A SIMPLIFIED METHOD FOR ANALYZING POLYBROMINATED DIPHENYL ETHERS IN SOIL USING ACCELERATED SOLVENT EXTRACTION TECHNIQUE

Choi KK, Wong YL

Government Laboratory, 7/F, Ho Man Tin Government Offices, 88 Chung Hau Street, Ho Man Tin, Kowloon, Hong Kong Special Administrative Region.

Introduction

Polybrominated diphenyl ethers (PBDEs) are persistent and bioaccumulative compounds used as fire retardants in polyurethane foams, plastics, and textiles. Common products that contain PBDEs include furniture cushions, carpet backings, electrical insulation, and computer and television casings.[1] These chemicals are structurally similar to polychlorinated biphenyls (PCBs), which are known as neurotoxicants. The three most widely used PBDEs are penta-BDE, octa-BDE, and deca-BDE.[2] PBDEs are not chemically bound to foam or plastic and can be released into the environment. PBDEs were first discovered in the environment in 1979 and their presence today is found around the world.[3] Owing to the widespread use of PBDEs as flame-retardants, they have been found throughout the environment, in animals and humans. With the increasing concern on PBDEs by environmentalists and government bodies, monitoring of this class of compounds has become a timely topic. The Government Laboratory, HKSAR had received analytical requests for PBDEs in soil samples in past year. The determinations were performed by high resolution gas chromatography – high resolution mass spectrometry (HRGC-HRMS) using analytical method referenced to Draft USEPA Method 1614[4] and Draft International Standard ISO/DIS 22032.[5] Very recently, we had further reviewed our in-house method for the analysis of PBDEs in soil samples. The studies mainly focused on optimization of capillary columns and GC temperature program for the separation of the 27 interested PBDE isomers, their elution order and profile in florisil, multi-layers silica gel and activated alumina column chromatographic clean-up, and comparison of extraction efficiency using conventional Soxhlet extraction and accelerated solvent extraction (ASE) techniques. Details of these findings are discussed in this article.

Materials and Methods

An inter-laboratory proficiency test soil sample (SETOC 764) was used throughout the study. The sample was used as received. Native PBDEs and their ^{13}C-labelled internal standards were purchased from Wellington Laboratories Inc. Silica gel and alumina were activated at 550 °C for at least 8 hours. All solvents used were of analytical grade and distilled before used. Sample extractions were conducted using Soxhlet apparatus or DIONEX ASE 200 accelerated solvent extractor. GC-MS analysis was carried out using SIM mode with Agilent 6890N gas chromatograph / Agilent 5973 mass spectrometer or Finnigan MAT95S HRMS equipped with a Hewlett-Packard 5890 gas chromatograph.

The soil sample (SETOC 764) was spiked with 100 μL native PBDEs mixed standard at concentrations ranging from 1.0 – 5.0 ng/μL. Sample extraction was either conducted on an accelerated solvent extractor (ASE) using dichloromethane (DCM) or Soxhlet extraction setup using DCM or toluene as extraction solvent. The extract was concentrated to about 2 mL using a rotary evaporator. The concentrated extract was ready for column chromatographic clean-up. Details of column packing materials, elution volume and collection fractions are summarized in Table 1. The collected fractions were combined, concentrated, made up to small volume and added with recovery standard {[$^{13}C_{12}$]-PBDE isomer 138}. The extract was ready for GC-MS analysis. Details of instrumental setup are listed in Table 2.

Results and Discussion

It is well recognized that Soxhlet extraction is one of the classical and reliable extraction techniques for solid samples such as soil, sediment and biota. However, this technique requires relatively long extraction time (normally ≥ 16 hours) and very much depends on the solvent properties, e.g. boiling point and solvent polarity. We found that a simplified and fast extraction technique – accelerated solvent extraction (ASE) – could be employed for the PBDEs analysis of soil samples using DCM as extraction solvent. The technique involved the use of a short-bed florisil (~ 3.5 g) packed under the soil sample (~ 1 g) inside the extraction cell (11 cm), that functioned as a pre-cleaning step during the extraction process. This would further increase column clean-up efficiency of the later steps using silica gel and activated alumina since some interferences due to polar compounds / matrix impurities might be removed in this step. Total run time for each extraction cycle is approximately 15 min (Table 3), which is significantly shorter than the conventional method using Soxhlet extraction, and the said solvent extraction technique had shown comparable extraction efficiency. Similar approach had been employed for PCB analysis in soil / sediment in our laboratory for years. The results of the present study had shown promising evidence for the analysis of PBDEs in soil. Table 4 shows the comparison of recovery data of two different extraction techniques with different solvents. In summary, recovery data ranging from 81-103 % were obtained from ASE technique, which were found comparable to those using Soxhlet extraction.

Elution profile of PBDE isomers on column chromatographic clean-up is another area of our study. The elution profiles of the 27 PBDE isomers using florisil, multi-layer silica gel (from top to bottom: neutral, acidic, neutral basic and neutral) and activated alumina were extensively studied either in individual or combined conditions. Table 5 summarizes the results of elution order and spectra of the 8 target PBDE isomers (**28**, **47**, **99**, **100**, **153**, **154**, **183** and **209**). It is worth noting that these PBDE isomers cover a relatively wider elution spectra than the seventeen 2,3,7,8-chlorine substituted dibenzo-*p*-dioxins and dibenzofurans (dioxins) and the 12 dioxin-like PCB congeners. The optimized chromatographic clean-up condition using florisil, and combined multi-layers silica gel / activated alumina columns is summarized in Table 1.

For the separation of PBDE isomers, there is a requirement of baseline separation between tetra-BDE isomers 49 and 71 in Draft USEPA Method 1614. In our studies, a GC temperature program using DB-1 (30 m, 0.32 mm i.d., 0.25 μm) capillary column for the separation of 27 PBDE isomers, namely 3, 7, 15, 17, **28**, **47**, 49, 66, 71, 77, 85, **99**, **100**, 119, 126, 138, **153**, **154**, 156, **183**, 184, 191, 196, 197, 206, 207 and **209**, was successfully established. Baseline separations for ALL these isomers were achieved. Some other columns such as DB-XLB (15m, 0.25mm i.d., 0.25μm) and DB-1701 (30m, 0.25mm i.d., 0.25μm) had also been investigated. However, separation of some of these isomers with the use of such columns was found not as satisfactory as that obtained from DB-1. Moreover, results obtained from DB-5MS (30m, 0.25mm i.d., 0.25μm) could achieve better separation of the two isomers (49 and 71) while compared with DB-1 and with shorter run time. Owning to column polarity, some isomers with high substituted number of bromo group, in particular for nona- and deca-bromo substituted PBDEs, were retained in the column while using DB-5MS, thus resulting in certain limitation on the applicability of this column.

Table 1 Optimized column chromatographic clean-up conditions for PBDEs analysis in soil samples

	Column Packing Material	Eluent	Elution Volume (mL)
Step 1	**Florisil** [#]		
	Portion 1	DCM	30
Step 2	**Silica Gel***		
	Portion 1	Hexane	50
	Activated Alumina*		
	Portion 2	5 % DCM in Hexane	20
	Portion 3	10 % DCM in Hexane	40
	Portion 4	40 % DCM in Hexane	40

Florisil column chromatographic clean-up is only applied on sample extract obtained from Soxhlet extraction.
* Multi-layers silica gel column is used [from top to bottom: neutral (1 cm), acidic (7 cm), neutral (0.5 cm), basic (3 cm) & neutral (0.5 cm)]. Activated alumina (8 cm) column is used. Dimensions of glass chromatographic columns for clean-up: 100 / 200 mm length, 8 mm i.d. with coarse-glass frit.
Portions 3 & 4 are combined together, rotary evaporated to small volume, concentrated to near dryness under a fine stream of nitrogen, and made up to certain volume for GC-MS analysis.

Table 2 GC and MS ion source conditions

Column	:	DB-1 (30m, 0.32mm i.d., 0.25μm film thickness)
Injector temperature	:	250 °C
Carrier gas, flow rate	:	He, 1.8 mL/min
Oven programming	:	100 °C (1 min) → 10 °C/min to 200 °C (2 min) → 5 °C/min to 300 °C (20 min) → 20 °C/min to 320 °C (5 min)
Injection mode	:	Splitless
Transfer line temperature	:	300 °C
Source temperature	:	230 °C
Electron energy	:	~ 70 eV

Remark: Under the GC conditions as specified above, baseline separations of 27 PBDE isomers were achieved.

Table 3 Operating conditions of the accelerated solvent extractor for the extraction of PBDE in soil samples

Oven temperature	:	100 °C
Pressure	:	2000 psi
Oven heat-up time	:	5 min
Static time	:	5 min
Flush volume	:	60 % cell volume
Nitrogen purge	:	60 sec at 150 psi
Static cycle	:	1

Table 4 Comparison table on recoveries studies using different extraction techniques (accelerated solvent extraction and Soxhlet extraction).

Entry	Isomer No.	Concentration (ng/μL)	Recovery (%)*		
			Accelerated Solvent Extraction [§]	Soxhlet Extraction[§,#]	Soxhlet Extraction[†,#]
1	28	1.0	86	91	80
2	47	1.0	92	99	84
3	99	1.0	100	114	97
4	100	1.0	91	94	59
5	153	2.0	99	105	90
6	154	2.0	81	85	46
7	183	2.0	102	104	92
8	209	5.0	103	116	89

* 100 μL PBDEs mixed native standard was spiked to a soil sample (SETOC 764). Clean-up procedures as described in Table 1 were employed.

§ Extraction solvent: dichloromethane
† Extraction solvent: toluene
# Extraction details: 16 hours at 4 cycles per hour

Table 5 Elution profile of the 8 target PBDE isomers in soil using the chromatographic condition as specified in Table 1

Isomer No.	P1	P2	P3-1	P3-2	P3-3	P3-4	P3-5	P3-6	P3-7	P3-8	P4-1	P4-2	P4-3
28	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	–
47	–	–	–	✓	✓	✓	✓	✓	–	–	–	–	–
99	–	–	–	✓	✓	✓	–	–	–	–	–	–	–
100	–	–	✓	✓	✓	✓	–	–	–	–	–	–	–
153	–	–	–	✓	✓	✓	–	–	–	–	–	–	–
154	–	–	✓	✓	✓	–	–	–	–	–	–	–	–
183	–	–	–	✓	✓	✓	✓	–	–	–	–	–	–
209	–	–	–	–	–	–	–	–	✓	✓	✓	–	–

Notes:
P1 denotes the portion obtained using hexane (50 mL) as eluent.
P2 denotes the portion obtained using 5 % DCM in hexane (20 mL) as eluent.
P3 denotes the portion obtained using 10 % DCM in hexane as eluent collected at 5 mL each portion.
P4 denotes the portion obtained using 40 % DCM in hexane as eluent collected at 10 mL each portion.
✓ means that the analyte was present in the specified portion.

Acknowledgements

The authors are grateful to Dr. T. L. Ting, the Government Chemist of HKSAR, for his encouragement and kind support of this work.

The contents of this paper do not necessarily reflect the views of the Government of the Hong Kong Special Administrative Region, nor does mention of trade names or commercial products constitute endorsement or recommendations of use.

References

1. Health Canada Fact Sheet – PBDEs, May 2004.
2. McDonald TA. *Chemosphere* 2002;46:745.
3. Lahey A. *Canadian Geographic* 2003;123:88.
4. Draft EPA Method 1614, August 2003.
5. Draft International Standard ISO/DIS 22032.