Talanta 118 (2014) 217–223

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

Kenneth O Amayo [a,b,*], Andrea Raab [a], Eva M Krupp [a,c], Jörg Feldmann [a,**]

[a] Trace Element Speciation Laboratory (TESLA), Department of Chemistry, Meston Walk, University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK
[b] Department of Chemistry, Ambrose Alli University, Ekpoma, Nigeria
[c] ACES Aberdeen Centre of Environmental Sustainability, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK

ARTICLE INFO

Article history:
Received 13 July 2013
Received in revised form
26 September 2013
Accepted 30 September 2013
Available online 22 October 2013

Keywords:
Arsenolipids
Cod-liver oil
RP-HPLC
ES-Orbitrap-MS
ICP-MS

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

* Corresponding author at: Trace Element Speciation Laboratory (TESLA), Department of Chemistry, Meston Walk, University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK. Tel.: +44 1224 27 2911.
** Corresponding author.
E-mail addresses: kennethamayo@yahoo.com (K. Amayo), j.feldmann@abdn.ac.uk (J. Feldmann).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.09.056

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 (30×6 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; 4.6×150 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 ± 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; 4.6×150 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%