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Analytical Methods

Inorganic arsenic in seafood: Does the extraction method matter?

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ABSTRACT

Nine different extraction methods were evaluated for three seafood samples to test whether the concentration of inorganic arsenic (iAs) determined in seafood is dependent on the extraction method. Certified reference materials (CRM) DOLT-4 (Dogfish Liver) and TORT-2 (Lobster Hepatopancreas), and a commercial herring fish meal were evaluated. All experimental work described here was carried out by the same operator using the same instrumentation, thus eliminating possible differences in results caused by laboratory related factors. Low concentrations of iAs were found in CRM DOLT-4 (0.012 ± 0.003 mg kg^{-1}) and the herring fish meal sample (0.007 ± 0.002 mg kg^{-1}) for all extraction methods. When comparing the concentration of iAs in CRM TORT-2 found in this study and in the literature dilute acids, HNO_3 and HCl, showed the highest extracted iAs wheras dilute NaOH (in 50% ethanol) showed significantly lower extracted iAs. However, most other extraction solvents were not statistically different from one another.

1. Introduction

Arsenic speciation is toxicologically important since the toxicity differs between arsenic species, where inorganic arsenic, occurring in the oxidation forms As(III) and As(V), is more toxic than organic arsenic species (ATSDR., 2007; World Health Organisation (WHO)., 2001). To date, over 50 arsenic species have been identified (European Food Safety Authority, 2009). The levels of total arsenic (totAs) found in different food commodities can vary by a factor of 1000, where the highest concentrations are usually found in seafood and algae (European Food Safety Authority, 2009; Francesconi, 2010; Urgast, Adams, Raab, & Feldmann, 2010). However, the concentration of inorganic arsenic (As(III) + As(V) = iAs) is not correlated with the total As concentration (Francesconi, 2010; Urgast et al., 2010), e.g. seafood generally has low concentrations of iAs since the arsenic is mainly found as non-toxic organic arsenic species such as arsenobetaine (AB) (Edmonds & Francesconi, 1993; European Food Safety Authority, 2009). Several proficiency tests on iAs in different food commodities (e.g. rice, seafood, algae, wheat and vegetables) have been conducted recently to assess the validity of the methods used (Baer et al., 2011; de la Calle et al., 2011, 2012). Briefly, test material was sent out to participating laboratories, where the amount of iAs was measured with their method of choice. The determination of iAs in rice, wheat and vegetable food showed consistent values, however, a wider spread of results was found for seafood and algae (Baer et al., 2011; de la Calle, Linsinger, Emteborg, Charoud-Got, & Verbist, 2010; de la Calle et al., 2012). For the different food commodities used in the proficiency testing, a set of expert laboratories established the concentration of iAs, for which no certified values exist at present. For the algae, the expert laboratories were able to agree on a value for iAs, however, only 20% of those reported satisfactory results, compared to 60–85% for the wheat and vegetable food that was measured in the same survey (de la Calle et al., 2012). For seafood, the expert laboratories could not even agree on a value for iAs (Baer et al., 2011). As stated above, in contrast to terrestrial food, marine food can contain high concentrations of total arsenic, but generally relatively low levels of iAs. Along with the non-toxic AB, the remainder of the As can be found as numerous other organoarsenic species (Borak & Hosgood, 2007; Francesconi, 2010). Here, it is a challenge to obtain separation of the analyte of interest i.e. the toxic iAs, from the multitude of organic arsenic species. High-performance liquid chromatography (HPLC) with online detection by inductively coupled plasma-mass spectrometry (ICP-MS) is a very common method for separation of arsenic species (Francesconi & Kuehnelt, 2004), but to do so beyond doubt of co-elution with any other As compound is often difficult to achieve. Here, the addition of an extra step of hydride generation (HG) to HPLC-ICP-MS for arsenic speciation was shown to be an excellent option (Pétursdóttir et al., 2012).

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However, there has been an on-going debate within the scientific community whether the iAs concentration determined in food commodities is dependent on the analytical procedure (extraction/instrumental setup). A recent study showed that three different instrumental setups (HPLC-HG-AFS and HPLC-(HG)-ICP-MS) resulted in similar results for the certified reference material (CRM) TORT-2, indicating that the difference was independent of the detection method (Petursdottir et al., 2012). Another study using HPLC-HG-ICP-MS vs HPLC-HG-AFS showed no significant difference in the concentrations of different As species (including As(III) and As(V)) for environmental samples such as oysters, seawater and CRM TORT-1 (Gomez-Ariza, Sanchez-Rodas, Giraldez, & Morales, 2000) Furthermore, in a proficiency testing for animal feed of marine origin, the iAs concentration in TORT-2 found by the use of two different instrumental setups was reported (Sloth et al., 2011): The laboratories taking part in the proficiency testing (solid phase extraction (SPE) HG-AAS), resulted in a mean value sufficiently precise and accurate compared to the value of iAs found by the expert laboratory participating in the proficiency testing (HPLC-ICP-MS). Further comparison of SPE-HG-AAS and HPLC-ICP-MS showed that for a set of 20 samples of marine food and feed no statistical difference was found (Rasmussen, Hedegaard, Larsen, & Sloth, 2012). Since the iAs concentration appears to be independent on the instrumental setup, the objective of the present study is to investigate whether the determination of the iAs concentration in seafood samples is dependent on different extraction methods.

2. Experimental procedures

2.1. Chemicals and reagents

Ultrapure water (>18 MΩ cm) was used for all analytical purposes. For calibration of total As and speciation with hydride generation, a 1000 mg As L^{-1} certified As stock solution (as H_3AsO_4 in 0.5 M HNO_3) was supplied by Merck, UK. Quantification for speciation using HPLC-ICP-MS was performed with sodium dimethylarsinic acid (DMA, 98%, ChemService (USA)). Rhodium (Specpure, Alfa Aesar, Germany) and germanium (Aldrich Chemical Company, UK) were used as internal standards. Nitric acid (HNO_3, 69%) and trifluoracetic acid (TFA, >98.0%) were supplied by Fluka (UK). Ammonium nitrate (98 + %) and hydrobromic acid (HBr, 48%) were obtained from Sigma–Aldrich (UK). Sodium arsenite (As(III)), ammonium solution (28%) and hydrazine sulphate ($NH_2NH_2H_2SO_4$, 99%) were supplied from BDH (UK). Hydrogen peroxide (H_2O_2, >30% w/v), ammonium phosphate ($NH_4H_2PO_4$), sodium hydroxide (NaOH, Laboratory reagent grade (LR)), chloroform ($CHCl_3$, LR grade) and hydrochloric acid (HCl, 32%, LR grade), used for the hydride generation reaction, were obtained from Fisher Scientific (UK). Sodium persulfate (98 + %) and sodium borohydride ($NaBH_4$, 99%) were purchased from Acros organics (UK). HCl (37%) was supplied by VWR (UK). Certified reference material SPS-WW2 (waste water, Spectrapure standards, Norway) was used for monitoring the performance of the Agilent ICP-MS on a day-to-day basis. All chemicals used were at least of analytical grade unless otherwise stated.

2.2. Samples and sampling

Three samples were used throughout the study: Two certified reference materials for trace metals, Dogfish liver (DOLT-4), Fish protein (DORM-3) and Lobster Hepatopancreas (TORT-2) were obtained from the National Research Council Canada. The third sample material was a herring (*Clupea harengus*) fish meal sample, a commercial fish feed ingredient obtained from Síldarvinnslan í Neskaupsstað, an industrial producer in Iceland.

2.3. Sample preparation

Nine different commonly used extraction methods used for extraction of iAs, referred to as methods M1–M9, were applied as summarised in Table 1. For **M1**–**M3** and **M5**–**M9** an extractant was added to the dry, accurately weighed homogenised sample (~0.2 g), and the extraction facilitated by microwave assisted extraction (MAE) or sonication (SON), see Table 1. Method **M4** was however considerably different from the other extraction methods, and was therefore omitted from Table 1, but is described in detail in the text below.

The procedure **M4** was carried out as follows: 1.64 mL H_2O and 7.36 mL of HCl (conc. 37%) were added to the sample (~0.2 g) in a 50 mL Teflon tube, and the solution was mechanically agitated for 1 h and left standing overnight. A reducing agent was added (0.8 mL of 48% HBr + 0.4 mL of 1.5% (w/v) hydrazine sulphate) and the sample vials manually shaken for 30 s. 4 mL of chloroform were added and shaken for 3 min. The mixture was centrifuged at 3500 rpm (1643*g*) for 5 min and the two phases separated. The extraction was subsequently repeated twice. The remnants of the acid phase were removed by aspiration. Subsequently the samples (chloroform phase) were filtered through 0.45 μm filters with a PTFE membrane (0.8 mL of $CHCl_3$ was added to remove the remaining sample from the filter), and then back extracted into 4 mL of 1 mol L^{-1} HCl. Back extraction was repeated once. All references in the text to the extract, refer to the this last step of the extraction process in **M4** (1 M HCl), since this phase should contain the extractable iAs, whereas most organoarsenic species should have stayed in the conc. HCl phase.

Prior to analysis with HPLC-(HG)-ICPMS, all samples were adjusted to pH 7 ± 1, if needed, by adding appropriate amounts of NH_3 or HNO_3. The amounts added were small, (ranging from 5 to 50 μL) so this did not significantly dilute the samples. All samples were centrifuged at 13,000 rpm (13226*g*) for 10 min prior to analysis with HPLC-HG-ICP-MS. The total arsenic determination of the 1 M HCl phase for **M4** is generally used solely for determination of iAs with this method (Baer et al., 2011; Munoz, Velez, & Montoro, 1999). Therefore, additionally to the determination of the extract with HPLC-HG-ICP-MS, the total concentration of the extract was further determined here using high resolution (HR)-ICP-MS.

For the determination of total arsenic concentration in all the other extracts (except M4) 1 mL subsample of the extract was diluted to 10 mL prior to analysis with ICP-MS (Agilent 7500c).

2.4. Analytical method

2.4.1. HPLC-HG-ICP-MS

The separation and detection of anionic arsenic species in all sample extracts was carried out on a Hamilton PRP-X100 column (10 μm, 4.6 × 250 mm) with a flow rate of 1 mL min^{-1}. The mobile phase, 6.2 mM ammonium nitrate and 6.5 mM phosphoric acid adjusted to a pH of 6.0 with ammonia was used. An Agilent 1100 HPLC system was connected directly to a continuous flow HG system, as previously described (Pétursdóttir et al., 2012). Briefly, acid (3 M HCl, 1.4 mL min^{-1}) and $NaBH_4$ (1.5% w/v, in 0.1 M NaOH, 0.9 mL min^{-1}) were mixed with the sample post column (reaction coil: Teflon (~1.3 m length)) and passed into a gas–liquid separator. The gaseous products were transported via an argon flow (0.1 L min^{-1} (make-up gas from the ICP-MS)) to the ICP. The sample gas flow was mixed with the nebulised continuous internal standard (20 ng mL^{-1} rhodium) creating stable wet plasma conditions and allowing for instrument sensitivity drift.