SUPERCRITICAL FLUID EXTRACTION OF POLYBROMINATED DIPHENYL ETHERS, PBDEs, FROM LONG-FINNED PILOT WHALE (*GLOBICEPHALA MELAS*) FROM THE ATLANTIC

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Introduction

Polybrominated diphenyl ethers (PBDEs) are persistent and lipophilic compounds used as flame retardants in electronic equipment, plastic material and synthetic fibbers among other things. The PBDEs are mainly used as Deca-BDE and Bromokal 70-5DE, a mixture of tetra-, penta- and hexa-BDE[1]. Due to its chemical and physical properties PBDEs, especially TeBDEs, tend to bioaccumulate. PBDEs were first reported in sediments in USA[2], and in fish from a Swedish river[3]. More recently PBDEs have also been reported in seals[4,5], birds[4], mussels[6], whales[7,12] and humans[1, 5, 8, 9]. The aim of this study was to develop an SFE-method for rapid analysis of PBDEs in whale blubber and so determinate the concentrations of these compounds in whale samples of different age and sex caught in the Faroe Islands in 1997.

Material and Methods

Samples of long finned pilot whales were taken in connection with the traditional drive kills in Torshavn, Faroe Islands, 24. September 1997. Blubber samples were taken ventrally at a level aligned approximately to the caudal end of the dorsal fin. The samples were kept frozen at -20°C until analysis. For analysis the samples from the long-finned pilot whale (*Globicephala melas*) were homogenised in a mixer with sodium sulphate (1:5). About 3 g of the homogenised tissue was packed in a Hewlett Packard standard extraction vessels (7 ml). An internal standard consisting of ^{13}C-labeled PBDE #77 was added before the SFE extraction. On the top of the sample around 4.5 g basic aluminium oxide (AlOx) was added as a fat retainer. The extractions were carried out on a Hewlett-Packard 7680T SFE using CO_2 as the supercritical fluid. The chamber temperature was 40°C and the pressure 281 bar during extraction, resulting in a supercritical fluid density of 0,9 g/ml and a flow rate of 2 ml/min. All the analytes were trapped on a C18 solid sorbent (ODS, Octadecylsilica). The nozzle and trap temperatures were kept at 45°C and 40°C respectively. After completion of the extraction the trap was rinsed with 2 ml hexane and 2 ml methylene chloride at a rate of 2 ml/min. Each solvent fraction was collected in a separate vial. After addition of the recovery standard containing ^{13}C-labeled PCB #178 in tetradecane the sample volume was reduced to 30 µl, producing an extract ready for GC/MS analysis. Separate lipid determination was performed by applying a part (~1g) of the homogenate on a small column and quantitatively extract with methylene chloride and hexane (1:1). The weight of the extracted lipids was determined gravimetrically.

Selected ion SIR HRGC/MS spectra were recorded using a Fisons GC 8000 gas chromatograph coupled to a MD800 mass spectrometer. Chromatographic separation was achieved by splitless injection of 2 µl on a non-polar DB-5 column using helium as the carrier gas. The GC oven was programmed as follows: 180°C initial hold for 2 min. increase at a rate of 15°C/min to 205°C, followed by an increase of 3,7°C/min to 300°C, final hold at 300°C for 15 minutes. The two most intense ions of the molecular ion cluster were monitored for TeBDE (*m/z* 483.7, 485.7), PeBDE (*m/z* 563.6, 565.6) and HxBDE (*m/z* 641.5, 643.5) in addition to masses for the ^{13}C-labeled internal standard (*m/z* 495.71, 497.68). The quantification standard consisted of TeBDE, PeBDE and HxBDE, and detected PBDEs were quantified against a PBDE of the same bromination level closest to its retention time.

Results and discussion

Lipid carry over creates a problem during GC/MS analyses. Considering human samples AlOx was successfully used as a fat retainer during SFE extraction of PCBs and Pesticides[10, 11]. The composition of fatty acids in pilot whale blubber is different. To study the lipid carry over, the extraction time was varied and the results are presented in figure 1. After 20 minutes extraction the lipid carry over increases rapidly. To avoid lipid carry over 20 minutes was chosen as the extraction time.

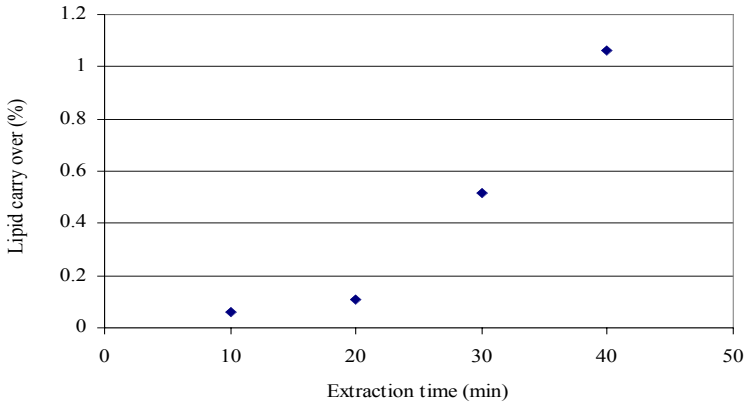

Figure 1. Lipid carry over (%) after adding 4.5 g AlOx to the extraction vessel when the extraction time was varied.

The recoveries of the used internal standards varied between 85-153% for ^{13}C-labeled PBDE #77 during the 20 minutes of extraction time. This indicates that the extraction conditions are suitable for PBDEs. The levels obtained by SFE-LC were also compared with the traditional analysis[12] and showed good agreement.

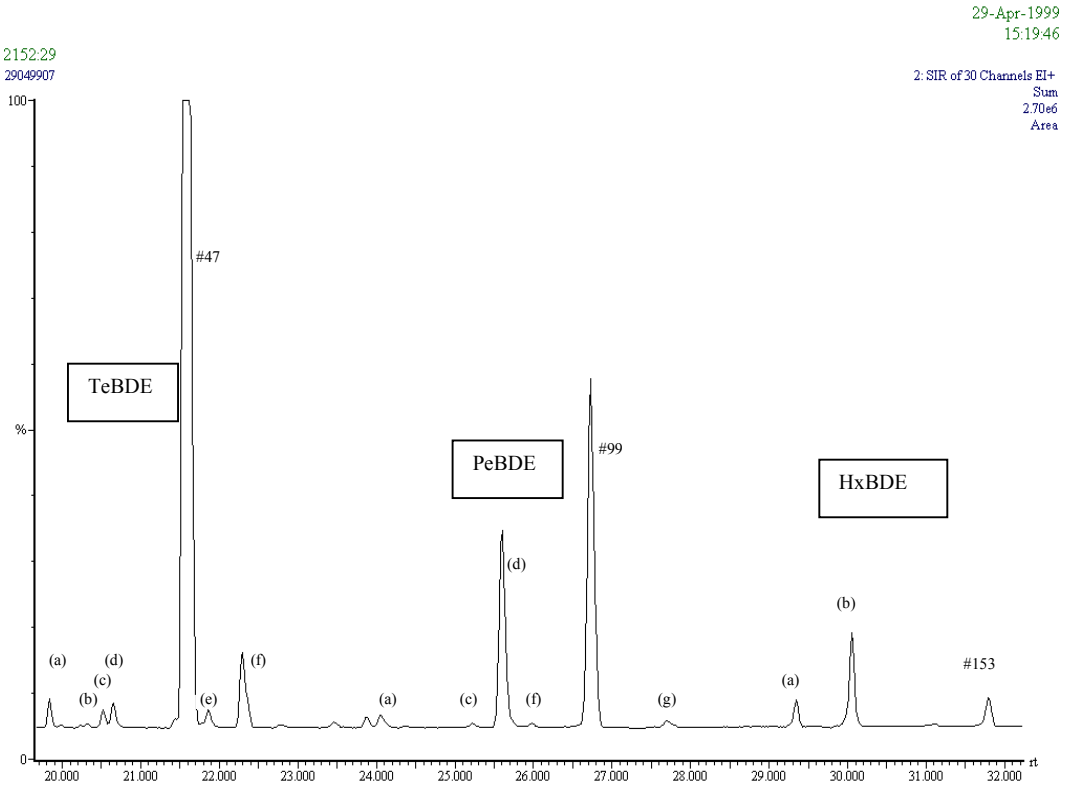

Figure 2. A reconstructed ion chromatogram (m/z 485.70, 565.60, 643.50) of a juvenile male pilot whale sample. The retention times for TeBDEs are 19.8-22.3 min, PeBDEs 24.1-28.9 min and for HxBDEs 29.4-34.5 min. The unidentified PBDEs are denoted as Lindström et al.[12]

In Table 1 the results for 12 samples of blubber from pilot whales are presented. The sum of PBDEs varied between 125 and 1246 ng/g lipids. The congener with the highest concentration in each sample was TeBDE #47 (66-864 ng/g). Among the PeBDEs the highest concentration was detected for PeBDE #99 (23-169 ng/g) and for HxBDEs the not yet confirmed congener (b) (9.8-42 ng/g). The concentrations are lower than for pilot whales caught in Vestmanna 1996[12]. This difference is probably a result of the large variation among pilot whale individuals. In the different age and sex classes the lowest concentrations were found in the adult females and the highest in juvenile females. Adult and juvenile males were having about similar concentrations, the levels in juveniles being somewhat higher. PBDEs are transported lactationally from the female to the offspring. This may explain the low concentrations in adult females and the to some extent higher concentrations in juveniles[12]. The relations between the concentrations in the different age and sex classes and also the patterns in the chromatograms at the different levels of bromination agree with earlier publications [7,12].

Table 1. Concentrations (ng/g lipid) of PBDEs in pilot whale blubber from Torshavn 24 September 1997. ND = Not detected. Concentrations are represented for three individuals per category. Unidentified PBDEs are denoted as Lindström et al.[12].

	Adult males			Adult females			Juvenile males			Juvenile females		
Lipids	78%	71%	52%	79%	85%	71%	85%	69%	82%	80%	81%	78%
TeBDE (a)	1.6	1.6	1.7	1.3	1.5	0.5	1.8	1.2	3.5	3.7	2.0	3.9
TeBDE (b)	ND	0.3	ND	0.3	0.4	ND	0.3	ND	0.4	ND	0.4	0.4
TeBDE (c)	1.0	0.8	1.4	0.8	0.5	ND	0.9	0.8	1.8	2.7	1.1	2.7
TeBDE (d)	1.9	3.4	2.2	3.1	3.4	2.1	2.5	1.7	3.2	2.6	2.8	2.8
TeBDE #47	366.4	271.0	468.6	211.7	166.9	66.0	332.3	249.4	557.1	864.2	247.1	749.1
TeBDE (e)	1.6	1.0	2.6	1.3	0.8	ND	1.6	1.0	2.5	4.2	1.6	3.9
TeBDE (f)	7.1	9.6	8.1	7.8	6.5	3.2	8.2	5.9	15.0	17.5	11.0	14.4
PeBDE (a)	2.9	1.5	3.3	1.5	1.4	0.9	2.5	2.7	4.5	5.9	2.0	5.8
PeBDE (b)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PeBDE (c)	ND	ND	ND	0.6	ND	ND	0.6	ND	1.2	1.5	0.7	ND
PeBDE (d)	45.4	28.2	50.4	26.0	16.5	12.4	42.2	34.0	59.9	97.7	33.5	82.8
PeBDE (e)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PeBDE (f)	ND	0.4	ND	0.7	ND	ND	0.8	ND	1.0	ND	1.1	ND
PeBDE #99	74.8	54.5	92.9	51.1	31.6	23.9	72.0	67.1	112.5	169.3	67.3	159.5
PeBDE (g)	1.0	1.3	1.7	0.0	0.2	0.3	ND	1.9	2.5	3.5	2.4	3.8
PeBDE #85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HxBDE (a)	13.4	4.2	7.1	4.7	2.7	3.4	10.1	10.0	4.5	17.3	5.9	16.3
HxBDE (b)	35.0	14.9	22.7	11.8	6.9	9.8	24.6	25.9	20.3	42.4	16.5	39.9
HxBDE (c)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HxBDE #153	10.9	4.7	6.4	3.7	2.1	3.0	8.3	9.7	5.3	13.5	6.5	12.6
HxBDE #138	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sum BDE	**562.9**	**397.2**	**669.2**	**326.3**	**241.4**	**125.6**	**508.7**	**411.2**	**795.2**	**1246.3**	**401.9**	**1097.8**

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