

Horizontal Standardisation of Brominated Flame Retardants (BFRs)

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Analysis of Polybrominated Diphenyl Ethers (PBDEs) in soils and
sewage sludge

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants. They are measured in several matrices and for environmental purposes it is necessary that the methods applied in these matrices are comparable and making use of the same principles and instrumentation. The project HORIZONTAL has been started to develop horizontal and harmonised European standards in the field of sludge, soil and contaminated soil. The suggested method is here described. However, depending on the properties of the matrix, different or slightly different steps in the method can be necessary. In this work we describe the methods applied on soils and sewage sludge.

Soils

6 PBDE congeners, from tetra- to hepta-BDEs are included in the analytical work: one tetraBDE (BDE-47), two pentaBDEs (BDE-99, BDE-100), two hexaBDEs (BDE-153, BDE-154) and one heptaBDE (BDE-183).

Pre-treatment

Samples are transferred to the laboratory at a temperature of 4°C, and then frozen at -20°C before being frozen dried. The lyophilised samples are ground and homogenized by sieving and stored in sealed containers at -20°C until analysis. Before extraction, surrogate standard are added to 1.0 g of dry weight sample. Spiked samples are kept over night to equilibrate.

Extraction and Clean-up

Method A: Soxhlet extraction+ SPE clean-up

Soxhlet extraction was accomplished in cellulose thimbles containing 1 g of soil. 2 g of copper were added to soil to remove sulfur interference. Extraction was done using 75 mL of a mixture of hexane:dichloromethane (1:1) for 24 h. In order to test the efficiency of the Soxhlet extraction, a subsequent extraction was performed. After extraction, the extracts and the rinses of the Soxhlet were combined, concentrated to a few mL by rotary evaporation and then subjected to the SPE clean-up procedure.

Five grams alumina SPE cartridges were used. SPE cartridges were conditioned with 20 mL hexane. The sample volume loaded was ~ 1 mL, and the elution step was performed with 30 mL hexane:dichloromethane (1:2). Samples were finally concentrated to incipient dryness and

re-dissolved with the recovery standard (PCB-209) prior to the analysis by GC-NCI-MS.

Method B: Selective Pressurized liquid extraction (PLE)

PLE was carried out using a fully automated ASE 200 system (Dionex, Sunnyvale, CA, USA). Alumina was selected as sorbent in the extraction cell. A 22 mL extraction cell was loaded by inserting two cellulose filters into the cell outlet, followed by 6 g of alumina. Spiked soil samples of 1 g were ground with alumina (2 g) and cooper (2 g). The mixture was loaded into the extraction cell on top of alumina. The dead volume was filled with Hydromatrix, and the cell was sealed with the top cell cap. The extraction cell was filled with hexane:dichloromethane (1:1) mixture until the pressure reached 1500 psi, and heated to 100°C. After an oven heat-up time of 5 min. under these conditions, two static extractions of 10 min. at constant pressure and temperature were developed. After this static period, fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. The flush volume amounted to 100% of the extraction cell. The extraction was cycled twice. The volume of the resulting extract was about 35 mL. Samples were finally concentrated to incipient dryness and re-dissolved with the recovery standard (PCB-209) prior to the analysis by GC-NCI-MS.

Gas chromatography-mass spectrometry

GC-NCI-MS analyses are performed on a gas chromatograph Agilent 6890 connected to a mass spectrometer Agilent 5973 Network (Agilent). A HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) containing 5% phenyl methyl siloxane (model HP 19091S-433) capillary column is used for the determination of congeners from of tetra- to heptaBDEs. The temperature program is from 110°C (hold for 1 min) to 180°C (hold for 1 min.) at 8°C/min., then from 180°C to 240°C (hold for 5 min) at 2°C/min., and then from 240°C to 265°C (hold for 6 min) at 2°C/min., using the splitless injection mode during 1 min. The operating conditions are as follow: ion source temperature = 250°C, ammonia as chemical ionisation moderating gas at an ion source pressure of $1.9 \cdot 10^{-4}$ torr. The injection volume was 2 µL, injector temperature = 275°C and the interface temperature = 250°C. The experiments are carried out monitoring the two most abundant isotope peaks from the mass spectra corresponding to $m/z = 79$ and 81 ($[Br]^-$).

Confirmation criteria for the detection and quantification of PBDEs should include the following: (i) retention time for all m/z monitored for a given analyte should maximize

simultaneously ± 1 s, with signal to noise ratio ≥ 3 for each; (ii) the ratio between the two monitored ions should be within 15% of the theoretical. Quantification of tetra- to hepta-BDEs are carried out by internal standard procedure with the PCB-209 as internal standard.

Results

Table 1 show the mean values as well as the associated relative standard deviation of the two methods studied. Recoveries obtained using the optimized SPLE method ranged from 53 to 75%, whereas those obtained by conventional method were in the range of 61 and 82%. In general, similar results were obtained using both systems. As regards relative standard deviations, the values obtained for the SPLE method were lower than those obtained using the conventional method. The low standard deviations obtained with SPLE method could be the result of the automation of the system.

Table 1. PBDE recoveries obtained using the conventional Soxhlet-SPE method as well as the optimized SPLE method.

	Soxhlet-SPE method		SPLE method	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
Tetra-BDE-47	81	4	74	2
Penta-BDE-100	70	8	60	1
Penta-BDE-99	82	9	75	2
Hexa-BDE-154	67	9	61	3
Hexa-BDE-153	63	13	68	5
Hepta-BDE-183	61	12	53	10

The method detection limits (LOD) obtained using SPLE followed by GC-NCI-MS in SIM mode are shown in Table 2. The values were compared with those obtained using the conventional Soxhlet-SPE-GC-NCI-MS method. The LODs were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyses of a spiked soil, and on minimal value of signal-to-noise ratio of 3. The LOD obtained with the developed method were between 8 and 36 pg/g, showing similar values to those found for Soxhlet-SPE followed by GC-NCI-MS method (from 8 to 31 pg/g).

Table 2. PBDE detection limits (expressed as pg/g) obtained by SPLE-GC-NCI-MS and Soxhlet-SPE-GC-NCI-MS.

	Soxhlet-SPE method		SPLE method	
	LOD	LOQ	LOD	LOQ
Tetra-BDE-47	13	42	15	50
Penta-BDE-100	22	72	15	51
Penta-BDE-99	8	26	31	103
Hexa-BDE-154	36	121	8	25
Hexa-BDE-153	13	43	11	35
Hepta-BDE-183	15	48	13	43

Sewage Sludge

10 PBDE congeners, from tetra- to deca-BDEs are included in the analytical work: one tetraBDE (BDE-47), two pentaBDEs (BDE-99, BDE-100), two hexaBDEs (BDE-153, BDE-154), one heptaBDE (BDE-183), three octaBDEs (BDE-196, BDE-197, BDE-203) and the decaBDE (BDE-209).

Pre-treatment

Samples are transferred to the laboratory at a temperature of 4°C, and then frozen at -20°C before being frozen dried. The lyophilised samples are ground and homogenized by sieving and stored in sealed containers at -20°C until analysis. Before extraction, surrogate standard are added to 1.0 g of dry weight sample. Spiked samples are kept over night to equilibrate.

Extraction and Clean-up

Optimization of Sample amount and SPE cartridge

Soxhlet extraction was accomplished in cellulose thimbles containing 0.5 g or 1.0 g of sewage sludge. To remove sulfur interference 1.0 g and 2.0 g of copper are added to sewage sludge 0.5 g and 1.0 g, respectively. Extractions were done using 100 mL of a mixture of hexane:dichloromethane (1:1) for 24 h. After extraction, the extracts and the rinses of the Soxhlet are combined and then subjected to the clean-up procedure.

Both 0.5 g and 1.0 g sewage sludge samples extracts were treated with concentrated sulfuric acid (2×25 mL) in separatory funnels and subsequently purified with two and five grams of alumina cartridges. SPE cartridges are conditioned with 20 mL hexane. The sample volume loaded are ~ 1 mL, and the elution step is performed with 30 mL hexane:dichloromethane (1:2). Samples were finally concentrated to incipient dryness and re-dissolved with the recovery standards (PCB-209 and 4'-chloro-2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether (Cl-BDE-208)) prior to the analysis by GC-NCI-MS.

Optimization of Acid treatment procedure

Soxhlet extraction was accomplished in cellulose thimbles containing 1.0 g of sewage sludge. Two g of copper were added to sewage sludge. Extractions were done using 100 mL of a mixture of hexane:dichloromethane (1:1) for 24 h. After extraction, the extracts and the rinses of the Soxhlet are combined and then subjected to the clean-up procedure.

The volumes Soxhlet extracts were reduced to 10 ml and treated with concentrated sulfuric acid (2×10 mL) in centrifugations tubes. The organic layers were combined and further cleaned up on a column containing activated silica impregnated with conc. sulfuric acid (1 g, 2:1, w/w) by elution with 15 ml of dichloromethane:hexane (1:1). Subsequently the extracts were purified on five grams of alumina cartridges as described above. Samples were finally concentrated to incipient dryness and re-dissolved with the recovery standards (PCB-209 and 4'-chloro-2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether (Cl-BDE-208)) prior to the analysis by GC-NCI-MS.

Gas chromatography-mass spectrometry

GC-NCI-MS analyses are performed on a gas chromatograph Agilent 6890 connected to a mass spectrometer Agilent 5973 Network (Agilent). A HP-5MS (30 m \times 0.25 mm i.d., 0.25 μ m film thickness) containing 5% phenyl methyl siloxane (model HP 19091S-433) capillary column is used for the determination of congeners from of tetra- to heptaBDEs. The temperature program is from 110°C (hold for 1 min) to 180°C (hold for 1 min.) at 8°C/min., then from 180°C to 240°C (hold for 5 min) at 2°C/min., and then from 240°C to 265°C (hold for 6 min) at 2°C/min., using the splitless injection mode during 1 min. The operating conditions are as follow: ion source temperature = 250°C, ammonia as chemical ionisation moderating gas at an ion source pressure of 1.9×10^{-4} torr. The injection volume was 2 μ L,

injector temperature = 275°C and the interface temperature = 250°C. The experiments are carried out monitoring the two most abundant isotope peaks from the mass spectra corresponding to $m/z = 79$ and 81 ($[Br]^-$).

For octa- to decaBDEs, a DB-5MS (15 m × 0.25 mm i.d., 0.10 µm film thickness) containing 5% phenyl methyl siloxane capillary column is used with helium as the carrier gas at 6 psi. The temperature program is from 140°C (hold for 2 min.) to 325°C (hold for 10 min.) at 10°C/min., using the splitless injection mode during 1 min. The interface temperature was set at 270°C on these 15 m column.

Confirmation criteria for the detection and quantification of PBDEs should include the following: (i) retention time for all m/z monitored for a given analyte should maximize simultaneously ± 1 s, with signal to noise ratio ≥ 3 for each; (ii) the ratio between the two monitored ions should be within 15% of the theoretical. Quantification of tetra- to hepta-BDEs are carried out by internal standard procedure with the PCB-209 as internal standard, whereas Cl-BDE-208 was used as internal standard for octa- to deca-BDEs.

Results

Optimization of Sample amount and SPE cartridge

Table 3 show the mean values as well as the associated relative standard deviation of the different tests carried out for the optimization of sample amount as well as SPE cartridge. The best recoveries were obtained using 1.0 g of sample amount, and with SPE Alumina cartridges of 5 g. For these conditions, recoveries ranged from 54 to 100%, being lower the values obtained for the high brominated compounds. As regards relative standard deviations, values were lower than 10%, with the exception of deca-BDE-209, which presented a 20% of RSD.

Optimization of Acid treatment procedure

Table 4 show the mean values as well as the associated relative standard deviation of the different tests carried out for the optimization of acid treatment procedure. Results obtained corresponded to 1.0 g of sample Soxhlet extracted and SPE Alumina cartridges of 5 g. The acid treatment in a separatory funnel was compared with acid treatment with centrifugation. Recoveries obtained using an acid treatment with centrifugation were higher; however, very high relative standard deviations were obtained, up to 38% for the deca-BDE-209.

Table 3. PBDE recoveries (mean and % RSD) obtained for the optimization of sample amount and SPE cartridge.

	0.5 g sample		1.0 g sample	
	SPE - 2 g (n=3)	SPE - 5 g (n=2)	SPE - 2 g (n=3)	SPE - 5 g (n=3)
Tetra-BDE-47	69 (8)	82 (7)	86 (7)	92 (3)
Penta-BDE-99	72 (5)	82 (7)	83 (6)	92 (5)
Penta-BDE-100	71 (5)	79 (7)	82 (6)	88 (5)
Hexa-BDE-153	73 (7)	82 (9)	82 (8)	91 (4)
Hexa-BDE-154	80 (6)	88 (6)	91 (7)	100 (5)
Hepta-BDE-183	77 (9)	93 (5)	86 (6)	94 (5)
Octa-BDE-196	59 (9)	65 (3)	60 (8)	62 (6)
Octa-BDE-197	79 (12)	86 (5)	75 (7)	75 (3)
Octa-BDE-203	74 (7)	65 (1)	77 (6)	61 (9)
Deca-BDE-209	16 (19)	29 (9)	52 (26)	54 (20)

Table 4. PBDE recoveries (mean and % RSD) obtained for the optimization of acid treatment procedure.

	Separatory funnel	Centrifugation
Tetra-BDE-47	92 (3)	139 (8)
Penta-BDE-99	92 (5)	116 (3)
Penta-BDE-100	88 (5)	106 (4)
Hexa-BDE-153	91 (4)	94 (5)
Hexa-BDE-154	100 (5)	105 (11)
Hepta-BDE-183	94 (5)	101 (6)
Octa-BDE-196	62 (6)	82 (18)
Octa-BDE-197	75 (3)	93 (27)
Octa-BDE-203	61 (9)	91 (27)
Deca-BDE-209	54 (20)	89 (38)

The method detection limits (LOD) obtained using Soxhlet extraction (1.0 g of sample) followed by acid treatment in a separatory funnel, followed by SPE cartridges (Alumina, 5g.) and GC-NCI-MS in SIM mode are shown in Table 5. The LODs were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyses of spiked sewage sludge, and on minimal value of signal-to-noise ratio of 3. The LOD obtained with the developed method were between 30 and 1230 pg/g.

Table 5. PBDE detection limits (expressed as pg/g) obtained by Soxhlet-Acid treatment (separatory funnel)-SPE-GC-NCI-MS.

	LOD	LOQ
Tetra-BDE-47	30	90
Penta-BDE-100	180	610
Penta-BDE-99	230	760
Hexa-BDE-154	170	570
Hexa-BDE-153	180	610
Hepta-BDE-183	380	1280
Octa-BDE-196	411	1371
Octa-BDE-197	197	656
Octa-BDE-203	336	1121
Deca-BDE-209	1230	4100