The Measurement of Hydroxylated Polychlorinated Biphenyls without Derivatization using a high-resolution gas chromatograph / high-resolution mass spectrometer

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Introduction

Polychlorinated biphenyls (PCBs) are persistent and bioaccumulative substances, the usage and disposal of which were banned in 2004 under the Stockholm Convention because they are persistent organic pollutants. Recently, various kinds of PCB metabolites have been detected in the environment. Mainly, the PCB metabolites are formed by the metabolism by living organisms of PCBs in the environment. Hydroxylated PCBs (OH-PCBs) are well known as metabolites of PCBs. OH-PCBs show a high affinity for thyroid hormone transport protein in the human blood, resulting in a reduced quantity of thyroid hormone. OH-PCBs have been detected in the blood and tissues of humans and several wildlife species and in environmental media.

In general, hydroxyl groups of OH-PCBs are derivatized to methoxy groups, OH-PCBs are measured using a high-resolution gas chromatograph / high-resolution mass spectrometer (HRGC/HRMS) as methoxylated PCBs (OMe-PCBs). However, the reaction efficiency of the derivatization depends on the substitution position of the hydroxyl group and the number of chlorine atoms. Moreover, because the stability of the reaction affects the measurement accuracy, it is desirable to measure OH-PCBs without derivatization. Our goal is to develop an analytical method for measuring the concentration of OH-PCBs in foods. In this study, we sought to determine which capillary column was most suitable for the measurement of OH-PCBs without derivatization using HRGC/HRMS.

Materials and methods

Chemicals

The standard solutions of 38 kinds of OH-PCB and 7 kinds of OMe-PCB were purchased from Accustandard, Inc. (CT, USA). The OH-PCBs standard for the evaluation of capillary columns and the labeled PCBs standard for the internal standard were purchased from the Wellington Laboratories, Inc. (ON, Canada). These standard solutions are listed in Table 1.

HRGC/HRMS

Identification of OH-PCBs and OMe-PCBs was performed using HRGC/HRMS (Agilent Technology, USA, 6890 series /Waters, UK, Autospec-Ultima) above 10,000 resolution.

Table 1 List of the standard solutions

The standard solution of 38 kinds of OH-PCB	
6-OH-CB2	3'-OH-CB65
4-OH-CB1	4'-OH-CB50
4-OH-CB2	4'-OH-CB61
4'-OH-CB3	4'-OH-CB69
2'-OH-CB9	4'-OH-CB72
3'-OH-CB9	4'-OH-CB65
4'-OH-CB9	6'-OH-CB106
4-OH-CB14	6'-OH-CB112
2'-OH-CB5	4'-OH-CB86
2'-OH-CB12	4'-OH-CB93
2'-OH-CB30	4'-OH-CB106
6'-OH-CB18	4'-OH-CB112
3'-OH-CB30	4'-OH-CB121
4'-OH-CB26	3'-OH-CB101
4'-OH-CB30	4'-OH-CB101
2'-OH-CB61	6'-OH-CB101
2'-OH-CB65	4'-OH-CB159
6'-OH-CB69	4'-OH-CB165
3'-OH-CB61	4'-OH-CB172
The standard solution of 7 kinds of OMe-PCB	
4'-OMe-CB3	4'-OMe-CB101
4'-OMe-CB9	4'-OMe-CB159
4'-OMe-CB26	4'-OMe-CB172
4'-OMe-CB72	
The OH-PCBs standard solution for the evaluation of capillary column	
4-OH-CB#54	4-OH-CB#146
4'-OH-CB#104	4-OH-CB#187
4-OH-CB#107	4'-OH-CB#172
3'-OH-CB#138	
The internal standard solution	
$13C_{12}$-PCB#70	$13C_{12}$-PCB#138
$13C_{12}$-PCB#111	$13C_{12}$-1,2,3,4,6,7,8-HpCDF

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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Reference

1. Matsumoto K., Iseki N., Kameda H., Kashima Y., Shiozaki T.(2006); Bulletin of JESC,33,49-54 (in Japanese)

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.

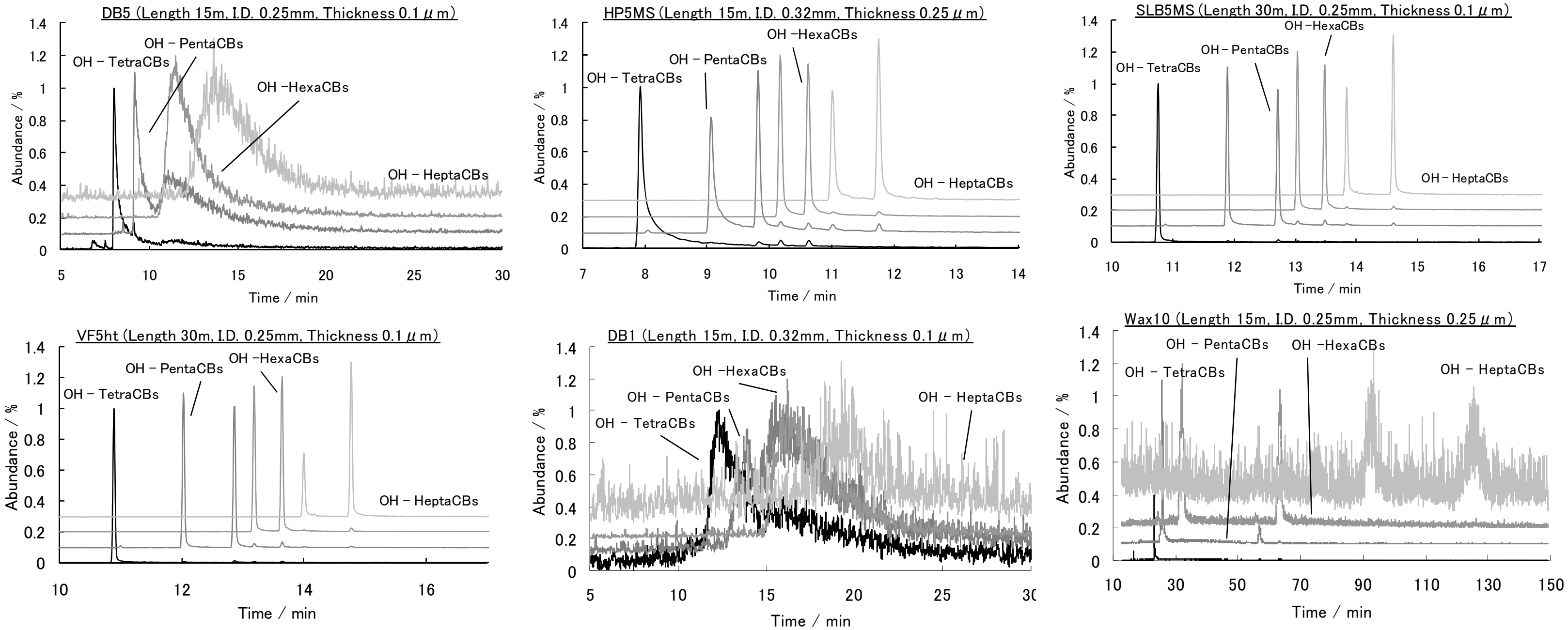

Fig. 1 Typical examples of OH-PCB chromatograms

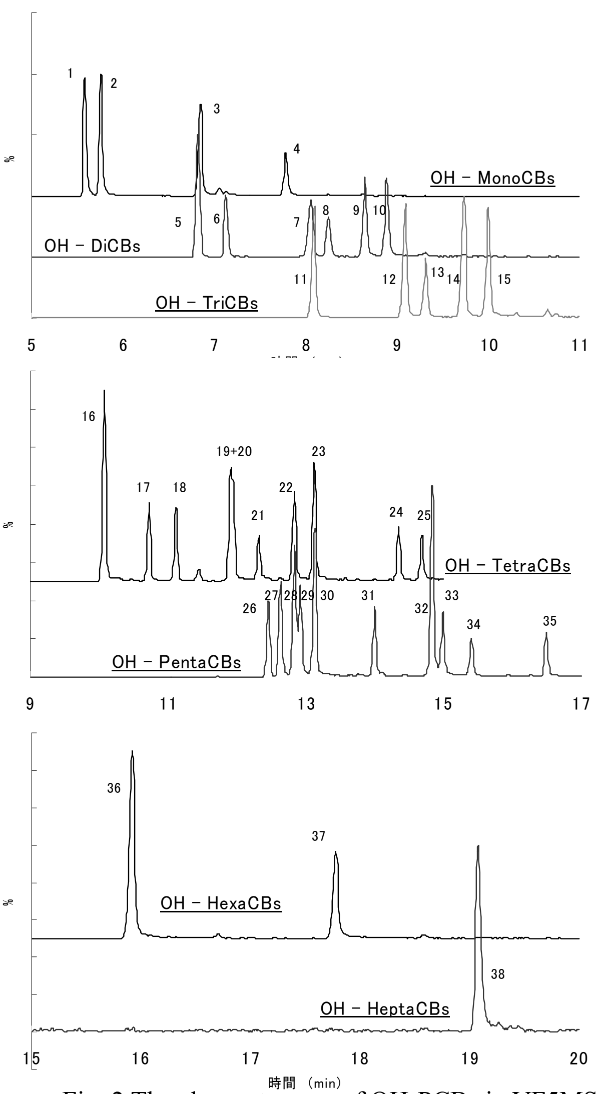

Fig. 2 The chromatogram of OH-PCBs in VF5MS

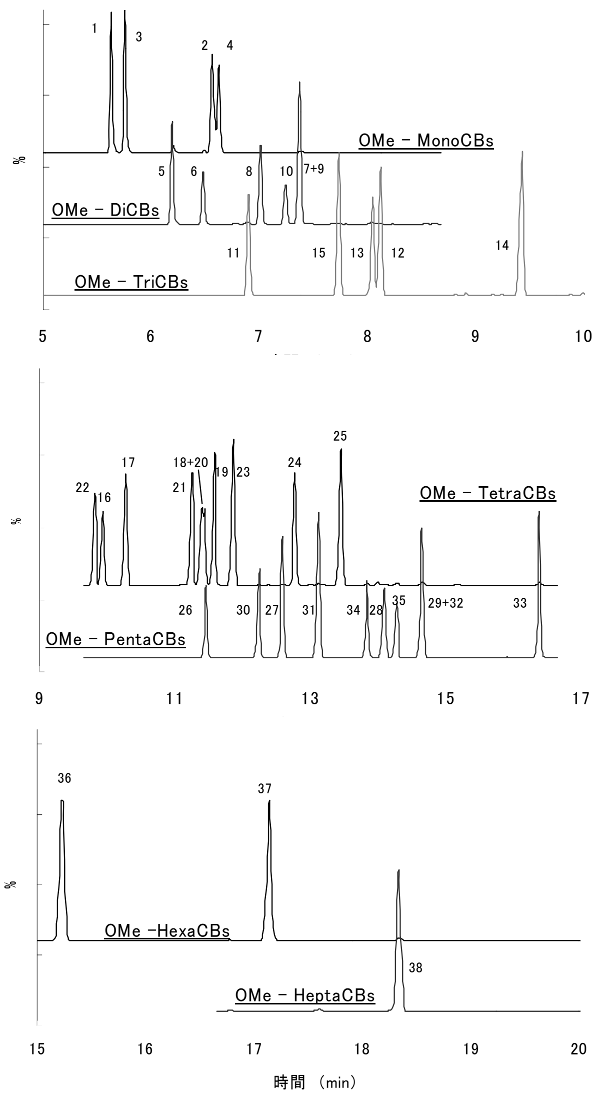

Fig. 3 The chromatogram of OMe-PCBs in VF5MS

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)