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# Development of a matrix solid-phase dispersion method for the screening of polybrominated diphenyl ethers and polychlorinated biphenyls in biota samples using gas chromatography with electron-capture detection

A. Martínez, M. Ramil, R. Montes, D. Hernanz, E. Rubí, I. Rodríguez*, R. Cela Torrijos

*Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain*

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**Abstract**

A low cost method for the screening of six polybrominated diphenyl ethers (PBDEs) and seven polychlorinated biphenyls (PCBs) in biological samples containing up to 100% of fat is presented. Compounds are extracted from the sample and isolated from lipids using a matrix solid-phase dispersion (MSPD) cartridge and 20 ml of *n*-hexane as elution solvent. PBDEs and PCBs are fractionated on a second cartridge containing 2 g of a normal phase sorbent. The potential of neutral silica, Florisil and basic alumina to separate PBDEs and PCBs in two independent fractions has been evaluated. The best results were obtained using silica. PCBs are eluted, in a first fraction, using *n*-hexane. PBDEs are further recovered with *n*-hexane–dichloromethane. The applicability of the method for the screening of PBDEs and PCBs, in samples containing both groups of compounds, has been demonstrated using spiked, certified and real polluted samples from different biota materials. Globally, recoveries higher than 75% and quantification limits around 0.4 ng/g have been achieved using gas chromatography with electron-capture detection (GC-ECD).


*Keywords:* PBDEs; PCBs; Fractionation; Biota samples; Matrix solid-phase dispersion

## 1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardant additives in polymers and textiles. From these materials they are easily released into the surrounding environment [1]. Commercial PBDEs preparations (named as penta-BDE, octa-BDE and deca-BDE mixtures) contain only a few number of the theoretically possible 209 congeners. The less brominated congeners (tetra-, penta- and hexa-BDEs), which appear in commercial penta-mix preparations, are considered as the most persistent and bioaccumulative PBDEs [2]. A recent review has shown a worldwide steady increase in the concentrations of 2,2′4,4′-tetra-BDE (BDE47), 2,2′,4,4′,5-penta-BDE (BDE99), 2,2′,4,4′,6-penta-BDE (BDE100), 2,2′,4,4′,5,5′-hexa-BDE (BDE153) and 2,2′,4,4′,5,6′-hexa-BDE (BDE154) in different environmental compartments during last 30 years [3]. A similar tendency has been observed in a ten years monitoring campaign focussed in a particular geographic area [4]. The highest PBDEs levels have been found in biota samples, particularly in fatty tissues from marine mammals, fish, filter-feeding organisms and, in general, top predator animals [5–7].

On the other hand, polychlorinated biphenyls (PCBs) were employed, as complex commercial mixtures containing more than one hundred congeners, in many industrial applications [8]. Nowadays, their production has been stopped; however, they are still ubiquitous environmental pollutants. Normally, their highest levels are also detected in fatty tissues [9].

Therefore, biota and food samples might be polluted with PBDEs and PCBs [10]. Even more, it has been proposed the existence of correlations between their concentrations in certain samples [2,11,12]. PBDEs and PCBs have also been

* Corresponding author. Tel.: +34 981 563100x14387; fax: +34 981 595012.
*E-mail address:* qnisaac@usc.es (I. Rodríguez).

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detected in human tissues and fluids [13,14]. Dietary intake of polluted food probably constitutes one of their main input routes to the human body.

Methods for the determination of PBDEs and PCBs usually involve three basic steps: (1) extraction, (2) removal of co-extracted lipids and/or non-volatile compounds, e.g. using an oxidative treatment and/or size exclusion purification, and (3) selective determination by gas chromatography based techniques. Soxhlet, column elution, supercritical fluids (SFE), microwave assisted (MAE) and pressurized solvent extractions (PLE) have been successfully applied to the extraction of PCBs and PBDEs from biota and food samples [6,12,15–19]. Alternatively to those techniques, matrix solid-phase dispersion (MSPD) has been proposed for the simultaneous extraction and purification (fat removal) of PCBs from fish, seafood and fat samples [20,21]. MSPD uses moderate volumes of organic solvents and does not require the acquisition of expensive instrumentation. However, from our knowledge, no applications have been reported for PBDEs.

Regarding the determination step, PBDEs and PCBs are normally analysed by gas chromatography (GC) in combination with mass spectrometry (MS). Instruments equipped with electron impact ionisation sources and low resolution mass analysers (EI-LR-MS) are worldwide available; however, they lack of enough sensitivity for the determination of PBDEs in most biological samples. Electron impact high resolution mass spectrometry (El-HR-MS) [4,22], tandem mass spectrometry (MS-MS) [11,23], or low resolution mass spectrometry using negative chemical ionisation (NCI-LR-MS) [6,15,16], are required for the sensitive and selective determination of PBDEs after their GC separation. Unfortunately, some of them are costly techniques not available in many laboratories. A low cost and sensitive alternative for the screening of halogenated pollutants is the electron-capture detector (ECD). The major drawback of GC-ECD is the risk of analytes misidentification, since retention times of tetra-, penta- and hexa-BDEs fall in the same region than those of hepta to deca CBs [2,11]. This problem can be solved if both groups of compounds are previously fractionated. Reported studies have proved that PBDEs are more strongly retained on silica than PCBs [24–27]. Therefore, they can be sequentially eluted from this sorbent using organic solvents with increasing polarities. Additional fractionation attempts have also been done with other normal phase materials [11].

The aim of this work is the development of a simple method for the screening of PBDEs and PCBs in biological samples, with different fat contents, avoiding the use of expensive sample preparation and determination techniques. Matrix Solid-Phase Dispersion was used for the extraction of PBDEs and PCBs from biological samples. Lipids were removed in the same MSPD cartridge. After extraction and before chromatographic analysis, the possibilities of alumina, silica and Florisil to fractionate PBDEs and PCBs were systematically investigated in order to allow their more reliable determination using GC-ECD.

## 2. Experimental

### 2.1. Reagents and materials

Sulphuric acid, organic solvents for trace analysis (*n*-hexane, dichloromethane, and isooctane), anhydrous sodium sulphate, Florisil (150–250 μm), basic alumina (100 μm) and neutral silica with 2 different particle sizes (63–200 μm and 40–63 μm) were purchased from Aldrich (Milwaukee, WI, USA) or Merck (Darmstadt, Germany). Silica, alumina and Florisil were activated at 130 °C for at least 48 h before using them. Acidic silica (44% sulphuric acid) was prepared by mixing neutral silica with concentrated sulphuric acid. Polyethylene solid-phase extraction cartridges (15 ml capacity) and 20 μm frits were purchased from International Sorbent Technology (Mid Glamorgan, UK).

### 2.2. Standards and samples

*PCBs:* Individual congeners of several non-coplanar PCBs (CB28, CB52, CB118, CB138, CB153, CB180, CB101 and CB209, IUPAC nomenclature) and three coplanar congeners (CB77, CB126, CB169) were purchased from Dr. Ehrensdorfer (Augsburg, Germany). A test mixture containing 62 CB congeners was obtained from Wellington Laboratories (Ontario, Canada). Individual solutions of each CB and mixtures of them were prepared in isooctane. Further dilutions were made in isooctane (when used as calibration solutions) and *n*-hexane (when used to prepare spiked samples). CB30 (Dr. Ehrensdorfer) was dissolved in isooctane. This compound was used as injection standard to compensate any variation in the final volume of the sample extracts.

*PBDEs:* A penta-mix standard with a total PBDEs concentration of 10 μg/ml in cyclohexane was purchased from Dr. Ehrensdorfer. Individual congeners of BDE47, BDE100, BDE99, BDE154 and BDE153 (50 μg/ml in nonane) were purchased from Wellington Laboratories. 2,2′,3,4,4′-penta-BDE (BDE85), 50 μg/ml in isooctane, was obtained from Accustandard (New Haven, CT, USA). Diluted solutions of the individual congeners and the penta-mix standard were prepared in isooctane and *n*-hexane.

Samples. Beef fat, chicken fat, turbot fish *(Psetta maxima)* muscle and dogfish *(Scyliorhinus canicula)* liver were directly acquired in local supermarkets. Samples were cut in small pieces and homogenised. Sub-samples of 10 g were spiked with solutions of PBDEs and/or PCBs in *n*-hexane, and thoroughly mixed. Then, the organic solvent was allowed to evaporate for 12 h. Spiked and non-spiked sub-samples of those materials were stored at −18 °C. Spiked samples were aged for at least one week before extraction.

Reference materials of pork fat (IRMM 446), containing certified concentrations of seven CBs and an indicative level of BDE47, and freeze-dried naturally contaminated fish tissue (WELL-WMF-01), with certified concentrations of five BDEs, were obtained from the Institute for Reference

Materials and Measurements (Geel, Belgium) and Wellington Laboratories, respectively.

### 2.3. Sample preparation

*Extraction.* Spiked and non-spiked samples, 0.5 g, were thoroughly dried with 2 g of anhydrous sodium sulphate and dispersed on 1.5 g of Florisil in a glass mortar with a pestle for 5 min [21]. The mixture was then transferred to the top of a SPE cartridge containing 5 g of acidic silica. PCBs and PBDEs were eluted using 20 ml of *n*-hexane. A fraction of lipids and other interfering compounds contained in the sample were partially retained on the Florisil sorbent particles. The rest was oxidized in the acidic silica layer, placed at the bottom of the MSPD cartridge.

*Fractionation of PCBs and PBDEs:* The extract from the MSPD cartridge was reduced to 0.5 ml using a Turbo Vap concentrator and added on top of a second SPE cartridge containing 2 g of neutral silica (40–63 μm). PCBs were eluted with *n*-hexane (20 ml). PBDEs were recovered in a second fraction with *n*-hexane–dichloromethane (80:20, 12 ml). Both fractions were spiked with 200 μl of a CB30 solution in isooctane (ca. 70 ng/ml) and evaporated to approximately 0.5 ml using the Turbo Vap system. After that, they were transferred to GC autosampler vials and additionally concentrated to 0.2 ml with a gentle stream of nitrogen. Extracts were stored at 4 °C until being analysed.

### 2.4. Apparatus

Determinations of PCBs and PBDEs were carried out, in independent chromatographic runs, using a HP 5890 series II Plus gas chromatograph (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a $^{63}$Ni electron-capture detector. Separations were performed using a HP-5 capillary column (30 mm × 0.25 mm i.d., 0.25 μm film thickness) purchased from Agilent (Wilmington, DE, USA). Nitrogen was employed as column carrier gas at a constant pressure of 45 kPa, and also as auxiliary gas in the ECD (50 ml/min). Standard solutions and sample extracts were injected in the splitless mode (1–2 μl, purge time 1 min). Chromatographic separations were carried out using the following oven program: 90 °C (1 min), first ramp at 15 °C/min to 220 °C, second ramp at 8 °C/min to 300 °C (held for 10 min). Injector and detector temperatures were fixed at 260 and 300 °C, respectively.

A GC-MS ion trap instrument, operating in the MS-MS mode, was used to verify the quality of the fractionation between PCBs and PBDEs. The system consisted of a Varian CP 3900 gas chromatograph (Walnut Creek, CA, USA), equipped with an identical column than that used in the GC-ECD, connected to a mass spectrometer (Varian Saturn 2100). The oven temperature program was the same as in the GC-ECD system. The helium column flow was adjusted to 1 ml/min. The trap temperature was fixed at 220 °C.

### 2.5. Quantification

Levels of PBDEs and PCBs in spiked and non-spiked samples (including certified reference materials) were determined comparing their normalised responses (peak area/CB 30 peak area) in sample extracts against calibration curves obtained for mixtures of PCBs or PBDEs in isooctane. The same amount of CB 30 was added to sample extracts and calibration mixtures.

## 3. Results and discussion

### 3.1. Performance of GC-ECD detection

Fig. 1 shows a chromatogram for a standard solution containing six PBDEs and seven PCBs. As expected, the pair CB180/BDE47 was not baseline resolved [11]; moreover, a similar behaviour was observed for the couple CB209/BDE85. Using the 62 CB congeners test mixture, it was also found that CB194 and BDE100 showed identical retention times, figure not shown. The separation of CB180/BDE47 and CB209/BDE85 could be improved using longer columns or slower oven temperature programs. For the pair CB194/BDE100, their chromatographic separation does not seem to be possible using non-polar coated columns.

The performance of the GC-ECD system was evaluated using two sets of standard solutions in isooctane: one containing the six PBDEs and the other with the seven PCBs. The linearity of the response was investigated in the range 2–200 ng/ml for PDBEs and 2–500 ng/ml for PCBs. Peak areas for each compound were divided by the CB30 peak area and represented versus its concentration. Correlation coefficients higher than 0.998 were obtained for all species. Relative standard deviations for 5 consecutive injections of

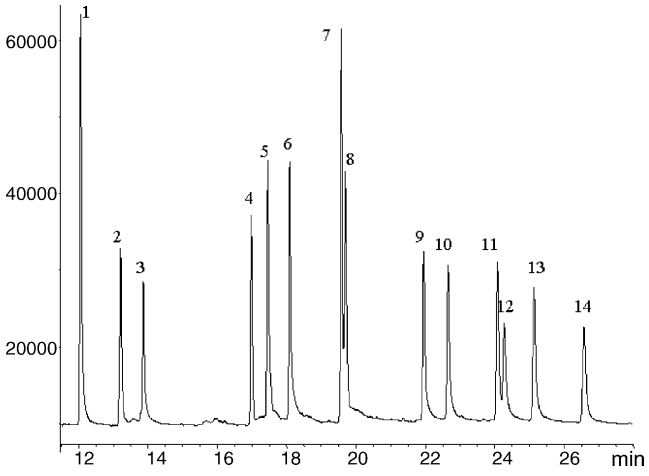


Fig. 1. GC-ECD chromatogram for a mixture of seven PCBs and six PBDEs. (1) CB30 (IS, 70 ng/ml); (2) CB28; (3) CB52; (4) CB118; (5) CB153; (6) CB138; (7) CB180; (8) BDE47; (9) BDE100; (10) BDE 99; (11) CB209; (12) BDE85; (13) BDE154; (14) BDE153, 50 ng/ml each one.

PCBs and PBDEs standard solutions (10 ng/ml level) ranged from 2 to 4%. Instrumental quantification limits of 1 ng/ml were obtained for PCBs and PBDEs.

### 3.2. Extraction of PBDEs from biota samples

In a recent paper, we have proved the applicability of MSPD for the extraction of PCBs from biological samples [21]. Briefly, samples were dried with anhydrous sodium sulphate, dispersed on Florisil and analytes extracted using 15–20 ml of *n*-hexane. In addition, a normal phase material (Florisil or acidic silica) was required as co-sorbent to retain lipids and other co-eluted interferences.

This procedure was extrapolated to the determination of PBDEs in spiked beef fat samples. Florisil and acidic silica (40–63 μm) were initially considered as fat retainers. Four fractions of *n*-hexane (10 ml each one) were collected from the MSPD cartridge. They were concentrated to 0.2 ml and injected in the GC-ECD system. Using acidic silica, PBDEs were recovered in the first two *n*-hexane fractions. In the case of Florisil, they appeared distributed in the four fractions, Table 1. For both sorbents, the percentage of fat in the *n*-hexane fractions was gravimetrically determined. It always remained below 0.05% of the sample intake.

In view of the above results, acidic silica and 20 ml of *n*-hexane were employed to recover PBDEs from the dispersion cartridge (PCBs are also eluted with this volume of solvent [21]). Additional experiments were carried out in order to investigate the influence of the co-sorbent amount and particle size on the lipids removal efficiency. Obtained values are given in Table 2. For fish muscle, 1 g of acidic silica was enough to obtain a GC analysable extract. In the case of pure fat samples, up to 5 g were necessary to reduce the percentage of eluted lipids below 0.1% of the sample intake (0.5 g). Similar fat removal efficiencies were achieved independently of the silica particle size: 40–63 μm or 63–200 μm. The last was used in further experiments, since the largest the particle size, the faster the gravimetric elution of the MSPD cartridge is.

Table 1
Normalized peak areas in the consecutive 10 ml *n*-hexane fractions obtained from the MSPD cartridge as function of the fat retainer

| BDE congener | Fat retainer (5 g) | Fraction number | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 47 | Acidic silica (40–63 μm) | 96 | 4 | | |
| 100 | | 94 | 6 | | |
| 99 | | 96 | 4 | | |
| 85 | | 100 | | | |
| 154 | | 100 | | | |
| 153 | | 100 | | | |
| 47 | Florisil | | 25 | 52 | 23 |
| 100 | | 15 | 45 | 34 | 6 |
| 99 | | 11 | 42 | 39 | 8 |
| 85 | | | | | 100 |
| 154 | | | 75 | 25 | |
| 153 | | | 72 | 28 | |

Empty cells correspond to non-detected compounds. Beef fat samples (0.5 g) spiked only with PBDEs.

Table 2
Percentage of fat in *n*-hexane extracts (20 ml) obtained from the MSPD cartridge

| Sorb, mass (g) | Part, size (μm) | Fat percentage (g of lipids/100 g of sample) | | |
|---|---|---|---|---|
| | | Beef fat | Chicken fat | Fish muscle |
| 1 | 40–63 | 5.6 | 11.5 | 0.02 |
| 3 | 40–63 | 3.6 | 0.2 | 0.03 |
| 5 | 40–63 | 0.08 | 0.07 | 0.03 |
| 5 | 63–200 | 0.06 | 0.06 | 0.03 |

Silica was used as fat retainer. Sample intake was 0.5 g.

Absolute recoveries for a sample of beef fat spiked only with PBDEs (200 ng/g of the penta-mix standard) were 89 ± 3% (BDE47), 89 ± 3% (BDE100), 94 ± 3% (BDE99), 94 ± 3% (BDE85), 95 ± 3% (BDE154) and 95 ± 3% (BDE153), $n = 4$ replicates.

### 3.3. Fractionation of PBDEs and PCBs

As stated in a previous review article [28], the two aromatic rings of PBDEs are disposed in a 120° angle. In the case of PCBs, both rings are aligned in the same or in a different plane depending on the congener. As a consequence, PBDEs show a higher polarity than PCBs and therefore, theoretically, they could be separated on the basis of this property. This possibility was systematically investigated using 2 g of the following sorbents: Florisil, neutral silica (40–63 μm) and basic alumina packed in a polyethylene SPE cartridge. Two hundred microliters of a standard solution of PCBs (containing seven non-coplanar and three coplanar congeners, 1 μg/ml) or PBDEs (1 μg/ml of the penta-mix) were added on top of each sorbent. Five fractions of *n*-hexane (10 ml volume for silica and alumina and 5 ml in the case of Florisil) followed by one of *n*-hexane–dichloromethane (80:20, 10 ml volume) were collected from the fractionation cartridges. Each fraction was concentrated to 0.2 ml and injected in the GC-ECD system. Results for non-coplanar PCBs and PBDEs are given in Table 3.

As predicted, the three sorbents showed a higher affinity for PBDEs than for PCBs. Particularly, the pairs of compounds: CB180/BDE47 and CB209/BDE85 could be relatively well separated with all of them. However, a selective separation of all considered PCBs and PBDEs in two independent fractions was more difficult to achieve. The worst results corresponded to Florisil. The second and third *n*-hexane fractions from this sorbent contained considerable amounts of both groups of compounds. In the case of alumina, PBDEs were mainly recovered in the fraction of *n*-hexane: dichloromethane. PCBs were distributed in the five previous *n*-hexane fractions. The exception was CB28 which was also found in the *n*-hexane–dichloromethane fraction. Silica was the sorbent with the highest selectivity: the seven

Table 3
Capabilities of Florisil, basic alumina and neutral silica (2 g of each sorbent) to fractionate PCBs and PBDEs

| Sorbent | Compound | Fraction number | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Florisil[a] | CB28 | 21 | 47 | 23 | 7 | 2 | |
| | CB52 | 39 | 43 | 13 | 4 | 1 | |
| | CB118 | 26 | 43 | 21 | 6 | 2 | 2 |
| | CB153 | 62 | 29 | 5 | 3 | 1 | |
| | CB138 | 34 | 42 | 15 | 5 | 2 | 2 |
| | CB180 | 66 | 26 | 4 | 2 | 1 | 1 |
| | CB209 | 91 | 7 | 1 | 1 | | |
| | BDE47 | | | 1 | 2 | 2 | 95 |
| | BDE100 | | 3 | 5 | 8 | 9 | 75 |
| | BDE99 | | 3 | 4 | 8 | 9 | 76 |
| | BDE85 | | | | | | 100 |
| | BDE154 | | 12 | 13 | 13 | 18 | 44 |
| | BDE153 | | 12 | 13 | 11 | 15 | 49 |
| Alumina | CB28 | | 6 | 12 | 30 | 28 | 24 |
| | CB52 | | 37 | 39 | 20 | 4 | |
| | CB118 | 4 | 35 | 34 | 12 | 15 | |
| | CB153 | 36 | 62 | 2 | | | |
| | CB138 | 2 | 20 | 37 | 26 | 12 | 3 |
| | CB180 | 25 | 70 | 5 | | | |
| | CB209 | 75 | 20 | 5 | | | |
| | BDE47 | | | | | | 100 |
| | BDE100 | | | | | 2 | 98 |
| | BDE99 | | | | | | 100 |
| | BDE85 | | | | | | 100 |
| | BDE154 | | | | | 2 | 98 |
| | BDE153 | | | | | | 100 |
| Silica | CB28 | 12 | 88 | | | | |
| | CB52 | 15 | 85 | | | | |
| | CB118 | 79 | 21 | | | | |
| | CB153 | 99 | 1 | | | | |
| | CB138 | 69 | 31 | | | | |
| | CB180 | 99 | 1 | | | | |
| | CB209 | 99 | 1 | | | | |
| | BDE47 | | | 1 | 24 | 38 | 37 |
| | BDE100 | | | 37 | 50 | 12 | 1 |
| | BDE99 | | | 1 | 33 | 44 | 22 |
| | BDE85 | | | | | 26 | 74 |
| | BDE154 | | | 25 | 48 | 25 | 2 |
| | BDE153 | | | 10 | 42 | 42 | 6 |

Normalized peak areas in each 10 ml fraction (experiments were carried out in duplicate). Fractions 1–5, *n*-hexane. Fraction 6, *n*-hexane–dichloromethane (80:20). Empty cells correspond to non-detected compounds.
[a] The volume of fractions 1–5 was 5 ml.

non-coplanar PCBs were eluted in the two first fractions of *n*-hexane; PBDEs began to elute after the third fraction. These results are in agreement with the capacity factor values obtained for PCBs and PBDEs using normal phase HPLC silica columns and *n*-hexane as mobile phase [25]. Regarding the coplanar PCBs (CB 77, 126 and 169) they eluted together with the non-coplanar congeners using silica. In the case of alumina and Florisil, they were mainly recovered in the *n*-hexane–dichloromethane fraction, data not shown. In further experiments, 2 g of neutral silica were employed to fractionate the extract from the MSPD cartridge. PCBs were recovered with 20 ml of *n*-hexane. After that, PBDEs were extracted using 12 ml of *n*-hexane–dichloromethane (80:20).

### 3.4. Recoveries and quantification limits

Absolute recoveries of the whole method, extraction followed by fractionation of PCBs and PBDEs, were evaluated using spiked (added level 200 ng/g for PCBs and 16 ng/g for PBDEs) biota materials with different fat contents. Blank subsamples of those materials were also processed. As shown in Table 4, average recoveries from 74 to 99% were obtained for all compounds. Globally, slightly higher recoveries and lower relative standard deviations were achieved for PCBs than for PBDEs, Table 4. In view of those results, a very high efficiency was achieved during sample extraction and the further fractionation of PCBs and PBDEs. Moreover, losses of the most volatile CB congeners (including CB 30), when extracts

Table 4
Average recoveries of the whole method for biota samples spiked with PCBs (200 ng/g) and PBDEs (16 ng/g)

| | Congener | Chicken fat | | Beef fat | | Fish muscle | |
|---|---|---|---|---|---|---|---|
| | | Rec (%) | R.S.D. (%) | Rec (%) | R.S.D. (%) | Rec (%) | R.S.D. (%) |
| *n*-Hexane fraction | CB28 | 90 | 3 | 84 | 2 | 75 | 2 |
| | CB52 | 86 | 2 | 84 | 2 | 82 | 1 |
| | CB118 | 94 | 2 | 98 | 2 | 87 | 3 |
| | CB153 | 89 | 1 | 96 | 3 | 88 | 4 |
| | CB138 | 91 | 2 | 97 | 2 | 90 | 2 |
| | CB180 | 87 | 2 | 98 | 2 | 86 | 3 |
| | CB209 | 84 | 3 | 99 | 2 | 84 | 3 |
| *n*-Hexane–dichloromethane fraction | BDE47 | 97 | 7 | 85 | 2 | 92 | 2 |
| | BDE100 | 90 | 7 | 83 | 2 | 84 | 9 |
| | BDE99 | 96 | 7 | 82 | 2 | 85 | 3 |
| | BDE85 | 94 | 10 | 74 | 3 | 80 | 2 |
| | BDE154 | 98 | 8 | 80 | 3 | 82 | 6 |
| | BDE153 | 99 | 9 | 75 | 5 | 81 | 3 |

$n = 3$ Replicates.

are reduced to 0.2 ml, were not noticed; otherwise recoveries over 100% would be obtained for the less volatile analytes. GC-ECD chromatograms corresponding to *n*-hexane and *n*-hexane–dichloromethane fractions from a spiked beef fat sample are shown in Fig. 2. Quantification limits of the proposed method, considering 0.5 g of sample and a final extract volume of 0.2 ml, corresponded to 0.4 ng/g for PBDEs and CBs 138,153, 180 and 209. In the earlier region of the GC-ECD chromatogram for the *n*-hexane fraction, a noisier baseline was observed. As a consequence, quantification limits around 3 ng/g were estimated for CBs 28, 52 and 118.

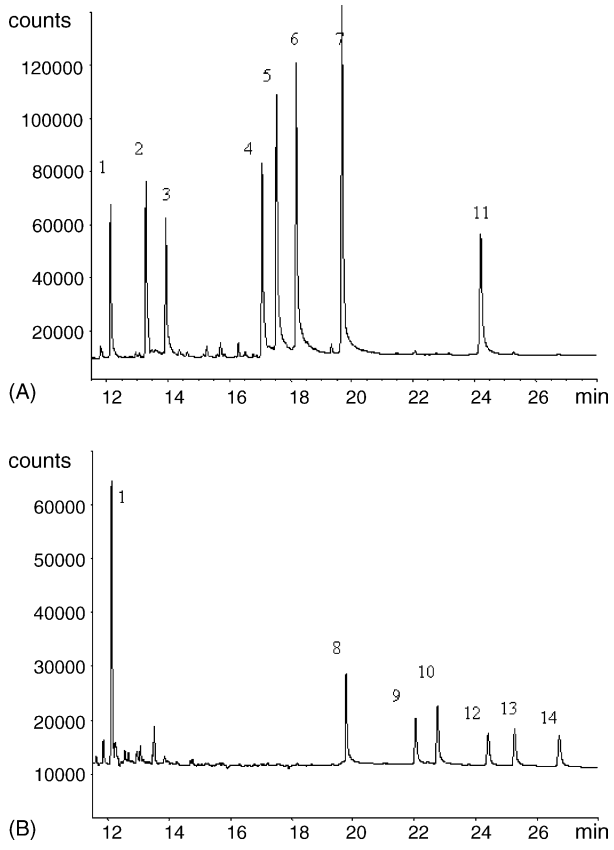


Fig. 2. GC-ECD chromatograms for a beef fat sample spiked with PCBs (200 ng/g) and PBDEs (16 ng/g). (A) *n*-Hexane fraction. (B) *n*-Hexane–dichloromethane fraction. For peak identification see Fig. 1.

### 3.5. Analysis of reference materials

The availability of biota materials with certified concentrations of PBDEs is relatively limited.

Two of them were used to asses the accuracy of the proposed method. Concentrations of PBDEs and PCBs in both materials were determined comparing their signals in sample extracts with those corresponding to calibration mixtures in isooctane. IRMM 446 is a laboratory-polluted pork fat with certified concentrations of seven non-coplanar CB congeners. An indicative value for the native level of BDE47 in this sample is also available. The congener CB101, which had not been considered during the optimisation of the method,

Table 5
Concentrations of PCBs and BDE 47 (ng/g) in reference material IRMM 446 (pork fat)

| Compound | Certified (conc. ± S.D.) | Measured (conc. ± S.D.) |
|---|---|---|
| CB28 | 29.6 ± 2.1 | 26.9 ± 0.6 |
| CB52 | 25.5 ± 1.8 | 24.6 ± 0.9 |
| CB101 | 30.0 ± 4.0 | 25.9 ± 0.6 |
| CB118 | 30.2 ± 2.7 | 25.2 ± 0.5 |
| CB153 | 30.8 ± 2.4 | 26.4 ± 0.6 |
| CB138 | 32.0 ± 4.0 | 26.5 ± 0.8 |
| CB180 | 29.8 ± 2.5 | 24.3 ± 1.5 |
| BDE47 | 6.1[a] | 4.3 ± 0.4 |

$n = 4$ Replicates.
[a] Indicative value, non-certified.

Table 6
Concentrations of BDEs (ng/g) in reference material WELL-WMF-01 (freeze-dried fish tissue), $n = 4$ replicates

| Compound | Certified (conc. ± S.D.) | Measured (conc. ± S.D.) |
|---|---|---|
| BDE47 | 123.2 ± 24.8 | 131 ± 30 |
| BDE100 | 35.9 ± 14.5 | 31.4 ± 5.9 |
| BDE99 | 37.5 ± 4.2 | 33.9 ± 4.9 |
| BDE154 | 19.8 ± 2.9 | 18.0 ± 1.4 |
| BDE153 | 17.0 ± 8.0 | 13.8 ± 0.5 |

was included in the calibration solution mixture and its concentration in IRMM 446 evaluated. For PCBs the measured concentrations represented between 82% (CBs153 and 180) and 96% (CB52) of the certified values, Table 5. For BDE47, the obtained value corresponded to 70% of the indicative one.

The WELL-WMF-01, from our knowledge, is the only biota material with certified concentrations of five PBDEs. Moreover, it corresponds to a naturally polluted sample. Apart from PBDEs it also contains PCBs; however, only the concentration of some mono-ortho and coplanar congeners are certified. In contrast to the rest of materials employed in this study, it is a freeze-dried fish tissue; therefore, samples were reconstituted using 0.5 ml of water previous to being submitted to the proposed method. For this sample, only the concentrations of PBDEs were quantified after extraction and fractionation. Measured values showed a reasonable agreement with the certified ones, Table 6. It should be however noted that uncertainties associated to the certified concentrations of some PBDEs represented up to 40% of the average value. Relative standard deviations of found concentrations were also higher (up to 23%) than those obtained for the spiked samples, Table 4.

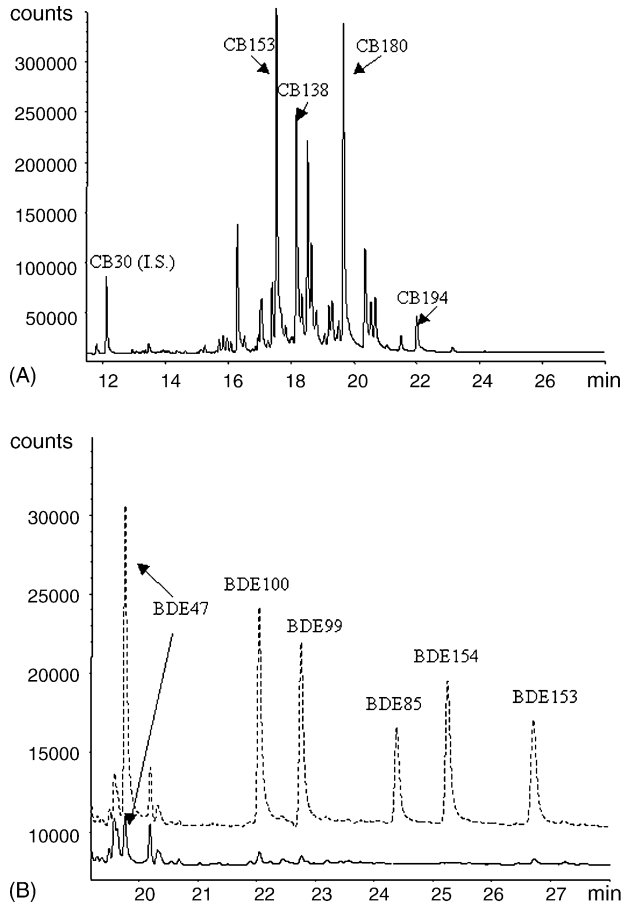


Fig. 3. GC-ECD chromatograms for dogfish liver sample. (A) Chromatogram of the *n*-hexane fraction for a non-spiked sample. (B) Overlay of chromatograms for the *n*-hexane–dichloromethane fractions of a non-spiked (solid line) and a sample spiked with PBDEs at 16 ng/g (dotted line).

### 3.6. Application to a real sample

Fig. 3A shows the GC-ECD chromatogram corresponding to the *n*-hexane fraction of a non-spiked dogfish liver sample (0.5 g). The GC-ECD profile matched quite well with that of the commercial Aroclor 1260 mixture, (chromatogram not shown). The most intense peaks were observed at retention times of CB153, CB138 and CB180. For the last one an estimated concentration of 370 ± 30 ng/g (3 replicates) was obtained. In addition, a peak at the retention time of CB194 was also observed, Fig. 3A. Obviously, the reliable identification of each peak in this fraction requires the use of a more efficient column than that used in this study. The GC-ECD chromatogram for the *n*-hexane–dichloromethane fraction showed a small peak at the retention time of BDE47, Fig. 3B. This signal could correspond either to a low level of BDE47 in the sample, or to a residual amount CB180 in this fraction. To confirm the identity of this peak an aliquot of the extract was analysed using GC-MS-MS. The *m/z* ratio 486 was selected as the BDE47 parent ion and MS-MS chromatograms were monitored using the 324 + 326 + 328 *m/z* ratios [23]. The presence of BDE47 in the extract was evident, Fig. 4. PBDEs concentrations in spiked and non-spiked sub-samples of the dogfish liver material are given in Table 7. Values measured for spiked sub-samples were in agreement with the native concentrations plus the added ones.

Table 7
Determination of PBDEs ($n = 3$ replicates) in spiked and non-spiked dogfish liver

| Sample congener | Non-spiked (conc. ± S.D.) | Spiked (16 ng/g per congener) (conc. ± S.D.) |
|---|---|---|
| BDE47 | 2.4 ± 0.1 | 18.6 ± 1.2 |
| BDE100 | [a] | 16.6 ± 1.3 |
| BDE99 | [a] | 16.0 ± 1.5 |
| BDE85 | [b] | 14.9 ± 1.5 |
| BDE154 | [b] | 15.6 ± **1.5** |
| BDE153 | [b] | 16.8 ± 2.0 |

Concentrations in ng/g.
[a] Under quantification limit (0.40 ng/g).
[b] Under detection limit (0.15 ng/g).

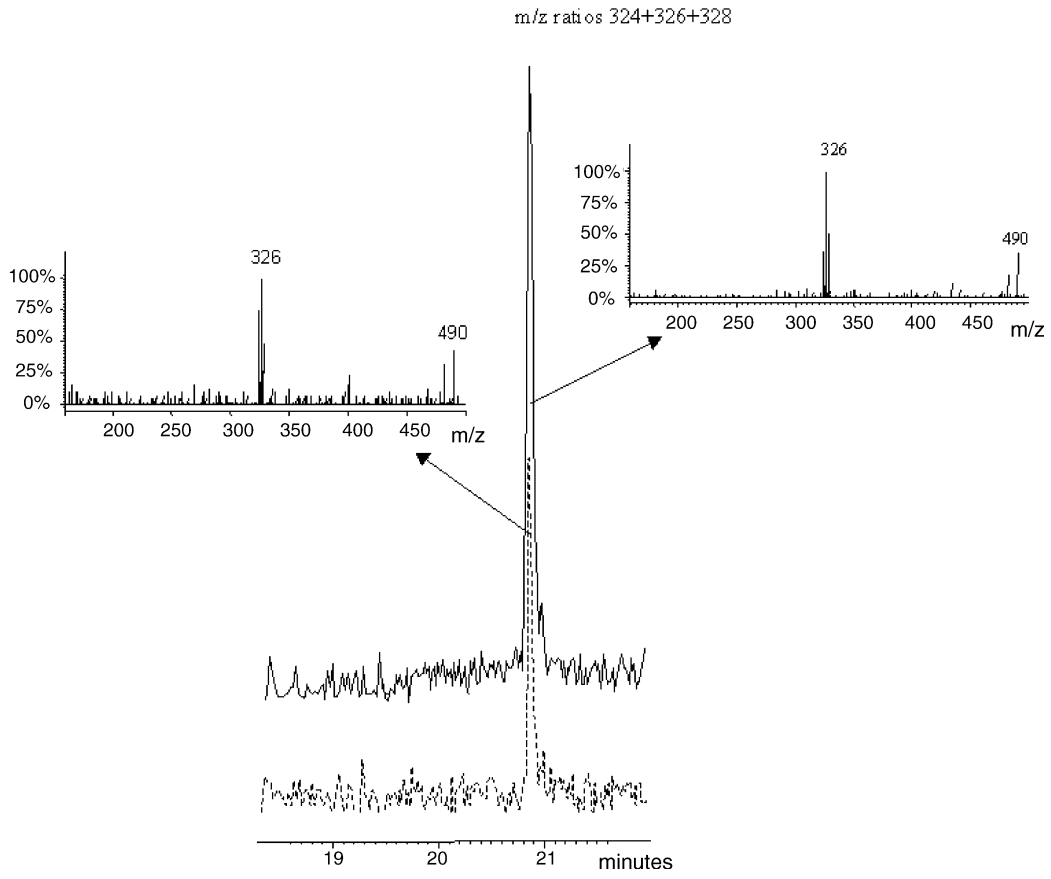


Fig. 4. GC-MS-MS chromatograms for a standard of BDE47 at 10 ng/ml (solid line) and the *n*-hexane–dichloromethane fraction of a non-spiked dogfish liver sample (dotted line).

## 4. Conclusions

A low cost method for the screening of tetra-, penta- and hexa-BDEs, as well as seven non-coplanar indicative PCBs (from tri- to deca-congeners), in biological samples has been optimised. MSPD allowed the extraction of the analytes from biota samples and the simultaneous removal of lipids. Therefore, a GC analysable extract was obtained in a single step with a low consumption of organic solvents. The screening of PBDEs in biota samples, using GC-ECD, requires their previous separation from PCBs. This can be achieved using 2 g of neutral silica. The proposed method showed recoveries higher than 75% for all considered compounds in samples with different fat contents; moreover, it allowed the determination of low PBDEs concentrations in matrices heavily polluted with complex PCBs mixtures. As presented, the method is useful for the fast screening of PCBs and PBDEs in biota samples. However, the reliable determination of a particular CB congener in the *n*-hexane fraction requires the use of a more efficient column.

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