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TECHNICAL NOTE

A method for screening arsenolipids in fish oils by HPLC-ICPMS

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We present a method for screening lipid-soluble arsenic compounds (arsenolipids) in fish oils by reversed-phase HPLC-ICPMS using a gradient elution with ethanol and acetate buffer at pH 6. Two different approaches were tested to reduce changes in arsenic response due to the carbon effect: addition of a supplementary methanol solution directly to the spray chamber or addition of methanol post-column through a T-piece. The latter method proved to be the best option for maintaining constant response for several arsenolipids covering a wide range of polarities. With the optimized method it is possible to perform a screening of at least three groups of arsenolipids with different polarities in 90 min with detection limits ranging from 5 to 11 μg As L^{-1}, depending on the analyzed compound. The method was applied to the screening of arsenolipids in fractions obtained from cod liver oil and capelin oil, which include arsenic-containing fatty acids, arsenic-containing hydrocarbons and another group of lower polarity and unknown character.

Introduction

Arsenic contamination is a major global environmental problem because of the presence of inorganic arsenic in drinking water and the consequent chronic health effects such as cancers, cardiovascular diseases and possibly diabetes.[1] Many arsenic species also occur in food, especially in food of marine origin, and research during the last three decades has identified more than 60 arsenic compounds in marine organisms. Most of the arsenic compounds identified so far are water soluble, and there is considerable information on the toxicity and occurrence of these water-soluble arsenic species in biological and environmental samples.[2]

Arsenic can also occur, however, as lipid-soluble arsenic species, but our knowledge in this area is limited. Although there is an early report of a lipid-soluble arsenosugar in a brown alga, the structures of the major arsenolipids have only been revealed over the last three years. These studies have identified arsenic-containing fatty acids in cod liver oil[3] and arsenic-containing hydrocarbons in capelin,[4–6] cod liver[7] and sashimi tuna fish.[8] The studies have also indicated the presence of many unknown arsenolipids in the fish samples, and highlighted the need for an analytical method to allow screening of food samples. Fig. 1 shows the structures of some of the identified lipid-soluble arsenicals such as As-containing fatty acids (C15-FA) and As-containing hydrocarbons (C15-HC).

Although HPLC-ICPMS has been proved to be a valuable technique for determining water-soluble arsenic species, it is not yet commonly used for lipid-soluble species. The method was successfully applied under normal or reversed-phase conditions with isocratic elution to quantify groups of arsenolipids in fish oils.[9] Separation of the many diverse naturally occurring arsenolipids, however, requires gradient elution HPLC with mobile phases of high and variable organic solvent content, and this creates problems for quantification because the arsenic signal is highly dependent on the amount of carbon entering the plasma.

Recently Amayo *et al.*[6] reported the identification and quantification of arsenolipids with gradient reversed phase chromatography separation and using a factor correction to compensate for changes in response due to the carbon effect. Another study

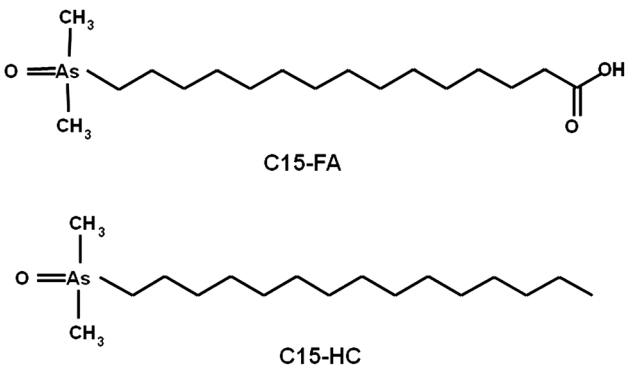

Fig. 1 Examples of an arsenic-containing fatty acid (C15-FA) and an As-containing hydrocarbon (C15-HC) identified in cod liver oil and capelin samples (ref. 3,4,6–8).

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has shown the advantages of gradient elution reversed-phase HPLC, with variable methanol content in the mobile phase, when dealing with arsenic species that are intrinsically water soluble, and reported a novel approach to improve quantification under gradient elution conditions.[10] This latest work encouraged us to investigate gradient elution HPLC-ICPMS to determine arsenolipids, which require high levels of organic solvent. The present study reports the optimization of a gradient separation method for the screening of arsenolipids in fish oils using ICPMS as an arsenic-selective detector.

Experimental

Standards and reagents

Water used throughout the present work was Milli-Q water (18.2 MΩ cm). Triphenylarsine oxide (Ph_3AsO) was synthesized according to Merijanian and Zingaro.[11] Dimethylarsinate (DMA; sodium dimethylarsinate trihydrate), methanol (p.a.), ethanol (p.a.), ammonium acetate (p.a.) and aqueous ammonia solution (25%, Suprapur) were purchased from Fluka (Buchs, Switzerland). The arsenic-containing fatty acid (Fig. 1, C15-FA, $C_{17}H_{35}AsO_3$)[3] and the arsenic-containing hydrocarbon (Fig. 1, C15-HC, $C_{17}H_{37}AsO$)[4] were synthesized in-house following the reported procedures. Diluted standards were prepared in methanol.

Instrumentation

HPLC-ICPMS: a Waters Atlantis dC_{18} reversed-phase column (1 × 150 mm) was used at 30 °C with ethanol/20 mM ammonium acetate pH 6 as a mobile phase at a flow rate of 100 μL min^{-1}. The gradient program was: 35% ethanol to 100% ethanol in 60 min and then maintained at 100% ethanol for 30 min. The injection volume was 1 μL. An Agilent 7500ce ICPMS equipped with an integrated sample introduction system (ISIS) served as an arsenic selective detector (sample cone Pt, skimmer cone Ni; rf power 1600 W, reflected power < 5 W); sampling depth 8 mm; plasma gas 15 L min^{-1} and auxiliary gas 1 L min^{-1}; optional gas flow rate 6–12% of carrier gas flow. The outlet of the HPLC column was directly connected with PEEK capillary tubing (0.125 mm i.d.) to the ICPMS equipped with a Burgener Ari Mist HP nebulizer and a Scott Type spray chamber operated at −5 °C. To prevent deposition of carbon on the interface cones, an optional gas (20% oxygen in argon) was introduced through a T-piece connecting the spray chamber and the torch (1 mm inner diameter).

Addition of a supplementary solution directly to the spray chamber. For this approach the system reported by Kovacevic and Goessler[12] and further developed for arsenic compounds by Raber *et al.*[10] was used whereby an organic solvent is added *via* a capillary in the end cap directly to the spray chamber so that the spray chamber is saturated with vapours of the organic solvent. Solutions containing different proportions of organic solvents (supplementary solutions) were tested: methanol, methanol/water (50 : 50, v/v), methanol/water (80 : 20, v/v), ethanol/water (80 : 20, v/v), and acetone/water (50 : 50, v/v). Optimization of the ICPMS was performed with 100% ethanol at a flow rate of 100 μL min^{-1}. The supplementary solution was pumped at ~0.3 mL min^{-1} into the spray chamber using the peristaltic pump of the ISIS. Fig. 2A shows a scheme of this approach. To ensure better stability of the peristaltic pump tubing towards organic solvents, we used tubings made from Tygon® MH2075. To avoid carbon deposition on the cones, 6–12% optional gas was used, depending on the organic solvent introduced. The amount of carbon arriving at the plasma was determined by monitoring the *m*/*z* 52 ($^{40}Ar^{12}C^+$) signal.

Post-column addition of a supplementary solution. For this approach, the supplementary solution was added through a T-piece after the column and before the nebulizer, using a peristaltic pump from the ISIS, as shown in Fig. 2B. The flow rate was fixed at 0.22 mL min^{-1}. Methanol and ethanol were tested as supplementary solutions. As mentioned above, tubings made from Tygon® MH2075 were used and 8–10% optional gas was used. The amount of carbon arriving at the plasma was determined by monitoring the *m*/*z* 52 ($^{40}Ar^{12}C^+$) signal.

Sample preparation

Fractions analyzed in the present work were obtained after solvent partitioning of different fish oils as follows:

Aqueous methanol fraction from cod liver oil. Crude cod liver oil was partitioned between hexane and aqueous methanol and the polar phase subjected to preparative chromatography with anion-exchange media to yield a fraction enriched in the acidic arsenolipids. The details of sample preparation are reported by Rumpler *et al.*[3]

Aqueous methanol fraction from capelin oil. Aqueous methanol fraction from capelin oil was obtained as reported by Taleshi *et al.*[4] Fish oil was partitioned in the same way as for the cod liver oil and the main arsenolipids were obtained in the basic/neutral fraction (as arsenic-containing hydrocarbons) after preparative anion-exchange column chromatography.

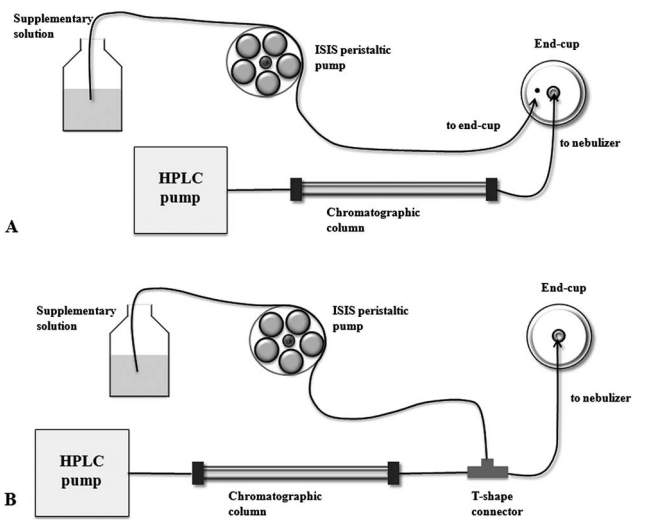

Fig. 2 Scheme of the approaches used in the present study. (A) Addition of a supplementary solution directly to the spray chamber and (B) post-column addition of a supplementary solution.

Isopropanol fraction of capelin oil. The hexane fraction obtained after the separation of the aqueous methanol fraction was partitioned into aqueous isopropanol to extract less-polar arsenolipids which were then further purified on a silica-gel column (M. S. Taleshi, unpublished work).

Results and discussion

Arsenolipid determination with HPLC-ICPMS

The chromatographic behaviours shown by the different lipid-soluble arsenic compounds identified so far necessitated the use of gradient elution conditions to effect the separation of the compounds in the major arsenolipid groups in a single run. A gradient program from 35% to 100% ethanol in 90 min was shown to be suitable for the separation/screening of both As-containing fatty acids and As-containing hydrocarbons, together with other arsenolipids with unknown structure and with less-polar properties. These gradient elution conditions, however, precluded compound independent quantification. Therefore, we tested two different approaches to get a constant amount of carbon into the plasma.

Addition of a supplementary solution directly to the spray chamber. To check arsenic sensitivity during the application of the gradient program, flow injection experiments were performed with a polar standard (DMA), a non-polar standard (Ph_3AsO), and two standards corresponding to the types of compounds found in the analyzed samples, namely an As-containing fatty acid (C15-FA, Fig. 1) and an As-containing hydrocarbon (C15-HC, Fig. 1). Every standard (1 μL injection containing approx. 1 ng As) was injected approximately every 2.5 minutes during a complete run of the gradient program. An example of the results obtained when pure methanol was used as supplementary solution is shown in Fig. 3A. The arsenic response changes by a factor of three during the gradient program. Moreover, changes in the *m*/*z* 52 are also observed towards the end of the gradient program, when high proportions of organic solvent are used. The other supplementary solutions tested (*e.g.* ethanol/water) had similar problems of variable arsenic response, and were further disadvantaged by showing poorer signal stability compared with methanol.

These experiments revealed that even when we compensate the carbon load in the plasma the addition of a supplementary solution with our setup is only useful for gradients with relatively low proportions of ethanol in the mobile phase. Thus, gradient compensation was only of limited use for the quantification of arsenolipids found in natural samples because of large polarity differences of these compounds.

Post-column addition of a supplementary solution. In this approach the supplementary solution was added through a T-piece using a peristaltic pump from the ISIS. Methanol and ethanol were tested as supplementary solutions. Methanol provided better results, possibly because its higher vapour pressure allowed it to better compensate for the volatility differences due to the different proportions of ethanol during the gradient program. Flow injection experiments were also performed with the four arsenic standards. In Fig. 3B the flow injection signals of

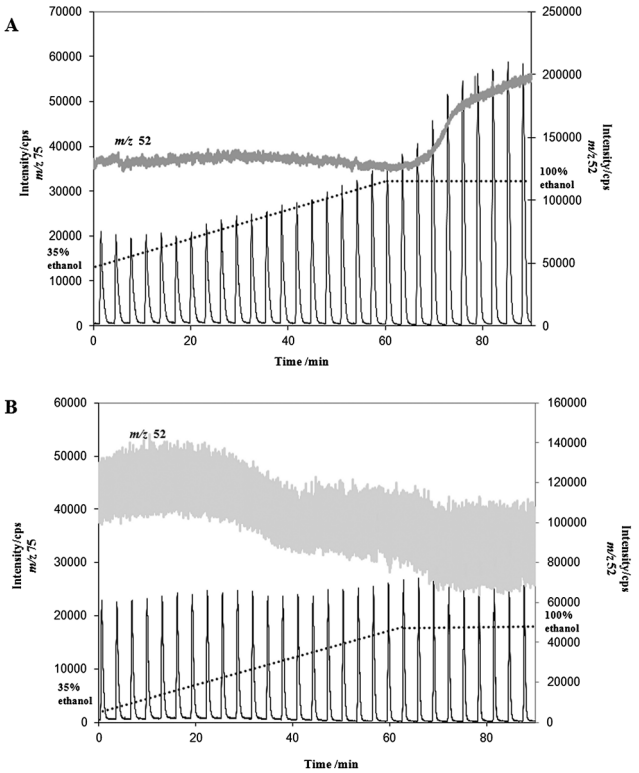

Fig. 3 Flow injection signals obtained for the As-hydrocarbon standard (C15-HC). (A) Using methanol as the supplementary solution added directly to the spray chamber and (B) using post-column addition of methanol as the supplementary solution. Signals were obtained after injecting 1 μL of the standard solution (containing 1 ng As) every 2.5 min while the gradient program is running.

the As-hydrocarbon standard are shown; the other three arsenic standards behaved similarly. The response of the arsenic signal is fairly constant during the whole gradient, with a coefficient of variation of the area during the whole gradient of ~6%. Although due to dilution effects with the supplementary solution sensitivity is reduced, the estimated instrumental detection limit by using this approach was in the low μg L^{-1} range (~5 μg L^{-1} for C15-FA and C15-HC). However, *m*/*z* 52 has higher noise than in the previous set-up. This is attributed to the fact that the supplementary solution is added directly through a T-piece and a peristaltic pump.

Application of the method. The method was used for the analysis of a fraction obtained from cod liver oil and two fractions from capelin oil. These three fractions obtained after solvent partitioning correspond to a methanol fraction from cod liver oil enriched in polar arsenolipids (As-containing fatty acids), a methanol fraction from capelin oil enriched in non-acidic arsenolipids (As-containing hydrocarbons), and an isopropanol fraction after partial purification by using a silica-gel column, which contains non-polar arsenolipids. We injected 1 μL of each fraction containing between 10 and 20 ng As. The overlaid chromatograms of the different fractions (Fig. 4) show that by using the method it is possible to analyze in the same run these three groups of arsenolipids which cover the range of arsenolipids found so far in biological and food samples. The

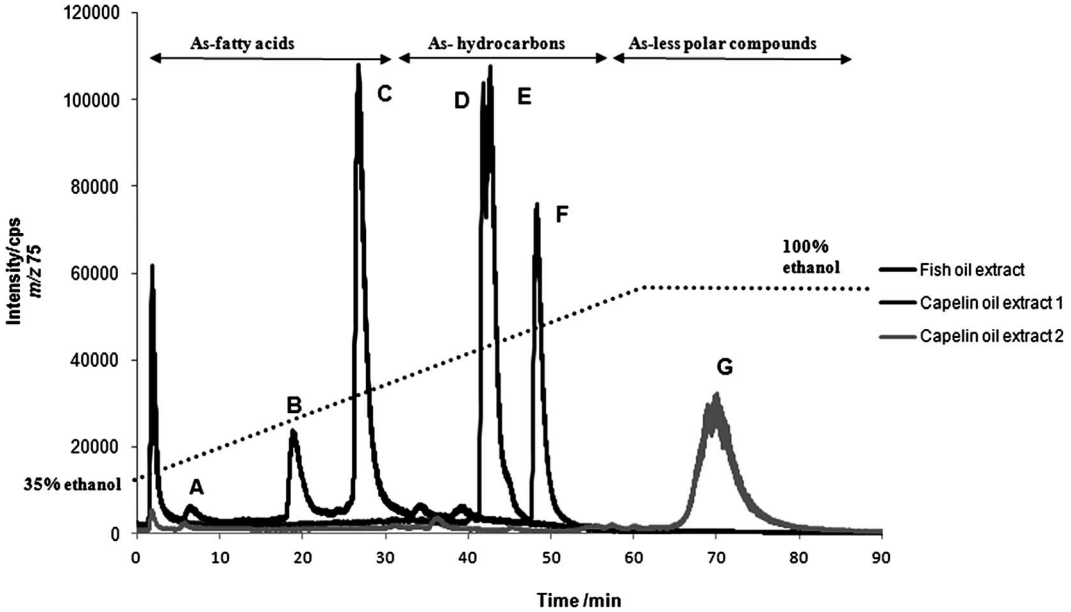

Fig. 4 Overlaid of three chromatograms obtained from the fraction of cod liver and capelin fish oils using post-column addition of methanol as the supplementary solution. (A) C13-fatty acid ($C_{15}H_{31}AsO_3$); (B) C15-fatty acid ($C_{17}H_{35}AsO_3$); (C) C17-fatty acid ($C_{19}H_{39}AsO_3$); (D) C15-hydrocarbon ($C_{17}H_{37}AsO$); (E) C17-hydrocarbon ($C_{19}H_{41}AsO$); (F) C21-unsaturated hydrocarbon ($C_{23}H_{37}AsO$); (G) unknown. For structures see Fig. 1 and ref. 3 and 4.

method allows the analysis of fractions obtained from simple partitioning steps, and thus provides a quick screening of various samples for the presence of these compounds.

Acknowledgements

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