JOURNAL OF ENVIRONMENTAL SCIENCES 49 (2016) 97–103

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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Preliminary studies on the stability of arsenolipids: Implications for sample handling and analysis☆

*Muslim Khan, Kevin A. Francesconi**

Institute of Chemistry-Analytical Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria

ARTICLE INFO

Article history:
Received 24 March 2016
Revised 8 April 2016
Accepted 10 April 2016
Available online 29 April 2016

Keywords:
Arsenolipids
Stability
Mass spectrometry

ABSTRACT

Human health risk assessments concerning arsenic are now estimating exposure through food in addition to exposure through drinking water. Intrinsic to this assessment is sample handling and preparation that maintains the arsenic species in the form that they occur in foods. We investigated the stability of three arsenolipids (two arsenic fatty acids, AsFA-362 and AsFA-388, and one arsenic hydrocarbon AsHC-332), common constituents of fish and algae, relevant to sample storage and transport, and to their preparation for quantitative measurements. The fate of the arsenolipids was followed by high performance liquid chromatography/electrospray triple quadruple mass spectrometry (HPLC/ESIMS) analyses. Storage of the compounds dry as pure compounds or mixed in fish oil at up to 60°C did not result in significant changes to the compounds, although losses were observed by apparent adsorption onto the plastic walls of the polypropylene tubes. No losses occurred when the experiment was repeated with glass tubes. When the compounds were stored in ethanol for up to 15 days under acidic, neutral, or alkaline conditions (each at room temperature), no significant decomposition was observed, although esterification of the fatty acids occurred at low pH. The compounds were also stable during a sample preparation step involving passage through a small silica column. The results indicate that these typical arsenolipids are stable when stored in glass at temperatures up to 60°C for at least 2 days, and that, consequently, samples of food or extracts thereof can be transported dry at ambient temperatures, i.e. without the need for cool conditions.

Introduction

Arsenolipids, naturally occurring arsenic compounds commonly found in marine organisms including many seafoods, have been shown recently to have cytotoxic properties (Meyer et al., 2014). Human health issues concerning arsenic are starting to take into account exposure through food in addition to the much-researched exposure through drinking water. Such assessments require analytical methods capable of distinguishing and quantifying the various forms of arsenic in food. Intrinsic to this assessment is sample handling procedures that maintain the arsenic species in the form that they occur in foods.

Quantitative methods, based mainly on high performance liquid chromatography (HPLC)/inductively coupled plasma mass spectrometry (ICPMS), are available for the measurement

☆ This paper is dedicated to Professor Bill Cullen in recognition of his outstanding contribution to our understanding of the chemistry of arsenic in the environment and human health.

* *Corresponding author*. E-mail: kevin.francesconi@uni-graz.at (K.A. Francesconi).

http://dx.doi.org/10.1016/j.jes.2016.04.004

of the major water-soluble arsenic species found in foods (Francesconi and Kuehnelt, 2004). The situation for arsenolipids, however, is more complex. There are at least six groups of arsenolipids encompassing arsenic-containing fatty acids (Rumpler et al., 2008; Amayo et al., 2011, 2014; Lischka et al., 2013; Arroyo-Abad et al., 2013, 2015; Taleshi et al., 2014), As-hydrocarbons (Taleshi et al., 2008, 2014; Amayo et al., 2013; Raab et al., 2013; Arroyo-Abad et al., 2015), As-fatty alcohols (Amayo et al., 2013), arsenosugar-phospholipids (García-Salgado et al., 2012; Raab et al., 2013), and As-phosphatidylcholines and As-phosphatidylethanolamines (Viczek et al., 2016). Identification of the arsenolipid groups and the many compounds within each group has usually been performed on extracts that have undergone some form of sample preparation, such as solvent partitioning at different pH and solid phase extraction using silica under acidic or basic conditions (Rumpler et al., 2008; Taleshi et al., 2008; Amayo et al., 2011; García-Salgado et al., 2012; Glabonjat et al., 2014). Possibly, the sample preparation might cause some changes to the original arsenolipids, and indeed, a recent investigation indicated that using a silica solid phase extraction step was not suitable for the analysis of some arsenolipids (Viczek et al., 2016). The fate of the arsenolipids undergoing such sample preparation steps, and later during storage before measurement, however, has not been systematically investigated; such information is integral to quantitative analysis.

We investigate possible changes taking place during sample handling and storage using three standard arsenolipids as model compounds (Fig. 1). We use quantitative HPLC/mass spectrometry to monitor the stability of the compounds during silica chromatography and storage in solution or as freeze-dried extracts. The studies provide basic data on sample handling valuable for quantitative analysis of arsenolipids in environmental and food samples.

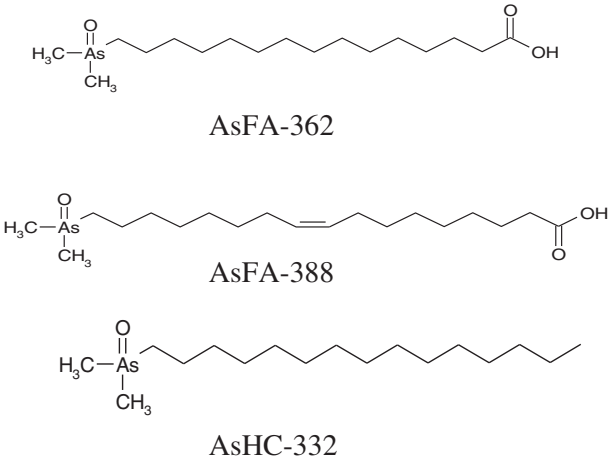

Fig. 1 – Structures of the three arsenolipids investigated in this study — two arsenic fatty acids (AsFA-362 and AsFA-388) and one arsenic hydrocarbon (AsHC-332). The compounds are referred to by the aforementioned abbreviation followed by the nominal molecular mass of the compound.

1. Experimental

1.1. Chemicals and reagents

Water used for all experiments was obtained from a Milli-Q system (18.2 MΩ cm, Millipore GmbH, Vienna, Austria). Methanol (≥99.9%, MeOH), ethanol (100%, EtOH), hydrochloric acid (ROTIPURAN 25%), ammonia (25%) and formic acid (≥98%) were obtained from Carl Roth GmbH (Karlsruhe, Germany); hexane (≥95%) and acetone (≥99.5%) were purchased from Sigma-Aldrich (Vienna, Austria); and silica gel 60 was obtained from Merck (Buchs, Switzerland). Fish oil capsules (Omega-3 Kapseln 1000, St. Rodegan) were purchased from Bio-Garten GmbH, Klagenfurt, Austria. Three naturally occurring arsenolipids (AsFA-362, AsFA-388 and AsHC-332, Fig. 1) were synthesized in-house (Taleshi et al., 2014) and their purity (>99%) was assessed by nuclear magnetic resonance (NMR) and HPLC/mass spectrometry. Stock solutions of the compounds (0.33 μmol/L, 25 μg As/L) were prepared in ethanol; further solutions were prepared by dilution in ethanol.

1.2. Instrumentation and operating conditions

Solvents were evaporated on a centrifugal lyophilizer (Christ RVC 2-33 CD plus, Martin Christ GmbH, Osterode am Hartz, Germany). Separation of arsenic species was carried out on an Agilent 1200 series HPLC system prior to online *m/z* measurement with an electrospray triple quadrupole mass spectrometer (Agilent 6460 system, Agilent Technologies, Waldbronn, Germany). For some samples, simultaneous molecular and elemental (As) mass spectrometry was performed by splitting the HPLC effluent and directing part of it to the Agilent 6460 and part to an ICPMS 7900 (Agilent Technologies, Waldbronn, Germany). The Agilent 7900 instrument was equipped with a MicroMist concentric glass nebulizer (Glass Expansion, West Melbourne, Australia) and a Peltier-cooled Scott-type double-pass spray chamber with the standard Ni interface cones. Total arsenic was measured by using an Agilent 7500ce series instrument equipped with an Ari Mist HP nebulizer (Burgerner, Mississauga, Canada).

1.3. Measurement of arsenolipids by HPLC/electrospray triple quadrupole mass spectrometry (ESIMS)

Chromatographic separation of arsenic species was carried out on an Asahipak reversed-phase C-8 column (4.6 × 150 mm, particle size 5 μm) at 40°C under gradient elution conditions using water (containing 0.1% formic acid) and MeOH (containing 0.1% formic acid) as mobile phases with the following gradient; 0 to 1 min, 30% MeOH; 1 to 4 min, 100% MeOH; 4 to 6 min, 100% MeOH, 6.1 to 16 min 30% MeOH; flow rate was 0.5 mL/min and injection volume was 10 μL. Mass spectra were recorded on an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. ESI was operated in the positive ion mode with a precursor ion scan (*m/z* 100–600) in which product ions at *m/z* 123 and 105 were measured at fragmentor voltage of 135 V and collision energies of 25 and 30 V, respectively. System control and data acquisition were controlled by Agilent Mass Hunter software. Detailed source

conditions were: Gas temperature: 100°C, gas flow 12 L/min, nebulizer pressure: 45 psi, sheath gas temperature: 400°C; sheath gas flow: 11 L/min, capillary voltage: 4500 V, nozzle voltage: 500 V. With these HPLC/ESIMS conditions, the limit of detection was 0.002 μg As/L for AsHC-332 based on 3^*SE_y, (standard error of the y-intercept) of a five-point calibration curve ranging from 0.01 to 0.05 μg As/L. The system showed good stability and precision: over the measurement period of 24 hr (measuring standards and samples), a 25 μg As/L standard of AsHC-332, measured every four hours, returned a mean value of 25.5 ± 0.9 μg As/L (Relative standard deviation = 3.6%, $n = 6$).

1.4. HPLC with simultaneous ESIMS and ICPMS detection

Samples of AsFA-388 and AsFA-362, which had been stored at pH 2, were also analyzed by HPLC/ICPMS/ESIMS in which the HPLC effluent was split whereby 10% was transferred to the ICPMS (7900) and 90% to the ESIMS (6460) using a fixed passive splitter (Analytical Scientific Instruments, Richmond, USA). For ICPMS measurements, a support flow of water containing 1% formic acid and 10 μg/L Ge, (0.5 mL/min) was introduced through a T-piece after the splitter. Carbon compensation (Raber et al., 2010) was performed by continuous addition of water/EtOH (9 + 1, V/V) delivered with an ISIS pump (0.02 r/min) to ensure a stable carbon content reaching the plasma. Water (containing 0.1% formic acid) and MeOH (containing 0.1% formic acid) were used for the mobile phase with the following gradient; 0 to 1 min, 30% MeOH; 1 to 15 min, increasing to 100% MeOH; 15 to 22 min, 100% MeOH; 22 to 29 min, decreasing to 30% MeOH. Injection volume was 20 μL for the splitting experiments. ESIMS conditions were the same as mentioned earlier. ICPMS signals were recorded at *m/z* 75 (^{75}As and $^{40}Ar^{35}Cl$) and *m/z* 77 ($^{40}Ar^{37}Cl$, for monitoring possible chloride interferences) at dwell times of 300 ms, and for the internal standard at *m/z* 74 (^{74}Ge), at dwell times of 100 ms. Data evaluation was carried out with chromatographic software Mass Hunter Version B.01.01 (Agilent, Waldbronn, Germany). Quantification was based on peak areas against external calibration with AsFA-362 and AsFA-388.

1.5. Total arsenic determination–acid digestion and ICPMS

Total arsenic was measured in fish oil from Omega-3 capsules and from 0 hr and 48 hr samples of AsHC-332. Fish oil (100 mg) and a portion (1 mL) of an ethanolic solution from 0 hr and 48 hr samples were weighed into quartz tubes (12 mL). Solvent was evaporated in a centrifugal lyophiliser, and water (2 mL) and HNO_3 (2 mL) were added to the samples before the tubes were covered with Teflon caps and transferred to the Ultraclave microwave system (MLS GmbH, Leutkirch, Germany). Argon (40 bar) was applied and the following temperature programme was started: 0 to 10 min, 80°C; 10 to 30 min, 150°C; 30 to 45 min, 250°C; 45 to 65 min, 250°C. The digests were then allowed to cool to room temperature before being transferred to polypropylene tubes (15 mL) and diluted with water to 9 mL (based on mass). Finally, 1 mL of a solution containing 100 μg/L Ge was added to all digested samples as an internal standard. Samples were analyzed for total arsenic in triplicate.

Determination of arsenic in the digested fish oil samples was carried out by using ICPMS in collision cell mode (He, 5 mL/min) to minimize polyatomic interferences from argon chloride ($^{40}Ar^{35}Cl$) on arsenic (^{75}As). Standards for calibration were prepared in 20% HNO_3 for matrix matching and contained 10 μg/L Ge as internal standard.

Three experiments were performed to check the stability of AsFA-362, AsFA-388 and AsHC-332.

1.6. Fate of arsenolipids during sample handling

1.6.1. Stability of arsenolipids on silica column

One set of triplicate samples (1 μg As each, called pre-silica) was dissolved in 10 mL of ethanol and directly measured. For another set of triplicate samples, three small silica columns were prepared by putting silica gel 60 into small glass Pasteur pipettes (150 × 5 mm, filled to a height of 4 cm); the column was conditioned with 4 mL of a MeOH/acetone (1 + 1, V/V) mixture containing 1% formic acid. The arsenolipid (1 μg As) was applied in 1 mL of ethanol to the silica column, then the column was washed with 2 mL of MeOH. Finally the arsenolipid was eluted by MeOH containing 1% NH_3 (8 mL); the solvent was evaporated in a centrifugal lyophilizer, and the dry residue was stored at −18°C. For measurement, each sample was re-dissolved in 10 mL of ethanol, and the solution was analyzed by HPLC/ESIMS.

1.6.2. Stability of arsenolipids in acidic, neutral and basic media

The arsenolipid (3 μg As) was added to 30 mL of a solution of 90% EtOH +10% H_2O (by mass) having pH 2, 7 or 12 (giving 100 μg As/L). The pH was adjusted with HCl or aq. NH_3. Each solution for each pH and compound was separated into triplicate subsamples (10 mL each); 1 mL of sample was taken at days 0, 1, 3, 7, and 15. Acidic samples were neutralized by adding 22 μL of aqueous NH_3 (2.5%) and the basic sample was neutralized by adding 69 μL of HCl (25%). The amount of acid or base required to neutralize the solutions was previously tested with a 10 mL solution of 90% EtOH + 10% H_2O at pH 12 or pH 2, respectively. The samples were dried in the centrifugal lyophiliser and kept at −18°C until measurement; at that time, the samples were extracted with 1 mL of ethanol, leaving behind the white residue of ammonium chloride, and the clear supernatant of ethanol was analyzed by HPLC/ESIMS. In addition, the samples from the arsenic fatty acids stored at pH 2 were analyzed by HPLC/ICPMS/ESIMS to give simultaneous elemental (As) and molecular mass detection.

1.6.3. Stability of arsenolipids at 60°C (dry and in fish oil)

Each of the arsenolipids (25 μg As) was dissolved in 1 mL of ethanol. Two sets (2 × 18, 1 μg As) of each compound were transferred to individual polypropylene tubes (15 mL capacity, total 36 tubes) and the ethanol was evaporated under a stream of N_2 gas. To one set of samples was added 1 g of fish oil; the other set was left "dry" (*i.e.*, only the arsenolipid was present). Both sets of samples were kept at 60°C (water bath) for up to 48 hr. At intervals, three samples from each set were removed and the contents partitioned between hexane and MeOH/water (9 + 1); the MeOH/water layer was evaporated to dryness and stored at −18°C. Later, the samples were

re-dissolved in 10 mL of ethanol and analyzed by HPLC/ESIMS.

1.6.4. Adsorption of AsHC-332

Two sets (2 × 15, 1 μg As) of AsHC-332 were transferred to individual polypropylene tubes (15 mL capacity) or glass tubes (12 mL); the ethanol was evaporated under a stream of N_2 gas, and the residue was left "dry" (*i.e.*, only the arsenolipid was present). One group of triplicate samples, in both plastic and glass tubes, were immediately stored at −18°C; the rest of the samples were placed at room temperature, 40 or 60 °C (water bath) for up to 6 hr. In a parallel experiment, triplicate samples, in tubes (plastic and glass) left open to the atmosphere were kept at 60°C (water bath) for 6 hr to check for possible losses owing to volatile degradation products. Later, the samples were re-dissolved in 10 mL of ethanol and analyzed by HPLC/ESIMS.

1.6.5. Solubilization of adsorbed AsHC-332

Triplicate samples of arsenolipid held at 60°C for 6 hr (plastic tubes) were extracted with 2 × 2 mL of ethanol; all traces of ethanol were removed by evaporation, and then the remaining contents in the tube were extracted by adding concentrated nitric acid and shaking overnight. The HNO_3 extracts were acid-digested in the Ultraclave, and the digests were analyzed for their arsenic content by using ICPMS as described above.

A second set of triplicate samples were extracted with EtOH at 60°C, and a third set with hexane at 60°C overnight. Solvent was evaporated; the residue re-dissolved in EtOH and the solution was measured by HPLC/ESIMS.

2. Results

Previous analytical studies of arsenolipids have used silica chromatography, with acidic and basic conditions, as a simple sample clean-up step (Amayo et al., 2011; García-Salgado et al., 2012; Glabonjat et al., 2014). In two experiments, we investigated the effects of this silica clean-up step on the recovery of three arsenolipids (AsFA-362, AsFA-388 and AsHC-332), and then their stability in ethanolic solution under acidic, neutral and basic conditions. When we applied the silica clean-up method individually to our three test arsenolipids, we found high recoveries for the AsHC-332 (0.95 ± 0.01 μg As, 95%, n = 3), but lower recoveries for the saturated AsFA-362 (0.88 ± 0.06 μg As, 88%, n = 3) and for the unsaturated AsFA-388 (0.82 ± 0.05 μg As, 81%, n = 3). We then examined the effect of pH when the arsenolipids are stored for up to 15 days (Appendix A Table S1). At pH 7 and at pH 12, all three arsenolipids were unchanged and no losses were observed after the 15-day storage period. Differences were observed, however, between the arsenolipids stored at pH 2 (Fig. 2); whereas the As-hydrocarbons remained unchanged, the quantities of the two As-fatty acids, began to diminish at pH 2 after 1 day and both decreased to ca 50% of their initial amount by the end of the storage period.

When we analyzed these samples by HPLC coupled simultaneously to both ICPMS (for arsenic detection) and ESIMS (for molecular detection), we could see that the loss of the initial fatty acid was accounted for entirely by one product, with no other arsenic species detected (Fig. 3). In each case, the single product was less polar than the corresponding starting acid, and the ESIMS data clearly showed that it was simply the ethyl ester of the arsenic fatty resulting from acid-catalysed esterification in the ethanolic solutions. For example, the product from AsFA-388 had m/z 417 $[M + H]^+$ and m/z 389 $[M + H - 28]^+$.

2.1. Stability of arsenolipids at 60°C under dry conditions and in added fish oil

The effect of temperature (60°C) on the lability of the arsenolipids was also investigated, both with pure compound

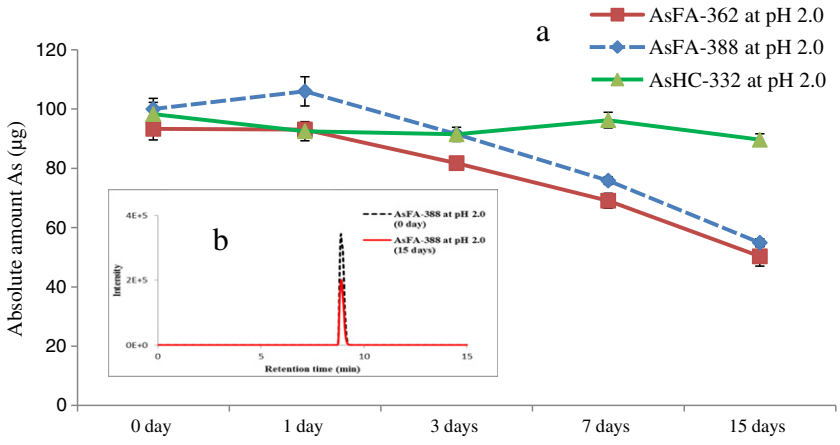

Fig. 2 – (a) Stability of AsFA-362, AsFA-388 and AsHC-332 stored in EtOH at room temperature (pH 2.0). Values represent the mean ± standard deviation (SD) of three replicate samples. (b) Reversed-phase chromatogram (HPLC/ESIMS) of AsFA-388 for 0 and 15 days samples at pH 2.0. Chromatographic conditions: column; Asahipak C-8 (4.6 × 150 mm, 5 μm); mobile phase water/MeOH sharp gradient (30%-100% MeOH, inc. 0.1% formic acid, over 4 min); flow rate 0.5 mL/min; column temperature 40°C; injection volume 10 μL. HPLC/ESIMS: high performance liquid chromatography/ESIMS: electrospray triple quadrupole mass spectrometry.

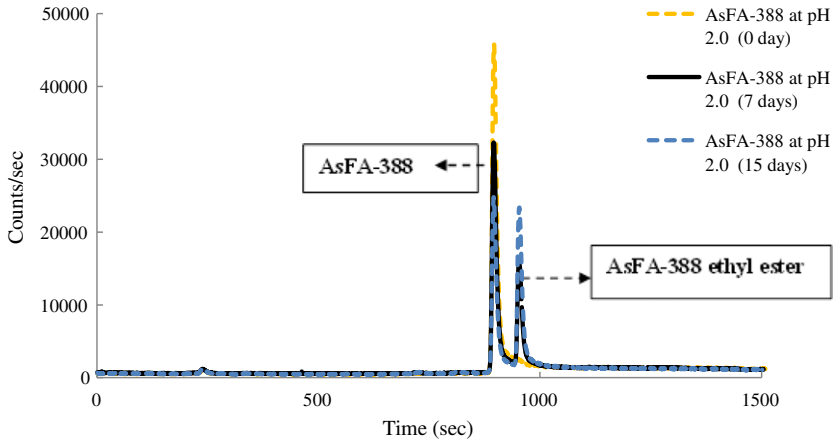

Fig. 3 – Reverse phase chromatogram (HPLC/ICPMS) of AsFA-388 for 0, 7 and 15 days samples at pH 2.0. Chromatographic conditions: column; Asahipak C-8 (4.6 × 150 mm, 5 μm); mobile phase water/MeOH slow gradient (30%–100% MeOH, inc. 0.1% formic acid, over 15 min); flow rate 0.5 mL/min; column temperature 40°C; injection volume 20 μL.

alone, and with pure compound spiked to fish oil. The total arsenic in fish oil was previously analyzed and shown to be negligible (<10 μg As/kg). The pure compound alone showed losses, and in the case of AsHC-332, the losses were dramatic (*ca.*, 80% loss) and already apparent after only 6 hr (Fig. 4). A repeat of this experiment with AsHC-332, again with three replicates, gave very similar results. These losses for AsHC-332 at 60°C were greatly reduced when fish oil was present together with the compound. The two fatty acids also showed losses on storage, but these losses were much smaller than those of the AsHC, and they were further slightly reduced when fish oil was also present (Fig. 4).

The reasons for the large losses of AsHC-332 were investigated by repeating the experiment in plastic tubes and glass tubes at room temperature, 40°C, and 60°C; losses were not observed for the glass tubes at any temperature (Fig. 5), and were negligible for both tube types at room temperature. No losses were observed from glass tubes whether they were open or closed. Losses were observed, however, for the plastic surfaces at 40°C and again at 60°C, with the losses being much higher at the higher temperature (Fig. 5).

When the tubes from the experiments where "missing" arsenic was observed were later extracted with nitric acid, no arsenic was found in the extract. However, when the tubes were re-extracted with ethanol at 60°C overnight we found that all of the "missing" arsenic was extracted back into solution; when hexane (60°C overnight) was used instead of ethanol, about 10% was extracted.

3. Discussion

The recent finding that some arsenolipids, in particular the AsHCs, have cytotoxic properties has created interest in regard to the human health implications of arsenolipids in food. Although there are many studies identifying arsenolipids, and new compounds are regularly being

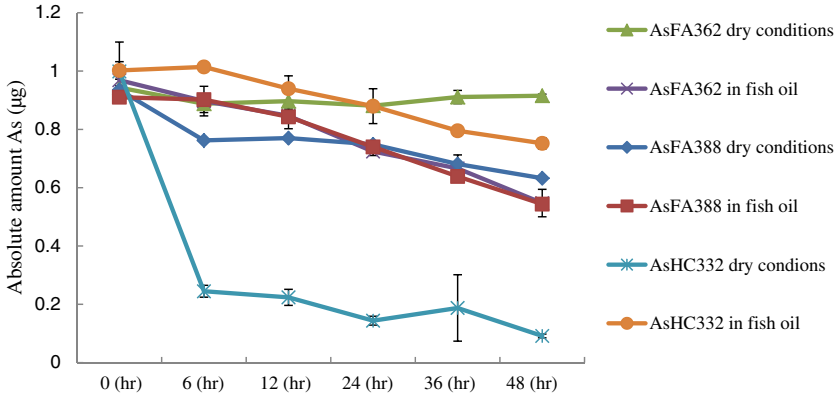

Fig. 4 – Losses of AsFA-362, AsFA-388 and AsHC-332 when the compounds were stored in polypropylene tubes (15 mL) at 60°C in fish oil and under dry conditions. Values represent the mean ± SD of three replicate sample treatments; measurements were by HPLC/ESIMS.

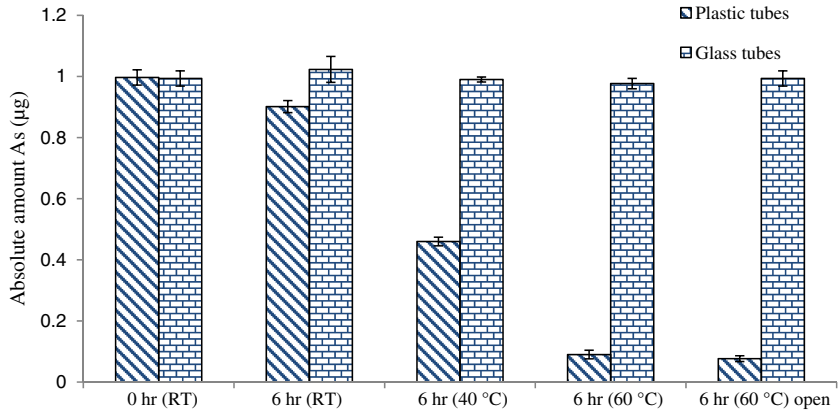

Fig. 5 – Losses of AsHC-332 when the compounds were stored in polypropylene tubes (15 mL) and glass tubes (12 mL) at room temperature (RT), 40°C and 60°C under dry conditions. Values represent the mean ± SD of three replicate treatments; measurements were by HPLC/ESIMS.

reported, quantification of the arsenolipids is proving difficult. Factors restricting progress in this area include the need to perform sample preparation before analysis, and the lack of knowledge of the stability of the analytes during common sample preparation steps.

A few studies have used silica chromatography as a clean-up step prior to arsenolipid measurements. Its effectiveness is based on the interaction between the basic O = AsR_3 group of the arsenolipids and the acidic silica, whereby the arsenolipids are retained on the column but normal lipids are not. For the edible alga, Hijiki, which contains most of its lipid-arsenic as As-hydrocarbons and arsenosugar phospholipids, the method provided good clean-up with little apparent losses (Glabonjat et al., 2014). In contrast, application of the procedure to the lipids in fish oil, which contain considerable amounts of As-fatty acid conjugates showed that many of the original compounds were changed by the procedure (Taleshi et al., 2014). In the experiments with pure compounds reported here, we found that AsHC-332 had negligible losses on the silica column, and that silica chromatography was entirely suitable for quantitative work for this arsenolipid; by inference, it is likely that this statement also holds for the other As-hydrocarbons found in seafoods. The two arsenic fatty acids were similarly found to be reasonably stable under these conditions (>80% recovery). The presence of a single double bond did not unduly exacerbate this stability issue as the recovery of AsFA-388 from the silica column was only slightly lower than that for AsFA-362.

The sample preparation steps for arsenolipids can involve sample extraction and partitioning in addition to silica chromatography. Long HPLC run-times (up to 60 min or more) are also common for these analytes. Thus, arsenolipids could be in solution for many hours before being measured, with increasing risk of decomposition of the original compounds. We tested the arsenolipid stability in ethanolic solution at two pH extremes and under neutral conditions. We used ethanol as the solvent because it is compatible with a range of arsenolipid polarities, and is also the major solvent used in reversed-phase HPLC of the arsenolipids (*e.g.*, Glabonjat et al., 2014). At pH 7 and 12, the three test arsenolipids were stable with no degradation products being observed even after 15 days. At pH 2, the AsHC was stable, but the two AsFAs underwent acid-catalysed esterification. Although this esterification was not noticeable in the first 24 hr, it was significant (*ca.*, 10%–15%) at $t = 3$ days. Thus, the limitations of ethanol when working with the arsenic fatty acids at low pH should be noted, and other solvents considered.

If the delay between extraction of arsenolipids, from a food sample for example, and measurement by HPLC/mass spectrometry is too long, one might prefer to store the samples as freeze-dried extracts. As a general rule, analysis involving organic solvents should avoid the use of plastics because of solvent incompatibility and extraction of chemicals from the plastics (*e.g.*, plasticisers) that could complicate later measurements by molecular mass spectrometry. Because arsenolipids are often determined by HPLC/ICPMS, and the ICPMS is a robust and very selective detector for arsenic, several groups including our group in Graz, have used plastic in the sample preparation steps and for storing extracts. We found, however, that storage of the AsHC-332 as the dry pure compound in polypropylene tubes resulted in severe losses (80% after 48 hr at 60°C) of the analyte, and that these losses were completely avoided when glass was used in place of polypropylene. The losses were also much less severe when the storage was at room temperature.

We presumed that the losses resulted from either volatilisation (*e.g.*, of a volatile product) or adsorption to the walls of the polypropylene tube. Possible volatilisation was tested and excluded by a simple experiment showing no differences in losses of AsHC-332 between open and closed tubes. Adsorption onto the walls of the plastic tube was then shown to be the more plausible explanation because when the tube was treated with EtOH at 60°C all the arsenolipid was recovered. A further point to note is that when the experiments were performed with the pure individual

compound in "arsenic-free" fish oil, the losses of analyte were greatly reduced. Presumably the other lipids in the fish oil outcompeted the arsenolipids for the adsorbing sites on the polypropylene surface. This unusual adsorption behaviour shown by AsHC-332, which requires further investigation, might be due to enhanced surface effects of the polypropylene tube at the higher temperature.

It remains to be seen if the other groups of arsenolipids (*e.g.*, arsenic-containing phospholipids and arsenic-phosphatidylcholines) present in marine foods are affected by sample preparation steps, containers and storage conditions. So far, the practical advantages of using plastics as labware and alcohols as solvents under acidic and basic conditions have outweighed any disadvantages. Analysts might be advised, however, to consider their use of solvents, clean-up conditions and storage containers when performing analyses for arsenolipids, and to use plastic rather than glass only when they are confident that losses will not be incurred.

Finally, we note that although previous investigations of arsenolipids, focussing on their identification, have used hybrid instruments with accurate mass capability such as Q-TOF or Q-Orbitrap, the low resolution QQQ mass spectrometric system used in our study was perfectly suited to the study goals. In addition, the precursor ion mode provided high selectivity, low detection limits (0.002 μg As/L), and stable responses over long analysis times (RSD 3.6% over a 24 hr runtime). These properties suggest that QQQ systems could be increasingly used for arsenolipid research, in particular for investigations where accurate mass measurements are not essential.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.04.004.

Acknowledgment

This research has been supported by the Austrian Science Fund (FWF) project number I 2412. We thank Ronald Glabonjat for ICPMS analyses and instrumental advice, and Melanie Ramböck for her contributions to related initial experiments.

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