methods
79 for a large range of food samples and arsenic species was also emphasized (European Food
80 Safety Authority, 2009). The increased focus on inorganic arsenic in food has led to several
81 initiatives towards development of methods for selective determination of inorganic arsenic
82 in seafood. For this purpose, the Institute for Reference Materials and Measurements
83 (IRMM) organised two proficiency tests (PT) in 2010 for measuring iAs, and trace metals
84 in seafood (IMEP-109 and IMEP-30). The determination of iAs in seafood test material
85 presented serious analytical problems. The expert laboratories were not able to agree on a
86 value for the iAs within a reasonable degree of uncertainty (Baer, Baxter, Devesa, Vélez,
87 Raber, Rubio, et al., 2011). It was concluded that more research in extraction and
88 chromatographic procedures was required to quantify the iAs in seafood (Baer et al., 2011).
89 The complexity of the seafood matrix requires accurate and robust procedures. However,
90 the analytical procedures used to date do not comply with these requirements (Feldmann &
91 Krupp, 2011).

92 Some authors reported inorganic arsenic values in several seafood CRM collected
93 from previously published studies (Leufroy et al. 2011; Pétursdóttir, Gunnlaugsdóttir,

94 Jörundsdóttir, Mestrot, Krupp, & Feldmann, 2012a; Pétursdóttir, Gunnlaugsdóttir,
95 Jörundsdóttir, Raab, Krupp, & Feldmann, 2012b; Pétursdóttir, Gunnlaugsdóttir, Krupp, &
96 Feldmann 2014). The results of iAs varied widely according to the extraction and detection
97 method. This emphasizes the need for the development of reliable methods for the
98 determination of iAs in seafood and a certified value of inorganic As in a seafood-based
99 reference material.

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

107

108 **2. MATERIALS AND METHODS**

109

110 **2.1 Instruments**

111 For total As, all measurements were carried out using an Agilent 7500ce ICP-MS
112 (Agilent, Germany) with a BURGNER Ari Mist HP type nebulizer. For As speciation,
113 LC-ICP-MS was used with an Agilent 1200 LC quaternary pump, equipped with an auto
114 sampler. The analytical columns Hamilton PRP-X100 (250 x 4.1 mm, 10 µm, Hamilton,
115 USA) and Zorbax-SCX300 (250 x 4.6 mm, 5 µm, Agilent, Germany) were protected by
116 guard columns filled with the corresponding stationary phases. The outlet of the LC column

117 was connected via PEEK capillary tubing to the nebulizer of the ICP-MS system. A
118 microwave (Milestone Ethos Touch Control) was used for digesting and extracting the
119 samples. The fish samples supplied by MAPA (Brazil) were lyophilized in a ModulyonD
120 Freeze Dryer lyophilizer (Thermo Electron Corporation, USA) and milled in an A 11 Basic
121 micro-mill (IKA – Werke, Germany).

122

123 2.2. Reagents and standards

124 Analytical grade reagents were used exclusively. Deionized water with a specific
125 resistivity of $18 \text{ M}\Omega \text{ cm}^{-1}$ from a Milli-Q water purification system (Millipore, Bedford,
126 MA, USA) was used for the preparation of all solutions. Formic acid (98%) (Panreac, p.a.,
127 Barcelona, Spain), ammonium dihydrogen phosphate (Panreac, p.a., Barcelona, Spain),
128 aqueous ammonia solution (25%) (Panreac, p.a., Barcelona, Spain), and pyridine (Scharlau,
129 p.a., Barcelona, Spain) were used for the preparation of mobile phases. The following
130 reagents were used for sample digestion and extraction: 31% H_2O_2 (Merck, Selectipur,
131 Darmstadt, Germany) and 69% HNO_3 (Panreac, Hiperpur, Barcelona, Spain). External
132 calibration standards for total As were prepared daily by dilution of a standard stock
133 solution traceable to the National Institute of Standards and Technology (Gaithersburg,
134 USA) with a certified concentration of $1001 \pm 5 \text{ mg As L}^{-1}$ (Inorganic Ventures Standards,
135 Christiansburg, USA). A solution of ^9Be , ^{103}Rh and ^{205}Tl was used as the internal standard
136 in ICP-MS measurements. An arsenate standard solution of $1000 \pm 5 \text{ mg As L}^{-1}$ (Merck,
137 Darmstadt, Germany) was used for external quality control in total arsenic and arsenic
138 speciation measurements. Stock standard solutions ($1000 \text{ mg As L}^{-1}$) for arsenic speciation
139 were prepared as follows: As(III), from As_2O_3 (NIST, Gaithersburg, USA, Oxidimetric

140 Primary Standard 83d, 99.99%) dissolved in 4 g L⁻¹ NaOH (Merck, Suprapure, Darmstadt,
141 Germany); As(V), from Na₂HAsO₄·7H₂O (Carlo Erba, Milano, Italy) dissolved in water;
142 MA, prepared from (CH₃)AsO(ONa)₂·6H₂O (Carlo Erba, Milano, Italy) dissolved in water;
143 DMA, prepared from (CH₃)₂AsNaO₂·3H₂O (Fluka, Buchs, Switzerland) dissolved in water.
144 Arsenocholine (AC) from (CH₃)₃As⁺(CH₂) CH₂OHBr⁻ was supplied by the “Service
145 Central d’Analyse” (CNRS Vernaison, Solaize, France) and trimethylarsine oxide (TMAO)
146 was prepared from (CH₃)₃AsO (Argus Chemicals, Vernio, Italy) dissolved in water. The
147 certified reference material of arsenobetaine (AB) from (CH₃)₃ As⁺CH₂COO⁻ was supplied
148 by NMIJ (Tsukuba, Japan) as a standard solution, NMIJ CRM 7901-a. For our internal
149 quality control, the As concentration in in-house prepared As speciation standards was
150 determined by ICPMS. For this, As(V), As(III), DMA, MA, AC, TMAO and AB were
151 standardized against two arsenic certified standard solutions (Merck, Darmstadt, Germany
152 and Inorganic Ventures, Christiansburg, USA) as well as against As₂O₃ solution. All stock
153 solutions were kept at 4 °C, and further diluted solutions for the analysis were prepared
154 daily.

155 ***2.3. Reference materials and samples***

156 The following certified reference materials (CRM) were used for method
157 development: DOLT-4 (Dogfish), TORT-2 (Lobster Hepatopancreas) (both from the
158 National Research Council, Canada); NIST SRM 2976 (Mussel Tissue) and NIST SRM
159 1566b (Oyster Tissue) (National Institute of Standards and Technology, Gaithersburg, MD,
160 USA); BCR-627 (Tuna fish), ERM-BC211 (Rice) and ERM-CE278 (Mussel Tissue)
161 (Institute for Reference Materials and Measurements of the European Commission’s Joint
162 Research Centre, Geel, Belgium). The reference material (RM) 9th PT on fish from the

163 Community Reference Laboratory-Istituto Superiore di Sanità (CRL-ISS, Rome, Italy) was
164 also analysed.

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

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

180 **2.4. Procedures**

181 **2.4.1. Moisture determination**

182 The moisture of fresh samples was determined in triplicate by drying 0.5 g aliquots
183 in an oven at $102 \pm 3^\circ\text{C}$ until constant weight. Moisture ranged from 45% to 94%, and all
184 results are expressed as dry mass.

185 **2.4.2. Total arsenic analysis**

186 The total arsenic content in seafood and CRM samples was determined by ICP-MS
187 following microwave digestion. Initially, 0.5 g and 2 g aliquots of lyophilized and fresh
188 samples, respectively, were weighed in digestion vessels, after which 8 mL of concentrated
189 nitric acid and 2 mL of hydrogen peroxide were added. The microwave digestion procedure
190 was carried out according to the following programme: 10 min from room temperature to
191 90 °C, maintained for 5 min at 90 °C, 10 min from 90 °C to 120 °C, 10 min from 120 °C to
192 190 °C and 10 min maintained at 190 °C. After cooling to room temperature, the digested
193 samples were diluted in water up to 25 mL. Helium gas was used in the collision cell to
194 avoid interference in the ICP-MS measurements. A solution of ⁹Be, ¹⁰³Rh and ²⁰⁵Tl was
195 used as the internal standard. The samples were quantified by means of an external
196 calibration curve from As(V) standards. Triplicate analyses were performed for each
197 sample. For quality control purposes, the standards of the calibration curve were run before
198 and after each sample series. The corresponding digestion blanks (one for each sample
199 digestion series) were also measured. Quality control standard solutions at two
200 concentrations were measured after constructing the calibration curve. To assess the
201 accuracy of the ICP-MS method, seven CRMs (DOLT-4, TORT-2, SRM 2976, SRM
202 1566b, BCR-627, ERM-BC211 and ERM-CE278) and one RM (9th PT) were analysed.

203

204

205 **2.4.3 Arsenic speciation analysis**

206 The extraction of As species was based on our previous study (Llorente-Mirandes,
207 Calderón, Centrich, Rubio, & López-Sánchez, 2014). For this, 0.2 g and 1.0 g aliquots of

208 lyophilized and fresh samples, respectively, were weighed in digestion vessels and 10 mL
209 of a solution containing 0.2% (w/v) of nitric acid and 1% (w/v) of hydrogen peroxide were
210 added to perform a microwave assisted extraction (MAE) at temperature of 95 °C. Samples
211 were cooled to room temperature and centrifuged at 3500 rpm for 25 min. The supernatant
212 was filtered through PET filters (Chromafil, Macherey–Nagel, pore size 0.45 µm).
213 Triplicate analyses were performed for each sample. This extraction method completely
214 oxidizes As(III) into As(V), without conversion of the other organoarsenic species into
215 inorganic arsenic (iAs). The iAs was identified and quantified as As(V) in the extracts by
216 comparing the chromatographic peak for the samples with the peak of As(V) standard
217 solution. Total arsenic in the extracts was determined by ICP-MS (as described previously).
218 Arsenic speciation was carried out in the extracts by LC-ICP-MS. Two chromatographic
219 separation methods were used for separation of the arsenic species. As(III), As(V), DMA
220 and MA were analysed by anion exchange chromatography. AB, AC and TMAO were
221 analysed by cation-exchange chromatography. The performance characteristics of anion-
222 exchange chromatographic system are previously described (Llorente-Mirandes, Calderón,
223 Centrich, Rubio, & López-Sánchez, 2014). The main chromatographic conditions of cation-
224 exchange chromatography were: mobile phase of 20 mM pyridine, pH = 2.6, flow rate at
225 1.5 mL min⁻¹, and injection volume of 50 µL. Arsenic species in extracts were identified by
226 comparison of retention times with standards. External calibration curves were used to
227 quantify MA, DMA, As(III), As(V), AB, TMAO and AC according to the corresponding
228 standards. Extraction blanks were also analysed by LC-ICP-MS in each work session. The
229 ion intensity at m/z 75 (⁷⁵As) was monitored using time-resolved analysis software.
230 Additionally, the ion intensities at m/z 77 (⁴⁰Ar³⁷Cl) and m/z 35 (³⁵Cl) were monitored to
231 detect possible argon chloride (⁴⁰Ar³⁵Cl) interference at m/z 75. In each speciation run, an

232 As(V) certified standard solution (Merck, Darmstadt, Germany) and a certified reference
233 material solution were measured every ten samples and at the end of the sequence to ensure
234 stable instrument sensitivity.

235

236 **3. RESULTS AND DISCUSSION**

237

238 3.1 Quality control

239 3.1.1 Analysis of the total As concentration

240 To evaluate the accuracy of the applied procedure, several CRMs were analysed.
241 Seafood CRMs (TORT-2, DOLT-4, SRM 2976, SRM 1566b, BCR-627, ERM-BC211 and
242 ERM-CE278) and one material reference (9Th) were analysed during the study. The
243 concurrent analyses of the CRMs listed above were used to measure the accuracy of the
244 determination of total As (Table 1). For quality control of acid digestion, a CRM was
245 analysed in every batch of samples measurements (total As concentration). The comparison
246 between each obtained value of total As with its corresponding certified value (Table 1)
247 showed no significant difference at a 95% confidence level when Student's *t*-test was
248 applied. The repeatability (six times within a day, n=6) was assessed for the results
249 obtained by analysis of different replicates of CRMs (Table 1). The RSD (%) values were:
250 4.9% for TORT-2 and 1.2% for DOLT-4. The detection (LOD) and quantification limits
251 (LOQ) were calculated as three times the standard deviation (3σ) and ten times the standard
252 deviation signal (10σ) of ten digestion blanks, respectively (Llorente-Mirandes et al.,
253 2014). The results obtained were as follows: 0.006 mg As kg⁻¹ dry weight basis for method
254 detection limit and 0.021 mg As kg⁻¹ dry weight basis for method quantification limit.

255

256 3.1.2 Analysis of As species

257 *Extraction efficiencies*

258 The extraction efficiency was evaluated by calculating the ratio between total
259 arsenic present in the samples, given by the acid digestion, and the total arsenic present in
260 the extracts. The extraction efficiencies are presented in Table 1 for the CRMs and Table 2
261 for the real samples. The efficiency obtained in this work varied between 73% and 104%
262 with an average of 89%, which is consistent with the literature (Amayo, Petursdottir,
263 Newcombe, Gunnlaugsdottir, Raab, Krupp, et al., 2011; Pétursdóttir et al., 2014; Zheng &
264 Hintelmann, 2004). Thus, the solution containing 0.2% (w/v) of HNO₃ and 1% (w/v) of
265 H₂O₂ proved to be an effective solvent in the extraction of As species in seafood. A recent
266 study compared nine extraction methods for determination of iAs in seafood, including the
267 HNO₃/H₂O₂ (Pétursdóttir et al., 2014). The highest extraction efficiency for all samples was
268 achieved by HNO₃/H₂O₂ method, which corroborate with this work. An average extraction
269 efficiency of 93% was obtained for most samples, with the exception of DOLT-4, ERM
270 CE278 and salmon-2, for which the average was 75%. According to Pétursdóttir et al.
271 (2012b) and Amayo et al. (2011) this difference in extraction efficiencies can be attributed
272 to the different amount of lipids in the samples. Salmon has a high lipid content and
273 possibly contained arsenolipids that could not be extracted by the present extractant. Zheng
274 & Hintelmann (2004) attributed the remaining arsenic (lower efficiencies in the extraction
275 procedures) to the arsenolipids, which is not soluble in the methanol/water solvent. For
276 DOLT-4 extraction efficiency, the value of 77% found in this work is similar to (78%)
277 reported by Pétursdóttir et al. (2014) that used the same extraction method. On the other
278 hand, whitefish and swordfish, which have low lipid content, had high extraction
279 efficiencies of 97% and 95%, respectively.

280

281 *Column recovery*

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

290

291 *Recovery of inorganic arsenic*

292 Standards of As(III) and As(V) were spiked in solid samples of red porgy, tuna-1,
293 clam-1, mussel and CRM TORT-2 and then homogenized. Samples were taken for
294 extraction 30 minutes after spiking. Quantitative oxidation of As(III) to As(V) was
295 achieved since only As(V) was found as iAs in the spiked samples. Thus, anion LC-ICP-
296 MS was used to quantify the As(V) as iAs in the samples. The recoveries found for red
297 porgy, tuna-1, clam-1, mussel and TORT-2 were 102 ± 2 , 100 ± 5 , 100 ± 4 , 101 ± 2 and
298 106 ± 2 (mean % \pm standard deviation, n=3), respectively. These recovery values were
299 calculated according to the literature (Llorente-Mirandes et al., 2014) and show good
300 recovery of iAs. As an example, Figure 1 and Figure 2 show the chromatograms of clam-1
301 and red porgy extracts, respectively. The clam-1 was fortified with $0.200 \text{ mg As kg}^{-1}$ of
302 As(III) and As(V); the red porgy with $0.250 \text{ mg As kg}^{-1}$ of As(III) and As(V). As can be
303 seen, iAs was recovered successfully as As(V) from the two samples.

304

305 *Accuracy*

306 In order to verify the accuracy of the proposed speciation method, two CRMs were
307 analysed and evaluated: BCR-627 (Tuna fish) and ERM-BC211 (Rice). The CRM BCR-
308 627 has a certified value of 3.9 ± 0.22 mg As kg⁻¹ for AB and 0.15 ± 0.02 mg As kg⁻¹ for
309 DMA. To assess the accuracy of the inorganic arsenic results, the ERM-BC211 rice
310 material was analysed because there is no CRM for measurement of inorganic arsenic in
311 seafood. The ERM-BC211 has a certified value of 0.124 ± 0.011 mg As kg⁻¹ for iAs and
312 0.119 ± 0.013 mg As kg⁻¹ for DMA. The values found for the ERM-BC211 and CRM BCR-
313 627 are shown in Table 1 and did not differ significantly from certified values at a 95%
314 confidence level.

315

316 *Limits of detection and quantification*

317 Limits of detection (LOD) and quantification (LOQ) were estimated for each As
318 species. To calculate these parameters, the standard deviation of the base line and the
319 chromatographic peak base of each analyte multiplied by 3 or 10 (LOD and LOQ
320 respectively) were interpolated in the slope of the height calibration curve. The instrumental
321 limits were converted to sample limits by multiplying by the extraction dilution factor. The
322 LODs for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0010, 0.0014, 0.0017,
323 0.0024, 0.0010, 0.0028 and 0.0018 mg As kg⁻¹ dry weight basis, respectively. The LOQs
324 for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0033, 0.0047, 0.0056, 0.0080,
325 0.0033, 0.0093, 0.0060 mg As kg⁻¹ dry weight basis, respectively.

326

327 3.2 Comparison of inorganic arsenic in seafood Reference Materials

328

329 The concentrations of iAs in TORT-2, DOLT-4, BCR-627 and SRM 1566b CRMs
330 found in the literature since 2005 are given in Table 3. These concentrations vary widely
331 according to the extraction and detection method. According to Table 3, the concentrations
332 of iAs ranged from 0.09-1.233 mg kg⁻¹ for TORT-2, 0.010-0.152 mg kg⁻¹ for DOLT-4,
333 0.004-1.161 mg kg⁻¹ for SRM 1566b and 0.015-0.192 mg kg⁻¹ for BCR-627. No iAs
334 concentrations were found in the literature for NIST SRM 2976, ERM-CE278 and 9th PT
335 RMs, however the concentrations found in this work are given in Table 1.

336 The international measurement evaluation programme (IMEP) and the EU-RL-HM
337 performed two proficiency tests in 2010 for the determination of trace metals,
338 methylmercury and iAs, in seafood. In these proficiency tests, CRM DOLT-4 was used as
339 the test material and the iAs values reported by expert laboratories using different
340 extraction methods and techniques (Baer et al., 2011) ranged between 0.040 and 0.152 mg
341 kg⁻¹ (Table 3), highlighting strong discrepancies among the reported results. In other words,
342 it was not possible to establish an assigned value for iAs, which was clearly more difficult
343 to analyse in the seafood matrix than other matrices (Baer et al., 2011). Due to these
344 problems, Pétursdóttir *et al.* have been published several works about determination of iAs
345 concentration in CRMs using different extraction and detection methods (Pétursdóttir,
346 2012a and 2012b; Pétursdóttir et al., 2014). In the most recent study, nine different
347 extraction methods were used to extract DOLT-4 and TORT-2 (Pétursdóttir et al., 2014).
348 The reported values ranged between 0.010–0.036 mg kg⁻¹ and 0.315–0.823 mg kg⁻¹ for
349 DOLT-4 and TORT-2, respectively (Table 3). This fact illustrates that solvent plays a role
350 in the extraction of iAs, and therefore, a difficulty in obtaining a consistent value of iAs in
351 DOLT-4 and TORT-2. The concentrations of iAs found in the present study for DOLT-4

352 (0.020 ± 0.003 mg kg⁻¹) and TORT-2 (0.71 ± 0.04 mg kg⁻¹) are concordant with
353 Pétursdóttir et al. (2014) work (0.017 ± 0.003 mg kg⁻¹ and 0.714 ± 0.092 mg kg⁻¹ for DOLT-
354 4 and TORT-2, respectively), which used a similar extraction method (MAE, 2% HNO₃ in
355 3% H₂O₂). On the other hand, Leufroy et al. (2011) used two MAE methods (water and
356 methanol/water) and found a mean concentration of 1.183 mg kg⁻¹ iAs for TORT-2 that is
357 higher than found in HNO₃/H₂O₂ extraction method. For CRM BCR-627, the concentration
358 found in this study was 0.02 ± 0.002 iAs. Leufroy et al. (2011) found 0.074 ± 0.014 mg kg⁻¹
359 iAs with water and 0.192 ± 0.071 mg kg⁻¹ iAs with methanol/water. Santos, Nunes,
360 Barbosa, Santos, Peso-Aguiar, Korn, et al. (2013) using MAE (methanol/water) method
361 found 0.325 mg kg⁻¹ iAs. Sloth & Julshamn (2008) using MAE (ethanol/NaOH) method
362 found 0.015 mg kg⁻¹ iAs. The latter concentration was the most similar to that found in this
363 work. In relation to SRM 1566b, the concentration of iAs found was 0.05 ± 0.001 mg kg⁻¹,
364 different from that reported by Santos (1.161 mg kg⁻¹) and Sloth (0.004 mg kg⁻¹) (Santos et
365 al., 2013; Sloth & Julshamn, 2008).

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

374

375 3.3 Total arsenic in samples

376 Total As was determined in 22 seafood samples, four of which were Brazilian fish
377 samples and the remainder Spanish seafood samples. The samples were classified as fish
378 (n=14), crustaceans (n=3) and bivalves (n=5) and the values found for total As in seafood
379 samples are reported in Table 4. The concentration of total As ranged from 1.2–35.2 mg kg⁻¹
380 dry mass. Crustaceans and bivalves contained more total As than fish (with the exception
381 of three fish samples). A mean of 10.2 mg kg⁻¹ dry mass (dm) was found in fish, while in
382 bivalves and crustaceans the mean were 15.0 and 2.2 mg kg⁻¹, respectively. These results
383 are consistent with the literature (Baeyens, Gao, De Galan, Bilau, Van Larebeke, &
384 Leermakers, 2009; Fontcuberta, Calderon, Villalbí, Centrich, Portaña, Espelt, et al., 2011;
385 Leufroy et al., 2011; Moreda-Piñeiro, Peña-Vázquez, Hermelo-Herbello, Bermejo-Barrera,
386 Moreda-Piñeiro, Alonso-Rodríguez, et al., 2008; Sirot, Guérin, Volatier, & Leblanc, 2009).
387 The 2004 EU SCOOP report (European Commission, 2004) and Sirot et al. (2009)
388 highlighted the importance of geographical, seasonal and environmental factors in the large
389 variation in arsenic levels in seafoods. Two Brazilian fish samples (whitefish and red
390 porgy) and one Spanish fish sample (forkbeard) showed the highest levels of total As: 35.2
391 ± 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
392 As in oyster and mussel samples were 24.6 ± 0.30 mg kg⁻¹ and 12.9 ± 0.74 mg kg⁻¹,
393 respectively. Leufroy et al. (2011) found similar values in five different oyster samples
394 (average of 20.4 mg kg⁻¹ for total As) and ten different mussel samples (average of 11.3 mg
395 kg⁻¹ for total As). The Brazilian government, through the Ministry of Agriculture, Livestock
396 and Food Supply (MAPA), established a reference value of 1 mg kg⁻¹ for total As in fish
397 (National Program for Residue and Contaminant Control, 2012). The values found in this
398 work are above the values recommended by the Brazilian government. Although the
399 seafood samples had high levels of total As, the dominant species was AB (approximately

400 66% for oyster and mussel, and 95% for fish, Table 2), which is considered non-toxic. In
401 contrast, Zheng & Hintelmann (2004) found lower levels of AB in samples collected from
402 the Moira Lake (less than 16% of total arsenic). Those data demonstrate the need to carry
403 out speciation in seafood samples as the total amount of As does not provide enough
404 information about the toxicity of the analysed sample.

405

406 3.4 Arsenic species in samples

407 A selection of 22 seafood samples including crustaceans, bivalves and fish, were
408 analysed for their content of As species. The results are reported in Table 2.

409 AB was found the main arsenic species in all analysed samples as expected
410 (Leufroy et al., 2011; Sirot et al., 2009) ranging from 48 to 95% of the total arsenic. DMA
411 was also detected as minority compounds in mussels, clams and prawns, as reported in the
412 literature (Cao, Hao, Wang, Yang, Chen, & Wang, 2009; Cava-Montesinos, Nilles,
413 Cervera, & Guardia, 2005; Leufroy et al., 2011; Moreda-Piñeiro et al., 2008; Sirot et al.,
414 2009; Súnier, Devesa, Clemente, Vélez, Montoro, Urieta, et al., 2002). DMA was found in
415 73% of samples, and MA appeared in 36% of samples (prawns, shrimp, cockles and
416 oysters). DMA was found at higher levels than MA in fish samples which is in agreement
417 with other published studies (Cava-Montesinos et al., 2005; Leufroy et al., 2011; Sirot et
418 al., 2009; Súnier et al., 2002). TMAO and AC were found in 50% and 18% of all samples
419 respectively. As mentioned before, an interesting study was carried out by Zheng &
420 Hintemmann (2004), which reported an unusual distribution of As species in fresh water fish
421 samples. In this study, high concentration of DMA was found in a predatory fish sample
422 and a high TETRA content was observed in the muscle tissue of pumpkinseed (34.9%) and
423 largemouth bass (24.4%).

424 An unknown compound with a retention time of 279 s was found using the cationic
425 column (UC-A, ranged from 0.6% to 27% of total arsenic) (Figure 1), along with a second
426 unknown compound (UC-B, ranged from 0.3% to 6% of the total arsenic) with a retention
427 time of 360 s. These unknown cation species could be attributed to
428 trimethylarsoniopropionate (TMAP) and tetramethylarsonium ion (TETRA), respectively,
429 according to Kirby, Maher, Ellwood, & Krikowa (2004). However, it was not possible to
430 check this attribution due to the lack of appropriate standards.

431 In terms of anionic species, two unknown compounds, UA-A and UA-B, with a
432 retention time of 148 and 251 s respectively, were found as minor species in crustacean and
433 bivalve samples (Figure 1). These unknown peaks ranged from 0.4% to 0.9% and from
434 0.2% to 15% of the total arsenic, for UA-A and UA-B, respectively. These peaks could
435 correspond to arsenosugar compound such as dimethylarsinoysugarglycol and
436 dimethylarsinoysugarphosphate, which were identified in fish and molluscs (Nischwitz &
437 Pergantis, 2005). Due to the lack of appropriate standards, this attribution was not checked.

438 The inorganic arsenic was extracted, identified and quantified as As(V), and
439 selectively separated from other arsenic compounds. It was found in 36% of all samples
440 being always below 3.3% of the total arsenic. For fish samples, the inorganic arsenic
441 content is in all cases below the limit of detection. (n=14). This is illustrated in Figure 2a,
442 which shows that inorganic arsenic was not detected in red porgy extracts (continuous line),
443 and also shows that the all the spiked iAs was successfully recovered as As(V) (dotted
444 line). The extraction method not converted the other organoarsenic species into inorganic
445 arsenic (iAs). Figure 2b shows that the major arsenic compound in red porgy extracts was
446 arsenobetaine. Low concentrations for iAs ($<0.037 \text{ mg kg}^{-1}$) in fish have been reported in
447 other studies which are in agreement with the results found in the present study

448 (Fontcuberta et al., 2011; Larsen, Engman, Sloth, Hansen, & Jorhem, 2005; Leufroy et al.,
449 2011). However, iAs was found in bivalves and crustaceans at concentrations of up to 0.35
450 mg kg^{-1} . In all samples analysed in this work, iAs accounted for less than 3.3% of the total
451 arsenic and was below the limits allowed by Australia/New Zealand (Australia New
452 Zealand Food Authority, 2013) and China (MHC, 2005). The highest concentration of iAs
453 ($0.35 \pm 0.009 \text{ mg kg}^{-1}$) was found in the clam-1 sample, followed by cockle (0.27 ± 0.008
454 mg kg^{-1}). Chromatograms of the clam-1 extract from anion exchange (a) and cation
455 exchange (b) are shown in Figure 1. Inorganic arsenic was found in the clam-1 sample (Fig.
456 1a, continuous line), which was fortified with As(III) and As(V), and as can be seen, iAs
457 was recovered successfully as As(V) (Fig. 1a, dotted line). The lowest concentration of iAs
458 ($0.033 \pm 0.003 \text{ mg kg}^{-1}$) was found in shrimp, as previously observed (Baeyens et al., 2009;
459 Leufroy et al., 2011; Sirot et al., 2009; Sloth, Larsen & Julshamn, 2005).
460 The present results showed a wide variability in the arsenic species found in seafood
461 samples, highlighting the need to carry out speciation to discern the toxic from the non-
462 toxic species.

463

464 **4. CONCLUSIONS**

465 The differences found in the literature among the concentrations of iAs in several
466 CRMs reinforce the need to develop reliable methodology to its determination. Therefore, a
467 method for the determination of inorganic arsenic as well as for AB, DMA, MA, AC and
468 TMAO species in seafood was proposed. Regarding the advantages of the proposed
469 method, the conversion of As(III) to As(V) which allows the quantification of iAs as As(V)
470 is the most notable factor. As(III) elutes near the void volume in the anion-exchange

471 column and it could co-elute with other cationic species usually found in seafood (specially
472 AB). Therefore, the oxidation of As(III) to As(V) allows the determination of iAs as As(V)
473 which is well separated from other As species. Also it is remarkable that is not necessary to
474 quantify two peaks to determine iAs, so errors are minimized. Thus, the present method
475 allows an accurate quantification of iAs and could be a valuable tool for food control
476 laboratories which assessing the iAs in seafood samples.

477 To assess the applicability of the method, total arsenic and arsenic species in
478 different seafood samples, including fish, crustaceans and bivalves, were determined. AB
479 was the predominant arsenic species in all samples. Inorganic arsenic content was below
480 the detection limit in all fish samples, whereas it was found in all bivalves and crustacean
481 samples ranged from 0.02 to 0.71 mg As kg⁻¹ of iAs.

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

489

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491

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502

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639

Table 1

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*	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	21.6 ± 1.8														
DOLT-4*	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	9.66 ± 0.62														
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	6.07 ± 0.13														
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	7.65 ± 0.65														
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	13.30 ± 1.8														
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	0.23 ± 0.01	<LOD	0.16 ± 0.03	<LOD	5.73 ± 0.36	98	83	
Assigned value ^b	6.65 ± 0.71														
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	

Certified value ^a	4.80 ± 0.3	0.15 ± 0.02	3.9 ± 0.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.262 ± 0.01	101	99
ERM-BC211	0.263 ± 0.011	0.265 ± 0.010	0.128 ± 0.006	0.016 ± 0.004	<LOD	0.119 ± 0.005	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Certified value ^a	0.260 ± 0.013	0.119 ± 0.013	0.119 ± 0.011	0.124 ± 0.011	0.119 ± 0.005	0.124 ± 0.011	0.119 ± 0.005	0.124 ± 0.011	0.119 ± 0.005	0.124 ± 0.011	0.119 ± 0.005	0.124 ± 0.011

^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

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	Extraction Efficiency (%)	Column Recovery (%)	
		DMA	MA	UA-A ^a	UA-B ^b	iAs	AB	TMAO	AC	UC-A ^c	UC-B ^d				
<i>Fish</i>															
White fish	34.3 ± 0.89	<LOD	0.014 ± 0.001	<LOD	<LOD	<LOD	33.5 ± 2.95	0.04 ± 0.005	<LOD	<LOD	<LOD	0.1 ± 0.004	33.6 ± 2.96	97	98
Red porgy	33.8 ± 1.84	<LOD	0.010 ± 0.001	<LOD	<LOD	<LOD	33.2 ± 2.71	0.04 ± 0.004	<LOD	<LOD	<LOD	<LOD	34.1 ± 2.77	97	101
Hake-1	6.70 ± 0.16	<LOD	<LOD	<LOD	<LOD	<LOD	6.58 ± 0.19	0.03 ± 0.004	<LOD	<LOD	<LOD	<LOD	6.65 ± 0.39	94	99
Hake-2	3.80 ± 0.03	0.13 ± 0.02	0.012 ± 0.001	<LOD	<LOD	<LOD	3.2 ± 0.20	<LOD	0.07 ± 0.026	<LOD	<LOD	<LOD	3.41 ± 0.25	90	89
Forkbeard	27.6 ± 1.22	0.24 ± 0.02	<LOD	<LOD	<LOD	<LOD	20.3 ± 1.12	<LOD	<LOD	<LOD	<LOD	<LOD	25.0 ± 1.43	86	89
Sardine	6.88 ± 0.27	0.16 ± 0.015	<LOD	<LOD	<LOD	<LOD	5.27 ± 0.13	0.07 ± 0.003	<LOD	<LOD	<LOD	<LOD	6.0 ± 0.14	93	87
Salmon-1	1.45 ± 0.04	0.012 ± 0.0010	<LOD	<LOD	<LOD	<LOD	1.18 ± 0.04	0.024 ± 0.015	<LOD	<LOD	<LOD	<LOD	1.21 ± 0.056	86	85
Salmon-2	1.38 ± 0.08	0.03 ± 0.006	<LOD	<LOD	<LOD	<LOD	0.86 ± 0.08	0.03 ± 0.007	<LOD	<LOD	<LOD	<LOD	0.93 ± 0.009	76	70
Tuna-1	1.41 ± 0.09	0.05 ± 0.008	<LOD	<LOD	<LOD	<LOD	0.90 ± 0.037	0.08 ± 0.002	<LOD	<LOD	<LOD	<LOD	1.08 ± 0.054	98	77
Tuna-2	1.71 ± 0.06	0.02 ± 0.006	<LOD	<LOD	<LOD	<LOD	1.43 ± 0.09	0.01 ± 0.009	<LOD	<LOD	<LOD	<LOD	1.46 ± 0.10	94	86
Louvar	4.65 ± 0.07	0.04 ± 0.008	<LOD	<LOD	<LOD	<LOD	4.15 ± 0.31	<LOD	<LOD	<LOD	<LOD	<LOD	4.3 ± 0.32	104	93
Swordfish-1	5.20 ± 0.08	0.16 ± 0.008	<LOD	<LOD	<LOD	<LOD	4.20 ± 0.16	0.008 ± 0.006	0.02 ± 0.005	0.35 ± 0.05	<LOD	<LOD	4.73 ± 0.22	102	91

Swordfish-2	3.00 ± 0.11	0.05 ± 0.009	<LOD	<LOD	<LOD	<LOD	1.73 ± 0.02	0.01 ± 0.002	<LOD	0.89 ± 0.02	<LOD	2.68 ± 0.05	93	104
Swordfish-3	2.58 ± 0.05	0.05 ± 0.007	<LOD	<LOD	<LOD	<LOD	1.96 ± 0.05	<LOD	<LOD	0.16 ± 0.04	<LOD	2.17 ± 0.09	90	84
<i>Crustaceans</i>														
Prawn-1	2.0 ± 0.07	0.06 ± 0.008	0.08 ± 0.009	<LOD	<LOD	<LOD	1.44 ± 0.023	<LOD	<LOD	0.01 ± 0.004	<LOD	1.66 ± 0.12	87	83
Prawn-2	2.9 ± 0.05	<LOD	0.012 ± 0.002	<LOD	0.007 ± 0.001	0.037 ± 0.002	2.21 ± 0.039	<LOD	0.016 ± 0.003	0.054 ± 0.002	0.040 ± 0.001	2.37 ± 0.050	94	82
Shrimp	1.0 ± 0.09	<LOD	0.016 ± 0.001	<LOD	<LOD	0.033 ± 0.003	0.61 ± 0.017	<LOD	0.005 ± 0.001	0.020 ± 0.002	0.016 ± 0.002	0.70 ± 0.024	83	70
<i>Bivalves</i>														
Clam-1	16.8 ± 0.94	0.25 ± 0.006	<LOD	0.18 ± 0.02	2.07 ± 0.08	0.35 ± 0.009	11.7 ± 0.73	<LOD	0.29 ± 0.03	0.33 ± 0.06	<LOD	15.4 ± 0.91	99	92
Clam-2	10.5 ± 0.06	0.14 ± 0.02	<LOD	<LOD	1.86 ± 0.44	0.20 ± 0.005	7.93 ± 0.27	<LOD	0.02 ± 0.009	0.04 ± 0.006	0.03 ± 0.004	10.21 ± 0.13	86	97
Mussel	10.3 ± 0.08	0.07 ± 0.007	<LOD	0.04 ± 0.005	0.65 ± 0.10	0.08 ± 0.006	8.79 ± 0.07	<LOD	0.08 ± 0.006	0.26 ± 0.009	0.03 ± 0.009	10.0 ± 0.10	80	97
Cockle	7.5 ± 0.45	<LOD	0.13 ± 0.009	<LOD	0.16 ± 0.008	0.27 ± 0.008	4.01 ± 0.193	<LOD	<LOD	0.38 ± 0.011	0.50 ± 0.024	5.5 ± 0.24	90	73
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	0.06 ± 0.005	0.46 ± 0.076	<LOD	17.1 ± 0.84	88	79

^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

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	
TORT-2	HPLC-ICP-MS	MAE/(HCl/H ₂ O ₂)	0.648	Pétursdóttir et al., 2012	
		MAE/(HNO ₃)	0.663		
		MAE/(NaOH/EtOH)	0.417		
	HPLC-HG-ICP-MS	MAE/(HCl/H ₂ O ₂)	0.614		
		MAE/(HNO ₃)	NM ^a		
		MAE/(NaOH/EtOH)	0.453		
	IEC/ICP-MS	MAE/(H ₂ O)	1.133		Leufroy et al., 2011
		MAE/(MeOH/H ₂ O)	1.233		
	HPLC-ICP-MS	MAE(MeOH/H ₂ O)	0.320		Foster et al., 2007
		MAE/(HNO ₃)	0.780		
	HPLC-ICP-MS	MAE/(H ₂ O)	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		0.340	Pétursdóttir et al., 2012	
	HPLC-HG-ICP-MS	MAE/(EtOH/NaOH)	0.470		
	HPLC-HG-AFS		0.369		
	HPLC-ICP-MS	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/(H ₃ PO ₄)	0.450	Geng et al., 2009	
CT-HG AAS	Alkaline digestion/(NaOH)	ND ^b			
HPLC-HG-ICP-MS		MAE/(HCl/H ₂ O ₂)	0.614	Pétursdóttir et al., 2014	
		MAE/(H ₂ O/MeOH)	0.676		
		SON and MAE/(TFA /H ₂ O ₂)	0.315		
		Described in reference	0.331		

		MAE/(HNO ₃)	0.823		
		MAE/(HNO ₃ /H ₂ O ₂)	0.714		
		MAE/(H ₂ O)	0.611		
		SON/(H ₂ O)	0.470		
		MAE/(NaOH/ EtOH)	0.453		
DOLT-4	HPLC-ICP-MS	MAE/(HCl/H ₂ O ₂)	0.039	Pétursdóttir et al., 2012	
		MAE/(HNO ₃)	0.028		
		MAE/(NaOH/EtOH)	0.027		
	HPLC-HG-ICP-MS	MAE/(HCl/H ₂ O ₂)	0.011		
		MAE/(HNO ₃)	0.011		
		MAE/(NaOH/EtOH)	0.010		
	HPLC-ICP-MS	MAE/(HCl/H ₂ O ₂)	<0.040		Baer et al., 2011
		MAE/(MeOH/H ₂ O)	ND		
		SON/(Trifluoroacetic acid/H ₂ O ₂)	0.047		
	FI-HG-AAS	Shaking/(H ₂ O/HCl/HBr/Hydrazine sulphate)	0.075		
HR-ICP-MS	Shaking/(H ₂ O/HCl/HBr/Hydrazine sulphate)	0.152			
		MAE/(HCl/H ₂ O ₂)	0.011		
		MAE/(H ₂ O/MeOH)	0.012		
		SON and MAE/(Trifluoroacetic acid/H ₂ O ₂)	0.011		
		Described in reference	0.036		
	HPLC-HG-ICP-MS	MAE/(HNO ₃)	0.011	Pétursdóttir et al., 2014	
		MAE/(HNO ₃ /H ₂ O ₂)	0.017		
		MAE/(H ₂ O)	0.011		
		SON/(H ₂ O)	0.010		
		MAE/(NaOH/ EtOH)	0.010		

BCR 627	IEC/ICP-MS	MAE/(H ₂ O)	0.074	Leufroy et al., 2011
		MAE/(MeOH/H ₂ O)	0.192	
	IEC/ICP-MS	MAE/(MeOH)	0.100	Dufailly et al., 2007
	HG-AFS	SON/(HNO ₃ /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/H ₂ O)	0.080	Moreda-Piñeiro et al., 2008
	IC-ICP-MS	MAE-enzymatic/(pronase/lipase)	ND ^b	Reyes et al., 2009
	LC-ICP-MS	MAE/(MeOH/H ₂ O)	0.325	Santos et al., 2013
	HPLC-HG-AFS	Shaking/(H ₃ PO ₄)	ND ^b	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/(H ₂ O)	ND ^b	Nischwitz & Pergantis, 2005
	LC-ICP-MS	MAE/(MeOH/H ₂ O)	1.161	Santos et al., 2013

^aNM not measured ^bND not detected

MAE Microwave Assisted Extraction SON Sonication

Table 4

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

Figure 1
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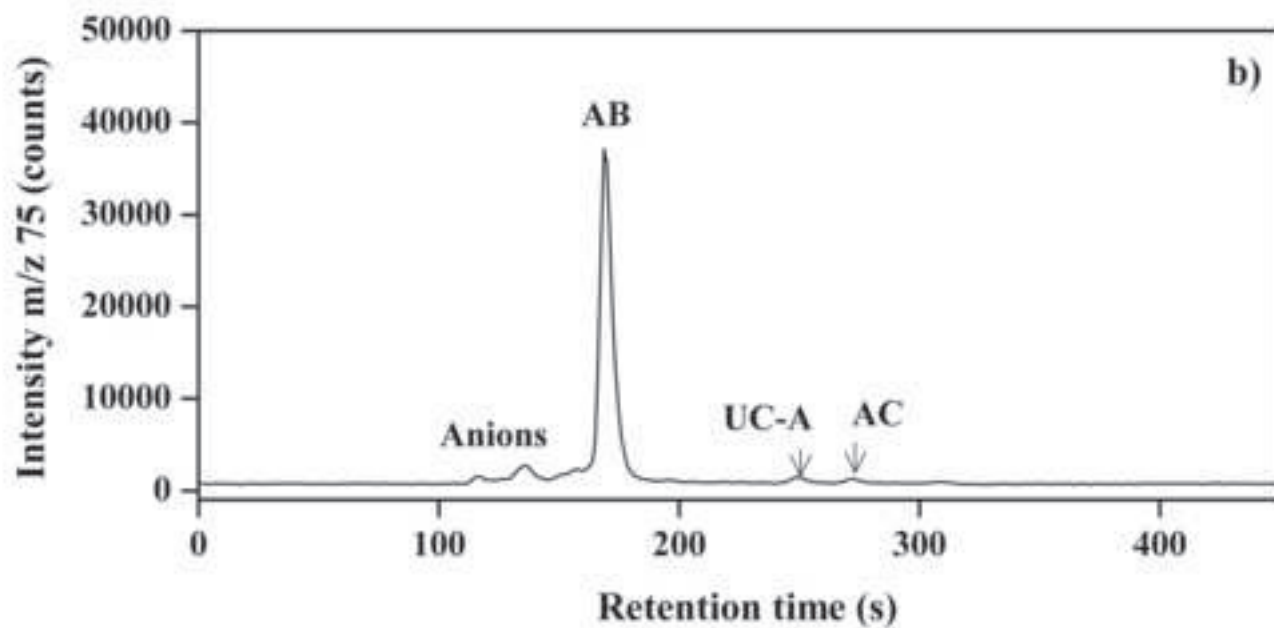
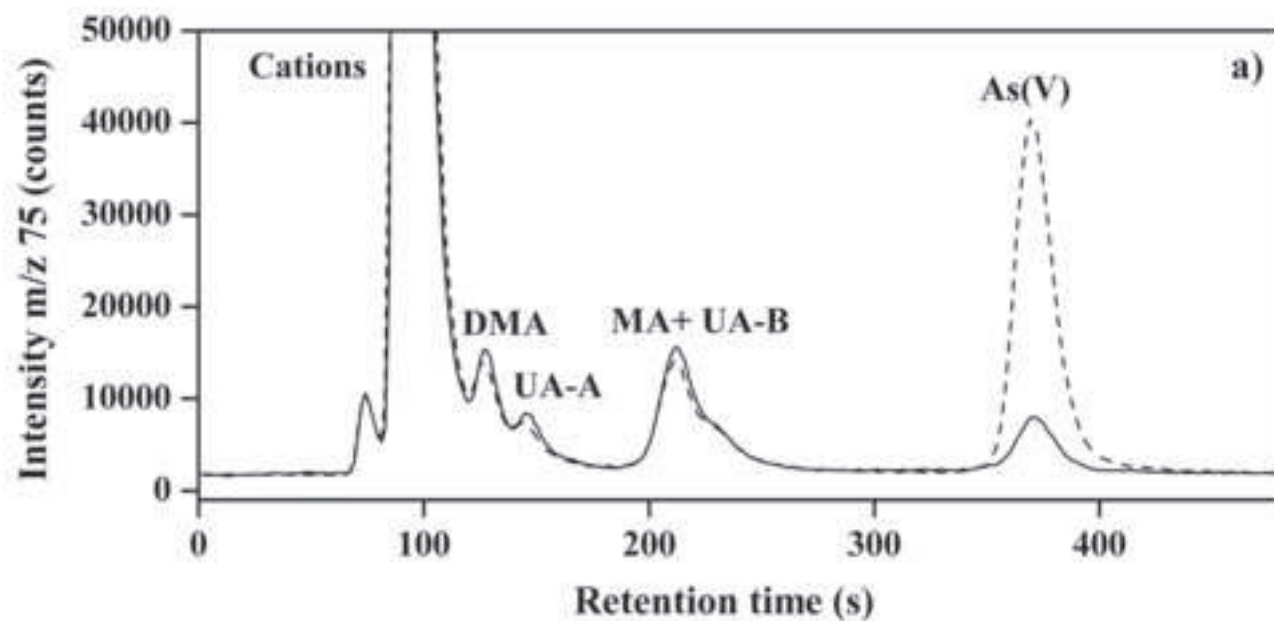


Figure 2
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