Analysis Of Polybrominated Diphenyl Ethers By GC/MS With Large Volume Injection

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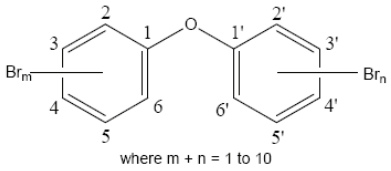
Abstract

Polybrominated Diphenyl Ethers (PBDEs) are a class of brominated flame-retardants that have been used over the past 30 years in the production of textiles, high impact plastics, and electronic circuitry. Recently PBDEs have gained increased attention as emergent chemicals because of their persistence in the environment and potential for bioaccumulation, as well as their similarity in structure to PCB's, Dioxins and Furans. Because of the potential toxicity of these chemicals, various methods have been developed for the extraction and analysis of these compounds. For solid matrices such as tissues, sediments, and soils, a high degree of extraction efficiency for PBDEs can be achieved by automated Soxhlet extraction, while continuous liquid-liquid extraction techniques have been used for the efficient extraction from aqueous samples. Improved selectivity for PBDEs is achieved by performing silica gel and acid cleanups. Sample extracts can then be analyzed using high-resolution gas chromatography and mass spectroscopy (HRGC/MS). By utilizing a large volume injector, inert ion source technology, and by acquiring data in selected ion-monitoring (SIM) mode, background interference for all matrices can be reduced, leading to an increase in instrumental sensitivity. The efficiency of combining these extraction and analytical methods has led to method detection limits for this class of analytes in the low part per trillion range for all matrices.



where m + n = 1 to 10

Introduction

PBDE's are a class of compound where bromine atoms are attached to the diphenyl ether molecule. (Figure 1) There are 209 possible compounds for PBDE's which are called congeners. The target compound list developed at Columbia Analytical is based on the most environmentally significant and lipophilic congeners. The target list is derived from the primary components of the major technical products currently in use.

PDBE's have been found in every sector of the environment, with particularly high levels in North America. PDBE's have been detected in sediments, sewage sludge, crops, meat and dairy products, fish, chicken eggs, mammals, human tissue, human breast milk and human blood. Levels detected in North America have been in the range of 10-100 times higher than in Europe, where the government is taking action to reduce exposure.[1] Some studies indicate that levels of PDBE's in breast milk of North American women are doubling every two to five years.[2]

Traditional approaches to the analysis of PBDE's have been with the use of high-resolution mass spectrometers. While this technique generally produces very low detection limits, it can be expensive. As evidence continues to mount regarding the bioaccumulative and toxic effects of PBDE's, the need for a sensitive, rugged, lower cost method is needed.

The analytical portion of the PBDE determination is performed by EPA Method 8270C using high-resolution gas chromatography and mass spectroscopy (HRGC/MS). Sensitivity for the target compounds was improved through the use of an ATAS Optic Large Volume Injector (LVI) that allows a 20µl injection into the gas chromatograph. After the residual solvent is evaporated, a large pressure pulse is applied while the port is rapidly heated to transfer the analyte. This is a splitless injection technique that allows for a greater mass of the target analyte to be transferred to the GC column. The Mass Spectroscopy detector is an Agilent "inert" ion source and the data is acquired in the selected ion-monitoring (SIM) mode, resulting in improved sensitivity.

Materials & Methods

Sample Preparation

- Solid samples are prepared using EPA Method 3541- Automated Soxhlet Extraction
- 20 grams for soils and sediments
- 10 gram for tissues (may be freeze dried)

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- 2 ml final extract volume
- Liquid samples are prepared using EPA Method 3520C – Continuous Liquid-Liquid Extraction
- One liter sample size
- 2ml final extract volume
- Prior to analysis sample extracts undergo silica gel and acid cleanup - EPA Methods 3630C and 3665.
- Method Detection Limit studies were prepared for water, soil and tissue matricies
- Initial Precision and Recovery (IPR) studies were prepared for water, soil and tissue matricies.

Standards

- Individual PBDE congeners @ 50µg/ml, Accustandard, Inc.
- Surrogate standards of PBDE-47($^{13}C_{12}$99%) and PBDE-99($^{13}C_{12}$99%), Cambridge Isotope Laboratories, Inc.
- Internal standard of Dibenzo(a,e)pyrene($^{13}C_{6}$99%), Cerillant
- Each standard and samples are spiked with 100ng/ml of internal standard prior to analysis.
- A calibration curve was then prepared at concentrations of 1ng/ml to 200ng/ml with PBDE 206 and PBDE-209 at 100ng/ml to 2000ng/ml.

Injector Parameters

- ATAS Optic 2 Programmable Capillary Injector
- 20ul injection volume

Temperature profile:
Initial temperature: 50°C

Ramp	Rate(°C)	Final Temp(°C)	Isothermal time (min)
1	0	50	0.50
2	10	390	10.60

Pressure Ramps:

Ramp	Start Pressure (PSI)	Step Time(min)	Target Pressure(PSI)
1	10.00	0.50	10.00
2	50.00	2.50	50.00
3	10.50	5.00	22.00
4	22.00	3.67	29.80

Split State:

Time (min)	Split State
Initial	Vent
0.45	Closed/Splitless
3.0	Open/Split

GC/MS Run Conditions

Column:	Phenomenex ZB-5 (5% phenyl, 95% dimethyl polysiloxane)
Length:	15m
Diameter:	0.25mm
Film Thickness:	0.1µm
Oven:	Agilent 6890 100°C initial temp for 3.0min 40°C/min to 300°C for 0.0min 15°C/min to 340°C for 1.0min Run Time – 11.67 minutes
Detector:	Agilent 5973 MSD with Inert Ion Source
Transfer Line:	325°C
Quad Temp:	150°C
Solvent Delay:	5.50 minutes

Figure 1. PDBE-209

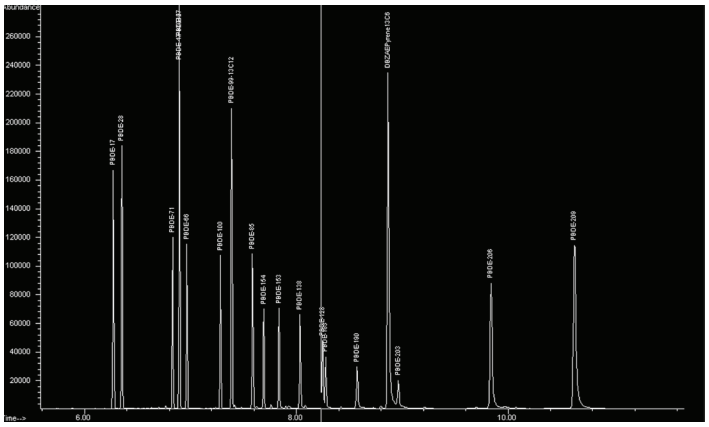

Figure 2. EICP of PBDE-47, 66 and 71 at 1ng/ml

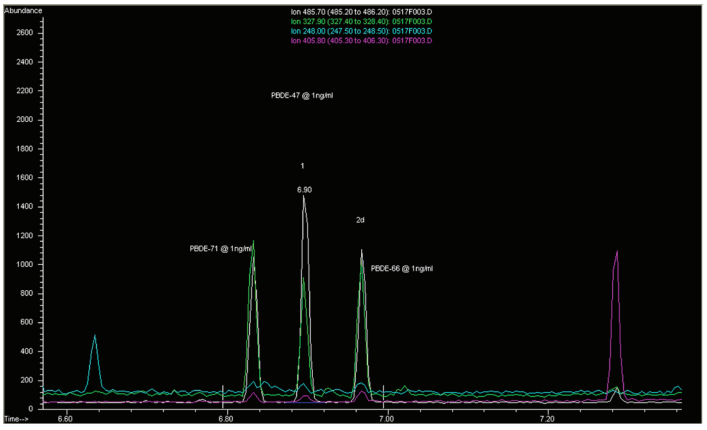

Figure 3. EICP of PBDE-209 at 200ng/ml

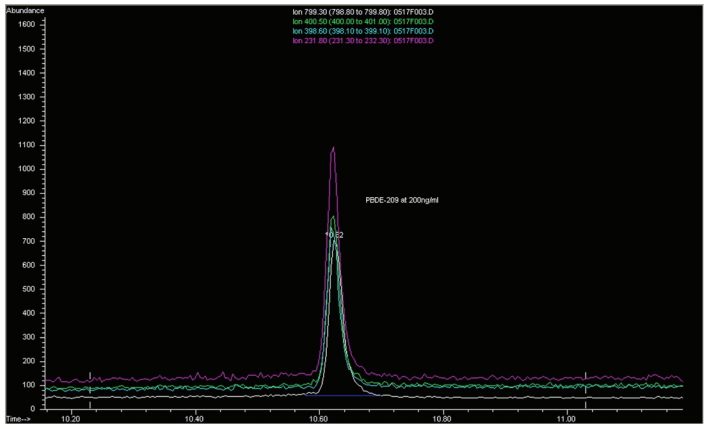

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Table 1. Calibration Summary

Compound Name	Calibration Range (ng/ml)	% RSD	R^2 Value
PBDE-17	1.0 – 200	3.0	0.999
PBDE-28	1.0 – 200	3.4	0.999
PBDE-47	1.0 – 200	4.2	0.999
PBDE-66	1.0 – 200	4.1	0.999
PBDE-71	1.0 – 200	3.5	0.999
PBDE-85	1.0 – 200	6.3	0.999
PBDE-99	1.0 – 200	4.4	0.999
PBDE-100	1.0 – 200	2.8	0.999
PBDE-128	1.0 – 200	14.2	0.999
PBDE-138	1.0 – 200	9.9	0.999
PBDE-153	1.0 – 200	7.9	0.999
PBDE-154	1.0 – 200	6.0	0.999
PBDE-183	1.0 – 200	10.9	0.999
PBDE-190	5.0 – 200	19.3	0.999
PBDE-203	1.0 – 200	15.6	0.997
PBDE-206	100-2000	21.8	0.995
PBDE-209	200-2000	20.9	0.995

SIM Parameters:

Group 1
Start time: 5.50 minutes

Ions/Dwell:	Mass, Dwell	Mass, Dwell	Mass/Dwell
	248.0, 10	327.0, 10	337.9, 10
	405.8, 10	417.8, 10	485.7, 10
	497.7, 10	563.6, 10	575.7, 10
	643.5, 10	721.1, 10	

Group 2
Start time: 8.75minutes

Ions/Dwell:	Mass, Dwell	Mass, Dwell	Mass/Dwell
	306.1, 40	308.2, 40	563.6, 40
	643.5, 40	799.3, 40	

Group 3
Start Time: 9.60

Ions/Dwell:	Mass, Dwell	Mass, Dwell	Mass/Dwell
	231.8, 20	398.6, 20	400.5, 20
	563.6, 20	643.5, 20	721.4, 20
	1799.3, 20		

Results and Discussion

The analysis of PBDE's by GC/MS with large volume injection shows excellent chromatography of each congener including PBDE-209 (Fig. 1).

The use of the LVI proved critical to the success of the analysis. Major gains in sensitivity were realized when a significant pressure pulse was applied after solvent evaporation. By injecting into a cold injection port, thermal breakdown was minimized and further reduced by using a shorter, thin film column. The response of the low calibration levels indicates very good signal to noise

Table 2. Initial Precision and Recovery Study for Soil

Analyte	Spike Level µg/Kg	Results IPR 1	IPR 2	IPR 3	IPR 4	Average Percent Recovery	% RSD
PBDE-17	2	1.64	1.757	1.735	1.611	84.29	4
PBDE-28	2	1.646	1.759	1.727	1.621	84.41	4
PBDE-71	2	1.657	1.773	1.767	1.635	85.40	4
PBDE-47	2	1.596	1.743	1.727	1.573	82.99	5
PBDE-66	2	1.672	1.794	1.792	1.643	86.26	5
PBDE-100	4	3.502	3.685	3.671	3.418	89.23	4
PBDE-99	4	3.405	3.628	3.54	3.275	86.55	4
PBDE-85	4	3.51	3.754	3.717	3.518	90.62	4
PBDE-154	8	7.002	7.693	7.473	7.54	92.84	4
PBDE-153	8	7.145	7.784	7.468	7.425	93.19	4
PBDE-138	5	4.222	4.694	4.67	4.391	89.89	5
PBDE-128	5	3.91	4.38	4.346	4.141	83.89	5
PBDE-183	5	4.273	4.674	4.701	4.508	90.78	4
PBDE-190	5	3.826	4.28	4.319	3.989	82.07	6
PBDE-203	5	3.65	4.105	4.104	3.82	78.40	6
PBDE-206	20	16.115	17.833	17.865	16.711	85.66	5
PBDE-209	20	19.263	20.452	20.402	20.211	100.41	3

Table 3. Initial Precision and Recovery Study for Water

Analyte	Spike Level ng/L	Results IPR 1	IPR 2	IPR 3	IPR 4	Average Percent Recovery	% RSD
PBDE-17	40	32.32	32.8	32.88	32.62	81.64	1
PBDE-28	40	32.02	32.56	32.44	32.88	81.19	1
PBDE-71	40	31.8	32.44	32	33.94	81.36	3
PBDE-47	40	31.36	31.68	31.84	32.78	79.79	2
PBDE-66	40	32	32.98	32.18	34.04	82.00	3
PBDE-100	80	66.3	69.22	67.98	72.7	86.31	4
PBDE-99	80	64.04	65.98	65	68.38	82.31	3
PBDE-85	80	65.52	68.52	69	69.9	85.29	3
PBDE-154	160	153.34	148.3	150.04	150.36	94.07	1
PBDE-153	160	152.6	147	147.54	149.56	93.23	2
PBDE-138	100	86.7	80.26	87.12	92.72	86.70	6
PBDE-128	100	78.24	73.08	81	84.9	79.31	6
PBDE-183	100	87.64	81.46	88.86	91.94	87.48	5
PBDE-190	100	75.76	73.26	77.34	82.26	77.16	5
PBDE-203	100	70.96	66.54	74.64	79.06	72.80	7
PBDE-206	400	318.84	304.02	327.5	338.54	80.56	5
PBDE-209	400	384.88	390.86	394.48	399.82	98.13	2

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at a level of 1ng/ml for PBDE-47, PBDE-66, PBDE-71 and 200ng/ml for PBDE-209 (Figures 2 and 3).

The calibration of each congener showed good linearity with most under 10% RSD. As the bromination level increased, linearity decreased slightly, but was still within EPA criteria. Table 1 summarizes calibration results of each analyte. See Table 1.

The accuracy and precision was evaluated for each matrix through the analysis of replicate studies and produced RSDs within EPA's criteria. Results are summarized in Tables 2-4.

Table 5 lists achievable method reporting limit (MRL) and method detection limit (MDL).

Table 4. Initial Precision and Recovery Study for Tissue

Analyte	Spike Level µg/Kg	Results IPR 1	IPR 2	IPR 3	IPR 4	Average Percent Recovery	% RSD
PBDE-17	10	14.076	14.082	15.148	12.994	140.75	6
PBDE-28	10	13.856	13.944	15.018	12.942	139.40	6
PBDE-71	10	13.898	13.832	15.162	12.806	139.25	7
PBDE-47	10	13.258	13.288	14.402	12.484	133.58	6
PBDE-66	10	14.056	14.004	15.234	13.158	141.13	6
PBDE-100	20	28.64	28.47	31.074	26.962	143.93	6
PBDE-99	20	26.544	26.412	28.776	24.914	133.31	6
PBDE-85	20	27.564	26.69	30.086	25.246	136.98	7
PBDE-154	40	54.068	54.32	57.98	51.636	136.25	5
PBDE-153	40	51.426	72.326	55.346	49.298	142.75	18
PBDE-209	100	128.692	137.186	137.404	132.722	134.00	3

Conclusions

- Use of the ATAS Large Volume Injector provides a very efficient analyte transfer to the column with minimal mass discrimination and thermal breakdown.
- Short, thin film columns are necessary to further reduce analyte reactivity.
- Accuracy and Precision meets requirements of USEPA.
- LVI-GC/MS-SIM provides excellent sensitivity and is a viable alternative to a high resolution GC/MS analysis.
- Cost of analysis is less than half of GC/HRMS analysis.

References

1. Cynthia de Wit, Swedish Environmental Agency, Brominated Flame Retardants, Report 5065, 2000.
2. Travis Madsen et al, Environment California Research and Policy Center, Growing Threats Toxic Flame Retardants and Children's Health, 2003.
3. Emmit Soffey, Agilent Technologies, Oral presentation on the analysis of Poly Brominated Diphenyl Ethers, Columbia Analytical Services Inc., 2003.

Table 5. Polybrominated Diphenyl Ether Method Reporting Limits/Detection Limits

Compound Name	BZ#	Sediment (µg/Kg)		Tissue (µg/Kg)		Water (ng/L)	
		MRL	MDL	MRL	MDL	MRL	MDL
2,2′,4-TriBDE	PBDE-17	0.1	0.03	0.2	0.06	1.0	0.2
2,4,4′-TriBDE	PBDE-28	0.1	0.03	0.2	0.09	1.0	0.2
2,2′,4,4′-TetraBDE	PBDE-47	0.1	0.03	0.2	0.04	1.0	0.5
2,3′,4,4′-TetraBDE	PBDE-66	0.1	0.02	0.2	0.03	1.0	0.3
2,3′,4′,6-TetraBDE	PBDE-71	0.1	0.02	0.2	0.03	1.0	0.4
2,2′,3,4,4′-PentaBDE	PBDE-85	0.1	0.02	0.2	0.05	1.0	0.2
2,2′,4,4′,5-PentaBDE	PBDE-99	0.1	0.05	0.2	0.05	1.0	0.8
2,2′,4,4′,6-PentaBDE	PBDE-100	0.1	0.03	0.2	0.1	1.0	0.3
2,2′,3,3′,4,4′-HexaBDE	PBDE-128	0.1	0.03	0.2	0.04	1.0	0.2
2,2′,3,4,4′,5′-HexaBDE	PBDE-138	0.1	0.02	0.2	0.02	1.0	0.2
2,2′,4,4′,5,5′-HexaBDE	PBDE-153	0.1	0.02	0.2	0.03	1.0	0.2
2,2′,4,4′,5,6′-HexaBDE	PBDE-154	0.1	0.02	0.2	0.04	1.0	0.2
2,2′,3,4,4′,5′,6-HeptaBDE	PBDE-183	0.1	0.02	0.2	0.03	1.0	0.2
2,2′,3,3′,4,4′,5,6-HeptaBDE	PBDE-190	2.0	0.02	0.2	0.05	1.0	0.1
2,2′,3,4,4′,5,5′,6-OctaBDE	PBDE-203	2.0	0.02	0.2	0.02	1.0	0.1
2,2′,3,3′,4,4′,5,5′,6-NonaBDE	PBDE-206	1.0	2.0	2.0	2	10	30.9
Decabromodiphenyl ether	PBDE-209	1.0	2.0	2.0	2	10	5