IMPROVEMENT OF METHODS FOR ANALYZING BROMINATED FLAME RETARDANT IN FOOD

Ashizuka Y[1], Yasutake D[1], Nakagawa R[1], Shintani Y[1], Hori T[1], Tsutsumi T[2], Matsuda R[2]

[1]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu-shi, Fukuoka, Japan;
[2]National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, Japan

Introduction

Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), have been widely used in plastics and textile coatings throughout the world. The major commercial products made with the PBDEs primarily used are penta-BDE, octa-BDE and deca-BDE (DeBDE). In Japan, although the use of low-brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. Therefore, they are easily released into the environment from waste products. It is predicted that, in Japan, the amount of waste Br from the plastics used in electrical appliances will increase until at least 2020 due to the increasing size of TV sets there[1)]. This prediction suggests an urgent need to monitor these brominated compounds and to manage them in waste. For PBBs, the commercial products are mixtures containing hexa-BB, octa-BB, nona-BB and deca-BB. Products made with PBBs have not been produced in Japan, but PBBs have been detected in environment samples[2)]. It is suspected that the contaminant came from imported products or impurities in other BFRs. Decabromodiphenyl ethane (DBDPE) and bis(2,4,6-tribromophenoxy)ethane (BTBPE) are relatively new brominated flame retardants that came to market in the 1990s as alternatives to DeBDE. There is very little information about their toxicity or contamination levels.

In relation to BFRs, it is problematic that *de novo* synthetic compounds, such as polybrominated dibenzo-p-dioxins, dibenzofurans (PBDD/DFs) and coplanar polychlorinated/brominated biphenyls (Co-PXBs) have been found in market fish[3)4)] and human samples[5) 6)]. Co-PXBs may also be formed from BFRs and have toxicity levels similar to those of Co-PCBs due to their structural similarities.

It is important to investigate the levels of these brominated organic compounds in foods and to estimate their effects on humans. In our previous study, we developed a method for simultaneously analyzing PBDEs and brominated dioxins[7)], and we analyzed brominated dioxins, PBDEs, Co-PXBs and PBBs in fish samples and market basket samples in Japan[8)9)]. In the present study, we examined instrumental and sample cleanup conditions, aiming to improve the simultaneous-analysis method for brominated compounds including newly BFRs such as DBDPE and BTBPE.

Materials and Methods

Chemicals

DBDPE, BTBPE and PBDE analytical standards were purchased from Wellington Laboratories (Guelph, ON, Canada). The PBB analytical standards were purchased from Wellington Laboratories and AccuStandard (New Haven, CT, USA). Dichloromethane, *n*-hexane and toluene used for extraction and cleanup were of dioxins analysis grade (Kanto Chemical, Tokyo, Japan). Acetonitrile was PR grade and was purchased from Wako Pure Chemical Industries (Osaka, Japan). DMSO was dioxins analysis grade (Kanto Chemical). Silica gel (Wako Pure Chemical Industries) was heated for 3 h at 130˚C. Florisil (Kanto Chemical) was heated for 3 h at 130˚C and deactivated with 1% water. A sulfoxide cartridge column (6 g, 20 g glassware) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Analytical Methods and Instrumentation.

The concentrations of DBDPE, BTBPE, PBDEs, Co-PXBs and PBBs were determined using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 1.

HRGC/HRMS analysis was performed on a Micromass Autospec Ultima (Waters, Milford, MA, USA) connected to an HP6890 GC (Agilent). Further, we examined the analytical conditions of DBDPE, BTBPE and PBDEs using a model 1200 GC/MS/MS (Varian, Palo Alto, CA, USA).

Sample Preparation.

The analytical method for the brominated compounds was as follows. Each 50 g sample was frozen dried using a model AD 2.0ES-BC (VirTis, Warminster, PA, USA) freeze dryer. Dried samples were extracted with 10% (v/v) dichloromethane/*n*-hexane by an accelerated solvent extractor ASE300 (Dionex, Sunnyvale, CA, USA). The extraction temperature was 100˚C; the time was 10 min. Extracts were treated with sulfuric acid three times and applied to a silica gel column. The column was prewashed with 100ml *n*-hexane, and brominated compounds were eluted with 150 ml of 10% (v/v) dichloromethane/*n*-hexane. The eluate was evaporated and dissolved in 1 mL of *n*-hexane and treated with a sulfoxide cartridge column to remove the matrix. The eluted fraction was concentrated to a final volume of approximately 25 µl, and the samples were analyzed by HRGC/HRMS.

Partitioning with Acetonitrile/n-Hexane.

Two milliliters of acetonitrile (*n*-hexane-saturated) was added to 1 ml of sample solution and shaken vigorously. After the hexane layer was separated from the acetonitrile layer, the latter was collected in another tube. Thus, sample solution was extracted with 2 ml of acetonitrile three times. Then, 30 ml of water was added to 6 ml of the acetonitrile layer and extracted with 5 ml of hexane three times. After 15 ml of the collected hexane layer was dried on anhydrous sodium sulfate, the dried hexane layer was concentrated to a final volume.

Partitioning with DMSO/n-Hexane.

Two milliliters of DMSO (*n*-hexane-saturated) was added to 1 ml of sample solution and shaken vigorously. After the hexane layer was separated from the DMSO layer, the DMSO latter was collected in another tube. The operation after this was the same as in the case of partitioning with acetonitrile/*n*-hexane.

Table 1 Analytical conditions of HRGC/HRMS

	Column	Injection temp.	Injection type /volume	Oven temp.	HRMS conditions
DBDPE BTBPE	DB-5 (Agilent) 15 m, 0.25 mm (i.d.), 0.1 µm film	260˚C	Splitless 1 µl	100˚C - (20˚C/min) - 200˚C - (10˚C/min) 320˚C (7 min)	Electron energy 38 eV Filament current 750 µA Ion source temp. 270˚ C Resolution 10,000
PBDEs	DB-5 (Agilent) 15 m, 0.25 mm (i.d.), 0.1 µm film	260˚C	Splitless 1 µl	100˚C - (20˚C/min) - 200˚C - (10˚C/min) 320˚C (7 min)	
PBBs Co-PXBs	DB-5 (Agilent) 15 m, 0.25 mm (i.d.), 0.1 µm film	280˚C	Splitless 1 µl	130˚C(1 min) - (20˚C /min) - 170˚C(10 min) - (4˚C/min) - 210˚C - (10˚C/min) - 300˚C (3 min)	

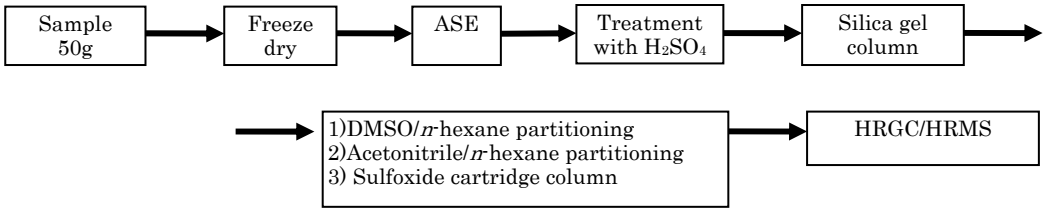

Figure1. Analytical flow of BFRs (PBDEs, PBBs, DBDPE and BTBPE)

Sulfoxide Column.

A sulfoxide column (6 g, 20 mL, glass) was prewashed with 20 mL of acetone and 20 mL of *n*-hexane. After prewashing, 1 mL of sample solution (*n*-hexane solution) was loaded. The column was washed with 12 mL of *n*-hexane. In the next step, the fraction of target brominated compounds was eluted with 25 mL of 50% (v/v) acetone/*n*-hexane. The elute was concentrated to a final volume.

Results and Discussion

We have already measured PBDEs, PBBs and Co-PXBs in fish and other food samples using HRGC/HRMS[8)9)]. In this study, we examined the instrumental condition of brominated compounds including newly BFRs such as DBDPE and BTBPE. We measured DBDPE and BTBPE using a 15 m x 0.25 mm, 0.1 µm film thickness, DB-5 column. The LOD (Limits of Detection) of DBDPE and BTBPE on HRGC/HRMS were approximately 1 pg. Figure 2 shows HRGC/HRMS chromatograms of DBDPE and BTBPE standards. In addition to congeners of PBDE and PBBs, it is possible to determine DBDPE and BTBPE using only one kind of column, the 15 m DB-5. Further, we examined DBDPE and BTBPE measurement conditions using GC/MS/MS. The detectable molecular weight of Varian model 1200 GC/MS/MS was below 800. We used monitor ions, which were 486 > 406 and 486 > 327 for DBDPE and 364 > 284 and 364 > 278 for BTBPE. The peak intensities of DBDPE and BTBPE on GC/MS/MS were high. However, it seems that more studies are needed in order to determine the GC/MS/MS measurement conditions, because the low-concentration standard had low intensity.

Table 2 shows the recoveries of PBDEs, DBDPE and BTBPE in the cleanup step of column or liquid-liquid partitioning. For the silica gel column, the recoveries of all of the congeners were in the range of 60%-120% using 10% (v/v) dichloromethane/*n*-hexane. For the Florisil column, although PBDEs and DBDPE were eluted in the first fraction, BTBPE was eluted in the second fraction, the same as brominated dioxins. When brominated dioxins are measured, it is better to use a Florisil column after silica gel column cleanup to separate PBDEs from the brominated dioxin fraction, because PBDEs affects the peaks of brominated dioxins. However, when we measure only BFRs (PBDEs, PBBs, DBDPE and BTBPE) without measuring brominated dioxins, it seems unnecessary to use a Florisil column after the silica gel column. Instead, for the step after silica gel column, further cleanup to remove fat is needed for the analysis of fatty food such as fish or meat.

Iwamura et al.[10)] reported the application of a sulfoxide cartridge column for PBDE analysis in biological and sediment samples. We examined three purification steps: partitioning with acetonitrile/*n*-hexane, partitioning with DMSO/*n*-hexane and the application of a sulfoxide cartridge column (Table 2). Although satisfactory recoveries (40%-120%) were obtained in each step, use of the sulfoxide cartridge column was the easiest and fastest step. We thus decided to use sulfoxide column cleanup. This method, combining silica gel and sulfoxide cartridge columns, we analyzed DBDPE and BTBPE in three fish samples. BTBPE was not detected in any of the samples, but DBDPE was detected at 6.38 pg/g wet weight (ww) in karei and 5.86 pg/g ww in anago. The recoveries of surrogates were in the range of 40%-120%, so this method is acceptable for determining the concentrations of newly BFRs. These results show that the improved method is an effective cleanup method for measuring BFRs in food samples. We could perform the rapid and effective analysis for determination of BFRs, including DBDPE and BTBPE. This method would be useful for purifying foods that contain a lot of matrix, such as fish or meat.

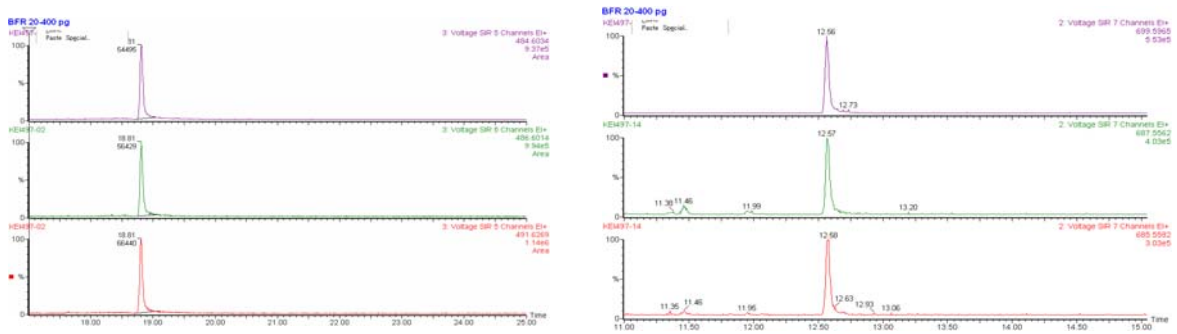

Figure 2. HRGC/HRMS chromatograms of DBDPE and BTBPE standards

Table 2. Recoveries of brominated compounds on purification (%)

	Silica gel column[1)]	Florisil column[2)]		DMSO/ *n*-hexane	Acetonitrile /*n*-hexane	Sulfoxide column
		1	2			
2,2',4-TriBDE(#28)	75.4	77.8	0.2	88.6	88.2	90.4
2,2',4,4'-TeBDE(#47)	78.7	75.9	0.3	88.3	86.8	86.6
3,3',4,4'-TeBDE(#77)	81.6	75.6	0.7	92.5	88.2	88.5
2,2',4,4',6-PeBDE(#100)	113.6	110.9	0.7	97.7	92.3	94.6
2,2',4,4',5-PeBDE(#99)	99.3	106.4	0.8	100.8	94.4	98.8
3,3',4,4',5-PeBDE(#126)	104.7	91.3	1.0	106.9	92.9	107.0
2,2',4,4',5,6'-HxBDE(#154)	85.2	84.4	0.3	90.7	79.7	95.8
2,2',4,4',5,5'-HxBDE(#153)	92.7	85.5	3.2	97.2	83.5	94.4
3,3',4,4',5,5'-HxBDE(#169)	95.7	86.4	1.1	94.9	77.0	103.4
2,2',3,4,4',5',6-HpBDE(#183)	102.5	98.6	0.6	104.0	86.9	88.2
2,2',3,3',4,4',6,6'-OcBDE(#197)	111.6	112.7	0.5	91.1	90.7	103.2
2,3,3',4,4',5,5',6-OcBDE(#205)	99.1	101.0	3.2	85.4	93.4	119.4
2,2',3,3',4,4',5,6,6'-NoBDE(#207)	90.7	78.0	0.1	101.1	80.2	102.9
DeBDE(#209)	96.2	83.7	0.1	86.7	71.8	101.0
DBDPE	63.9	67.7	3.1	56.3	53.3	40.2
BTBPE	94.4	0	121.5	92.8	90.9	82.6

1) The column was prewashed with 100ml *n*-hexane, and brominated compounds were eluted with 150ml of 10% (v/v) dichloromethane / *n*-hexane.
2) The first fraction (fraction 1) was obtained by elution with 150 ml of *n*-hexane, and the second fraction was obtained by elution with 200 ml of 60% (v/v) dichloromethane/*n*-hexane (fraction 2).

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