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# Determination of inorganic arsenic in seafood: Emphasizing the need for certified reference materials*

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*Abstract*: To evaluate the accuracy and robustness of an extraction method, utilizing an alkaline-ethanolic solution and microwave heating, the certified reference material (CRM) TORT-2 was subjected to three different instrumental methodologies: high-performance liquid chromatography (HPLC), coupled with and without post-column hydride generation; inductively coupled plasma-mass spectrometry (ICP-MS); and HPLC-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS). The three methods gave a consistent value of inorganic arsenic (As) which is near the mean value of the reported values in the literature, which, however, range by a factor of 10. Inorganic As, defined here as all As species that do not have an As–C bond, that is, the sum of arsenite and arsenate and any thiol-bound As, was found to be less than 4 % of total As concentration in 12 samples of fish meal when subjected to this extraction method followed by HPLC-ICP-MS. To date, there is no certified value of inorganic As in a seafood-based reference material to compare to in order to validate the findings. This illustrates the difficulties in quantitative determination of inorganic As in seafood and the need for a reference material for inorganic As and proficiency tests in order to introduce legislation for a maximum level of inorganic As in seafood and feed.

*Keywords*: analytical chemistry; arsenic; food chemistry; speciation.

## INTRODUCTION

More than 50 naturally occurring arsenic (As) species have been identified in the biosphere with the toxicity being dependent on the species [1]. Human exposure to As is mainly through intake of food and beverages [2]. A large portion of As in seafood is present in the form of the organic compound arsenobetaine (AB), which is considered innocuous [3,4]. Other As species are generally present in lower concentrations in marine biota and the most toxic, the inorganic As species, arsenite, As(III), and arsenate, As(V), usually do not exceed 3–4 % of the total As in fish and crustaceans [5]. Human health risk assessment indicates that inorganic As can have serious effects, including cancer, both from acute toxicity to long-term effects of exposure of lower dosages [6]. Seafood has a naturally high concentra-

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tion of total As compared to, e.g., vegetables and grains. Speciation of As compounds present in seafood is important as bioavailability and toxicity of As is dependent on its chemical form. Today, legislation for inorganic As in seafood already exists in China [7], Australia, and New Zealand [8]. Although no limits exist in the European Union (EU) on As in seafood and other food commodities, they do exist for feed, including fish meal, where these limits exist for total As concentration without differentiation of toxic As species [9]. However, a footnote in these EU regulations states that the responsible operator must perform an analysis to demonstrate that the content of inorganic As is lower than 2 mg $kg^{-1}$ upon request of the competent authorities [9]. Apart from this disguised limit on inorganic As in selected feedingstuffs, the regulatory body of the EU seems to be reluctant to establish a maximum permissible level for As because of As's complex chemistry and speciation in seafood.

In a recent proficiency test of inorganic As concentration in rice, a wide range of sample pretreatment methods, including extraction into water, acid, or basic extraction, etc., with different instrumental set-ups [including hydride generation-atomic absorption spectrometry (HG-AAS), high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS), and electrothermal atomic absorption spectrometry (ETAAS)] were applied [10]. The results show that, from an analytical point of view, there is no reason to postpone the introduction of a maximum level of inorganic As in food regulations of rice [10,11].

For marine food and feed, the situation is different, as fish and seafood can contain up to 100 times more As than rice. However, the inorganic As is usually only a small proportion among a variety of other organoarsenic compounds. The IRMM (Institute for Reference Materials and Measurements) further attempted a proficiency test for inorganic As in seafood, however, the determination of inorganic As in the seafood used as test material presented serious analytical problems, unlike the proficiency testing for the rice. The expert laboratories were not able to agree on a value for the inorganic As within a reasonable uncertainty [12].

Samples of seafood origin require different and more complex speciation analysis where the current analytical procedure for seafood samples is not sufficiently robust and accurate due to complicated matrix effects of seafood [13]. Despite the fact that a clause regarding the determination of inorganic As in seafood-based feedingstuffs is in current EU regulations, a reliable, robust, simple and affordable method for the determination of inorganic As in seafood is currently not available.

To date, a variety of different analytical methods have been reported in the literature for the determination of inorganic As in seafood [14–20]. One reported method includes extraction of inorganic As with chloroform, prior to microwave-assisted digestion with concentrated $HClO_4$ and $Fe_2(SO_4)_3$, determination by ETAAS [15]. Another method involves a reduction of As(V) to As(III), which was extracted with hydrochloric acid (HCl) as $AsCl_3$, followed by an extraction into chloroform and a back extraction into dilute HCl, then quantified by HG-AAS. The risk of co-extraction of methylarsonate (MA) and trimethylarsine oxide (TMAO) can lead to overestimation of the level of inorganic As [16]. The most commonly reported analytical approach is a nonspecific solvent extraction of As, using different mixtures of methanol/water, by, e.g., microwave-assisted heating, sonication, or agitation, followed by an HPLC separation of the As species and often coupled to ICP-MS as As detector [17–19,21]. The question has been raised [20] whether this methodology is suitable for a quantitative extraction of inorganic As, including As(III) which may be strongly bound to thiol groups in proteins via an As–S bond [16,22]. In order to liberate As(III) from the sample matrix, it has been suggested that a more energetic solubilization/extraction is necessary [16,20]. An extraction method utilizing an alkaline-ethanolic solution is supposed to be energetic enough to liberate the As(III), while other As species of interest [AB, MA, As(V)] are stable during the treatment [20]. At present, the various extraction methods result in a high variability of the reported concentration of inorganic As in certified reference materials (CRMs) [23], which, however, do not have any value assigned for inorganic As.

The extraction of inorganic As is not the only challenge regarding speciation in seafood. Seafood, including fish meal, can contain a large number of As species, thus achieving a chromatographic separation of the species of interest poses an analytical challenge, as it can be hard to exclude co-elution of

As species. One way to monitor whether co-elution of organic As species with inorganic As is present is the use of post-column HG. As(V), when treated with $NaBH_4$ at acidic conditions forms volatile arsine quantitatively, whereas most other organoarsenic compounds do not produce volatile As-containing product (e.g., AB) or only with low efficiency (e.g., arsenosugars) [24,25].

It is necessary to increase the knowledge of how inorganic As is bound and what measures are needed to quantify it. Furthermore, a certified value of inorganic As in reference materials, including seafood-based materials, is crucial in order to check the robustness of developed methods.

The inorganic As fraction referred to in this work will comprise all As species that do not have an As–C bond, that is, the fraction is defined as the sum of arsenite, arsenate, and other possible thio-binding As species, abbreviated as iAs.

The objective of this study is twofold: To measure and report the total and iAs concentration in fish meal samples and for quality control to measure iAs in a reference material with three different analytical methods. The focus will be on finding the robustness of different detection systems for analysis, not the extraction method, which clearly has to be investigated in detail, but was beyond the scope of this study. The results will then be compared with values reported in the literature.

## EXPERIMENTAL PROCEDURES

### Chemicals and reagents

Ultrapure water (>18 MΩ cm) was used for all analytical purposes. For calibration of total As, a 1000-mg As $L^{-1}$ certified As stock solution was supplied by CPI, Peak performance (USA). Quantification for speciation was performed with sodium dimethylarsinic acid (DMA, 98 %, ChemService, USA), and disodium hydrogen arsenate heptahydrate [As(V), BDH, UK]. Indium (CPI, Peak performance, USA) and rhodium (High Purity standards Charleston, USA) were used as internal standards. AB, nitric acid ($HNO_3$, 69 %), orthophosphoric acid (85 %) were supplied by Fluka (UK). Ammonium nitrate (98+ %) was obtained from Sigma-Aldrich. Sodium arsenite [As(III)], ammonium solution (28 %), and ammonium carbonate were supplied from BDH (UK). Hydrogen peroxide ($H_2O_2$, >30 % w/v), hydrochloric acid LR grade (HCl, 32 %), and sodium hydroxide LR grade (NaOH) were obtained from Fisher Scientific. Sodium persulfate (98+ %) and sodium borohydride (99 %) were from Acros organics. Dogfish Muscle (DORM-2), Fish Protein (DORM-3), and Lobster Hepatopancreas (TORT-2) CRMs for Trace Metals were obtained from the National Research Council Canada. All chemicals used were at least of analytical grade unless otherwise stated.

### Samples and sampling

Fish meal samples were collected from industrial producers in Iceland. Fish used for the meal was caught in Icelandic waters, mainly in 2008 and 2009, just prior to meal production. During sampling emphasis was laid on traceability of the samples so that the sampling site and season is known and documented (Table 1). The fish meal samples analyzed were from herring (*Clupea harengus*), capelin (*Mallotos villosus*), and blue whiting (*Micromesistius poutassou*) species. Four selected samples of each fish meal type were analyzed for As speciation and total As concentration.

 

**Table 1** Information about fishing date and fishing site of the fish meal samples measured in this study.

| Samples | Fishing date | Fishing site |
|---|---|---|
| **Herring** | | |
| H1 | Jun-08 | 64°N8°V–64°N10°V |
| H2 | Mar-09 | 63°N20°V |
| H4 | Dec-08 | 65°N22°V |
| H5 | Aug-08 | 72°N6°V |
| **Capelin** | | |
| C1 | Jan-08 | 67°N14°V |
| C2 | Jul-04 | 67.5°N22°V |
| C4 | Mar-08 | 64°N22°N |
| C5 | Feb-08 | 63.5°N17°V |
| **Blue whiting** | | |
| B1 | May-08 | 61°N8°V |
| B2 | Jun-05 | 63.5°N11°V |
| B3 | Feb-08 | 60°N5°V |
| B4 | Nov-04 | 65°N9°V–65°N10°V |

## Sample preparation

For determination of total As concentration, subsamples (approximately 0.2 g) were microwave-digested in 3.0 mL concentrated nitric acid and 1.5 mL of 30 % w/w $H_2O_2$ in XP1500 vessels in CEM Mars microwave system. Prior to analyses, the samples were diluted to the final volume of 30 mL with deionized water. Each sample was prepared in triplicate. For determination of total As concentration in the extracts for speciation 1 mL subsample of the extract was diluted to 10 mL in 5 % (v/v) ethanol. For the determination of iAs, an extraction method for the analysis of various seafood samples and marine animal feedingstuffs [26,27] was modified and applied. Briefly, a subsample (0.3 g) was accurately weighed in a 50-mL vial, and 10 mL of 1.5 mg $mL^{-1}$ NaOH in 50 % ethanol was added. The vial was placed in the microwave oven, and the temperature program set to 85 °C, just below the approximate boiling point of the mixture, and held for 5 min. After the microwave extraction, 0.1 mL of $H_2O_2$ was added to 0.9 mL of the mixture and left to react overnight in order to fully oxidize all arsenite to arsenate. Prior to analysis, the samples were centrifuged for 10 min at 13 000 rpm. For samples analyzed with HG-AFS, the ethanol was further removed under a stream of nitrogen before analysis, as samples with ethanol showed large interferences in the baseline signal.

## Analytical method for total arsenic concentration

Standard solutions for total As determination were freshly prepared each day of analysis in a matrix matched solution of 10 % (v/v) nitric acid by appropriate dilution of a stock solution of 1000 mg $L^{-1}$ As. The ICP-MS was optimized for optimal sensitivity and stability on As on a day-to-day basis. The *m*/*z* 75 for As and *m*/*z* 115 for Ir were checked. To avoid interferences, analysis were performed with a reaction cell ($H_2$, 3 mL $min^{-1}$). Standard ICP-MS conditions were used with the Agilent 7500ce as As detector. With every batch of samples, a blank and CRMs, DORM-2 or TORT-2, was measured.

 

## Analytical method for arsenic speciation analysis

### *HPLC-ICP-MS*

The response in the ICP-MS is element-specific, rather than molecular-specific, therefore for calibration a stock solution of DMA was diluted with deionized water to appropriate concentrations and an external calibration was performed before and after the sample measurements. To exclude matrix effects, this was further confirmed with a standard addition calibration using As(V) in CRM TORT-2. The separation and detection of anionic As species in the fish meal extracts were carried out on a Hamilton PRP-X100 column (10 μm, 4.6 × 250 mm) with a flow rate of 1 mL $min^{-1}$ using an Agilent 1100 HPLC coupled to an Agilent 7500c ICP-MS. As mobile phase, either an aqueous solution of 25 mM ammonium carbonate (pH 8.5) was prepared or 6.2 mM ammonium nitrate and 6.5 mM phosphoric acid adjusted to a pH of 6.0 with ammonia. CRMs, TORT-2 and DORM-3, were analyzed for speciation.

### *HPLC-HG-ICP-MS*

For quantification, an external As(V) calibration was used. The Agilent 1100 HPLC system, with the PRP-X100 column (flow rate of 1.0 mL $min^{-1}$, mobile-phase aqueous 6.2 mM ammonium nitrate, and 6.5 mM phosphoric acid adjusted to a pH of 6.0 with ammonia), was connected directly to a continuous-flow HG system. Acid (3M, HCl) and $NaBH_4$ (1.5 % w/v, in 0.1 M NaOH) were mixed with the sample post-column via two T-pieces. The flow of the acid and $NaBH_4$ was regulated with a separate peristaltic pump (7.5 rpm, approx. 0.9 mL $min^{-1}$ $NaBH_4$ and 1.3 mL $min^{-1}$ HCl). The sample then passed through a reaction coil [Teflon, 500 μL (~1.3 m)] into a gas liquid separator. The gaseous products were transported with an Ar flow (0.1 L $min^{-1}$) obtained from the make-up gas outlet of the ICP-MS to a glass T-piece connected directly to the torch and the cyclonic spray chamber of the Agilent 7500c ICP-MS. The flow was then mixed with the nebulized solution of a continuous internal standard (20 ng $mL^{-1}$ Rh) creating wet plasma conditions. This was done to confirm the identification of arsenate using its ability to volatilize as $AsH_3$.

For analyses with both methods (HPLC-(HG)-ICP-MS), the ICP-MS was optimized for optimal sensitivity and stability on As on a day-to-day basis. In addition to *m/z* 75 for As and *m/z* 103 for Rh, the possible chloride interference ($^{40}Ar^{35}Cl^+$) on *m/z* 75 was checked on *m/z* 77 ($^{40}Ar^{37}Cl^+$ or $^{77}Se$) and on m/z 82 ($^{82}Se$).

### *HPLC-HG-AFS*

Additional measurements were done under previously described conditions [24,28] with a HG-AFS Millenium Excalibur (PS Analytical, Kent, UK) using an As lamp (Superlamp 803S, Photron Pty, Ltd.) as detector after the HPLC [Hamilton PRP-X100 column (10 μm, 4.6 × 250 mm), flow rate of 1.0 mL $min^{-1}$, mobile-phase aqueous 6.2 mM ammonium nitrate and 6.5 mM phosphoric acid adjusted to a pH of 6.2 with ammonia]. During analysis, the HPLC effluent was acidified with HCl (3 M) solution and $NaBH_4$ (1.5 %) was added to form volatile $AsH_3$. Standard addition with As(V) was used for quantification for the CRM TORT-2.

## RESULTS AND DISCUSSION

### Quality control and robustness test using certified reference materials

#### *Analysis of the total arsenic concentration*

For quality control of the acid digestion a CRM was analyzed with every batch of samples measured for total As concentration. The measured concentrations were for DORM-2: 17.7 ± 1.3 mg $kg^{-1}$ ($n$ = 22) [certified: 18.0 ± 1.1 mg $kg^{-1}$] and TORT-2: 22.0 ± 1.1 ($n$ = 6) [certified: 21.6 ± 1.8 mg $kg^{-1}$]. All total As concentrations are given with the standard deviation (SD). To evaluate the precision of the measurement, a blue whiting sample was analyzed, in triplicate, at eight different days with independent calibration, where the concentration varied from 14.3 to 17.5 mg $kg^{-1}$. The results showed that the SD cal-

culated from the triplicate of each analysis was an underestimation of the total measurement uncertainty. The uncertainty can lie in the sample preparation step, the sample inhomogeneity and/or because of variations in the efficiency of the ICP-MS between different days of analysis.

### *Speciation analysis for inorganic arsenic*

*Identification*: The iAs was identified as As(V) by comparing the samples with standard solutions, with spiking experiments for CRMs and fish meal samples. The iAs in the CRM TORT-2 was also measured by using HG-AFS and HPLC-HG-ICP-MS as additional methods for identification as well as additional verification of the concentration.

*Extraction*: Extraction efficiency of the alkaline-ethanolic extraction method, based on certified values for total As, were the same for both DORM-3 ($n$ = 3) and TORT-2 ($n$ = 4); 91 ± 2 % for both reference materials. The efficiency of the alkaline-ethanolic extraction for four different biological samples of each fish meal was: herring 74 ± 6 % (69–85 %), blue whiting 89 ± 12 % (79–102 %), and capelin 76 ± 6 % (66–79 %). Herring and capelin have a similar extraction efficiency of 75 % on average, however, more As was extracted for the blue whiting, of approximately 90 % of the total As. This difference could be a result of physiological difference of the fish as blue whiting, e.g., has low lipid content in the flesh, whereas herring and capelin store lipids in the flesh. Lower extraction efficiency was expected for fish meal with higher lipid content as lipid-soluble arsenicals were not expected to be extractable with this method [29].

*Column recovery*: The column performance was evaluated with the sum of all eluting species, where percentage recoveries were based on the total As concentration in the extracts. The sum of all species for TORT-2 and DORM-3 was 25.9 ± 1.5 mg $kg^{-1}$ ($n$ = 10) compared to certified value of total As of 21.6 ± 1.8 and 8.8 ± 1.0 mg $kg^{-1}$ ($n$ = 3) and 6.88 ± 0.30 mg $kg^{-1}$, respectively. The column recovery for the fish meal was 87 ± 19 % for herring, 76 ± 20 % for capelin, and 127 ± 14 % for blue whiting. High column recoveries of the blue whiting and for the TORT-2 might be due to the high total As concentrations in those samples that were out of range of the calibration range, which was tailored toward the low concentration of iAs as As(V).

*Standard addition of iAs*: Spiking experiments before extraction were undertaken where As(III) and As(V) were spiked individually in a physiological concentration (approx. 3 µg As $L^{-1}$) to TORT-2, DORM-3, and the three fish meal types, before extraction. The concentration of the iAs was quantified as As(V), after oxidation of the sample, and determined with anion HPLC-ICP-MS. The recoveries in Table 2 show that all of the As(III) and As(V) is recovered successfully.

**Table 2** Recovery rates for samples spiked before extraction, average values and SD ($n$ = 3).

| | Recovery As(III) (%) | Recovery As(V) (%) |
|---|---|---|
| Herring | 82 ± 5 | 101 ± 13 |
| Capelin | 101 ± 27 | 110 ± 21 |
| Blue whiting | 78 ± 28 | 81 ± 23 |
| TORT-2 | 101 ± 3 | 103 ± 20 |
| DORM-3 | 91 ± 6 | 108 ± 14 |

*Calibration method*: The detection of the ICP-MS is element-specific, rather than molecular-specific, and therefore when using a nongradient mobile phase, it is enough to use a single elemental standard as a calibrant for all species. To test the robustness of DMA as an external calibrant for quantification of iAs in the samples (HPLC-ICP-MS) an additional calibration method was applied for comparison: The iAs in the TORT-2 was quantified both with standard addition method [As(V)] and an external DMA calibration where both calibrations were performed on the same day of analysis. Standard addition yielded a concentration of 0.333 ± 0.023 mg $kg^{-1}$ of iAs in the TORT-2 compared to

0.313 ± 0.021 mg $kg^{-1}$ iAs by using the DMA calibration for quantification. The difference between the two calibration methods was not significant, justifying using the less time-consuming method of quantification by DMA calibrant. As a test of the robustness of the calibration and the analytical method (including sampling), the TORT-2 was measured for iAs on a separate day of analysis, and prepared on a separate day as well, giving 0.390 ± 0.007 mg $kg^{-1}$. The combined uncertainty, taking into consideration the sample weighing, calibration, and analysis, was 18.5 %, hence the results were not significantly different.

*Method comparison for inorganic arsenic in TORT-2*: The possibility of co-eluting As compounds with the targeted arsenical cannot easily be excluded. This is in particular prominent when samples measured may contain a wide diversity of As species of different concentration. Therefore, in addition to the HPLC-ICP-MS, further verification of the iAs concentration was carried out using post-column online hydride generation atomic fluorescence spectrometric (HG-AFS) detection; a method that converts all separated hydride generation active As species into volatile As species.

The chromatogram shown in Fig. 1 illustrates that the intense signal detected at an early retention time when HPLC-ICP-MS was applied, was not seen when HPLC-HG-AFS detection was applied. This is in accordance with what would be expected since the major As compound in the extract, AB, does not form volatile As. Two separated As species present in low concentration were detected early in the chromatograph, for the TORT-2 (Fig. 1b). The first eluting peak may result from small amounts of DMA, or it could possibly originate from an arsenosugar since a conversion of arsenosugars into volatile As may occur with low efficiency [25,30]. Spiking of the TORT-2 sample showed that the peak eluting at retention time 750 s, both with HPLC-ICP-MS and HPLC-HG-AFS, came at the same retention time as arsenate (Fig. 1). Limit of quantification (LOQ) was high in this sample matrix when the HPLC-HG-AFS was used, 0.1 mg $kg^{-1}$, and therefore 11 of 12 fish meal samples in Table 4, would fall below LOQ. HG-AFS was not suitable to measure the low concentrations of iAs in the fish meal samples in this sample matrix. The HPLC-HG-ICP-MS set-up, applied to TORT-2, as a lower LOQ (0.026 mg $kg^{-1}$) and would be suitable to measure the iAs in the fish meal samples. Table 3 summarizes the range of iAs in the literature compared to values obtained in this work with three different instrumental set-ups.

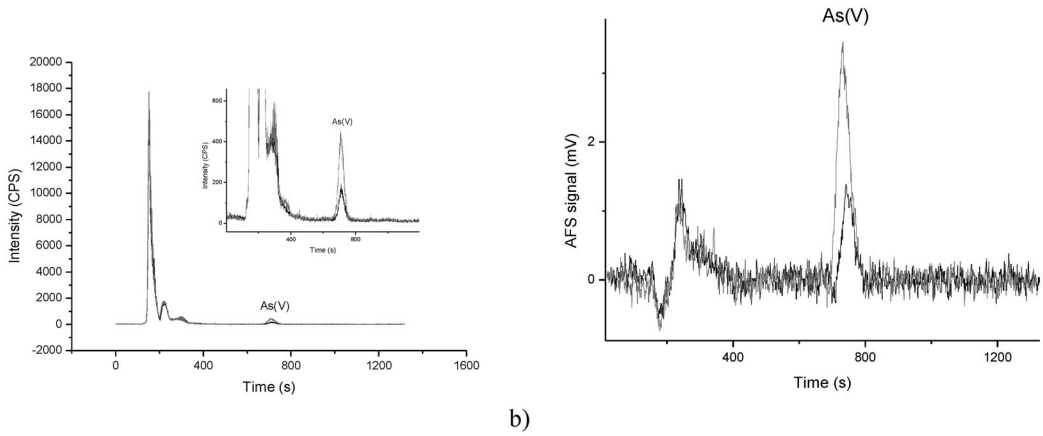


**Fig. 1** TORT-2 both spiked with As(V) (gray) and not spiked (black) analyzed on Hamilton PRP X100 (6.2 mM ammonium nitrate and 6.5 mM phosphoric acid, pH 6) coupled to (a) HPLC-ICP-MS, *m/z* 75 (b) HPLC-HG-AFS monitored on As.

**Table 3** Comparison of selected values reported in literature for iAs in TORT-2 as well as the iAs concentration found with different instrumental set-ups.

| **TORT-2** | | Conc. iAs (mg $kg^{-1}$) |
|---|---|---|
| Wahlen et al. [31] | HPLC-ICP-MS | 0.093 ± 0.037 |
| Larsen et al. [20] | HPLC-ICP-MS | 0.188 ± 0.014 |
| This work | HPLC-ICP-MS | 0.340 ± 0.034 |
| This work | HPLC-HG-AFS | 0.369 ± 0.018 |
| Kirby et al. [17] | HPLC-ICP-MS | 0.410 ± 0.030 |
| This work | HPLC-HG-ICP-MS | 0.470 ± 0.057 |
| Munoz et al. [16] | HG-AAS | 0.581 ± 0.055 |
| Leufroy et al. [23] | HPLC-ICP-MS | 1.133 ± 0.095 |

Leufroy et al. [23] recently compiled an extensive overview of the concentration of As species in CRMs, DORM-2, TORT-2, and BCR 627, reported in the literature over the last decade. The iAs concentration for TORT-2 ranged by a factor of 10, while for DORM-2 the reported values were even greater and ranged by a factor of 100.

The lowest value reported [31], in Table 3, is obtained with an extraction method of 20 min sonication in $H_2O$ at room temperature (re-extracted 3 times), which might not be energetic enough to extract the iAs from the sample matrix. The study by Larsen et al. [20] employed a similar extraction method as reported in this work, and it is uncertain why a lower concentration is found, part of it could be that no extra oxidizing agent was added to the sample prior to analysis, and perhaps not all As(III) was oxidized to As(V), as claimed. Most papers report HPLC-ICP-MS as detection method, and the differences between reported concentrations for iAs can be because of different chromotography methods (different LC columns, different eluents, etc.); if any co-eluting organoarsenic species were present the concentration could be overestimated. It is possible to say with a high degree of certainty that the value reported in this work is not too high because of co-elution, as the post-column HG excludes that. Munoz et al. report a method based on a different way of detection; the As is extracted in HCl (as $AsCl_3$) and back-extracted into chloroform, this however, opens up the risk of co-extraction of MA and TMAO, which could lead to overestimation of the iAs [16]. Leufroy et al. [23] report approximately more than 3 times higher iAs concentration in TORT-2 (Table 3), and in DORM-3 (0.328 ± 0.024 mg $kg^{-1}$) compared to this work (0.073 ± 0.008 mg $kg^{-1}$).

Figure 2 depicts iAs, found with different extraction methods and different detection methods, reported for TORT-2 in the literature [16,23,32], with values from this work. The second highest value of iAs shown in Fig. 2 was found with a MW extraction in 2 % $HNO_3$, where the same study found twice as low concentration of iAs when using methanol/water/$H_2O$ as extraction solvent [33]. The amount of iAs is not only dependent on the extraction solvent, as interestingly, the work that reports the lowest and highest concentration of iAs, both use 100 % $H_2O$ as extraction solvent [23,31]. Foster et al. [33] accredit the $HNO_3$ for the liberation of more iAs from the sample matrix. In the work where the highest amount of iAs is reported, $HNO_3$ was used as mobile phase with a gradient program, where high concentration of $HNO_3$ was used at the time of the elution of As(V) [23].

The three different instrumental set-ups used in this study did not differ significantly at a 95 % confidence level for the measured iAs concentration in TORT-2. Furthermore, the concentration found fitted close to the average of reported values in the literature.

HPLC-ICP-MS was used to determine the iAs concentration in 12 fish meal samples as well as an additional CRM analyzed for speciation, DORM-3. The DORM-3 gave a concentration of 0.073 ± 0.008 mg $kg^{-1}$ ($n$ = 3), however, DORM-3 has not been in general use as a CRM for a long time, thus limited data has been reported to date.

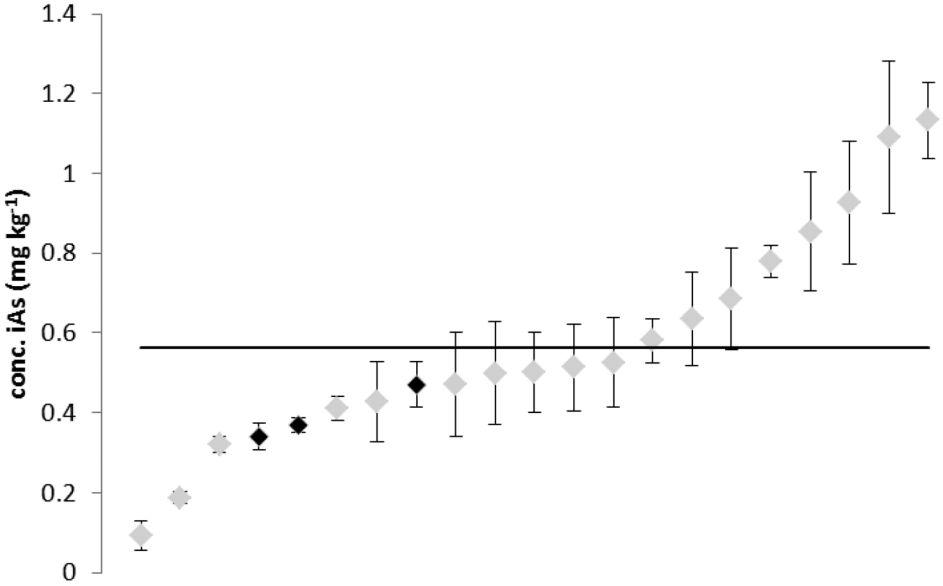


**Fig. 2** iAs concentration in TORT-2 reported in the literature (gray) [16,23,32] and concentration found in this work (black), the error bars represent the reported error. The black line represents the average concentration.

## Total and inorganic arsenic in fish meal

An example chromatogram, presented in Fig. 3, shows capelin fish meal with 4.1 mg $kg^{-1}$ total As and 0.05 mg $kg^{-1}$ iAs. The iAs, quantified as As(V) after the oxidation of As(III), elutes at a retention time of approximately 750 s, and a full baseline separation from other organoarsenic compounds is achieved.

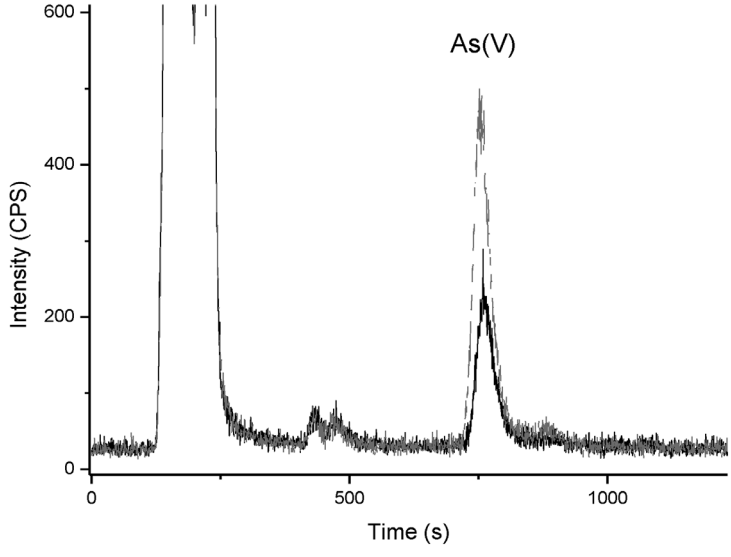


**Fig. 3** As speciation of a capelin fish meal sample analyzed with anion-exchange HPLC-ICP-MS, *m*/*z* 75, mobile-phase 25 mM aqueous ammonium carbonate. The sample is also spiked with As(III) before extraction, quantified as As(V) (gray).

 

The results for 12 samples of different fish meals are presented in Table 4. The data reveals that even though the total As concentration ranges from 2.5 to 16.2 mg $kg^{-1}$ the iAs concentration is below 0.2 mg $kg^{-1}$. The iAs concentration in most fish meal samples is under 1.5 % of total As concentration, and the iAs concentration does not exceed 4 % of total As concentration for any sample.

**Table 4** Total As and inorganic concentration in 12 fish meal samples.[a,b]

| | Conc. iAs (mg $kg^{-1}$) | *n* | Total As conc. (mg $kg^{-1}$) | *n* | % iAs of total |
|---|---|---|---|---|---|
| **Herring** | | | | | |
| H1 | 0.037 ± 0.018 | 5 | 4.35 ± 0.06 | 3 | 0.8 |
| H2 | 0.082 ± 0.009 | 3 | 2.51 ± 0.04 | 3 | 3.3 |
| H4 | 0.029 ± 0.003 | 3 | 3.45 ± 0.15 | 3 | 0.8 |
| H5 | 0.037 ± 0.009 | 3 | 5.31 ± 0.15 | 3 | 0.7 |
| **Capelin** | | | | | |
| C1 | 0.198 ± 0.008 | 3 | 5.17 ± 0.20 | 3 | 3.8 |
| C2 | 0.050 ± 0.018 | 9 | 4.11 ± 0.06 | 3 | 1.2 |
| C4 | 0.047 ± 0.005 | 3 | 2.95 ± 0.11 | 3 | 1.6 |
| C5 | 0.036 ± 0.009 | 3 | 4.42 ± 0.29 | 3 | 0.8 |
| **Blue whiting** | | | | | |
| B1 | 0.072 ± 0.003 | 3 | 16.3 ± 1.0 | 18 | 0.4 |
| B2 | 0.051 ± 0.003 | 3 | 8.38 ± 0.13 | 3 | 0.6 |
| B3 | 0.051 ± 0.004 | 3 | 12.4 ± 0.6 | 3 | 0.4 |
| B4 | 0.041 ± 0.016 | 9 | 14.0 ± 0.3 | 3 | 0.3 |

[a]Water content of fish meal samples ranges from 4.5 to 8.3 %, concentration given on a product weight basis.
[b]LOQ ≥ 0.014 mg $kg^{-1}$.

This is in accordance with previously reported literature values of iAs in seafood [16,19,21,23,26,27,34]. From the data obtained, a correlation between total and inorganic concentrations cannot be found.

## CONCLUSIONS

By using methods with two different detectors (ICP-MS and AFS), it is possible to exclude interferences such as spectral interferences, furthermore, by adding the HG step to the HPLC-ICP-MS, co-elution of other non-HG active organoarsenic species can be excluded. However, the possibility of co-elution is only excluded for the samples tested since fish meal samples of the same type could contain different As species, owing to biological diversity and the difference in the life cycle of the fish depending on, e.g., the season or location. Therefore, whether it can be extrapolated to other marine fish meal samples remains to be seen and needs further investigation. By applying more methods than one, the iAs concentration can be reported with more confidence with regard to the robustness of the instrumental method. However, without a certified value of iAs, it is not possible to evaluate the accuracy of the reported concentration and the efficiency of the applied extraction method. Further work on the comparison of different extraction methods would be a valued contribution to the topic, and a matter of high interest.

The concentration of iAs in the CRM TORT-2 is almost an order of magnitude higher than the average concentration found in the fish meal presented here, and therefore for quality control in fish meal and fish, a certified value of iAs in the same concentration range and the same matrix is urgently needed. Even though all fish meal samples analyzed were below the maximum level of total As in fish

 

meal (25 mg $kg^{-1}$), they were all above the threshold value for iAs in feed (2 mg $kg^{-1}$) [9]. To prove that the iAs concentration is below this threshold value, a validated method is needed. However, in order to validate methods a certified value of iAs in a reference material must be available, underlining the pressing need of further proficiency testing and reference materials certified for inorganic As.

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