The possible role of sample extraction for the determination of lipids and persistent organic pollutants in biological samples

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Introduction

During the last decade strong efforts have been laid upon the development of new selective and sensitive detection methods for the analyses of persistent organic pollutants (POP) in various environmental samples. New capillary gas chromatographic methods coupled with high sophisticated mass spectrometric detectors, allow reliable measurements into the fg/g (10^{-15}g/g) range. Despite the substantial progress made in developing new high sensitive detection methods, no comparable progress has been made with respect to sample preparation, extraction and clean-up procedures. Sodium sulphate (Na_2SO_4) homogenisation is often used for the preparation of biological samples for POP analyses due to easy handling, low costs and easy accessibility of the method. For further clean-up a variety of different extraction methods are used, such as cold column extraction, soxhlet extraction, accelerated solvent extraction, solid phase extraction and supercritical fluid extraction. A combination of lipid class and POP analyses is crucial for investigations of the importance of lipid dynamic on the accumulation and redistribution of POPs in arctic marine biota[1,2]. One of the most accepted methods for lipid extraction, originally described by Bligh and Dyer[3], is based on a solvent system of chloroform and methanol. The fact that gravimetric comparison of the extracts obtained by Bligh and Dyer's (B&D) method and standard extraction methods for the subsequent POP analysis, shows differences is known. The POPs are closely linked to the triacylglycerol (TAG) content of the extracted matter. As the TAG content is normally considered quantitatively extracted with standard work-up methods, it is generally accepted that the total POP content should be extracted by these procedures. However, the question still remains how important the differences in extraction capability are for the lipid-normalised determination of POPs. In the present study various methods for combined extraction of lipids and organochlorines from different tissues are investigated.

Materials and Methods

Three muscle samples of Arctic charr *(Salvelinus alpinus)* and three blubber as well as three liver samples from ringed seal (*Phoca hispida*) have been analysed. The ringed seal were

caught in the White Sea region in 1995 and the Arctic charr were collected in 1997 from Talvik (Finnmark county, North Norway). All solvents used were of Suprasolv quality purchased from Merck (Darmstadt, Germany). For standard cold extraction the tissue was homogenised and dried with anhydrous sodium sulphate treated at 650°C for two hours prior to use. The tissue samples were mixed with a 5-10 fold amount of sodium sulphate and homogenised in an IKA A10 analytical mill (Staufen, Germany). After a first homogenisation the tissue/Na_2SO_4-mixture was spread out on aluminium foil and dried for about two hours. Then a second homogenisation in the analytical mill was carried out. The homogenised samples were stored frozen at -20°C until extraction using a previously published method[4]. Different ratios of the solvent systems cyclohexane/acetone, n-hexane/iso-propanol as well as cyclohexane/ethyl acetate were used for extraction (Table 1). For the wet extraction cyclohexane/acetone and a modified Bligh and Dyer's chloroform/methanol method[3] was used. High-performance thin-layer chromatography with densitometric quantification was carried out using pre-coated silica plates as described previously[1].

Table 1. The extractable organic material (EOM) content (%) in Arctic charr muscle and ringed seal liver and blubber using different extraction methods.

Method	Solvent ratio	Na_2SO_4 addition	Seal liver	Seal blubber	Charr muscle
B&D	Chloroform/methanol/water (2:1:1)	No	5,3	92	9,1
	(modified Bligh and Dyer's method)		5,6	94	10,2
			5,3	95	8,9
C/A/W	Cyclohexane/acetone/water (2:2:1)	No	3.2	-	-
			3.6	-	-
			3.5	-	-
C/A 1:1	Cyclohexane/acetone 1:1	Yes	3.4	94	9.0
			3.6	98	9,4
			3.2	98	9,3
C/A 3:1	Cyclohexane/acetone 3:1	Yes	5.2	90	8,3
			4.6	89	8,3
			5.2	89	8,4
C	Cyclohexane	Yes	3,4	97	8,2
			3,6	103	7,6
			3,5	-	8,7
C/EA	Cyclohexane/ethyl acetate 1:1	Yes	3,8	90	-
			3,7	87	-
			3,3	90	-
H/IP	n-Hexane/iso-propanol 3:1	Yes	4,7	104	-
			5,0	98	-
			4,2	99	-

Results and Discussion

Two different types of extraction methods *viz.* wet extraction and cold column extraction after treatment with anhydrous sodium sulphate were tested on Arctic charr muscle and ringed seal blubber and liver. The solvent systems used included chloroform/methanol, cyclohexane, cyclohexane/acetone, cyclohexane/ethyl acetate and n-hexane/isopropanol. The gravimetric

comparison of extractable organic material (EOM) resulting from the different methods as well as abbreviations used, are presented in Table 1. The B&D-procedure is regarded as a selective and quantitative extraction method for lipids. This can be shown for the seal liver and charr muscle samples where the EOM content based on the modified B&D-extraction exceed the other methods applied. However, the EOM values for seal blubber based on extraction with cyclohexane and n-hexane/isopropanol were higher than the corresponding B&D-values. The EOM content obtained by wet extraction (C/A/W) is lower than the B&D-values and conform to the results obtained by the standard column extraction method using sodium sulphate homogenated samples and cyclohexane. These first findings indicate substantial differences in the EOM-content depending on sample preparation and solvents used for extraction.

For a more detailed look on these first findings, additional lipid class analyses were carried out by high-performance thin-layer chromatography (Table 2). The differences were especially marked for the more polar lipid classes, where a relative increase was observed in the extracted sodium sulphate homogenate compared to the B&D-method. The growing-up of compounds with low Rf-value indicate the presence of oxidation products. Compared to this only minor differences could be observed between the B&D and the wet extraction using cyclohexane/acetone (Table 2).

Table 2. Standard lipid class distribution (%) in charr and seal liver samples using different extraction methods (see Table 1). Abbreviations: MAG, monoacylglycerols; PL, phospholipids; OX, oxidation products; DAG, diacylglycerols; C, cholesterol; FFA, free fatty acids; TAG, triacylglycerols; CE, cholesterol esters; WE, wax esters.

Composition	Charr liver		Seal liver			
	B&D	C/A 3:1	B&D	C/A 3:1	C/A 1:1	C/A/W
MAG/PL/OX	13	26	18	30	25	16
DAG	11	11	<1	4	1	<1
C	9	11	16	17	19	17
FFA	36	32	42	36	43	55
TAG	27	14	<1	<1	<1	<1
CE/WE	2	5	13	7	7	11
Others	2	1	11	6	5	1

The differences in lipid composition can be explained by formation of oxidation products during sample work-up. The sodium sulphate homogenisation increases the surface of the tissue sample considerably. This increased exposure to oxygen and the elevated temperature makes the unsaturated fatty acids susceptible to autoxidation processes. Normally autoxidation does not take place during the B&D-procedure if it is conducted properly. Consequently, the degradation processes after the Na_2SO_4-treatment will change the lipid distribution and lead to additional faults in the gravimetric determination of the EOM-content.

As the sodium sulphate homogenisation lead to degradation of the unsaturated lipids, other preparation methods are recommended to insure the accuracy of sample work-up for quantification and identification of the lipid content. The number of analysed samples is small,

therefore only indication of differences can be drawn from the preliminary findings. However, these findings may result in a better understanding concerning sample preparation and extraction methods for the determination of POPs normalised to lipid content. They may also have implications on possible oxidative degradation processes on the POPs subjected to analysis.

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