Pressurized Liquid Extraction for the Simultaneous Analysis of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers from Soil by GC–TOF-MS Detection

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Abstract

Pressurized liquid extraction (PLE) system was optimized to simultaneously determine polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in soil samples by gas chromatography with time-of-flight mass spectrometry. PLE parameters (temperature, pressure, static time, and flush volume) and packing materials (activated copper and sorbents such as: Florisil, silica gel, and a combination of Florisil and silica gel) were studied to achieve a one-step extraction and cleanup that could be analytically rapid and reliable. Method detection limit was found to be in the range of 0.1–0.4 mg/kg for PCBs and 0.1–0.6 mg/kg for PBDEs with a relative standard deviation of 1.7–7.3% for PCBs and 2.6–6.3% for PBDEs. A standard reference material for PCBs, NIST-SRM 1939a, spiked with PBDEs standard, was analyzed to substantiate the validity of the optimized method. Experimental values agreed well with the certified values with recoveries of 71.6–117%, and the optimized PLE system has been proven to be useful for the simultaneous determination of PCBs and PBDEs with various congeners in soil samples.

Introduction

A great deal of attention has been focused on the monitoring and evaluation of dioxins and dioxin-like compounds in different environmental samples because of the health impacts that they pose (1,2). Among the dioxin-like compounds, polychlorinated biphenyls (PCBs) are reported to cause a wide variety of health effects, even at very low exposure levels. Although their production and use has stopped since 1977, studies show that they are still detected in the environment (3). On the other hand, conversely to what is observed for most anthropogenic organic pollutants, levels of polybrominated diphenyl ethers (PBDEs) in the environment have incessantly increased worldwide in the last 20 years, sometimes being higher than PCB levels (1,4,5). PBDEs are used in large quantities as flame-retardant additives in polymers, especially in the manufacturing of a great variety of electrical appliances, including televisions and computers, building materials, and textiles (6). Moreover, data suggest that lower PBDE congeners (tetra- to hexa-) are likely to be carcinogens, endocrine disrupters, and/or neurodevelopment toxicants (4). Thus, PBDEs have become a cause of growing concern nowadays, and more and more laboratories have to offer the capability to analyze this class of contaminant, which has begun to be included in monitoring programs (5,7).

PCBs and PBDEs exhibit similar behaviors due to the similarity in their chemical structure (8,9). They tend to persist in the environment. And because they are semivolatile and chemically stable, their resistance to biodegradation and photolysis has resulted in a "global distillation" and redistribution through the atmosphere. Transport of these compounds to the poles is thought to involve the cyclic process of wet/dry deposition and sublimation or evaporation combined with the net atmospheric flux of heat from equatorial regions (10). They tend to adsorb onto solid particles of soil and sediments. As a matter of fact, studies revealed that these compounds were detected in these matrices (4). Furthermore, PCBs and PBDEs have also been detected in meat, fish, sperm whale blubber, office air, and human blood (3,11,12). Even more, the existence of a correlation between the concentrations of PCBs and PBDEs in certain samples has been proposed (12).

Because these two groups of pollutants are ubiquitous, it is likely that they coexist in most environmental matrices (6). Therefore, simultaneous monitoring of their levels in environmental samples is highly desired in order to protect human health (11–13). A few studies were published regarding the simultaneous analysis of these toxic chemicals in a single sample (5), but none of them had completely separated PBDEs from PCBs, although it is known that they may interfere with each other (13). Most of the available methods nowadays involve two or

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three individual sample preparation procedures followed by instrumental analysis, which involves an enormous amount of time and resources (2). Therefore, a new method that will simplify the existing methodologies without sacrificing the quality of results would be advantageous. A procedure that would simultaneously determine these pollutants with minimal time and resources consumed will be very ideal, although this is not an easy task. The method for the determination of PBDEs and PCBs consists of three basic steps: extraction, cleanup, and selective determination by gas chromatographic techniques (12). Extractions are traditionally performed by means of Soxhlet or sonication. But these techniques demand large amounts of highly purified and hazardous organic solvents generating dirty extracts that require extensive cleanup steps before analysis (5,8,12). Moreover, Alaee et al. reported the possible coelution of PCBs and PBDEs, especially when using 5% diphenyls–95% dimethylsiloxane-type columns. It was noted that PCB180/PBDE 47, PCB 194/PBDE 120, and PCB 209/PBDE 85 coelutes (14). Thus, several measures are recommended to solve this problem. PBDEs were reported to be separated from PCBs in biological samples using matrix solid-phase dispersion (MSPD) followed by adsorption chromatography using silica (12). Liu et al. used multilayer silica gel chromatography to obtain three different fractions separating PCBs, PBDEs, and polychlorinated dibenzo-p-dioxins, and dibenzo-furans (PCDD/Fs) in environmental samples (13). A multi-step automated cleanup following extraction was also employed to separate PCBs, PBDEs and PCDD/Fs in fatty matrices (2). In all these cases, the sample preparation entails several steps before actual analysis. As a consequence, there has been an increasing demand for new technologies such as microwave assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE) that overcome these problems. Though MAE allows fast and multiple extractions, it also requires long cooldown periods and filtration to separate extracts from the solid materials once extraction is finished, whereas SFE is matrix dependent (15). PLE uses conventional organic solvents at high temperatures and pressure to extract solid samples rapidly using a small amount of solvent. By pressurizing the sample cell while using high temperature, solvents are maintained at their liquid state while in contact with the sample matrix. Moreover, elevated temperatures increase the solubility, diffusion rate, and mass transfer, which lowers viscosity and surface tension. This is probably the most important advantage of PLE in comparison to other conventional methods (16–18).

Many scientists have successfully used PLE for the isolation of different substances. PCBs have been successfully extracted using PLE in biota, soil, and sediment samples (16). Certain studies also indicated the use of PLE in the extraction of PBDEs in sediment samples (8). Although some works reported the direct analysis of PLE extracts without further cleanup, injection of crude extracts result in the deterioration of the chromatographic column and can give negative effects during the final analysis like matrix enhancement effect and coelution of analytes and interferents (16). Thus, there is a need to adapt a corrective measure to settle these problems. The use of sorbents in the PLE gives an encouraging possibility to solve this problem. Copper and alumina have been reported to be used in PLE for the extraction of PBDEs in sediment giving a clean extract without further cleanup (8). Gomez-Ariza et al. reported the use of Florisil as a sorbent in PLE analysis of PCBs in various biota samples (15). Alumina was also used as a retainer sorbent (19). Sulfuric acid impregnated silica gel was also used in the PLE cell (15).

In this study, we aim to propose a method for the simultaneous determination of PCBs and PBDEs in soil samples using a one-step extraction and cleanup procedure. It is also an objective to reliably quantify PCBs and PBDEs that were reported to coelute (i.e, PCB No.180 and PBDE No. 47; PCB No. 194 and PBDE No.100). Sixty-two PCB congeners from mono to decachlorobiphenyls, including the seven indicator PCBs (IUPAC Nos. 28, 52, 101, 118, 138, 153, and 180), and 24 PBDE congeners from mono to octabromodiphenyl ethers, including the seven PBDE congeners, which are usually monitored in most epidemiological studies (IUPAC Nos. 28, 47, 66, 99, 100, 153, and 154) (5) were separated. Although high-resolution mass spectrometry (HRMS) with a resolution of 10,000 is the method of choice in the analysis of dioxin and dioxin-like compounds (1), gas chromatography time-of-flight mass spectrometry (GC–TOF-MS) was used in this study. TOF-MS has shown capabilities in the analysis of PCBs in different typex of systems. It gives comparable results with high-resolution mass spectrometry (HRMS), which is the method of choice in the analysis of dioxin and dioxin-like compounds. Results were found to be acceptable and showed that TOF-MS allows analysis times that are at least an order of magnitude faster than HRMS without loss of qualitative and quantitative power. Thus, to amplify the rapidity in the analysis, GC–TOF-MS was utilized throughout the course of this investigation. NIST-SRM 1939a, a sediment CRM for PCBs, spiked with PBDE standards was used for the validation study.

The power offered by PLE and the feasibility of using different solvents and sorbent systems in the sample cell in combination with the fast sample analysis using GC–TOF-MS were explored to come up with a one-step extraction and cleanup method that is analytically acceptable as well as time- and resource-saving.

Materials and Methods

Reagents and samples

PCB standards (BP-MS), Carbon 13 (C13)-labeled PCB standards (MBP-CG), PBDE standards (BDE-MXE), and Carbon 13 (C13)-labeled PBDE standards were from Wellington Laboratories (Ontario, Canada).

BP-MS is a mixture of PCB congeners ranging from mono- to decachlorobiphenyls in nonane. The concentration was 2 µg/mL for each congener. MBP-CG, 5 µg/mL in nonane, is composed of mono- to decachlorobiphenyls. MBP-CG serves as a surrogate standard.

BDE-MXE, a PBDE mix from mono- to decabromodiphenyl ethers in nonane–toluene (v/v, 1:1), was used as PBDE standard. The concentrations were as follows: 1 µg/mL for mono- to pentabromodiphenyl ethers, 2 µg/mL for hexa- to octabromodiphenyl ethers, and 5 µg/mL for nona- to decabromodiphenyl ethers. Difficulties in the preliminary studies involving the highly brominated nona- and decabromodiphenyl ethers were encoun-

tered. Nona- and decabromodiphenyl ethers were not detected even on the 5 µg/mL level. However, lower brominated PBDEs might be of greater ecotoxicological concern than highly brominated nona- and decabromodiphenyl ethers because these seem to be easily incorporated in organisms and food webs (5,20). Because of this reason, we proceeded with our study for lower brominated PBDEs without quantifying nona- and decabromodiphenyl ethers. MBDE-MXC, the C-13 labeled PBDEs in 5 µg/mL concentration, was used as surrogate standards for PBDEs. Internal standard, phenanthrene-d_{10} (Supelco, Bellefonte, PA) in the concentration of 2000 µg/mL in methanol was used.

Florisil (60–100 mesh) was purchased from J.T. Baker (Phillipsburg, NJ). It was activated at 130°C overnight prior to use. Silica gel (75–150 µm) was from WAKO (Osaka, Japan). It was heated at 130°C for 16 h after which it was deactivated by adding 3.3% ultrapure water. Copper (Cu) powder was purchased from Yakuri (Kyoto, Japan). Cu oxides were removed by treating the Cu powder with dilute nitric acid followed by several rinsings of ultrapure water to remove all traces of acid. This step is followed by rinsing of acetone and drying under a stream of nitrogen. Sulfur powder was purchased from Yoneyama (Osaka, Japan). Diatomaceous Earth (DE), which is used to remove water, was from J.T. Baker.

Hexane (ultra-resi analyzed), acetone, and methylene chloride (ultra-resi analyzed) were purchased from J.T. Baker. Nitrogen gas used for all drying purposes was 99.9% pure. Ultrapure water was obtained from Milli-Q water purification system (Millipore, Molsheim, France).

The standard reference material, SRM 1939a, river sediment, was acquired from the National Institute of Standards and Technology (NIST) for Reference Materials and Measurements (Gaithersburg, MD).

Soil used throughout the experiment was air-dried, ground,

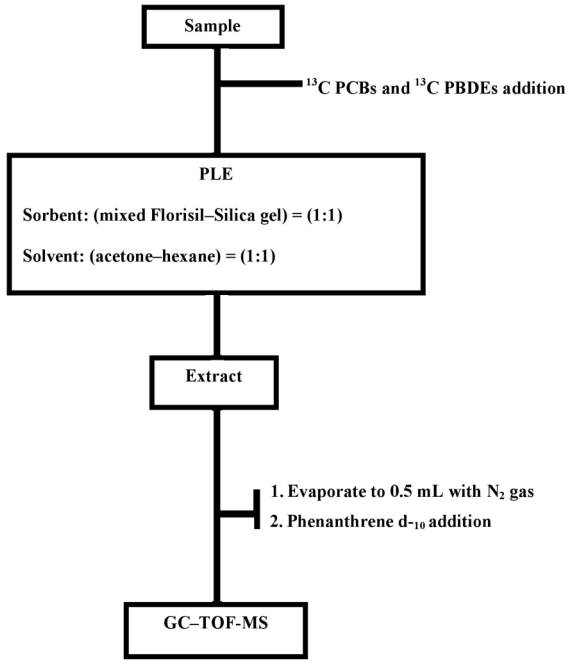

Figure 1. Scheme for the simultaneous analysis of PCBs and PBDEs in soil or sediment sample using PLE.

Table I. Comparison of Extraction Efficiency for Different Sorbent-Solvent Systems

Compounds	Florisil Recoveries (%)			Silica Gel Recoveries (%)			Mixed Florisil-Silica Gel Recoveries (%)			Layered Florisil-Silica Gel Recoveries (%)		
	Hex*	Hex: DCM†	ACE: Hex‡	Hex	Hex: DCM	ACE: Hex	Hex	Hex: DCM	ACE: Hex	Hex	Hex: DCM	ACE: Hex
Monochlorobiphenyls	51.6	62.0	69.7	41.7	64.4	68.9	41.1	66.5	81.3	54.0	53.5	69.1
Dichlorobiphenyls	66.9	71.3	78.4	59.3	85.6	76.6	57.7	55.5	87.0	76.0	62.9	77.5
Trichlorobiphenyls	71.9	78.6	84.8	68.4	85.6	83.0	60.6	69.3	89.0	80.6	71.8	74.8
Tetrachlorobiphenyls	68.3	78.0	85.9	68.4	90.9	86.0	58.4	65.5	87.7	77.6	66.8	73.5
Pentachlorobiphenyls	67.0	75.5	87.5	72.7	98.9	92.9	65.1	70.5	92.9	82.6	72.0	77.9
Hexachlorobiphenyls	65.3	70.3	80.4	74.2	101.4	98.5	65.0	72.8	94.1	85.9	74.9	80.8
Heptachlorobiphenyls	61.6	67.2	75.8	73.4	102.6	97.0	65.5	69.9	95.4	81.7	68.6	78.3
Octachlorobiphenyls	58.3	65.4	69.6	75.0	105.7	98.5	86.0	73.0	88.7	72.4	61.3	67.2
Nonachlorobiphenyls	57.6	67.8	69.0	79.0	118.9	106.9	67.6	74.0	104.8	74.5	61.0	68.5
Decachlorobiphenyls	48.5	59.2	60.0	76.5	114.7	107.0	63.6	75.2	100.8	75.6	62.1	66.6
Monobromodiphenyl ethers	72.6	70.8	79.2	62.3	78.5	80.4	58.1	60.8	88.0	78.5	61.5	75.0
Dibromodiphenyl ethers	88.0	88.0	94.2	85.0	100.5	107.0	99.5	103.6	111.0	87.9	82.1	84.4
Tribromodiphenyl ethers	70.2	76.5	83.2	71.3	105.4	103.0	80.3	88.1	99.9	84.8	78.6	84.1
Tetrabromodiphenyl ethers	55.8	63.1	67.7	73.0	107.2	106.3	61.3	70.9	95.1	81.6	70.7	83.6
Pentabromodiphenyl ethers	49.5	58.8	62.9	69.0	107.2	103.5	55.5	64.9	91.4	76.7	63.6	76.9
Hexabromodiphenyl ethers	31.2	50.7	56.7	67.4	111.9	102.6	41.9	60.1	85.4	73.6	57.3	64.8
Heptabromodiphenyl ethers	28.2	49.3	58.0	68.9	110.9	100.4	33.3	54.1	81.3	71.9	47.1	52.1
Octabromodiphenyl ethers	18.7	62.6	76.6	79.1	109.8	102.9	29.0	55.7	90.0	58.5	33.9	34.4

* Hex = Hexane
† Hex:DCM = Hexane:Dichloromethane
‡ ACE:Hex = Acetone:Hexane

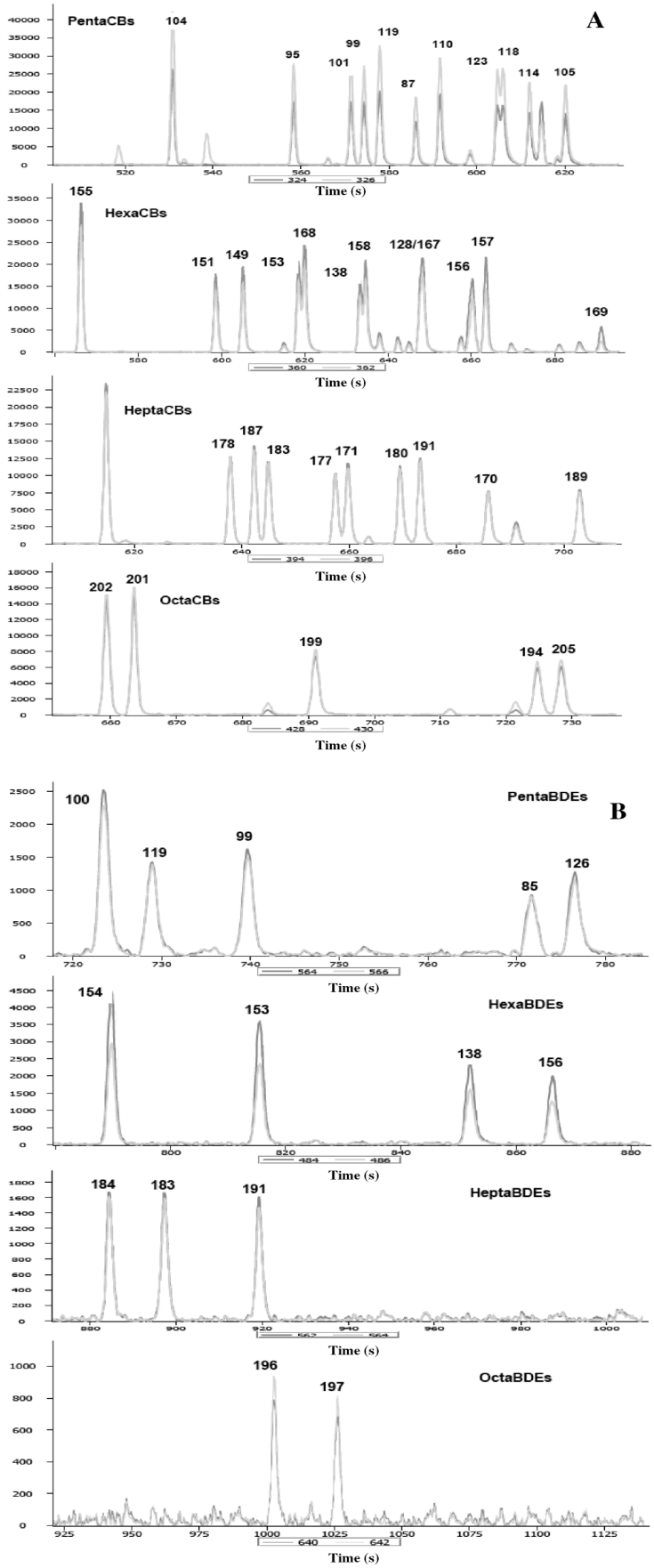

Figure 2. Representative chromatograms of PCBs using the optimized method. Quantification ion is represented by darker line; identification ion is represented by lighter line. PentaCBs = Pentachlorobiphenyls, HexaCBs = Hexachlorobiphenyls, etc. (A). Representative chromatograms of PBDEs using the optimized method. Quantification ion is represented by darker line; identification ion is represented by lighter line. PentaBDEs = Pentabromodiphenyl ethers, HexaBDEs = Hexabromodiphenyl ethers, etc. (B).

and sieved using Standard Testing Sieve # 35 with an aperture of 500 µm (Chung Gye Sang Sa, Seoul, Korea). These were consequently furnaced at 500ºC for at least 8 h and cooled at room temperature before using. Blank soil was analyzed for PCBs and PBDEs and no compound was found to be present, proving that the prepared soil (blank soil) is adequate for spiking experiments.

Chromatographic equipment and experimental conditions

GC was carried out using a 6890N Agilent gas chromatograph (Agilent Technologies, DE) using a DB-5 column (J&W Scientific) with dimensions of 10 m × 0.18 mm × 0.18-µm thickness. Helium (He) (99.9999% purity) at 0.8 mL/min was used as the carrier gas. The injector was operated in the pulsed splitless mode (0.1 MPa for 1.5 min.) at 280ºC. Oven temperature program used was as follows: 50ºC was initially set and held for 1 min, then raised to 250ºC at a rate of 20ºC/min, and finally raised to 300ºC at 10ºC/min and held for 10 min. Agilent 7683 autosampler was used and programmed to inject 2 µL of sample.

The GC system was attached to a Pegasus III TOF-MS (LECO, St. Joseph, MI). Mass spectra were obtained in the electron ionization mode (70 eV) in the range from 10–1000 *m/z*. The transfer line was heated to 280ºC while the ion source was set to 250ºC. Mass spectra were monitored using the extracted ion-monitoring mode. The acquisition rate of MS spectra was set at 10 spectra/s.

Pressurized liquid extraction system

The pressurized liquid extraction (PLE) system was ASE 200 from Dionex (Sunnyvale, CA). The PLE optimization conditions were done by extracting spiked soil samples. Ten grams of soil sample, spiked with 25 µL each of BP-MS and BDE-MXE, was homogeneously mixed with 2 grams of diatomeaceous earth (DE) throughout the experiment unless otherwise noted. Preliminary studies were done to select the best sorbent and solvent system that will give an acceptable soil extract. The 22-mL stainless PLE cell was prepared by placing a cellulose filter at the capped end of the cell, and then tightly packed following this order (from bottom to top): activated Cu powder, sorbent, cellulose filter, 10 grams of soil sample (spiked 25 µL each of BP-MS and BDE-MXE) with DE, and then cellulose filter. The merit of using 2 grams of activated copper powder for the elimination of interferents, primarily sulfur, in the soil was evaluated by comparing the total ion chromatograms (TIC) of samples which use copper powder in the cell and those which do not. Four grams each of Florisil and silica gel (1:1, w/w), mixture of Florisil and silica gel and (1:1, w/w) layered combination of Florisil and silica gel were tested for the optimization of sorbents. Different solvent systems were also tested in conjunction with these sorbents. Solvent systems assayed

were the following: hexane, hexane–methylene chloride (1:1, v/v), and hexane–acetone (1:1, v/v). In these investigations, the preliminary PLE conditions employed were: temperature of 100ºC, static time of 5 min (1 cycle), pressure of 1500 psi (10 MPa), flush volume of 60%, and purge time of 90 s.

The temperature parameter was tested at 60, 100, and 150ºC, respectively. Pressures of 1000, 1500, and 2000 psi (7, 10, and 14 MPa, respectively) were then evaluated next. Static time allowed for the solvent to reach the matrix pores. The shortest possible static time that can analytically extract the analyte is desirable. Five and 10 min were evaluated for this parameter. Also 20, 40, 60, 80, and 100% of the flush volume were finally tested.

After extraction, samples were concentrated to about 2 mL in a rotary evaporator. This was then transferred to a graduated test tube and concentrated to 0.5 mL by a stream of nitrogen. The internal standard was then added and finally injected in GC–TOF-MS. The final flow chart of the proposed methodology is depicted in Figure 1.

Results and Discussion

Extraction and cleanup

Elimination of interfering substances from the sample extract is the key to a successful organic trace analysis. Studies focused on obtaining a clean extract are very important to attain a low limit of quantitation, which was established by legislation for most toxic compounds and to protect the chromatographic system. In this vein, the advantage of using Cu powder in the PLE system was evaluated. The TICs of samples which uses Cu powder in the PLE cell revealed that an essential amount of sulfur was eliminated enough to correctly quantify PCBs and PBDEs simultaneously.

Florisil, silica gel, mixture of Florisil and silica gel (1:1, w/w) and lastly, layered Florisil and silica gel (1:1, w/w) were tested with different solvent systems to investigate their efficiency in extracting PCBs and PBDEs. Data revealed that mixed Florisil and silica gel with (1:1, v/v) acetone–hexane as the solvent

Table II. Method Validation Data for the PCB Congeners

PCB IUPAC #	Quantitation ion (*m/z*)	Correlation coefficient (R) *n* = 5	RSD (%)	MDL (ng/g) *n* = 7
1	Monochlorobiphenyl (*m/z* = 188)	0.9987	7.5	0.4
3		0.9975	10.1	0.5
10/4	Dichlorobiphenyl (*m/z* = 222)	0.9956	7.3	0.4
8		0.9991	7.2	0.3
15		0.9999	10.8	0.4
19	Trichlorobiphenyl (*m/z* = 256)	0.9999	3.5	0.2
18		0.9999	2.7	0.1
28		*0.9999*	*2.2*	*0.1*
33		0.9993	2.8	0.1
22		0.9994	2.3	0.1
37		0.9992	4.8	0.2
54	Tetrachlorobiphenyl (*m/z* = 290)	0.9999	2.6	0.1
52		*0.9999*	*0.9**	*0.09*
49		1.0000	2.3	0.1
44		0.9999	2.9	0.1
74		0.9993	4.1	0.2
70		0.9999	2.3	0.1
81		0.9913	13.2	0.6
77		0.9987	10.0	0.5
104	Pentachlorobiphenyl (*m/z* = 324)	0.9993	1.7	0.1
95		0.9993	3.0	0.1
101		*0.9987*	*2.1*	*0.1*
99		0.9998	3.6	0.2
119		0.9992	2.7	0.1
87		0.9993	7.5	0.3
110		0.9997	3.6	0.2
123		0.9996	6.2	0.3
118		*0.9997*	*3.2*	*0.1*
114		0.9980	3.7	0.2
105		0.9999	4.5	0.2
126		0.9993	5.2	0.3
155	Hexachlorobiphenyl (*m/z* = 360)	0.9996	3.0	0.1
151		1.0000	1.9	0.1
149		0.9994	1.1	0.0
153		*0.9956*	*1.0*	*0.1*
168		0.9998	1.1	0.1
138		*0.9998*	*9.0*	*0.4*
158		0.9992	2.9	0.1
128/167		0.9996	4.3	0.2
156		0.9994	5.2	0.3
157		0.9995	5.1	0.3
169		0.9998	4.6	0.2
188	Heptachlorobiphenyl (*m/z* = 394)	0.9999	2.7	0.1
178		0.9989	1.6	0.1
187		0.9998	2.7	0.1
183		0.9999	3.5	0.2
177		1.0000	2.1	0.1
171		1.0000	1.1	0.1
180		*0.9999*	*1.8*	*0.1*
191		0.9994	2.5	0.1
170		1.0000	5.3	0.3
189		1.0000	4.9	0.2
202	Octachlorobiphenyl (m/z = 428)	0.9998	2.2	0.1
201		0.9990	3.0	0.2
199		0.9996	2.0	0.1
194		0.9058	2.7	0.1
205		0.9978	3.1	0.1
208	Nonachlorobiphenyl (*m/z* = 462)	0.9997	2.6	0.1
206		0.9998	3.1	0.1
209	Decachlorobiphenyl (*m/z* = 498)	0.9997	2.1	0.1

* Data written in italics are part of the seven indicator PCBs. A 5-point calibration curve was generated in the range of 10–50 ng/g. MDLs were calculated using MDL = 1.943 SD (standard deviation)

system performed well in extracting the whole range of analytes (Table I). However, for this solvent-sorbent system, some analytes' recoveries exceed 100% suggesting that some unwanted contaminants, which have the similar polarities and mass spectra with the target analytes, might have coeluted with them. This observation was also reported by Dabrowski et al. (14). In our case, this phenomenon was most pronounced with the silica gel–hexane–dichloromethane solvent-sorbent system (Table I). And although this system gives a fairly good percentage of recovery, we did not choose this sorbent-solvent system because the percentage of recoveries for both monochlorobiphenyls and monobromodiphenyl ethers were lower than 80% (see Table I). Using percentage of recoveries as the criteria for the efficiency of extraction and the mixture of Florisil-silica gel with acetone–hexane as solvent was chosen to be used throughout the experiment. On the other hand, further investigation is required to establish the fact that mixed Florisil-silica gel performed much better than layered Florisil-silica gel.

Table III. Method Validation Data for the PBDE Congeners

PBDE IUPAC #	Quantitation ion (*m/z*)	Correlation coefficient (R) *n* = 5	RSD (%)	MDL (ng/g) *n* = 7
3	Monobromodiphenyl ether (*m/z* = 248)	0.9992	6.1	0.3
7	Dibromodiphenyl ether (*m/z* = 168)	0.9993	4.2	0.2
15		0.9976	5.3	0.3
17	Tribromodipheny lether (*m/z* = 246)	0.9994	4.1	0.2
28		*0.9995**	*2.6*	*0.1*
49	Tetrabromodiphenyl ether (*m/z* = 486)	0.9991	5.0	0.3
71		0.9984	5.0	0.3
47		*0.9983*	*2.3*	*0.1*
66		*0.9995*	*5.8*	*0.3*
77		0.9973	6.4	0.3
100	Pentabromodiphenyl ether (*m/z* = 564)	*0.9998*	*4.0*	*0.2*
119		0.9961	11.5	0.6
99		*0.9981*	*9.6*	*0.4*
85		0.9997	8.9	0.4
126		0.9981	8.4	0.3
154	Hexabromodiphenyl ether (*m/z* = 484)	*0.9968*	*5.9*	*0.6*
153		*0.9989*	*2.4*	*0.2*
138		0.9994	4.0	0.4
156		0.9998	5.8	0.6
184	Heptabromodiphenyl ether (*m/z* = 562)	0.9985	5.1	0.5
183		0.9974	2.2	0.2
191		0.9971	6.1	0.6
197	Octabromodiphenyl ether (*m/z* = 640)	0.9962	9.2	1.0
196		0.9962	5.7	0.6

*Data written in italics are part of the seven important PBDEs. A 5-point calibration curve was generated in the range of 20–200 ng/g. MDLs were calculated using MDL = 1.943 SD (standard deviation)

PLE

The parameters studied to extract PCBs and PBDEs simultaneously from soils were oven temperature, pressure, static time, and flush volume.

Three oven temperatures were evaluated: 60, 100, and 150ºC at a fixed pressure of 1500 psi (10 MPa). The oven temperatures tested were chosen from the literatures citing the use of PLE for extracting PCBs or PBDEs (4,8,14,16). 60ºC gave a slightly better result than 100ºC with recoveries ranging from 80.6–106.6% and 80.0–101.0%, respectively. A decrease in the recoveries for all analytes (31.6–94.9%), except for the mono- (103.4%) and diBDE (125.2%), was observed when the temperature was increased to 150ºC. An increase in temperature may have caused degradation of the analytes leading to low recoveries (16). On the other hand, the relationship between pressure and recoveries was established. Among the pressures studied: 1000, 1500, 2000 psi (7, 10, and 14 MPa respectively), 1500 psi (10 MPa) gave the highest recoveries (80.6–104.8%). A decrease in the recoveries (59.8–88.6%) when pressure was increased can be attributed to analyte degradation (16). The effect of extraction time indicated by the static time was also evaluated. A static time of 5 min gave percentage of recoveries in the range of 80.6–104.8%, while 10 min gave a percentage of recovery range from 71.3–98.9%.

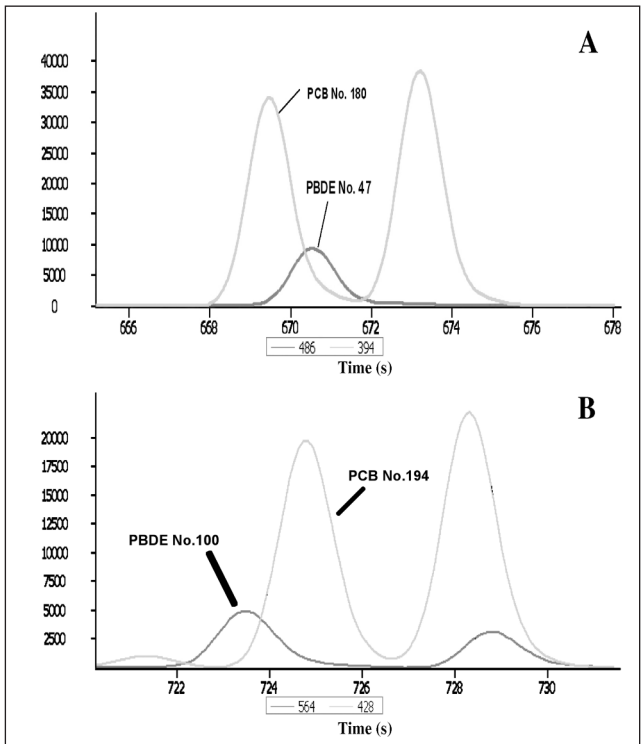

Figure 3. Chromatograms of PCB No. 180/PBDE No. 47, (A), and PCB No. 194/PBDE No. 100, (B). Proper identification and quantitation of partly overlapping peaks are resolved by deconvolution of peaks. The lines below represent the quantification ions of the selected compounds.

Doubling the extraction time from 5 to 10 min does not improve the extraction efficiency, and these results were consistent with the results found by E. Concha-Graña et al (17). A static time of 5 min was therefore selected.

Lastly, the solvent volume was evaluated by varying the flush volume of the PLE system. The results for the flush volume of 60% (~ 32 mL) (% recovery range of 78.2–101.2%) has been comparable with the results flush volume of 20% (approx. 24 mL) (% recovery range of 82.7–99.1), but for economical reasons, a flush volume of 20% was chosen to be used throughout the experiment.

As a conclusion, the optimal conditions selected were: 60°C for temperature, 1500 psi (10 MPa) for pressure, 5 min for static time, and 20 % for flush volume. Representative chromatograms for penta- to decaCBs and penta- to octaBDEs using the optimized method are shown in Figures 2A and 2B. The figures show the successful simultaneous extraction and separation of the selected PCBs and PBDEs.

Method evaluation

After establishing the optimum experimental parameters, linearity, recovery, method detection limits (MDL), and % relative standard deviations (% RSDs) were determined. The method was evaluated using the prepared soil. Five point-calibration curves were generated for PCBs and PBDEs in the range of 10–150 ng/g and 20–200 ng/g, respectively. Isotope labeled PCBs and PBDEs (MBP-CG and MBDE-MXC, respectively) were used as internal standards in the level of 10 ng/g soil. The calibration curves (ratio of analyte peak area to internal standard area versus concentration) were found to be linear at the concentration range tested with correlation coefficients (R) of 0.99 or better. The MDLs and RSDs were generated using seven replicates. Tables II and III showed that all % RSDs were below 15%, which indicates good method precision. MDLs were also found to be in the low ppb level for all analytes. These results are acceptable for most environmental analyses.

PBDEs that are mostly found predominantly in abiotic and biological samples require special attention. These PBDEs are usually monitored specially in epidemiological studies. PBDE Nos. 28, 47, 66, 99, 100, 153, and 154 comprise this group (5). From Table III, the optimized procedure gave reliable results for these compounds (% RSD = 2–10; % recovery = 94–109). Similarly, results for the seven indicator PCBs (PCB Nos. 28, 52, 101, 118, 138, 153, and 180) were good with % RSDs in the range of 0.9–3 (Table II). Recoveries in the range of 90–99% were found for these PCBs except for PCB No. 153, which gave a high % recovery of 130. The high recovery can be from the overlap of PCB No. 153 and PCB No. 168. Additional steps can be used to solve this problem, which requires additional analysis time. However, this was not done because it will sacrifice the time saved by this method. Data were accepted in favor of the rapidity of the method.

Because of the high selectivity of the proposed methodology, eluants can be simultaneously collected as one extract and separated using GC–TOF-MS. Just like our previous experiment (21), mono-ortho PCBs and coplanar PCBs, which were reported to be difficult to separate, were again successfully separated (21). PCB No. 126 (coplanar PCB) was successfully separated from mono-ortho PCB Nos. 114, 118, 123, 156, 157. With the optimized PLE and GC–TOF-MS, PCB Nos. 118 and 156 were separated with PCB Nos. 149 and 171, respectively, a task which is otherwise difficult unless one uses Lipidex (21). Most importantly, the proposed methodology was feasible to successfully quantitate PCB No. 180 and PBDE No. 47 (Figure 3A). In fact, these compounds were reported to coelute especially when one uses a 5% diphenyl 95%–dimethylsiloxane type column such as DB-5 (5,7,12,13). Moreover, PCB No. 194 and PBDE No.100, which was reported to be seemingly impossible to separate using non-polar coated column, was successfully determined (Figure 3B) (12). These evidently show that concurrent analysis of PCBs and PBDEs can be done using this simple technique.

Table IV. Recoveries of PCBs and PBDEs from SRM 1939a Using the Proposed Method

	IUPAC #	Certified value (µg/kg)	Experiment Results (µg/kg)	Recoveries (%)	RSD (%) (*n* = 3)
Chlorobiphenyl	44	1131 ± 74	810	71.6	2.0
	49	3740 ± 280	4215	112.7	1.6
	52	4320 ± 130	3857	89.3	1.2
	99	380 ± 96	290	76.3	5.9
	105	201 ± 28	200	99.3	3.0
	110	1068 ± 70	807	75.5	5.0
	118	423 ± 88	383	90.6	5.0
	151	192.1 ± 2.6	156	81.1	7.7
	156	37.0 ± 6.6	37	99.4	10.8
	170	107 ± 17	84	78.6	2.4
	180	140.3 ± 6.1	123	87.9	2.4
	183	47.3 ± 2.3	32	68.3	9.4
	194	35.5 ± 4.1	37	103.3	5.4
	206	29.7 ± 5.6	28	93.5	3.6
Bromodiphenyl ether	3	29.9 ± 0.4	26.0	87.1	5.8
	7	29.9 ± 0.4	24.1	80.5	1.7
	15	29.9 ± 0.4	29.3	98.0	3.1
	17	29.9 ± 0.4	21.4	71.6	3.7
	28	29.9 ± 0.4	30.8	102.9	3.2
	47	29.9 ± 0.4	28.3	94.7	5.3
	49	29.9 ± 0.4	27.9	93.4	4.6
	66	29.9 ± 0.4	30.5	102.0	8.5
	71	29.9 ± 0.4	30.6	102.6	4.6
	77	29.9 ± 0.4	31.7	106.1	3.8
	85	29.9 ± 0.4	27.4	91.7	11
	99	29.9 ± 0.4	29.6	99.1	3.1
	100	29.9 ± 0.4	32.6	109.0	4.9
	119	29.9 ± 0.4	31.0	103.9	6.8
	126	29.9 ± 0.4	30.1	100.7	10
	138	59.8 ± 0.8	52.9	88.6	3.02
	153	59.8 ± 0.8	60.0	100.4	5.2
	154	59.8 ± 0.8	64.5	108.0	2.8
	156	59.8 ± 0.8	66.2	110.8	4.4
	183	59.8 ± 0.8	62.3	104.2	6.8
	184	59.8 ± 0.8	69.8	116.9	1.1
	191	59.8 ± 0.8	58.4	97.7	0.41
	196	59.8 ± 0.8	51.8	86.8	5.0
	197	59.8 ± 0.8	61.9	103.7	5.8

The proposed method was used to analyze the standard reference material, SRM 1939a. Because of lack of appropriate reference material, river sediment with certified values for PCBs-SRM 1939a was spiked with PBDE standards in the level of 29.9 µg/kg for mono- to penta-BDEs and 59.8 µg/kg for hexa- to octa-BDEs for the final validation scheme. Although recoveries from spiked contaminants are not comparable to those from native samples, it provides reliable bench marks, which will be useful for further investigations (22). MBP-CG and MBDE-MXC were used as internal standards (10 ng/g) in these investigations. Results from the analysis conform excellently with the certified values with percentage of recoveries ranging from 68.3–116.9% and % RSDs from 0.41–11&, demonstrating that the optimized methodology can be used in the simultaneous analysis of PCBs and PBDEs in soil samples (Table IV). Moreover, these results are in accordance with the acceptance criteria specified in the US-EPA methods 1668A and 1614-draft for PCBs and PBDEs, respectively.

Conclusion

An automated, single-step extraction, and cleanup using PLE for the simultaneous analysis of PCBs and PBDEs in soil has been established. It was able to resolve reliably PCB No.180-PBDE No. 47 and PCB No.194-PBDE No.100, which were reported to coelute from one another. The scope of application of this method can be readily extended to sediment samples as shown by the successful analysis of the river sediment reference material. The proposed methodology offers a simple, easy, and economical alternative to the analysis of PCBs and PBDEs with sufficient accuracy and precision. This will allow the efficient study and monitoring of these pollutants available in different laboratories. In this experiment, we used one kind of matrix only; thus, the matrix variation cannot be ascertained. However, it has been shown by other researchers that matrix variation was encountered when using PLE (23); thus, the use of an internal standard is recommended to extend the application of the proposed method. This area needs further study.

Acknowledgements

This work was supported by grants (2N29640 and 2N30590) from the Korean Ministry of Education, Science and Technology.

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Manuscript received October 8, 2008;
Revision received December 24, 2008.