Development and evaluation of an Accelerated Solvent Extraction (ASE) method for the analysis of organic contaminants in biota.

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Introduction

Persistent organic pollutants such as chlorinated biphenyls (CBs) and polybrominated diphenyl ethers (PBDEs) are included on the OSPAR List of Chemicals for Priority Action and as such are often analysed as part of national and international monitoring programmes. Due to concerns about the environmental impact of CBs, production in the UK ceased in the 1970s. Authorisation for use in closed systems continued until 1986 when sales of CB formulations were prohibited in the UK. However, CBs still enter the marine environment following the destruction and disposal of industrial plants and equipment, or from emissions from old electrical equipment (for example from landfill sites). Their semi-volatile character and high environmental half-lives result in long-range atmospheric transport and CBs are, therefore, ubiquitous in the marine environment.

PBDEs were one of the most widely used groups of brominated flame retardants (BFR). PBDEs are used as additives to polymers and resins and are thought to be easily released to the environment Commercial PBDE mixtures are classified according to their degree of bromination. The penta BDE mixture is mainly used in furniture and upholstery, the octa mixture in plastics and the deca mixture in textiles. On 30 January 2001 the European Commission issued a proposal to ban the penta technical mixture. This ban was finally put in place on 15 August 2004, restricting the use of the penta and the octa technical mixtures (European Directive 2003/11/EC, 24th amendment of 76/769/EEC). Similar to CBs, atmospheric transportation is a major pathway for PBDEs into the marine environment and they have been found to concentrate in the Arctic.

The range of determinands on the OSPAR List of Chemicals for Priority Action continues to increase. If a separate analytical method is used for each determinand there may not be enough material, especially from a typical fish liver, to permit all the required analysis to be undertaken. Therefore a combined method, which can be used for a range of determinands, would be advantageous. The use of accelerated solvent extraction (ASE) for the combined extraction of CBs, organochlorine pesticides (OCPs) and PBDEs from marine biota was investigated. The results are presented here.

Materials and Methods

The total lipid content was determined using the Smedes method[2]. An appropriate amount of tissue, equivalent to a maximum 300 mg lipid, was mixed with anhydrous sodium sulphate (~ 40 g). This was spiked with appropriate recovery standards CB 35, CB 53, CB 112, CB 151, CB 198 and CB 209 for CB analysis and CB 198 for PBDE analysis and refrigerated overnight before being ground to a fine powder. Solvent washed ASE cells (100 ml) were packed as illustrated in Fig. 1. Thirty (30) g of 5% deactivated alumina was used as a fat retainer if PBDEs or CBs only were to be analysed. If OCPs were also required then only 15 g of 5% deactivated alumina was used. Samples were extracted at an oven temperature of 60^{o}C and a pressure of 1500 psi using an ASE 300 (Dionex,UK). Five minutes heating was followed by 2 x 5 min static cycles. The cell flush was 50% total cell volume with a 60 second purge with nitrogen at the end of the sample extraction. The extraction solvent used was *iso*-hexane. Following ASE extraction, the solvent extracts were concentrated by Turbovap to ~ 0.5 ml.

The concentration and composition of the PBDEs were determined by gas chromatography-negative chemical ionisation mass spectrometry (GC-NCIMS) using an HP6890 series gas chromatograph interfaced with a HP5973N MSD, fitted with a cool on-column injector. A Thames Restek STX-500 column (STX 500, 30 m x 0.25 mm i.d., 0.15 μm film thickness, Thames Restek, UK) was utilised , fitted with a Thames Restek Siltek (5 m, 0.53 mm i.d) guard column. The carrier gas was helium, set at a constant pressure of 15 psi. Methane was the reagent gas at a pressure of 1.6 bar. The transfer line was held at 280^{o}C and the ion source 150^{o}C. Injections were made at 120^{o}C, held for 2 minutes before elevation at 15^{o}C min^{-1} up to 205^{o}C. This was followed by a ramp at 6^{o}C min^{-1} up to a

final temperature of 330°C. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. The ions monitored were *m/z* 78.9 and 80.9 for all PBDEs (tri- to hepta-BDEs).

Samples which also required pesticide analysis were fractionated using column chromatography (alumina (3g) and silica (3 g)). The internal standards (2,4-dichlorobenzyl alkyl hexyl ether with C_6 and C_{16} (D16) alkyl chains) were added to the extract before concentrating using a TurboVap system. The concentration and composition of 23 CB congeners (CB 31, 28, 52, 44, 49, 70, 74, 110, 101, 149, 118, 153, 105, 157, 138, 158, 128, 156, 180, 187, 189, 170, 194) and 19 OCPs (α-HCH, γ-HCH, heptachlorobenzene, α-chlorodene, γ-chlorodene, heptachlor epoxide, oxychlorodane, γ-chlorodane, o,p'-DDE, α-cholordane, *trans*-nonachlor, dieldrin, o,p'-DDD, endrin, p,p'-DDD, o,p'-DDT, p,p'-DDT and p,p'-DDE) were determined by gas chromatography with electron capture detection (GC-ECD) using a Varian 3500 GC fitted with a cool on-column injector or GC-electron impact mass spectrometry (GC-EIMS). A medium polarity column was used for the analyses (HP 5, 60 m x 0.25 mm, 0.25 µm film thickness). The data was quantified using Totalchrom ver 6.2.1 (Perkin Elmer, Beaconsfield, UK). A laboratory reference material and procedural blank was analysed with each batch of samples and the results monitored on Shewhart control charts.

Results and Discussion

Traditionally the most common method for the extraction of CBs from environmental samples was Soxhlet. However, this method is very time-consuming and uses large volumes of organic solvents. Due to the volumes of solvents used, length of time required in evaporation steps and the number of steps involved there is the potential for contamination of samples and procedural blanks within the laboratory. An ASE method, using fat retainers, was developed and validated at FRS Marine Laboratory for the analysis of CBs, OCPs and PBDEs from biota. Alumina (30 g, 5% deactivated) was used as the fat retainer as it was found to give the greatest lipid retention and best CB and PBDE recoveries. If a higher alumina deactivation was used then lipid broke through while at a lower deactivation organohalogen congeners were retained in the ASE cell. A non-polar solvent was required to prevent lipid breakthrough, therefore, *iso*-hexane was used for the extraction.

OCPs could not be extracted using this method as the dieldrin was retained in the ASE cell. However, by reducing the amount of fat retainer (5% deactivated alumina) to 15 g, and introducing further clean-up steps, the analysis of OCPs was possible. CBs could be analysed either by GC-ECD or GC-EIMS (Fig 2a). If GC-ECD is used for the analysis, and/or OCPs are required, then the OCPs and CBs must be separated by using additional column chromatography. If OCPs are not required, then the CBs can be extracted along with the PBDEs using 30 g of alumina with no further clean up steps required and analysed directly by GC-ECD or GC-EIMS. Method validation data reported here for CBs and OCPs was determined by GC-ECD while PBDE data was determined by GC-NCIMS.

The precision of the method was determined by the replicate analysis, on separate days, of a laboratory reference material for CBs and OCPs and NIST CRM1946 Lake Superior fish tissue for PBDEs. NIST CRM1946 is not certified for PBDEs but these compounds are present in the material. CV% were generally <15% for all CBs, OCPs and PBDEs (Table 1).

The limits of detection (LoD) of individual CBs, OCPs and PBDE congeners were determined through the consecutive, same day repeat analysis of a low spiked sample. Mussels (*Mytilus edulis*, 8 g) were spiked with ~ 2 ng, 3 ng and 1 ng of each individual CB, OCP and PBDE congener, respectively, and left overnight and extracted as described in the Methods section. The mean concentration and the standard deviation (SD) for each congener were calculated and the LoDs determined (4.65 x Standard Deviation (SD); Table 1). The LoDs for the biota samples were between 0.05 µg kg^{-1} and 0.76 µg kg^{-1} for CBs, and 0.05 µg kg^{-1} and 0.07 µg kg^{-1} for PBDEs (Table 1).

Recoveries were calculated through replicate analysis, over several days, of CBs, OCPs and PBDEs in spiked salmon (*Salmo salar*) liver and mussels (Table 1). The mean recoveries for individual CB congeners were 88.5 – 98.2% (CV% 2.2 – 13.4, n = 4) and 74.2 – 101.8% (CV% 3.7-15.2, n = 6) for fish liver and mussels, respectively. The mean recoveries for PBDEs were 82.1-92.7% (CV% 14.1- 16.9, n = 6) and 66.9 -77.8 % (CV% 3.0-12.4, n = 4) for fish liver and mussels respectively (Table 1, Fig 2b).

With each batch of samples a procedural blank was analysed. The matrix/sodium sulphate mixture (Fig. 1) was replaced with sodium sulphate. The blank problems which occurred occasionally when Soxhlet was used for extraction were eliminated by using ASE. This was probably due to the reduction in solvent volumes, time for solvent evaporation and number of steps. Typically, blank concentrations were lower for the CBs from the ASE than from Soxhlet.

In conclusion, an ASE extraction method has been developed and validated for the analysis of CB and PBDEs in biota. For the analysis of CBs or PBDEs, 10 samples may be extracted and ready for analysis by GC-MS within one day. If OCPs are also to be analysed then less alumina must be used as a fat retainer in the cell and additional clean-up steps are required.

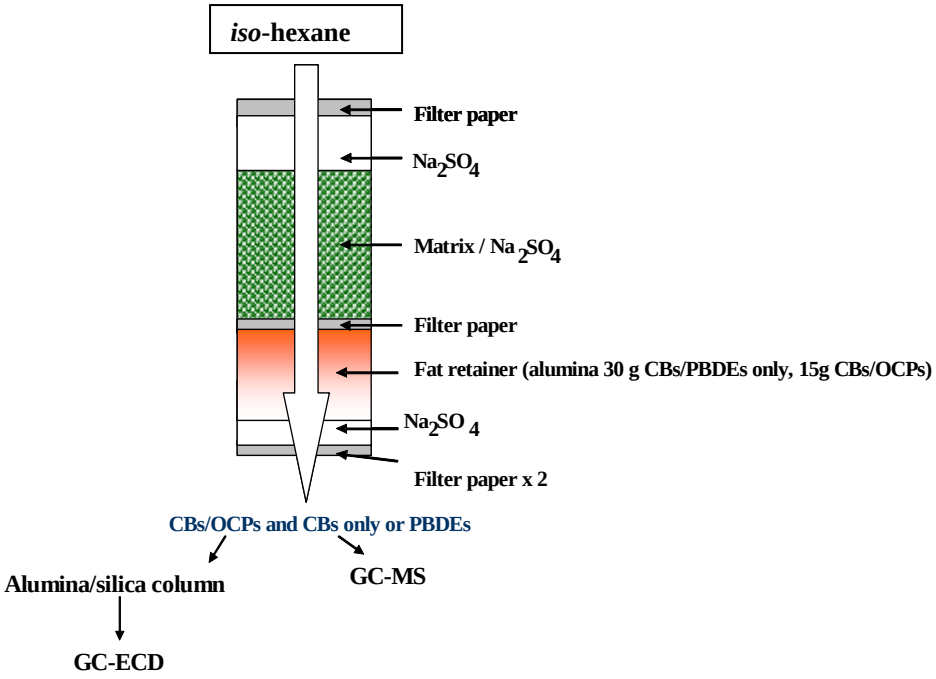
References

1. Fernandez P and Grimalt JO. 2003. *Chimia 2003;* 57: 514.
2. Smedes F. *Analyst* 1999;124:1711

Determinand	**% Recovery of spiked fish liver**	**CV%**	**% Recovery of Mussels**	**CV%**	**Precision (CV%)** LRM(CBs/OCPs) NIST 1946 (PBDEs)	**LoD** (μg kg^{-1} wet weight) Mussels
CB 31	93.4	16.6	74.2	12.1	22.4	0.05
CB 28	94.0	7.2	79.6	10.4	5.6	0.07
CB 52	92.9	11.3	83.4	11.6	4.9	0.48
CB 101	96.6	3.0	93.4	4.1	6.3	0.76
CB 105	78.8	13.4	86.9	15.2	11.4	0.23
CB 118	98.2	7.5	97.1	3.7	6.4	0.39
CB 138	88.5	7.4	98.5	4.0	7.1	0.33
CB 153	89.0	8.6	100.9	6.9	6.8	0.34
CB 156	95.4	9.2	101.8	5.7	6.3	0.08
CB 180	89.3	2.2	92.8	6.2	7.2	0.18
heptachlor epoxide	60.5	15.3	51.7	17.3	15.0	0.08
dieldrin	63.7	14.4	47.8	11.7	5.8	0.02
BDE17	85.4	14.3	71.4	4.2	21.8	0.05
BDE28	87.1	14.7	74.5	4.3	13.7	0.05
BDE47	82.7	14.7	66.9	3.5	3.5	0.07
BDE100	92.6	15.0	77.8	4.2	11.3	0.05
BDE99	90.9	14.4	77.2	3.0	6.2	0.06
BDE153	92.7	14.1	76.4	3.9	7.7	0.06
BDE190	87.2	16.9	73.2	12.4	23.1	0.05

Table 1. Method limit of detection (LoD) determined from the replicate analysis of a spiked mussel sample at low concentration (4.65 x S.D.), precision from replicate analysis of reference materials and recovery from spiked biota for a selection of CBs, OCPs and PBDEs.

Figure 1 Schematic of ASE cell for the extraction of CBs, PBDEs and OCPs from biota



(a) **(b)**

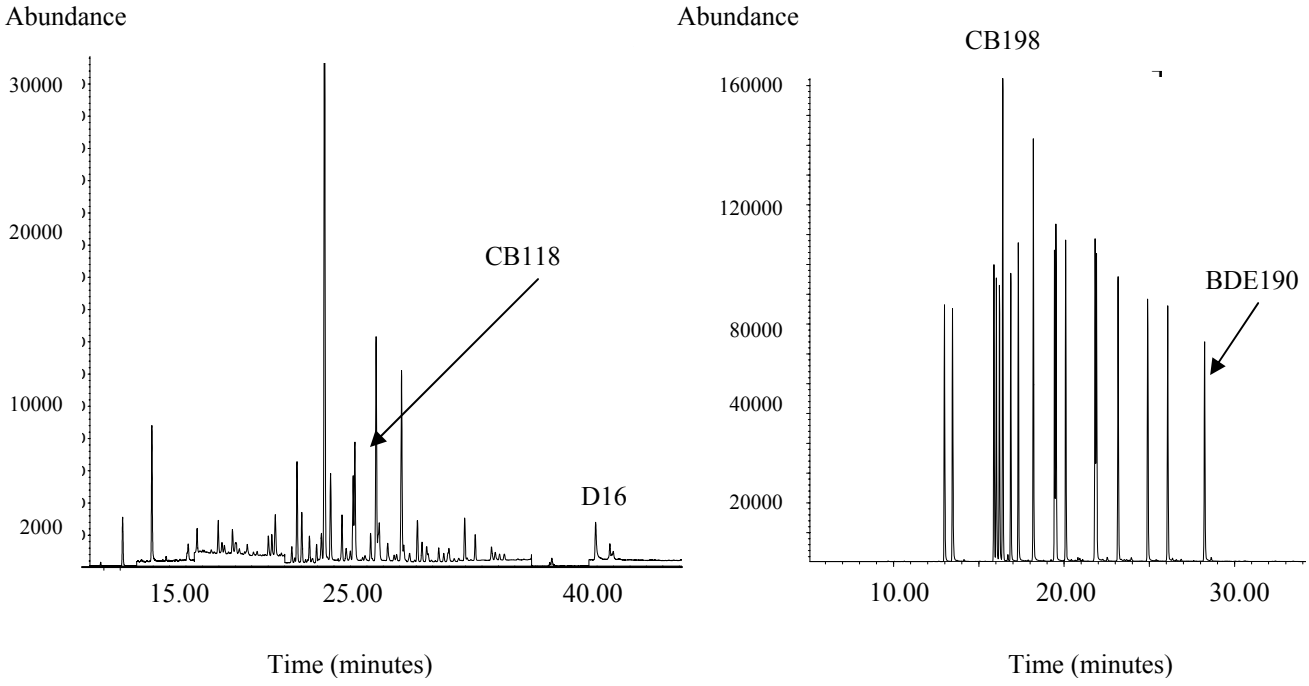


Figure 2 – Total ion chromatograms of (**a)** LRM analysed for CBs by GC-EIMS and **(b)** Spiked biota (salmon liver) analysed for PBDEs analysed by GC-NCIMS, which had been extracted by ASE. D16 is the internal standard for CB analysis and CB198 is the PBDE recovery standard.