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# Identification of arsenolipids and their degradation products in cod-liver oil

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ABSTRACT

Oils from marine samples are known to contain high concentrations of arsenolipids. However, their identification in lipid matrix poses a significant challenge especially when present in low concentrations. Here, we report the identification of sixteen arsenolipids in cod-liver oil. The fish oil was fractionated on a silica gel column and the fraction enriched with arsenic analysed using RP-HPLC online with ICP-MS and ES-Orbitrap-MS. Among the arsenolipids identified nine compounds have not been reported before. Structural assignment was achieved by arsenic signal from ICP-MS, retention time behaviour and accurate mass determination of fragment and molecular peaks. In addition, the unknown degradation products of arsenolipids eluting in the void volume were investigated using fraction collection, cation exchange chromatography and accurate mass determination, and were found to contain predominantly dimethylarsinic acid (DMA) with trace amounts of methylarsonic acid (MA), dimethylarsenopropanoic acid (DMAP) and dimethylarsenobutanoic acid (DMAB). This finding is essential in epidemiologic studies where urinary DMA and other arsenic metabolites have been used as biomarker in accessing human exposure to arsenic.



## 1. Introduction

Cod-liver oil is widely used as nutritional supplement due to health benefit associated with it as a source of vitamins A and D, and essential fatty acids for normal functioning of the brain, heart and the eye [1–3]. However, as with most fish oils, cod-liver oil also contains appreciable levels of lipid soluble arsenic compounds (arsenolipids) [4,5]. The presence of high concentrations of arsenolipids in marine oils has been known for many years. Sadolin in 1928 analysed two samples of cod for their concentrations of arsenic, and reported that the two samples analysed contain 0.4 and 0.8 ppm in the flesh, 0.7 and 3.2 ppm in the liver and 3.0–4.5 ppm in the liver oil respectively [6]. Lunde in 1968 reported the separation of arsenic in the lipid fraction of cod-liver and herring oils. The arsenic compounds were fractionated using gradient elution with methanol/chloroform on silicic column, and analysis of the fractions by means of neutron activation indicated the presence of two types of lipid soluble compounds [7,8]. Vaskovsky et al. (1972) also studied arsenic in the lipid extracts of marine invertebrates. The lipids were fractionated by means of thin layer chromatography and several different lipid-soluble organo arsenic compounds were shown to be present but their molecular structures could not be determined at the time [9].

However, even though the early work on arsenic in marine samples focussed on arsenolipid, the identification of the chemical forms proved difficult due to lack of suitable analytical techniques [5,10]. Contrary to the general belief that arsenic in fish is probably nontoxic [11,12], studies have shown that arsenolipids are biotransformed in humans mainly to DMA [13,14], and the implication of this might be that just like the highly toxic inorganic arsenic, it is possible that the arsenolipids are also producing toxic intermediates en route to DMA [12]. In addition, it has been also shown that DMA itself demonstrates unique toxicity and has been implicated as potential carcinogen [15,16]. Therefore, it seems reasonable to categorise arsenolipids as potentially harmful [17], which then underlines the need for speciation studies for accurate risk assessment and exposure from food, in particular fish oil being the major source of exposure.

Due to widespread use of cod-liver oil, it is of special interest to identify and quantify the arsenolipids present in it. Rumpler et al. (2008) reported the first identification of six arsenic containing long-chain fatty acids (AsFA) in cod liver oil using reversed-phase

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HPLC coupled to ICP-MS and structural assignment by accurate mass spectrometry. However, the compounds identified only accounted for about 20% of the total arsenolipids in fish oil, thus the need for further studies. Additionally, the presences of unknown degradation products of arsenolipids have been reported in previous works, which usually elute in the void volume of reverse phase chromatography [10,12,18]. In this study we report the identification sixteen arsenolipids and also the unknown degradation products of arsenolipids in cod-liver oil.

## 2. Experimental

### 2.1. Reagents, standards and sample

Ultra-pure water (18 MΩ cm, Elga, UK) was used throughout for sample preparation and for solutions of standards. Formic acid, sodium arsenite, Ge (ICP/DCP standard solution) and pyridine were supplied by Sigma-Aldrich (UK). Sodium dimethylarsinate ($DMA^V$), used as calibration standard for quantification of arsenic species and methyl arsenic acid were obtained from ChemService (USA). Hexane used for extraction, hydrogen peroxide ($H_2O_2$, 32%) and methanol were obtained from Fisher Scientific. Nitric acid ($HNO_3$, 65%) from Fluka (UK). Silica gel LC60A 40–63 μm used in vacuum liquid chromatography (VLC) column was obtained from Fluorochem, UK. Dimethylarsenopropanoic acid (DMAP) and dimethylarsenobutanoic acid (DMAB) used as reference standards were kindly donated by Kevin Francesconi, Karl-Franzens University, Graz, Austria and one sample of fresh pressed Icelandic cod-liver oil was donated by MATIS, Vinlandsleio 12, 113 Reykjavik

### 2.2. Sample digestion and determination of total arsenic by ICP-MS

About 0.1–0.5 g of cod-liver oil, cod-liver oil extract or certified reference material (CRM) were weighed precisely into a 50 mL Greiner tube, 1 mL of conc. $HNO_3$ added and left standing overnight for pre-digestion. Then, 2 mL of $H_2O_2$ was added and the samples subjected to open microwave digestion (Mars-5, CEM, UK) using a stepwise heating programme of: 5 min at 50 °C, 5 min at 75 °C and 25 min at 95 °C. Digests were diluted to 25 mL and total arsenic determined by ICP-MS (Agilent 7500c, Japan). $^{74}Ge$ was used as internal standard and quantification carried out against standard solutions of sodium arsenite. The possible $ArCl^+$ interference on $m/z$ 75 (As) was checked by measurement of $m/z$ 77 (Se) and $m/z$ 82 (Se) signals and no chloride interference was detected. The accuracy of the measurement was assessed by measuring the total arsenic in CRM (DORM-3); fish protein from National Research Council Canada. All measurements were carried out in triplicate and results expressed as mean values ± standard deviation ($X \pm SD$).

### 2.3. Fractionation of cod-liver oil using vacuum liquid chromatography (VLC)

The fractionation carried out here involved modifications of a previously reported method and the aim was to reduce the interference from the lipid matrix during analysis. [10] Approximately 10 g of raw Icelandic cod-liver oil was first extracted with water to remove the water soluble fraction and the residue containing the lipid soluble fraction was dissolved in 10 mL of hexane. The oil in hexane was mixed with silica gel 60 to absorb the arsenic compounds and left overnight to dry. Thereafter, the oil+silica gel mixture was transferred to a VLC column. The VLC consist of a glass column ($30 \times 6$ $cm^2$, i.d.) with an end-frit filled with silica gel 60, a sidearm of the outlet is connected to a vacuum line. Using a gradient of hexane, ethyl acetate and methanol as eluting agents the cod-liver oil was fractionated into nine fractions (F1–F9) as shown in Table S1 of supplementary information. Each fraction eluted with 300 mL of solvent was pre-concentrated using rotary evaporator and then evaporated to dryness under a stream of nitrogen gas. The residues were finally dissolved in methanol for analysis by reversed phase HPLC–ICP-MS.

### 2.4. Speciation of arsenolipids by RP-HPLC–HR-ICP-MS/ES-Orbitrap-MS

The arsenic species in VLC fractions were separated using a gradient of 0.1% formic acid in water and 0.1% formic acid in methanol on a reverse phase column (Agilent Eclipse, XBD-C18; $4.6 \times 150$ mm) as described elsewhere. [10] The eluent flow was split post-column for simultaneous detection with high resolution ICP-MS (Element 2, Thermo Scientific, Bremen, Germany) and ES-MS (LTQ Orbitrap Discovery; Thermo Scientific, Bremen, Germany). HR-ICP-MS (Bremen, Germany) was used in organic mode with platinum cones. $DMA^V$ was used as calibrant for quantification of the arsenic species and $^{74}Ge$ as internal standard to monitor fluctuations in intensities due to instability of the plasma. The instrument operating parameters are shown in Table 1.

### 2.5. Analysis of water soluble arsenic species

Water soluble arsenic compounds that eluted around the void volume during the reversed phase HPLC–ICP-MS analysis of arsenolipids was collected using Agilent 1100 fraction collector for further analysis. The fraction collector was configured for multiple collections at retention time 1–2 min. The fraction collected was evaporated to dryness, and then re-dissolved in 1 mL of ultrapure water and the arsenic species were separated and identified using cation exchange HPLC online with ICP-MS and ES-MS. Details of instrumental parameter are shown in Table 2.

## 3. Results and discussion

### 3.1. Total arsenic measurement and analysis of VLC fractions

The result of total arsenic measurement in the digest showed that $5.8 \pm 0.2$ μg As/g ($X \pm SD$, $n=3$) was originally present in the raw sample of cod-liver oil. The water extract constitutes a small

**Table 1**
HPLC–ICP-MS–ES-MS Parameters for arsenolipid speciation analysis.

| **HPLC** | **Thermo Scientific** |
|---|---|
| Column | Agilent Eclipse, XBD-C18; $4.6 \times 150$ $mm^2$ |
| Column temperature | 30 °C |
| Injection volume | 100 μL |
| Buffer A | 0.1% formic acid in water |
| Buffer B | 0.1% formic acid in methanol |
| Splitter ratio | 1:3 |
| Flow rate | 1 mL/min |
| Gradient | 0–25 min: 0–100%, 5 min 100% B |
| **ICP-MS** | **Element 2 ( Thermo Scientific)** |
| Mode | Organic mode |
| HF | 1570 W |
| Nebuliser | Micro-concentric |
| Nebuliser gas flow | 0.86 L/min |
| Optional gas flow | 20 mL/ min $O_2$ |
| Plasma gas flow | 0.89 L/min |
| Cooling gas | 14.9 L/min |
| **ES-MS** | **LTQ Orbitrap Discovery; Thermo Scientific** |
| Mode | Positive |
| Spray voltage | 4.5 KV |
| Normalised collision energy | 35% |

percentage (1.6%) of the total arsenic. Schmeisser et al. [19] reported 100% arsenolipid content in cod-liver oil. It is possible that the arsenic in the aqueous extract is accompanied by some hydrolysis products of arsenolipids [5]. The fractionation of the lipid fraction using VLC as described above enhanced the separation of the arsenic compounds from the oil matrix. Nine fractions F1–F9 were separated and analysed using reversed phase HPLC–ICP-MS. Only F9 was found to contain significant amounts of arsenic as shown in the chromatogram with arsenic containing peaks (A–J) in Fig. 1. No significant arsenic peaks were detected in other VLC fractions (F1–F8) and as such were not considered for further investigation. Since the analytical response under this condition is basically element specific and independent of the type of compound, quantification was done by measuring peak areas against standard $DMA^V$ giving a total concentration of 12.15 μg As/g ($n=1$) as shown in Table 3. A solution of 10 μg Ge/L was used as internal standard to check possible fluctuations in signal intensities due to instability of the plasma while the changes in arsenic response with methanol gradient was monitored by post-column addition of known concentration of arsenic and Ge as previously described [10]. A chromatographic recovery of 79% was calculated by comparing the total concentrations of arsenic (12.15 μg As/g extract) determined by HPLC–ICP-MS with the result obtained by measurement of total arsenic in the digest with ICP-MS following microwave-acid digestion giving 15.54 ± 0.67 μg As/g extract (X ± SD, $n=3$). Result for total arsenic in the digest was validated by measuring DORM-3 and the determined value, 6.85 ± 0.22 μg As/g showed good agreement with the certified value of 6.88 ± 0.30 μg As/g which corresponds to 99.6% recovery.

**Table 2**
Instrumental parameter for the analysis of water soluble fraction.

| **HPLC** | **Agilent 1100 Series** |
|---|---|
| Cation exchange column | Varian ionosphere C; 150 × 4.6 $mm^2$ |
| Injection volume | 100 μL |
| Column temperature | 20 °C |
| Mobile phase A | Double deionised water |
| Mobile phase B | 50 mM pyridinium formate |
| Gradient | (0–4 min: 1% B); (4.01–16 min: 10% B); (16.01–20 min: 50% B); (20.1–40 min: 1% B) |
| **ICP-MS** | **Agilent 7500C** |
| HF | 1570 W |
| Nebuliser | Meinhard |
| Cooling gas flow (Ar) | 15 L/min |
| Nebuliser flow (Ar) | 1.18 L/min |
| **ES-MS** | **Agilent 1100 MSD Trap XCT** |
| Capillary voltage | 4000 V |
| Drying gas flow ($N_2$) | 40 psi |
| Fragmentor voltage | 100 V |

### 3.2. Identification of arsenic species by RP-HPLC online with ICP-MS and ES-Orbitrap-MS

Arsenic species in the methanol fraction were separated using a water/methanol gradient (Table 1) with simultaneous detection by ICP-MS and high resolution Orbitrap-MS. The reversed phase HPLC–ICP-MS chromatogram shown in Fig. 1 revealed the presence of at least ten arsenic containing peaks (A–J). The arsenolipids eluted from the reversed phase column at 14–22 min corresponding to peaks (B–J). Peak A, was detected around the void volume at 1.6 min. A section of the chromatogram showing ICP-MS elemental specific signal for arsenic overlaid with the molecular signals from the Orbitrap-MS is shown in Fig. 2. Some of the arsenolipids (I1 and I2) and (J1–J3) that co-eluted in the ICP-MS to give single peaks I and J respectively were selectively detected by the high resolution Orbitrap-MS despite their limited chromatographic separation. Molecular information was generated by the Orbitrap-MS as illustrated in the mass spectrum in Fig. 3A with molecular formula and accurate masses of some arsenolipids. The elemental compositions generated for the molecular ions were validated by accurate mass measurement with Δm = 0.26–2.57 ppm (table 4).

The saturated arsenolipids identified are shown in Table 4, comprising two arsenic hydrocarbons (AsHCs) I2 and J2 previously reported in capelin oil, fishmeal and in tuna fish, [10,12,20,21] and the known arsenic fatty acids (AsFAs) B, D2, F, H1 identified in 2008 by Rumpler et al. [4] in cod-liver oil. In addition, six compounds; C, E, G, I1, J1 and J3 were also identified as saturated AsFAs. Proposed molecular structures were further supported by information generated from collision induced tandem mass spectrometry to give characteristic fragment ions. An example of MS/MS spectrum for AsHC is shown in Fig. 3B for the compound, I2. The presence of the ion peaks at $m/z$ 105 and 123 corresponds to the fragment ions $(CH_3)_2As^+$ and $(CH_3)_2AsOH_2^+$ respectively confirming the presence of dimethyl arsinoyl moiety in the molecule. Other characteristic features include the presence of prominent molecular peak $(M+H^+)$ at $m/z$ 333 and the ion peak at $m/z$ 315 in low relative abundance which correspond to $(M+H^+-H_2O)$. MS/MS analysis of arsenic fatty acids is similar to that of arsenic hydrocarbons with slight differences in fragmentation giving rise to loss of three molecules of water. The mass spectrum for AsFA, E, with protonated molecular formula 377 is shown in Fig. 3C. The ion fragments at $m/z$

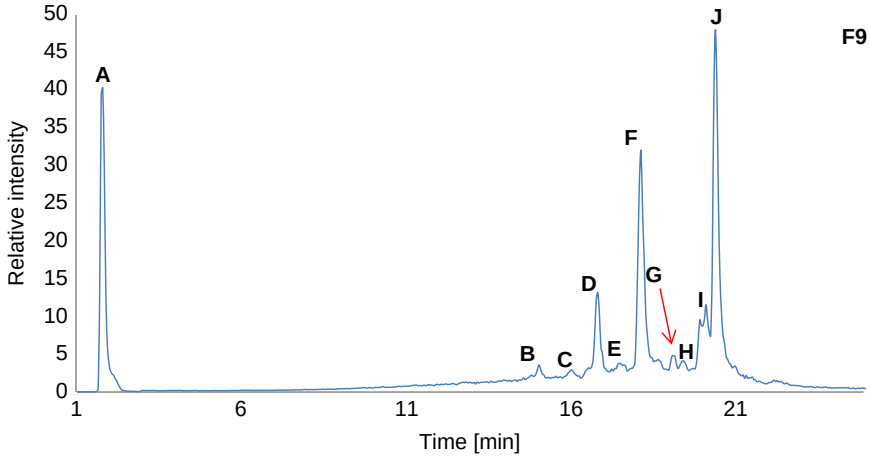


**Fig. 1.** Reversed phase HPLC–ICP-MS Chromatogram of cod-liver oil. VLC methanol fraction (F9).

**Table 3**
HPLC–ICP-MS quantification result of arsenic in μg As/g of the methanol extract (F9).

| Peak | A | B | C | D | E | F | G | H | I | J | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| μg As/g | 1.97 | 0.40 | 0.35 | 1.12 | 0.42 | 2.62 | 0.38 | 0.27 | 0.93 | 3.69 | 12.15 |
| % As | 16.25 | 3.33 | 2.86 | 9.24 | 3.42 | 21.54 | 3.13 | 2.23 | 7.64 | 30.34 | 100.00 |

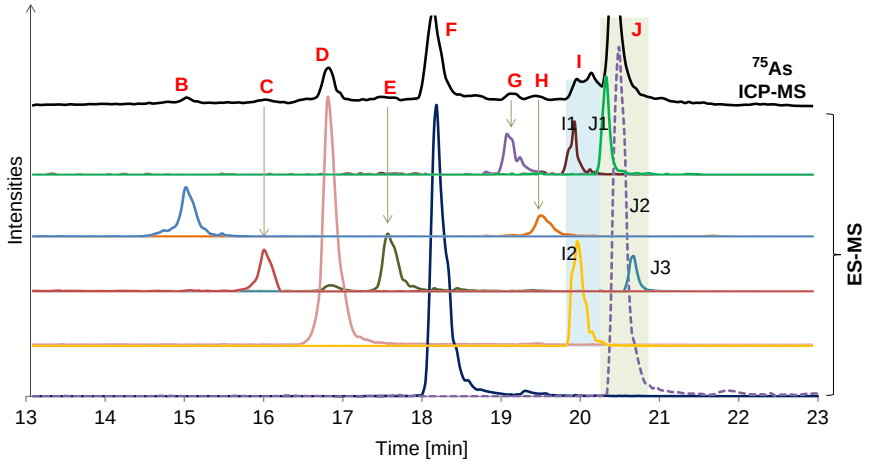


**Fig. 2.** A section of reversed phased chromatogram of cod-liver oil monitored online simultaneously by ICP-MS for arsenic at *m/z* 75 and molecular signals by the Orbitrap-MS. The compounds include AsFAs (B=*m/z* 335, C=*m/z* 349, D=*m/z* 363, E=*m/z* 377, F=*m/z* 391, G=*m/z* 405, H=*m/z* 419, I1=*m/z* 447, J1=*m/z* 475, and J3=*m/z* 503) and two AsHCs (I2=*m/z* 333 and J2=*m/z* 361).

105 and 123 further indicated the presence of dimethyl arsinoyl moiety. Other features include the ion peaks at *m/z* 359, 341 and 323 corresponding to $(M+H^+- -H_2O)$, $(M+H^+-2H_2O)$ and $(M+H^+-3H_2O)$ respectively and the assignment of fragments to the MS/MS spectrum was validated by accurate mass measurement with $\Delta m = \pm 0.1–3.1$.

Six AsFAs first identified in cod-liver oil contain equivalent of an even number of carbon atoms in their structures. The origin of these compounds was thought to be the result of the lack of fidelity in the biosynthetic mechanism for the generation of essential fatty acids that incorporates dimethylarsinoylpropionic acid (DMAP) in the synthetic mechanism [4]. Among the arsenolipids identified in this work, are three AsFAs (C, E and G) with the equivalent of an odd number of carbon atoms. A compound with the equivalent of an odd number of carbon atoms was first reported in capelin fishmeal [10]. The MS/MS for compound E is shown in Fig. 3C, and no significant difference was observed in the fragmentation pattern compared to those of AsFAs with the equivalent of an even number of carbon atoms. This possibly suggests that the only difference in their synthesis might be the starting material or the nature of arsenic moiety incorporated during their synthesis.

The twelve compounds reported in Table 4 are shown to be saturated compounds with proposed structures shown in Fig. 4. No clear arsenic peak was detected by ICP-MS for unsaturated arsenolipids possibly due to their low concentrations. However, the following unsaturated compounds with protonated molecular formula: $C_{19}H_{36}O_3As$, $C_{19}H_{38}O_3As$, $C_{26}H_{52}O_3As$ and $C_{21}H_{44}OAs$ were clearly shown to be present by Orbitrap-MS with $\Delta m = \pm$ (0.1–0.6 ppm), the mass spectra are available in online supplementary material (Figs. S6–S8). The accurate masses and the proposed structures based on elemental compositions are shown in Table 5 and Fig. 5 respectively.

### 3.3. Degradation products of arsenolipids

The chromatogram in Fig. 1 revealed an arsenic peak A that eluted around the void volume (1.6 min) of the reversed phase column indicating the presence of water soluble arsenic compound, although appearing in the non-polar phase of the VLC-fractionation. Similar arsenic peaks appearing in the void have been reported in the literature for reversed phase separation of arsenolipids. The arsenic compounds are not known but are considered to be degradation products of arsenolipids resulting from sample preparation. [10,18] Our investigation of this fraction using accurate mass derived elemental composition and CID-Tandem-MS revealed the presence of DMA as the major component with minor amounts of MA, DMAP, and DMAB. The elemental compositions with the accurate masses are shown in Table 6 and the mass spectra shown in supplementary material (Figs. S1 and S2). The molecular structures are shown in Fig. 6.

Since all the degradation products eluted at the same retention time (void volume) no chromatographic behaviour could be used to characterise them. Therefore, to enhance chromatographic separation and identification, the fraction was collected using fraction collector and pre-concentration as described in the experimental section. Analysis using cation exchange HPLC online with ICP-MS and ES-MS further confirmed the identity of the compounds. The HPLC–ICP-MS chromatogram is shown in Fig. 7 revealing arsenic peaks that are clearly separated. For identification purpose, the arsenic signals of the extract are also shown overlaid with signals of extract spiked with standard solution containing DMA, MA, DMAP and DMAB. The identities were further confirmed from the data generated by the ES-MS. Examples of the mass spectra at retention times 4.4 and 7.3 min are shown in the supplementary material (Fig. S3) with protonated molecular masses that correspond to DMA and DMAP respectively. The retention time at the ES is also shown to correspond with the time at the HPLC–ICP-MS were the compounds eluted in the chromatogram.

The speciation results presented here showed that DMA is the major degradation product that eluted in the void volume. This is in line with the work of Schmeisser et al., [5] where DMA was reported as the predominant species in aqueous extract of cod-liver oil. It was observed that the amount of water soluble arsenic,

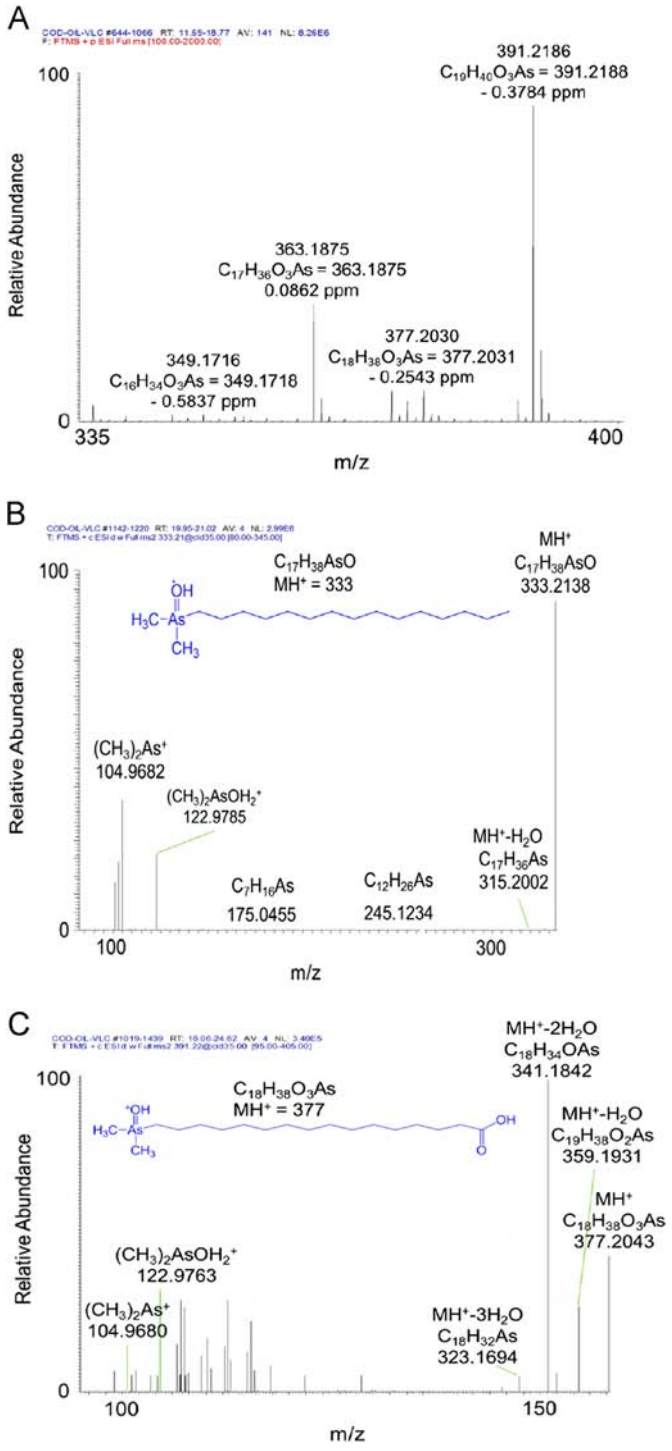


**Fig. 3.** Full scan ES-Orbitrap-MS spectrum in positive mode showing the elemental compositions and the accurate masses of four arsenolipids (a), MS/MS spectra showing some major fragments for arsenic hydrocarbon, I2 (b) and arsenic fatty acid, E (c).

predominantly DMA increased with time when the fish oil was subjected to prolong exposure to water at room temperature, suggesting that arsenolipids were slowly hydrolysing over time. DMA has also been reported in other studies as the major degradation product of unknown arsenolipids following hydrolysis. From such experiments, the presence of DMA-containing lipids was proposed [22,23]. In line with previous works, it thus appears that the water soluble degradation compounds eluting in the void volume might be a result of partial degradation of

**Table 4**
Saturated arsenolipids including six new compounds identified by RP-HPLC–ICP-MS/ES-Orbitrap-MS in positive scan mode.

| Peaks | Compd's (MH) | Exp. (MH) | Cal. (MH) | Δm (ppm) | References |
|---|---|---|---|---|---|
| B | $C_{15}H_{32}O_3As$ | 335.1563 | 335.1559 | −1.16 | [4] |
| C | $C_{16}H_{34}O_3As$ | 349.1718 | 349.1715 | −0.77 | This study |
| D | $C_{17}H_{36}O_3As$ | 363.1875 | 363.1871 | −1.03 | [4,31–33] |
| E | $C_{18}H_{38}O_3As$ | 377.2029 | 377.2027 | −1.03 | This study |
| F | $C_{19}H_{40}O_3As$ | 391.2187 | 391.2183 | −1.03 | [4,31,33] |
| G | $C_{20}H_{42}O_3As$ | 405.2340 | 405.2339 | −0.26 | This study |
| H | $C_{21}H_{44}O_3As$ | 419.2501 | 419.2495 | −1.54 | [4] |
| I1 | $C_{23}H_{48}O_3As$ | 447.2820 | 447.2807 | −1.54 | This study |
| I2 | $C_{17}H_{38}OAs$ | 333.2133 | 333.2129 | −1.03 | [21,32–34] |
| J1 | $C_{25}H_{52}O_3As$ | 475.3128 | 475.3119 | −1.54 | This study |
| J2 | $C_{19}H_{42}OAs$ | 361.2447 | 361.2441 | −1.54 | [33–35] |
| J3 | $C_{27}H_{56}O_3As$ | 503.3441 | 503.3431 | −2.57 | This study |

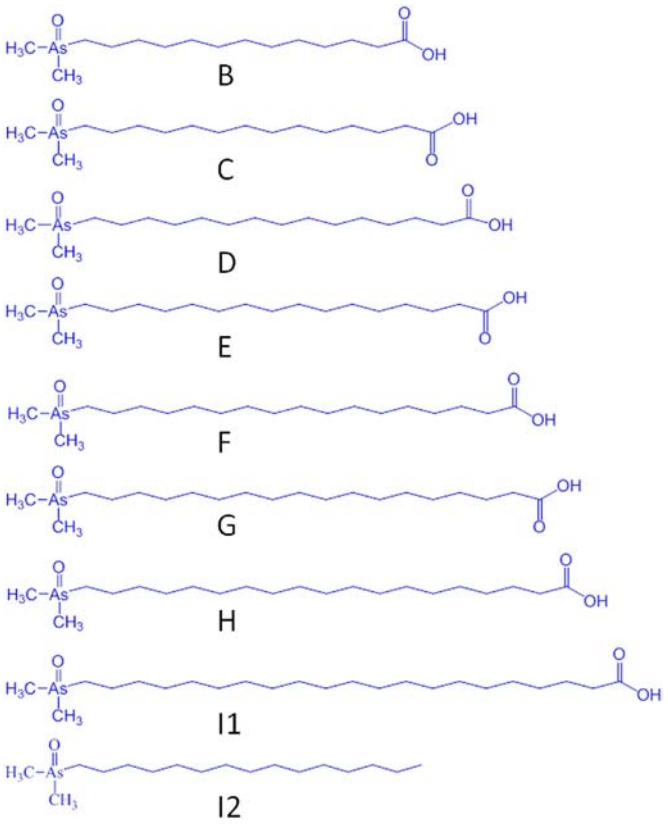


**Fig. 4.** Proposed structures of arsenolipids identified in cod-liver oil.

**Table 5**
The unsaturated arsenolipids including three AsFAs and one AsHC identified in the electrospray at low concentrations. Only one of the compounds has been reported before.

| N | Compd's (MH) | Exp. (MH) | Cal. (MH) | Δm (ppm) | Double bond | References |
|---|---|---|---|---|---|---|
| (I) | $C_{19}H_{36}O_3As$ | 387.1877 | 387.1875 | 0.57 | 2 | This study |
| (II) | $C_{19}H_{38}O_3As$ | 389.2031 | 389.2031 | −0.05 | 1 | [4,31] |
| (III) | $C_{26}H_{52}O_3As$ | 487.3130 | 487.3119 | −1.54 | 1 | This study |
| (IV) | $C_{21}H_{44}OAs$ | 387.2602 | 387.2603 | −0.10 | 1 | This study |

arsenolipids during sample preparation [18] and that the arsenolipids slowly hydrolyse mainly to DMA during storage. [5] Already, Lunde in 1972 suggested the presence of at least two arsenolipid compounds in marine oils, the water soluble fraction and fatty acid fraction following saponification of marine oils containing arsenic

[24,25]. Also, concerning the type of lipids to which arsenic is bound, Kohlmeyer et al., reported two types of arsenolipids including those which released water soluble arsenic species upon alkaline hydrolysis in marine tissues and those that do not release water soluble arsenic species upon strong alkaline hydrolysis in fish oil (non-saponifiable arsenolipids) [26]. Similarly, the result of this work indicates the occurrence of two types of arsenolipids, namely; the stable compounds including AsFAs and AsHCs, and the other group of unstable organo arsenic compounds as evident from their degradation products. Since peak A does not contain any arsenobetaine, which is the major water soluble arsenic species in cod, it is therefore unlikely that these water soluble compounds are in the oil as a result of micelle formation. Hence MA, DMA, DMAP and DMAB must be the degradation product of arsenolipids. So far monomethylated arsenic has not been identified bound in arsenolipids, only arsenolipids containing DMA. In the same vein, MA containing compounds have not been reported in more complex organo arsenicals except as hydrolysed arsenic species bound to the kidney and muscle lipids of seaweed eating sheep [27]. The identification of MA in the degradation products is of potential interest.

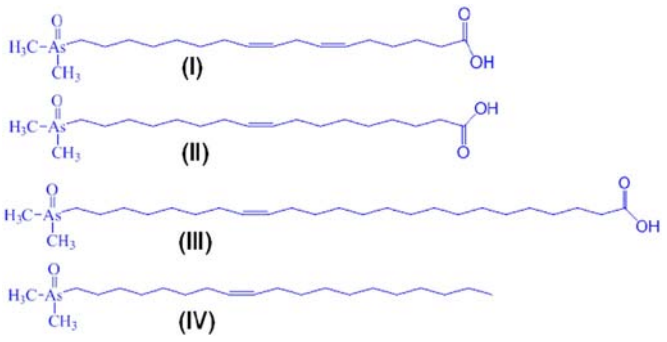


**Fig. 5.** Proposed molecular structures based on elemental composition and accurate mass measurement of unsaturated AsFAs and AsHC in Table 5.

**Table 6**
Elemental compositions and accurate masses of arsenic compounds identified in the void volume of reversed phase chromatography of arsenolipids methanol extract.

| Compd's | Formula (MH) | Cal. (MH) | Exp. (MH) | Δm (ppm) |
|---|---|---|---|---|
| MA | $CH_6O_3As$ | 140.9527 | 140.9524 | −2.11 |
| DMA | $C_2H_8O_2As$ | 138.9735 | 138.9734 | −0.72 |
| DMAP | $C_5H_{12}O_3As$ | 194.9997 | 194.9998 | 0.52 |
| DMAB | $C_6H_{14}O_3As$ | 209.0154 | 209.0155 | 0.48 |

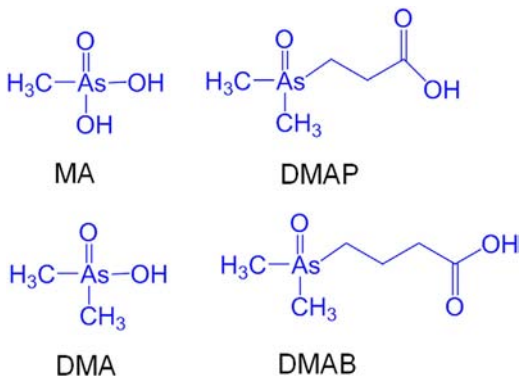


**Fig. 6.** Molecular structures of water soluble arsenic compounds identified by HR-Orbitrap-MS in the void volume of reversed phase chromatography.

DMA is a common metabolite of highly toxic inorganic arsenic, arsenosugars and arsenolipids [13,28–30]. Though the toxicity of arsenolipids has not been fully evaluated, the biotransformation to DMA could have important implication for human health [12]. The results presented here also have some implications in epidemiologic studies where urinary arsenic and species concentrations have been used to investigate exposure to arsenic and metabolic pathways. For instance, Schmeisser et al. reported that DMA is the major metabolite excreted in human urine following ingestion of cod-liver oil containing arsenolipids [13]. DMAB and DMAP are also among the urinary metabolite found in small concentrations. Similarly, the degradation products of arsenolipids identified here, mainly DMA may also potentially contribute directly to the total urinary arsenic concentration. This could lead to overestimation and misinterpretation of results from epidemiologic studies. This has serious implication in the assessment of metabolic pathways and the toxicity associated with arsenolipids exposure, and further suggests the need for proper evaluation of dietary sources of exposure in such studies.

## 4. Conclusion

In this study we report the identification of 16 arsenolipids including nine new compounds using VLC fractionation of the fish oil to separate the arsenic species from the interfering matrix and analysis by RP-HPLC–ICP-MS/ES-MS. In addition the degradation products of arsenolipids in the fish oil were also identified suggesting the presence of other classes of unknown and unstable arsenolipids. The information provided here will be useful in assessing the risk associated with arsenolipids and in epidemiologic studies where urinary metabolites similar to degradation products identified in this work have been used as biomarkers in assessing human exposure to arsenic.

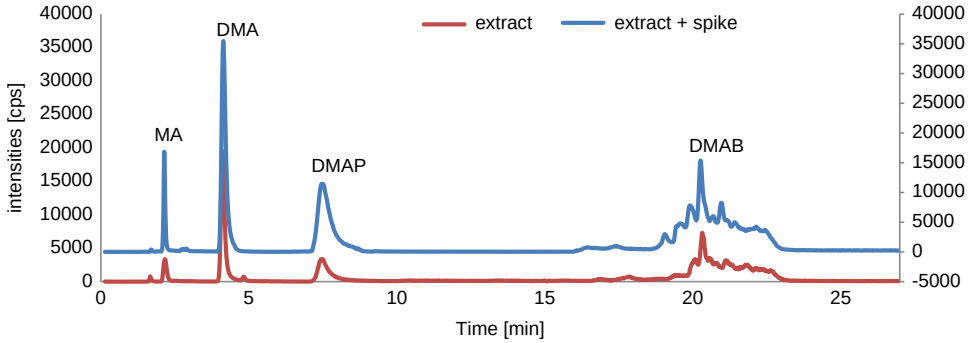


**Fig. 7.** HPLC–ICP-MS (cation exchange) chromatogram of water soluble extract overlaid with the same extract spiked with MA, DMA, DMAP and DMAB.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.09.056.

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