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Arsenolipids in fish oil determined by GC-ICPMS

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Background

Arsenic is a trace element found in particularly high concentrations in marine samples. It is an element forming a variety of inorganic and organic bound compounds that varies in abundance depending on the matrix. The water-soluble arsenic compounds have been studied for decades; while the lipid-soluble arsenicals – the arsenolipids, have been less studied. An interest for the arsenolipids has however been merging the last 10 years, and three major groups of arsenolipids have so far been identified: the arsenic-containing hydrocarbons, the arsenic-containing fatty acids and the arsenosugar-phospholipids. The presence of the arsenic-containing hydrocarbons in several fish oils will here be presented.

Introduction

Inductively Coupled Plasma Mass Spectrometry (ICPMS) is the preferred analytical technique for speciation analysis of arsenic. It is element specific and highly sensitive. Most speciation analysis of arsenic has been conducted on the water-soluble compounds using High Pressure Liquid Chromatography (HPLC) coupled to the ICPMS. Analysis of the lipid-soluble arsenicals has proven to be more challenging, due to the instability of the ICPMS when introducing organic solvents. However, the development within analytical techniques has resulted in the identification of arsenolipids using HPLC-ICPMS or Gas Chromatography (GC)-ICPMS.

Three major groups of arsenolipids have so far been identified; the arsenic-containing hydrocarbons (As-HC) [1], the arsenic-containing fatty acids (As-FA) [2] and the arsenosugar-phospholipids (As-SugPL) [3, 4]. The arsenolipids have been identified in marine oils, e.g. fish oils, cod liver oil, oils of sashimi tuna and lipid extract of fish meal. Large-scale preparative volumes and extensive analytical work-up schemes have been applied for the analysis of the arsenolipids.

The arsenic-containing hydrocarbons (Figure 1) are here analyzed in several fish oils by using a simple Solid Phase Extraction (SPE) clean up step followed by analysis by GC-ICPMS based on the principles reported in Raber *et al.* [5]. The total arsenic concentrations in the extracts were analysed by ICPMS after microwave assisted acid digestion. Commercially fish oils from various fish species typically used in fish feed production is analyzed, in addition to extracted oil of cod liver.

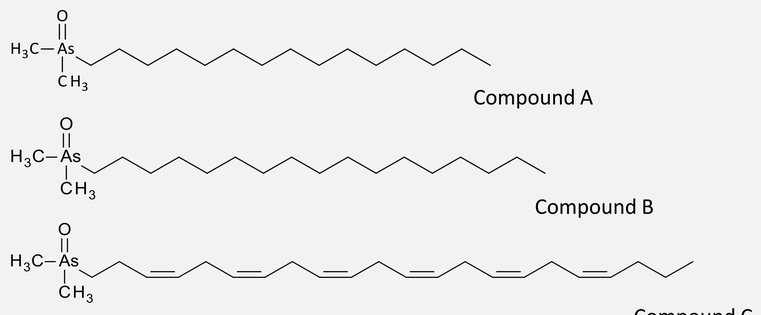

Figure 1. The arsenic-containing hydrocarbons.

Experimental Outline

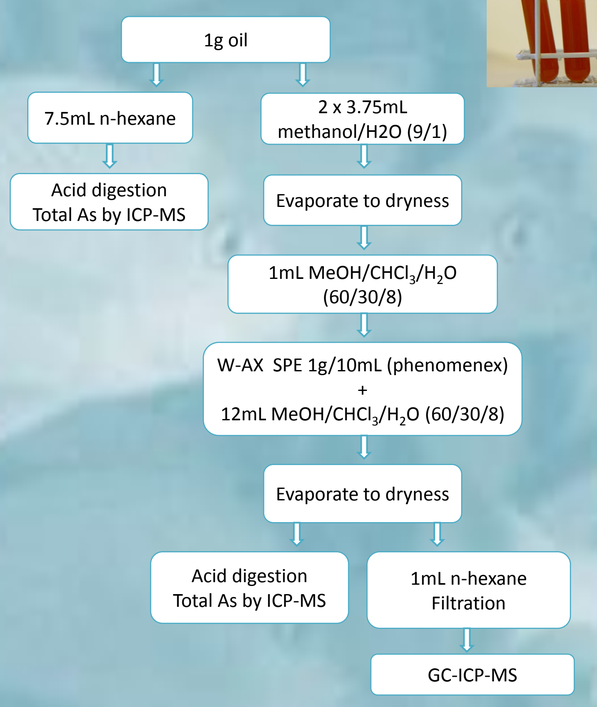

Table 1. GC and ICP-MS 7500a (Agilent Technologies) settings for analysis of arsenolipids. Triphenylarsin (Ph_3As) was used as external standard.

GC column	HP-5 (3m x 0.32mm, 0.25µm)
GC-program	Started at 50°C for 1min. 50°C to 180°C at 15°C/min, held for 1min. 180°C to 220°C at 3°C/min, held for 1.7min. 220°C to 280°C at 15°C/min, held for 8min.
Injector mode	Splitless injection,
Injection volume	1µL
Liner	Multibuffle
Injector temperature	280°C
Transfer temperature	180°C to 280°C at 4°C/min, held for 12min.
Carrier gas	Helium
Carrier gas flow	0.56L/min
RF power	800-1000W

Results

The methanol phase contained approximately 40% of arsenic present in the fish oils (Table 2.) and was relative concentrated (µg As/weight of extract). The methanol phase was further inverstigated using GC-ICPMS (Figure 2).

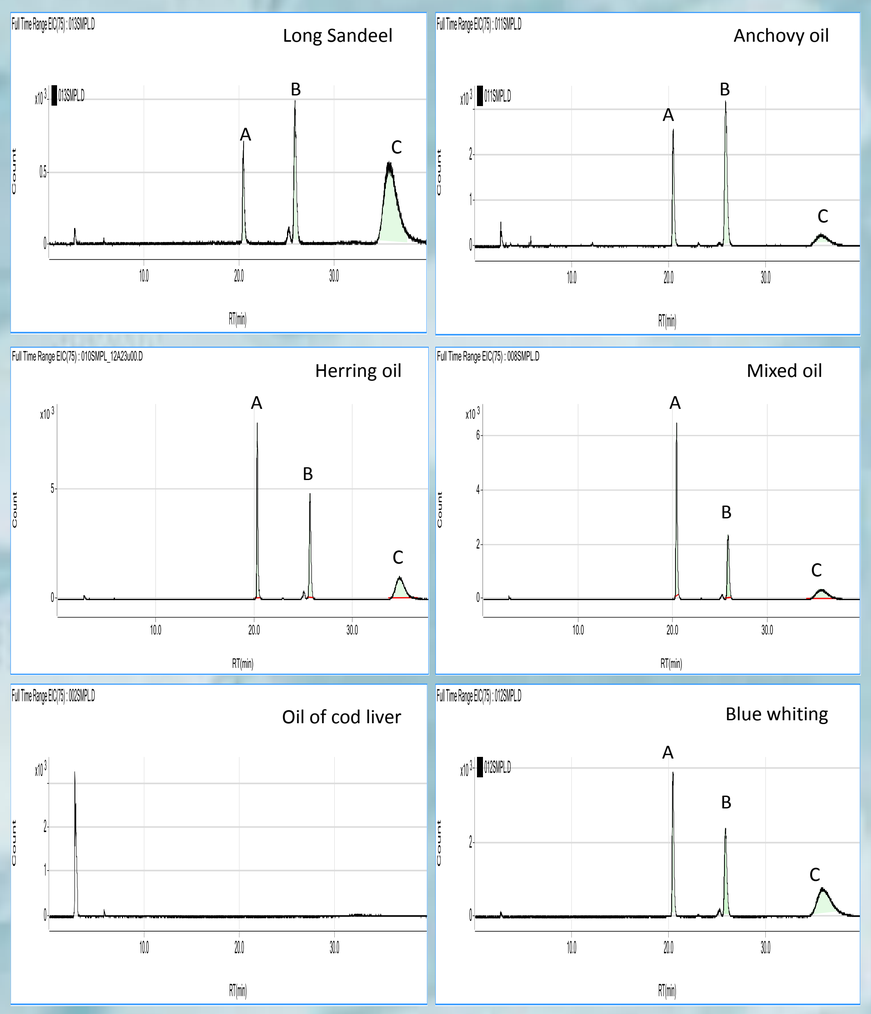

Figure 2. GC-ICPMS chromatograms of the methanol phase of fish oils. The three dominant peaks in all the fish oils, with exception of oil of cod liver, were confirmed by GC-MS/MS to be Compound A ($(CH_3)_2As(O)C_{15}H_{31}$), Compound B ($(CH_3)_2As(O)C_{17}H_{35}$) and Compound C ($(CH_3)_2As(O)C_{19}H_{31}$) (Figure 1.), all earlier identified by Raber *et al.* [5]. A fourth peak was also seen for the fish oils (r.t. 25min), with structure still unknown.

Table 2. Arsenic in the aqueous methanol fractions and in the SPE fractions.

		MeOH/H2O		SPE	
Sample	**As whole oil [mg/kg]**	**As (µg)**	**As (%) of whole oil**	**As (µg)**	**Recovery (%) of MeOH/H2O**
Long sandeel	7.96 ± 0.04	2.7 ± 0.3	34	2.40 ± 0.01	89
Herring	7.7 ± 0.1	2.34 ± 0.06	30	2.1 ± 0.3	88
Blue whiting	8.7 ± 0.1	2.9 ± 0.04	33	2.5 ± 0.2	85
Anchovy	8.6 ± 0.1	3.95 ± 0.05	46	2.9 ± 0.4	74
Mixed oil	8.3 ± 0.1	3.2 ± 0.1	38	3.0 ± 0.3	94
Cod liver A*	16.4 ± 1.2	*n.a.*	*n.a.*	*n.a.*	*n.a.*

n.a.: not analyzed.

Discussion & Conclusion

- Three dominant arsenic compounds were seen in the methanol fraction of the fish oils. The compounds were confirmed by GC-MS/MS to be Compound A, Compound B and Compound C, as identified by Raber *et al*. [5]. Additionally, a fourth peak (r.t. 25min) was seen for the fish oils , but this compound has not been identified.
- The recovery of arsenic in SPE fractions are between 74 and 94%. The remaining arsenic in the methanol fractions can be other arsenolipids, e.g. the arsenic-containing fatty acids, which have not been analyzed in this study.
- The chromatographic patterns vary for the different fish oils. The commercial fish oils are abundant in the arsenic-containing hydrocarbons, while the cod liver oil shows no signal for these compounds. This suggests that the presence of arsenolipids depends on the type of oil, whether the oil is extracted from whole fish or from liver of the fish.
- Future work will be aimed towards identification of the unknown peak and clean-up of the hexane phase prior to analysis by GC-ICPMS or HPLC-ICPMS.

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