Results and discussion

Investigation of the capillary column in OH-PCBs (non-derivatization) measurement

We evaluated 19 kinds of capillary column for OH-PCBs measurement. The OH-PCBs standard solution for the evaluation of the capillary columns, shown on Table 1, was measured under the GC/MS conditions shown in Table 2. Using the chromatograms for 19 kinds of capillary column, we evaluated each column's suitability for OH-PCBs measurement from the following three viewpoints: (i) each isomer was detected as a peak, (ii) the shape of the peak was symmetric, (iii) the peak was quantifiable. Table 3 shows the list of 19 kinds of capillary column and the evaluation results. Figure 1 shows typical examples of OH-PCB chromatograms. In the stationary phases of each capillary column, the increase in the number of chlorine atoms of OH-PCBs caused a peak tailing, like the chromatogram of DB5 (length 15m, i.d. 0.25 mm, 0.10 μm thickness) in Fig. 1. However, when the number of chlorine atoms of a PCB decreased in HP5 (length 15 m, i.d. 0.32 mm, 0.25 μm thickness), the peak tailing was also caused. The phenomenon of that peak tailing showed an opposite tendency compared with the peak tailing of other capillary columns. The OH-PCB measurements were difficult in DB5、HP5、DB5MS and ENV5MS, where a stationary phase occurred with (5%-phenyl)-methylpolysiloxane. The peak shapes were good in the chromatogram of SLB5MS, as shown in Fig. 1, but slight tailing existed. Therefore, OH-PCBs were quantifiable in HP5MS, VF5MS and VF5ht, as shown in Table 3 and Fig.1, and those capillary columns were used for the OH-PCB measurements without derivatization.

Table 2 GC/MS condition

		GC/MS condition
Injection Temparature		280 ℃
Injection Method		Splitless
Injection Volume		1 μL
Carrier for He		1.3 mL/min
Oven		120℃(1min)→10℃/min→310℃
Ion Source Temperatute		270 ℃
Transfer Temperature		280 ℃
Ion Monitoring	OH-TetraCB	307.9143
	OH-PentaCB	341.8754
	OH-HexaCB	375.8364
	OH-HeptaCB	409.7974

The confirmation of elution orders of OH-PCBs and OMe-PCBs in the VF5MS capillary column

In the VF5MS capillary column (length 30, i.d. 0.25 mm, 0.10 μm thickness), 38 kinds of OH-PCBs listed in Table 1 were measured with the GC/MS measurement conditions shown in Table 2. Figure 2 shows the chromatogram of OH-PCBs in VF5MS, and the elution order of 38 OH-PCB isomers in that column was confirmed. Using dimethyl sulfate[1], we derivatized 38 OH-PCB isomers to OMe-PCBs. The derivatized OMe-PCBs were measured under the same GC conditions as those for the OH-PCBs measurement. Figure 3 shows the chromatogram of OMe-PCBs in VF5MS, and the elution order of 38 OMe-PCB isomers in that column was confirmed.

Comparison of sensitivity of OH-PCB and OMe-PCB

The standard solution of 0.2 ng/mL of OH-PCBs and of 0.2 ng/mL of OMe-PCBs including the internal standard solution of 10 ng/mL shown in Table 1 were measured with GC/MS to calculate the minimum limit of detection for the apparatus. Table 4 shows the minimum limit of detection for the apparatus in each isomer of OH-PCBs and OMe-PCBs. When we compared the sensitivity of the OH-PCB with that of the OMe-PCB in the same PCB skeleton, we found that the sensitivities of the OH-PCB were high in the skeleton of PCB26 and the PCB72. The sensitivity of OH-PCB was equal to that of OMe-PCB in the skeleton of PCB101. In the skeletons of PCB3, PCB9, PCB159 and PCB172, the sensitivities of OMe-PCB were higher than that of OH-PCB. In particular, we noted that the sensitivity of 4'-OMe-CB172 was five times as high as that of 4'-OH-CB172. On the whole, the sensitivities of OMe-PCBs tended to be higher than that of OH-PCBs; however, the derivatization of OH-PCBs to OMe-PCBs led to a decline in sensitivity of the measuring method and increased measurement errors, considering the reactive efficiency and the reactive reproducibility of the derivatization[1]. Therefore, we concluded that the OH-PCBs measurement without derivatization has greater sensitivity, accuracy and measurement time than that with derivatization.

Acknowledgements

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Table 3 The list of 19 kinds of capillary column and the evaluation results.

Capillary Column	Length (m)	I.D. (mm)	Film Thickness (μm)	Stationary phase	Manufacturer	Evaluation results: Peak detection	Peak shape	Quantifiable peak	Remark
DB1	30	0.32	0.1	100% Dimethylpolysiloxane	Agilent Technologies	×	×	×	All undetectable
DB5	15	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	△	×	×	With increase of the number of chlorine atoms, some tailings exists.
DB5	30	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	△	×	With increase of the number of chlorine atoms, some tailings exists.
DB5	30	0.25	0.25	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	×	×	×	All undetectable
DB5	30	0.32	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	△	×	×	Undetectable
HP5	15	0.32	0.25	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	△	×	With increase of the number of chlorine atoms, some tailings exists.
HP5	30	0.32	0.25	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	△	×	With ***decrease*** of the number of chlorine atoms, some tailings exists.
DB5MS	30	0.32	0.25	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	△	×	×	Undetectable
HP5MS	30	0.25	0.25	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	○	○	Quantifiable
HP5MS	15	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	○	○	Quantifiable
SLB5MS	30	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Supelco	○	○	△	Some tailings exists in OH-heptaCBs
SLB5MS	30	0.25	1.0	(5%-Phenyl)-methylpolysiloxane	Supelco	○	○	×	With increase of the number of chlorine atoms, some tailings exists.
ENV5MS	30	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Kanto Chemical	×	×	×	With increase of the number of chlorine atoms, some tailings exists.
VF5MS	30	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	○	○	Quantifiable
VF5ht	30	0.25	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	○	○	Quantifiable
VF5ht	15	0.32	0.1	(5%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	○	○	Quantifiable
DB17	30	0.25	0.25	(50%-Phenyl)-methylpolysiloxane	Agilent Technologies	○	△	×	With increase of the number of chlorine atoms, some tailings exists.
007-65HT	25	0.25	0.1	(65%-Phenyl)-methylpolysiloxane	Quadrex	○	△	×	With increase of the number of chlorine atoms, some tailings exists.
WAX10	15	0.25	0.25	Polyethylene Glycol	Supelco	△	△	×	With increase of the number of chlorine atoms, some tailings exists. The measurement time is long, because the use temperature of the column is limited

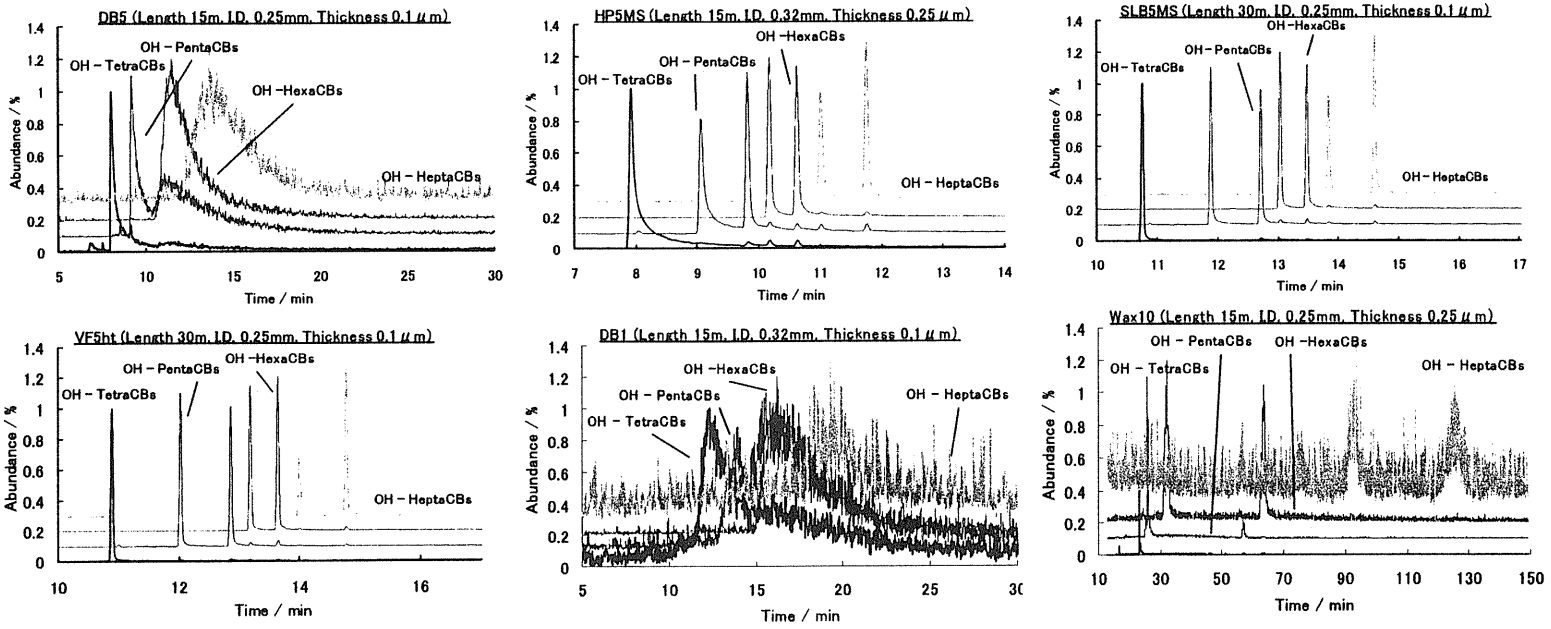

Fig. 1 Typical examples of OH-PCB chromatograms

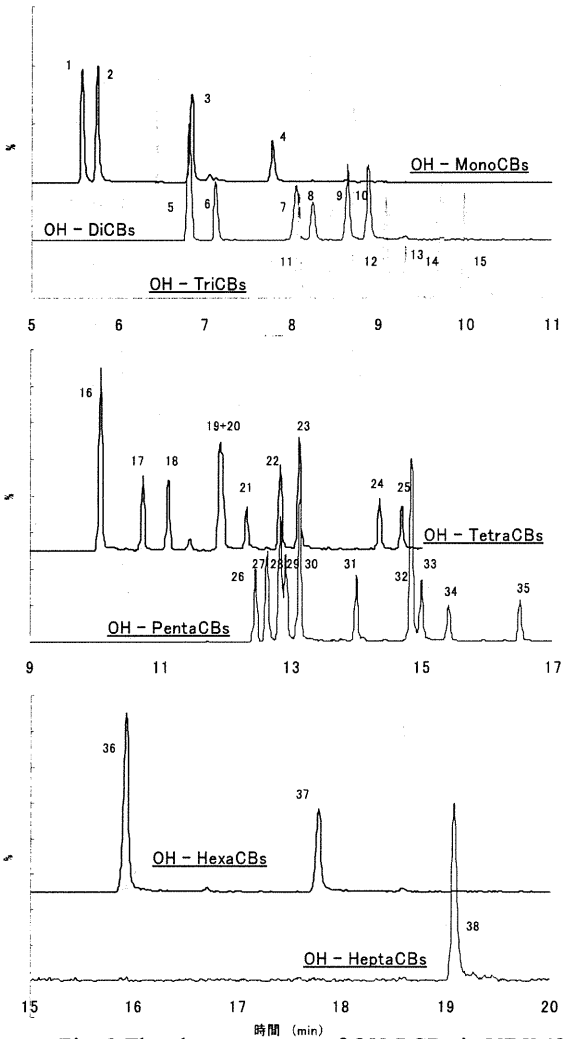

Fig. 2 The chromatogram of OH-PCBs in VF5MS

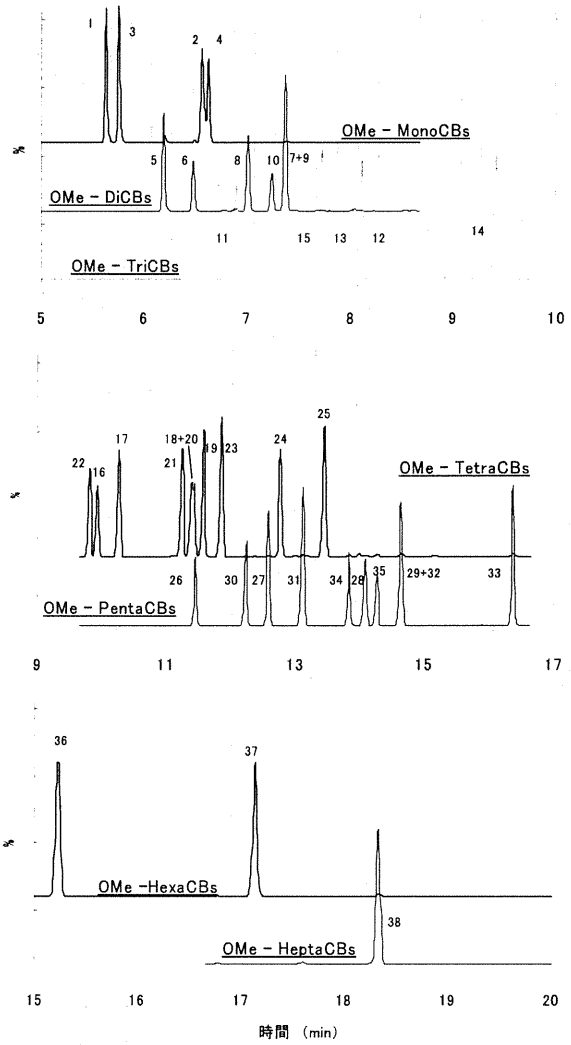

Fig. 3 The chromatogram of OMe-PCBs in VF5MS

Peak Number	OA*-PCBs	Peak Number	OA*-PCBs	Peak Number	OA*-PCBs	Peak Number	OA*-PCBs	Peak Number	OA*-PCBs	Peak Number	OA*-PCBs	Peak Number	OA*-PCBs
1	6-OA-CB2	7	4-OA-CB14	13	6'-OA-CB18	19	4'-OA-CB72	24	3'-OA-CB61	29	4'-OA-CB101	34	6'-OA-CB106
2	4-OA-CB2	8	2'-OA-CB12	14	4'-OA-CB18	20	4'-OA-CB50	25	4'-OA-CB61	30	4'-OA-CB112	35	4'-OA-CB86
3	4-OA-CB1	9	3'-OA-CB9	15	4'-OA-CB30	21	2'-OA-CB61	26	6'-OA-CB101	31	6'-OA-CB112	36	4'-OA-CB165
4	4'-OA-CB3	10	4'-OA-CB9	16	4'-OA-CB69	22	3'-OA-CB65	27	3'-OA-CB101	32	4'-OA-CB106	37	4'-OA-CB159
5	2'-OA-CB9	11	2'-OA-CB30	17	6'-OA-CB69	23	4'-OA-CB65	28	4'-OA-CB121	33	4'-OA-CB93	38	4'-OA-CB172
6	2'-OA-CB5	12	4'-OA-CB26	18	2'-OA-CB65								

*A: H (Hydorogen) or Me (methyl)

Table 4 The minimum limit of detection for the apparatus in each isomer of OH-PCBs and OMe-PCBs

PCB skeleton	Hydoxide	The minimum limit of detection	Methoxide	The minimum limit of detection
MonoCB	4'-OH-CB3	0.04	4'-OMe-CB3	0.02
DiCB	4'-OH-CB9	0.08	4'-OMe-CB9	0.02
TriCB	4'-OH-CB26	0.03	4'-OMe-CB26	0.05
TetraCB	4'-OH-CB72	0.02	4'-OMe-CB72	0.04
PentaCB	4'-OH-CB101	0.05	4'-OMe-CB101	0.04
HexaCB	4'-OH-CB159	0.08	4'-OMe-CB159	0.04
HeptaCB	4'-OH-CB172	0.2	4'-OMe-CB172	0.04

(pg)

IMPROVEMENT OF METHODS FOR ANALYZING BROMINATED FLAME RETARDANT IN FOOD

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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
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)	Ion source temp. 270° C Resolution 10,000
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)	

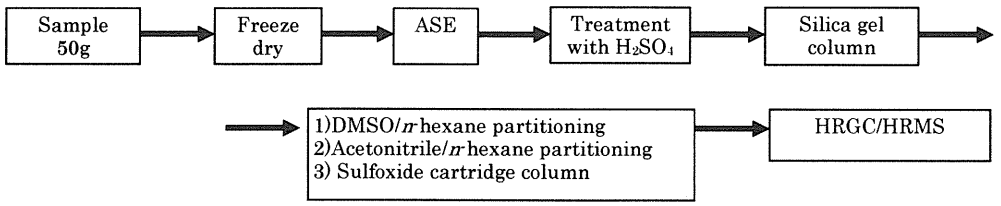

Figure1. Analytical flow of BFRs (PBDEs, PBBs, DBDPE and BTBPE)

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.

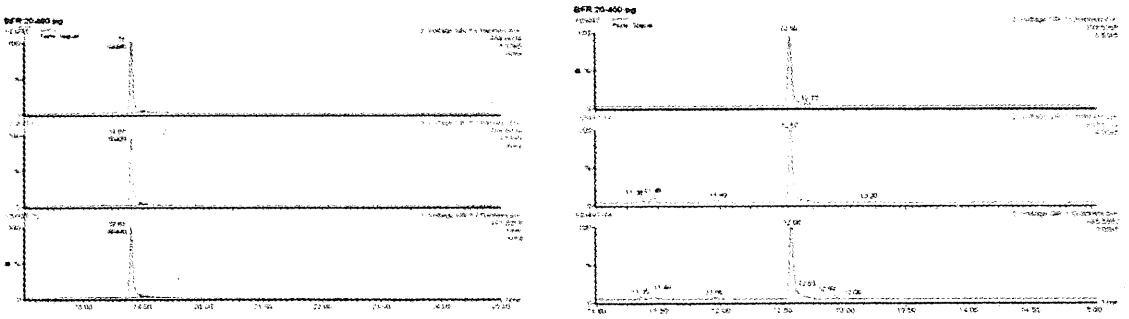

Figure 2. HRGC/HRMS chromatograms of DBDPE and BTBPE standards

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).

Acknowledgement
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