EVALUATION OF POLYBROMINATED DIPHENYL ETHERS (PBDEs) STANDARD SOLUTION BY ISOTOPE DILUTION METHOD USING HRGC/MS : A CASE STUDY

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Introduction

During last two decades, polybrominated diphenyl ether (PBDEs) and its related chemicals attracted scientists and environmental chemists at great concern due to their persistency and considerable bio-accumulation in the environmental samples, in wildlife and humans[1-2]. Only recently, some representative studies reported greater occurrence of PBDEs over global scale[(for example3-5)]. In general, PBDEs were used in various applications of high impact polymeric materials such as polystyrene, polyurethane foam, textile coatings, added to plastics, paints, machines and electronic devices (television and computer casing), building materials and textiles. Besides, leakage from households, traffic and other diffuse sources also another possible explanation[6]. The most recent production data figured that 40,000 ton/year has been manufactured worldwide. Therefore, there is a growing belief that this family may be the next generation of environmental contaminant of concern. Studies also showed that increasing level of PBDEs in humans[3] and in the organisms in the deep-sea ocean animals[7].

Toxicologically, mono-PBDE is most immunotoxic. They also affect mixed-function oxidase enzymes and ultra structure of rat and trout liver. Neonatal exposure of PBDE-99 and 47 can cause permanent aberrations in spontaneous behavior that seems to worsen with age. Furthermore, neonatal exposure of PBDE also affected learning and memory functions in adult animals. Mainly, cholinergic system seems to affect in mice. Recent toxicological studies have demonstrated that BFRs can elicit serious health effects such as thyrodiogenic, estrogenic and dioxin-like activity.

In order to examine the future analysis of all available congeners of PBDE group in environment, a case study with new standard solution containing all congeners (from mono- BDEs to deca-BDEs) is needed. Therefore, in this study we evaluate PBDEs by Isotope dilution method using HRGC/MS. It should worth mentioning that this is first of its kind of study with standard solution containing mono- through deca- brominated diphenyl ethers.

Materials and Methods

Standard Solution and Analysis

In general practice only tetra- through octa-BDEs were evaluated and analyzed until to-date. In this study, the standard solution of mono- through deca-BDEs used was gift from Wellington laboratories for the preliminary test purpose to evaluate PBDEs. The analytical procedures were adopted for Isotope dilution method, which is most efficient method for evaluation of standard solutions with precise and sophisticated analytical procedures. The standard used for analysis in this study were shown in Table 1 with congener number and 5 concentrations of native PBDE standard (BDE-MXC), $^{13}C_{12}$-PBDE standard (MBDE-MXC) and syringe spike (MBDE-139-IS) details. In addition, $^{13}C_{12}$-decaBDE was applied to evaluate their Relative Response Factor (RRF). The $^{13}C_{12}$ PBDE monitor ions (M+, M+2, M+4, M+6, M+8) mostly monitored mono-BDEs to the Hepta-BDEs in two or more monitor ions.

Table 1. Standard (Native, ^{13}C -labled) preparations for the analysis.

	BDE-No.	CS-1	CS-2	CS-3	CS-4	CS-5
Native PCDE (BDE-MXC)						
4-MBDE	3	1	5	20	100	400
2,4-DBDE	7	1	5	20	100	400
4,4'-DBDE	15	1	5	20	100	400
2,2',4-TrBDE	17	1	5	20	100	400
2,4,4'-TrBDE	28	1	5	20	100	400
2,2',4,4'-TeBDE	47	1	5	20	100	400
2,2',4,5'-TeBDE	49	1	5	20	100	400
2,3',4,4'-TeBDE	66	1	5	20	100	400
2,3',4',6-TeBDE	71	1	5	20	100	400
3,3',4,4'-TeBDE	77	1	5	20	100	400
2,2',3,4,4'-PeBDE	85	1	5	20	100	400
2,2',4,4',5-PeBDE	99	1	5	20	100	400
2,2',4,4',6-PeBDE	100	1	5	20	100	400
2,3',4,4',6-PeBDE	119	1	5	20	100	400
3,3',4,4',5-PeBDE	126	1	5	20	100	400
2,2',3,4,4',5'-HxBDE	138	1	5	20	100	400
2,2',4,4',5,5'-HxBDE	153	1	5	20	100	400
2,2',4,4',5,6'-HxBDE	154	1	5	20	100	400
2,2',3,4,4',5',6-HpBDE	183	1	5	20	100	400
DBDE	209	1	5	20	100	400
^{13}C -PBDE (MBDE-MXC)						
4-MBDE	3L	100	100	100	100	100
4,4'-DBDE	15L	100	100	100	100	100
2,4,4'-TrBDE	28L	100	100	100	100	100
2,2',4,4'-TeBDE	47L	100	100	100	100	100
2,2',4,4',5-PeBDE	99L	100	100	100	100	100
2,2',4,4',5,5'-HxBDE	153L	100	100	100	100	100
2,2',4,4',5,6'-HxBDE	154L	100	100	100	100	100
2,2',3,4,4',5',6-HpBDE	183L	100	100	100	100	100
Syringe spike (MBDE-139-IS)						
^{13}C -2,2',3,4,4',6-HxBDE	139L	100	100	100	100	100

While, (M+4-2Br, M+6-2Br, M+8-2Br, M+10-2Br) monitored penta-BDEs to deca-BDEs in two or more monitor ions. The monitor ion for mono-BDE and deca-BDE, were respectively ranged from, 247.9837 and 799.3338.

HRGC/MS conditions

The conditions of HRGC/MS was classified into 3 categories given as follows; 1) For mono- through hepta-BDEs the GC conditions were; The Micromass Autospec Ultima was used with the resolution of >10,000. The SIM mode was same to all the 3 class with two or more ions were monitored for each congener group in HEWLETT PACKARD HP 6890 Series GC system with a column size of DB-5MS 30m x 0.25 mm i.d. (0.25 µm). The column temperature was set as 100 °C for (1-min.) to 20 °C/min. as initial temperature to 200 °C (1-min.) to 10 °C/min. to 300 °C (8-min.) as exponential temperature and 10 °C/min. to 330 °C (15-min.) as a final temperature. Whereas, interface temperature and on-column injection temperatures were respectively, 300 °C and 110 °C in 0.1 min. to 100 °C/min. to 300 °C in 15 min. with the helium served as carrier gas with 1ml/minute. 2) For mono- through hepta-BDEs the Thermoquest Finnigan MAT 95 XL type GC-MS were also used with same resolution, SIM, column type and length, interface temperature and flow of helium gas used for Micromass machine. While, Column temperature was slightly varied with 150 °C for (2-min.) to 5 °C/ min. as initial temperature to 160 °C (1-min.) to 20 °C/min. to 300 °C (17-min.) as a final temperature. The on-column injection temperature was 170 °C to 100 °C /min. to 300 °C for 21.3 min. 3) In case of

Octa- through deca-BDEs, the column conditions given as follows; The GC machine was same as class 2 with same resolution, SIM condition and same helium gas flow. However, column temperature was set as 220 °C for (1-min.) to 10 °C/min as initial temperature to 295 °C (16-min.) as a final temperature. The recommended method of on-column injection temperature was 185 °C to 100 °C/min. to 300 °C for 16.3 min. Besides, the higher brominated DEs were degraded or de-bromonated with the final temperature of 300 °C film thickness of 0.25 µm and therefore, in class 3, the film thickness also changed as 0.1 µm which provide clear peak retention.

Table 2. Relative Response Factor (RRF) of Native and $^{13}C_{12}$-labled polybrominated diphenyls ethers.

Native (Cs)	BDE-No.		MAT 95 XL* (*n*=3)			MAT 95 XL Removed 1 pg/ul data			Micromass Autospec Ultima* (*n*=2)		
	Native	$^{13}C_{12}$	Average	STDEV	RSD (%)	Average	STDEV	RSD (%)	Average	STDEV	RSD (%)
4-MBDE	3	3	1.34	0.13	9.55	1.34	0.13	9.55	1.12	0.06	5.22
2,4-DBDE	7	15	0.59	0.04	7.22	0.59	0.04	7.07	0.58	0.02	2.98
4,4'-DBDE	15	15	0.16	0.04	3.67	1.16	0.04	3.79	1.12	0.02	2.16
2,2',4-TrBDE	17	28	0.99	0.05	4.96	1.00	0.05	4.56	0.92	0.04	4.00
2,4,4'-TrBDE	28	28	1.13	0.03	3.09	1.13	0.04	3.21	1.10	0.04	3.57
2,2',4,4'-TeBDE	47	47	1.17	0.04	3.57	1.17	0.04	3.72	1.19	0.03	2.37
2,2',4,5'-TeBDE	49	47	0.71	0.04	5.42	0.71	0.04	5.61	0.77	0.03	4.26
2,3',4,4'-TeBDE	66	47	0.72	0.03	4.00	0.72	0.03	3.82	0.68	0.04	6.50
2,3',4',6-TeBDE	71	47	0.75	0.03	4.34	0.75	0.03	4.36	0.73	0.02	3.11
3,3',4,4'-TeBDE	77	47	1.35	0.05	3.55	1.34	0.04	3.14	1.14	0.06	5.55
2,2',3,4,4'-PeBDE	85	99	0.96	0.10	**10.31**	0.93	0.04	4.35	0.80	0.05	6.47
2,2',4,4',5-PeBDE	99	99	1.02	0.05	4.95	1.02	0.05	5.12	1.04	0.05	4.32
2,2',4,4',6-PeBDE	100	99	1.06	0.09	8.59	1.05	0.09	8.51	1.32	0.05	3.94
2,3',4,4',6-PeBDE	119	99	1.13	0.06	5.01	1.13	0.05	4.79	0.86	0.05	5.45
3,3',4,4',5-PeBDE	126	99	0.20	0.02	8.92	0.20	0.02	7.99	1.19	0.08	6.89
2,2',3,4,4',5'-HxBDE	138	153	0.95	0.12	**12.27**	0.93	0.07	7.65	0.71	0.06	8.97
2,2',4,4',5,5'-HxBDE	153	153	1.07	0.05	4.83	1.05	0.03	2.44	1.04	0.03	2.58
2,2',4,4',5,6'-HxBDE	154	154	1.02	0.05	4.70	1.01	0.04	3.85	1.00	0.02	1.78
2,2',3,4,4',5',6-HpBDE	183	183	0.99	0.04	4.19	0.99	0.04	3.85	0.94	0.02	1.69
DBDE	209	**183**	0.06	0.02	**29.35**	0.06	0.02	**28.83**	0.06	0.02	**35.62**

* represents the concentration levels of 1, 5, 20, 100 and 400 pg/uL; $^{13}C_{12}$-100 pg/uL,
Note ; Note: Lower RRF values for PeBDE-126 was due to the data of MAT95XL M-2 Br cluster ions

Results and Discussion

The HRGC/MS results recorded that different average Relative Response Factor (RRF) in between Micromass Autospec Ultima and MAT 95 XL machines except the similarities of two MAT 95 XL machines with varied concentrations (Table 2). In general RRFs were slightly lower in Micromass than MAT 95 XL. Particularly, relative standard deviations (RSD%) for DBDEs calculated by $^{13}C_{12}$-HpBDE (#183) showed larger variation than rest of congeners despite, lowest RRFs. Collectively, 4-MBDE, 44'-DBDE, 244'-TrBDE, 22'44'-, 33'44'-TeBDE, 3 PeBDE and 2 HxBDE had great RRFs than rest of congeners (Table 1).

Considering the greater variation of DBDE RRFs, furthermore we subject this congener to analyze and compare the variations of native as well as carbon-isotope dilutions with different concentrations and the results have shown in Table 3. RRF vales for DBDE had proportional increase when they were calculated $^{13}C_{12}$-HpBDE standard concentrations (Table 3). In addition, when carbon-isotope $^{13}C_{12}$-DBDEs was subjected in to evaluation with similar concentration levels, proportional increase of native and internal standard area concentrations were apparent. While, relatively consistent values were noticed with increasing bromination (Table 3). On the whole, mean RRF was found to be 0.9882 with standard deviation of 0.0865 and CV% of 8.75. It should be worth mentioning that significant results based on our analysis were, when on-column temperature increased more than 290 °C with film thickness of 0.1 µm, we found lower RRF for DBDE due to thermal loss of these congener. Consequently, we recommend that on column injection technique would be recommended based on our

Table 3. Relative Response Factor (RRF) of native, $^{13}C_{12}$-labled standards at different concentrations.

Native Conc. DBDE	IS conc. $^{13}C_{12}$ 22'344'5'6-HpBDE	Native Area DBDE	I S Area IS-$^{13}C_{12}$-HpBDE	As/Ais X Cis/Cs
Cs (pg/ul)	**Cis (pg/ul)**	**As**	**Ais**	**RRF**
*5**	*100*	*140*	*79,845*	*0.0351*
*20**	*100*	*1117*	*87,336*	*0.0640*
*100**	*100*	*7,037*	*87,600*	*0.0803*
200	20	25,871	24,998	0.1035
1,000	20	181,478	31,747	0.1143
4,000	20	891,597	25,149	0.1773
20,000	20	7,936,218	26,803	0.2961
100,000	20	46,898,724	23,540	0.3985

Native Conc. DBDE	IS Conc. IS-$^{13}C_{12}$-DBDE	Native Area DBDE	I S Area IS-$^{13}C_{12}$-DBDE	As/Ais X Cis/Cs
5	500	1,835	157,350	1.1662
20	500	5,891	149,157	0.9874
100	500	29,407	153,999	0.9548
500	500	184,079	188,720	0.9754
2,000	500	1,009,048	258,297	0.9766
10,000	500	8,460,610	433,062	0.9768
50,000	500	55,126,640	626,205	0.8803
			Mean	**0.9882**
			SD	**0.0865**
			CV (%)	**8.75**

*indicates the Cis concentrations was 100 pg/ul determined in different dates as well as conditions by Micromass Autospec Ultima machine.

results that should not exceed 300°C with film thickness of 0.1 µm. More significantly, we found a formation of polybrominated dizbenzofurans (PBDFs) from the PBDEs in spiltless mode when high temperature 300 °C applied during injection steps. Therefore, we recommend on column injection method is best tool than the spiltless injection for DBDE analysis.

It is noteworthy that similar $^{13}C_{12}$-labled internal standard concentrations of DBDEs, the peak area increased drastically. This might have impacted by the native DBDE concentrations due to the DBDE was divided by HpBDE for recovery standard. This is one of most significant results of this study and we suggest carbon-isotope standards should be used to exact determination of higher brominated diphenyls ethers such as DBDEs for the samples like plastics, wastes and environmental samples with elevated PBDE concentrations.

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References

1. Sellstrom, & Jansson B. (1995) Chemosphere, 31, 3085
2. Sellstrom U., Kierkegaard A., de Wit C. and Jansson B. (1998) Environ. Toxicol. Chem. 17, 1065
3. Noren K. and Meironyte D. (2000) Chemosphere, 40,1111
4. Bergman A. (2000) Organohalogen Compounds, 47, 36de Wit C. (1999) Organohalogen Compounds, 40, 327
5. Watanabe I., Kawano M. and Tatukawa R. (1995) Organohalogen Compounds 24, 337
6. WHO (1998) Environmental Health Criteria 162: Brominated Diphenyl Ethers, (G.J. van Esch, ed. Geneva) pp.27-55.
7. De Boer J., Wester P.G., Klamer H.J.C., Lewis W.E. and Boon J.P. (1998) Nature 394, 28-29.