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Improvement of Measurement Method for Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in Blood Samples using LC/MS/MS

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Abstract Hydroxylated polychlorinated biphenyls (OH-PCBs) are well known as metabolites of PCBs in the human body. We improved a measurement method for OH-PCBs in blood samples using LC/MS/MS. A new 2μm particle column was used, and the analytical conditions for the LC/MS/MS measurements were optimized. The improved method is more sensitive than the conventional method for 5g blood samples. And, this method is effective at determining the concentrations of OH-PCBs, PCBs and dioxins from the same sample without special treatment of the sample such as derivatization.

Key words : Blood・OH-PCB・LC/MS/MS

Introduction

Hydroxylated polychlorinated biphenyls (OH-PCBs ; Fig. 1) are well known as metabolites of PCBs in the human body. Sakiyama et al.[1)] reported that OH-PCBs were derivatized with dimethyl sulfate, and the methoxylated PCBs were determined using HRGC/HRMS. Matsumoto et al.[2)] reported that methylation by trimethylsilyldiazomethane was an effective derivatization method. And, Yasutake et al.[3)] reported a measurement method without derivatization using HRGC/HRMS. On the other hand, R.J. Letcher et al.[4)] determined the concentrations of OH-PCBs in the plasma of Canadian polar bears using a liquid chromatography tandem mass spectrometry (LC/MS/MS) technique.

We previously developed an analytical method for measuring the concentrations of PCDDs,

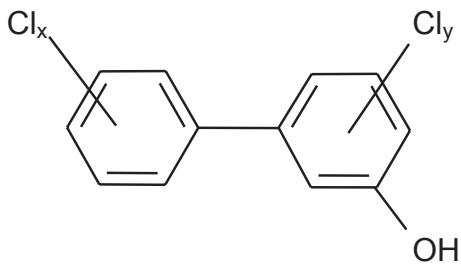

Fig. 1 Structure of hydroxylated polychlorinated biphenyls

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PCDFs and Co-PCBs in human blood samples.[5] And, we reported a measurement method for determination of OH-PCBs in human blood samples using LC/MS/MS with an electrospray ionization interface in a negative ion and selective reaction monitoring mode.[6]

In this study, we improved a measurement method for OH-PCBs in 5g blood samples using LC/MS/MS. This method is sensitive and effective at determining the concentrations of OH-PCBs, PCBs and dioxins from the same sample, and does not need a special treatment such as derivatization.

Materials and Methods

1. Chemicals and reagents

OH-PCBs standards were purchased from Wellington Laboratories, Inc., Ontario, Canada and Cambridge Isotope Laboratories, Inc., Massachusetts, US. These OH-PCBs standards are listed in Table 1. Each 1 mg/L standard solution was prepared by dilution with acetonitrile. Labeled standards of OH-[$^{13}C_{12}$]-PCBs, as internal standards, are listed in Table 2. 4-OH-2', 3, 3', 4', 5, 5'-HxCB (4'-OH-CB159) was used as a syringe spike. The standard solution for calibration curve contains all OH-PCB congeners as shown in Table 1 and 2.

Table 1 OH-PCBs standards

Compounds	Abbreviations	
4-OH-2,3,3',4',5-PeCB	4-OH-CB109	4H109
3-OH-2,2',3',4,4',5-HxCB	3'-OH-CB138	3H138
4-OH-2,2',3,4',5,5'-HxCB	4-OH-CB146	4H146
4-OH-2,2',3,3',4',5,5'-HpCB	4'-OH-CB172	4H172
4-OH-2,2',3,4',5,5',6-HpCB	4-OH-CB187	4H187

Table 2 OH-[$^{13}C_{12}$]-PCBs for internal standards

Compounds	Abbreviations	
4-OH-2,3,3',4',5-PeCB	4-OH-CB109	M4H109
4-OH-2',3,4',5,5'-PeCB	4'-OH-CB120	M4H120
3-OH-2,2',3',4,4',5-HxCB	3'-OH-CB138	M3H138
4-OH-2,2',3,4',5,5'-HxCB	4-OH-CB146	M4H146
4-OH-2',3,3',4',5,5'-HxCB	4'-OH-CB159	M4H159
4-OH-2,2',3,3',4',5,5'-HpCB	4'-OH-CB172	M4H172
4-OH-2,2',3,4',5,5',6-HpCB	4-OH-CB187	M4H187

Acetonitrile, methanol, Ammonium acetate and ultra pure water of LC/MS grade were purchased from Wako Pure Chemical Industries, Tokyo, Japan. A silver nitrate/silica gel, other reagents and solvents used in this study were of the analytic grade of dioxin that is commercially available. A cartridge of Envi-18 (500mg/6mL glass tube) was purchased from Sigma-Aldrich, Inc., Missouri, US.

2. Sample preparation

Each 5g blood sample was loaded into an extraction cell filled with Isolute. After 15 hours of freeze-drying with a freeze dryer (VirTis Co. Inc., New York, US), OH-[$^{13}C_{12}$]-PCBs, [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs and [$^{13}C_{12}$]-PCBs were added as internal standards. Acetone : n-hexane (1 : 4, v/v) was used as the extraction solvent for an accelerated solvent extractor (ASE-200, Thermo Scientific Dionex, California, US). After the extract was evaporated to near dryness, it was dissolved in n-hexane and treated with concentrated sulfuric acid overnight. The separated hexane layer was applied to a silver nitrate/silica gel column (0.5g). The first fraction containing PCDDs, PCDFs and Co-PCBs was eluted with 15mL of n-hexane. OH-PCBs were eluted with 15mL of 50% dichloromethane/n-hexane as the second fraction. The eluate was concentrated to near dryness with a multiple sample concentrator, and dissolved in 2mL of methanol. After the methanol solution was loaded onto an Envi-18 cartridge with 4mL of methanol, the eluate was concentrated under nitrogen flow and transferred to an LC injection vial with 0.2mL of methanol. A flow chart of this method is shown in Fig. 2.

3. LC/MS/MS measurement

All LC/MS/MS analysis was performed using an Alliance 2695 series high-performance Liquid Chromatograph Separations Module equipped with Quattro micro API mass spectrometer (Waters Corporation, Massachusetts, US). An

analytical column, L-column 2 ODS, 2.1 mm × 100 mm, 2 μm (CERI, Tokyo, Japan) was used under a linear gradient solvent condition with the flow rate set at 0.2mL/min. For conventional method, an L-column 2 ODS, 2.1 mm × 150 mm, 5 μm was used. The initial mobile phase was 40 : 60 methanol/2mM ammonium acetate in ultra pure water. The injection volume was 20μL.

Detection was performed on a quadrupole analyzer operated in negative electrospray ionization (ESI-) and in selected reaction monitoring acquisition mode (SRM). Nitrogen was used as the cone and desolvation gas. The potential applied onto the capillary was 1.0 kV. Cone and collision potentials were optimized for each molecule. Argon was used as the collision gas. Other analytical conditions for the LC/MS/MS measurements are summarized in Table 3.

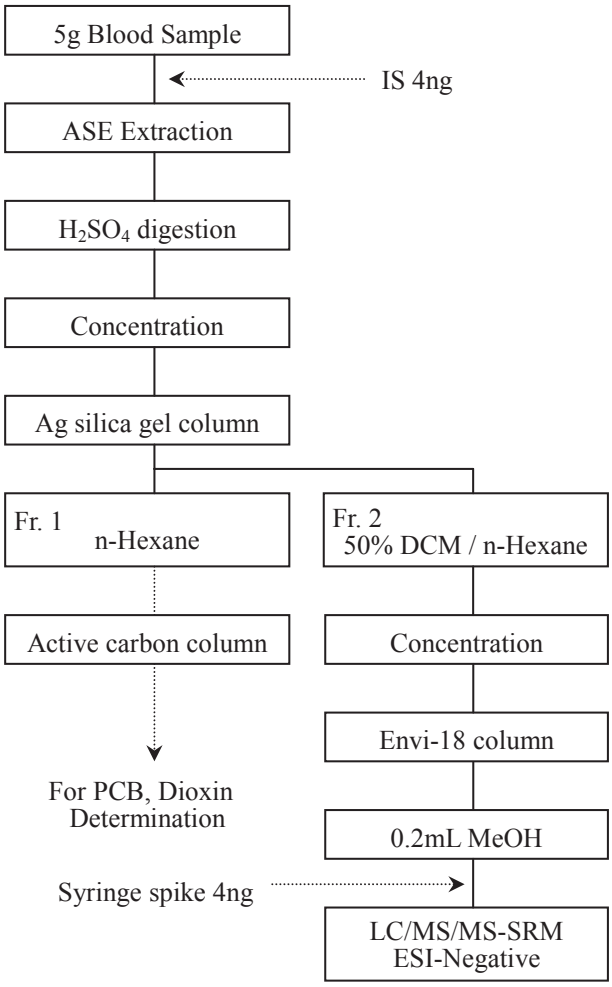

Fig. 2 Flow chart of the measurement method for OH-PCBs in blood samples

Results and Discussion

1. LC/MS/MS measurement

1-1. Optimization of collision energy and product ions

Figure 3 shows the product ion mass spectra of OH-HpCBs under different collision energy ; (A) stands for the collision energy of 20 eV, and (B) for that of 40 eV. A single peak of $[M-H]^-$ ions (*m/z* 408.7) was observed from the spectrum (A) in the negative ion scanning mode. It means that no product ion was produced. On the other hand, $[M-HCl]^-$ ions (*m/z* 372.7) and $[Cl]^-$ ions (*m/z* 34.8) were observed from the spectrum (B). Figure 4 shows the chromatograms of each product ion. Peak intensity of $[M-H]^-$ ions was the highest of the three. Though peak intensity of $[Cl]^-$ ions was the lowest of the three, the signal-noise ratio

Table 3 Analytical conditions for the LC/MS/MS measurement

Flow Rate		0.2 mL/min.
Injection Volume		20 μL
Column Temperature		50℃
Mobile Phase		2mM Ammonium acetate : Methanol = 60 : 40 → 5 : 95 linear gradient
Temperature ;	Source	120℃
	Desolvation	350℃
Gas Flow ;	Cone	Nitrogen, 50 L/hr
	Desolvation	Nitrogen, 600 L/hr
Voltage ;	Cone	30-50 V
	Capillary	1.0kV
Collision		Argon, 15eV
Ionization		ESI-Negative

was the best. The chlorine ion was selected as the product ion ; therefore, *m/z* 408.79 → 34.97 was monitored for OH-HpCBs. Other mass methods for the LC/MS/MS measurement are summarized in Table 4.

1-2. Comparison of LC columns

Chromatograms of OH-HpCBs in the standard solution were compared between the 2μm particle column and the conventional column. The chromatograms of the conventional column (2.1mm × 150mm, 5μm) and the 2μm particle column (2.1mm × 100mm, 2μm) are presented in Fig. 5 and 6, respectively. As a result, the signal-noise ratio of the chromatogram obtained using the 2μm particle column was better than that of the conventional column. It is considered that the analysis time can be set shorter using a 2μm particle column than a 5μm particle column.

1-3. Optimization of injection volume

Figure 7 shows the chromatograms of OH-HpCBs at various injection volumes (5-80 μL). The peak shape was normal up to 20μL, but collapsed from 40μL. Therefore, the injection volume was fixed to 20μL.

1-4. Calibration curve

Figure 8 shows the calibration curve of

Table 4 Analytical conditions for the LC/MS/MS measurement

Compounds		Precursor ion → product ion *m/z*
OH- PeCBs	$^{12}C_{12}$-	340.87 → 34.97
	$^{13}C_{12}$-	352.91 → 34.97
OH- HxCBs	$^{12}C_{12}$-	374.83 → 34.97
	$^{13}C_{12}$-	386.87 → 34.97
OH- HpCBs	$^{12}C_{12}$-	408.79 → 34.97
	$^{13}C_{12}$-	420.83 → 34.97

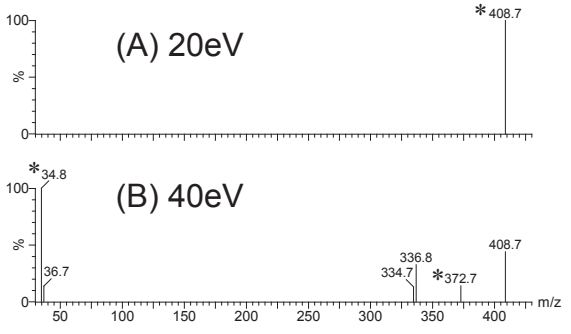

Fig. 3 Product ion mass spectra of OH-HpCBs under different collision energy

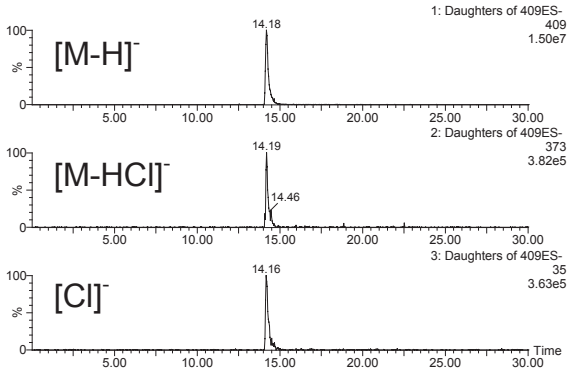

Fig. 4 Chromatograms of each product ion

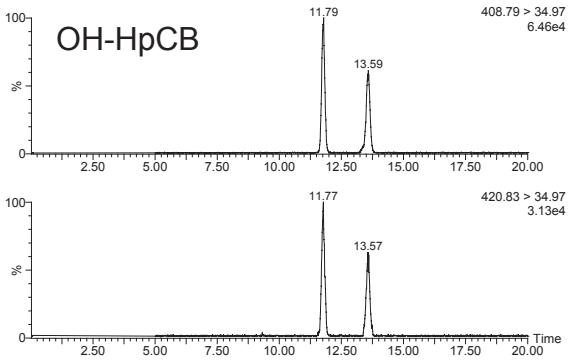

Fig. 5 LC/MS/MS chromatograms of OH-HpCBs with a column of L-column2 ODS (2.1mm × 150mm, 5μm)

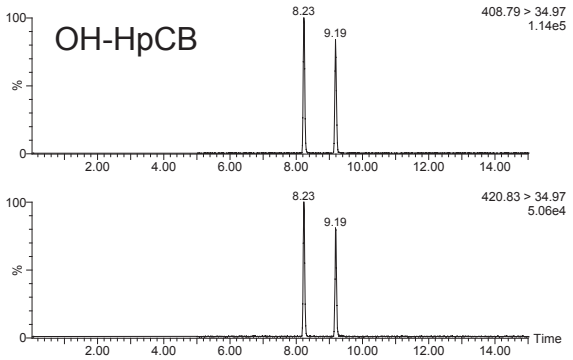

Fig. 6 LC/MS/MS chromatograms of OH-HpCB with a column of L-column2 ODS (2.1mm × 100mm, 2μm)

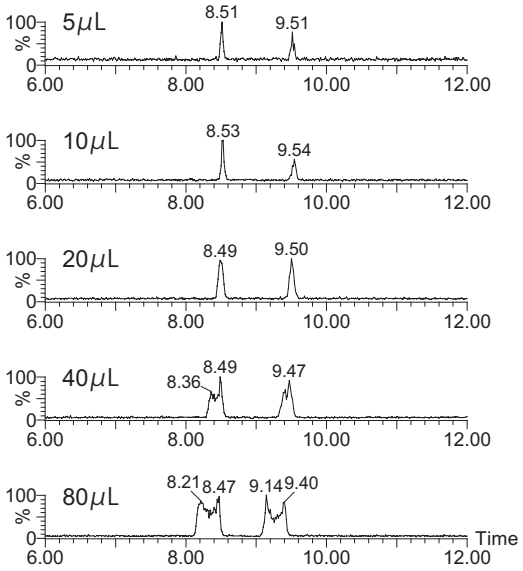

Fig. 7 Chromatograms of OH-HpCBs at various injection volumes

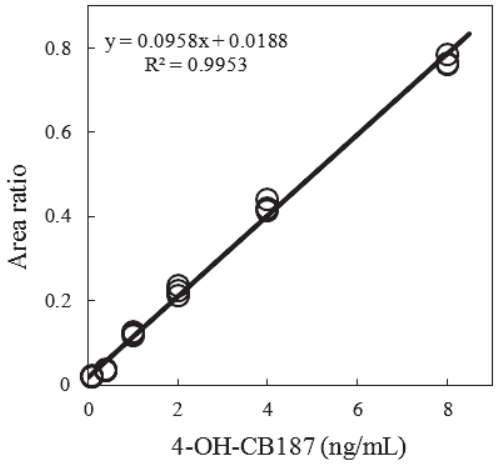

Fig. 8 Calibration curve of 4-OH-CB187 (0.1 ~8.0 ng/mL ; IS 4.0 ng/mL)

4-OH-CB187, which ranged from 0.1 to 8.0 ng/mL. The curve showed good linearity. The correlation coefficients were calculated from the concentration and the peak area ratio of 4-OH-CB187 to 4-OH-[$^{13}C_{12}$]-CB187.

2. Measurement of OH-PCBs in blood samples

Chromatograms of OH-HpCBs in blood sample were compared between the improved method and the conventional method. The chromatograms of the conventional method and the improved method are presented in Fig. 9 and 10, respectively. The signal intensity was high but background level was also high in Fig. 9. On the other hand, the signal intensity in Fig. 10 was relatively lower but the resolution of the chromatogram was much higher than conventional method.

Figure 11 illustrates the LC/MS/MS chromatograms of hydroxylated penta- through hepta-chlorinated biphenyls in the blood of Yusho patient. Peaks of 4-OH-CB109, 4-OH-CB146 + 3-OH-CB153, 4-OH-CB187 and 4'-OH-CB172 were detected, but 4-OH-CB146 and 3-OH-CB153 could not be separated in these analytical conditions, while 3'-OH-CB138 could not be observed because of low recovery. We suspected that 3'-OH-CB138 degrades under sulfuric acid treatment.

In conclusion, we improved measurement method for OH-PCBs in blood samples using

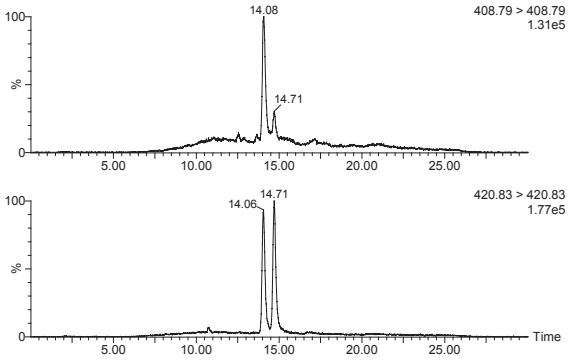

Fig. 9 Chromatograms of OH-HpCBs in blood under the conventional method

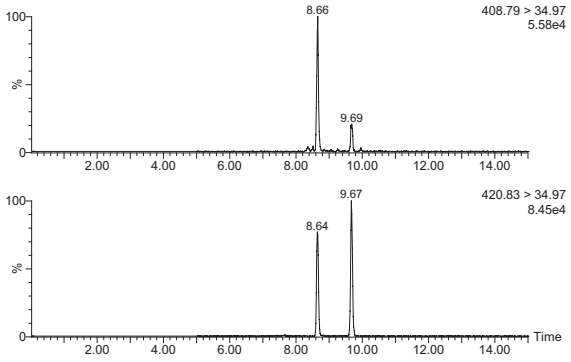

Fig. 10 Chromatograms of OH-HpCBs in blood under the improved method

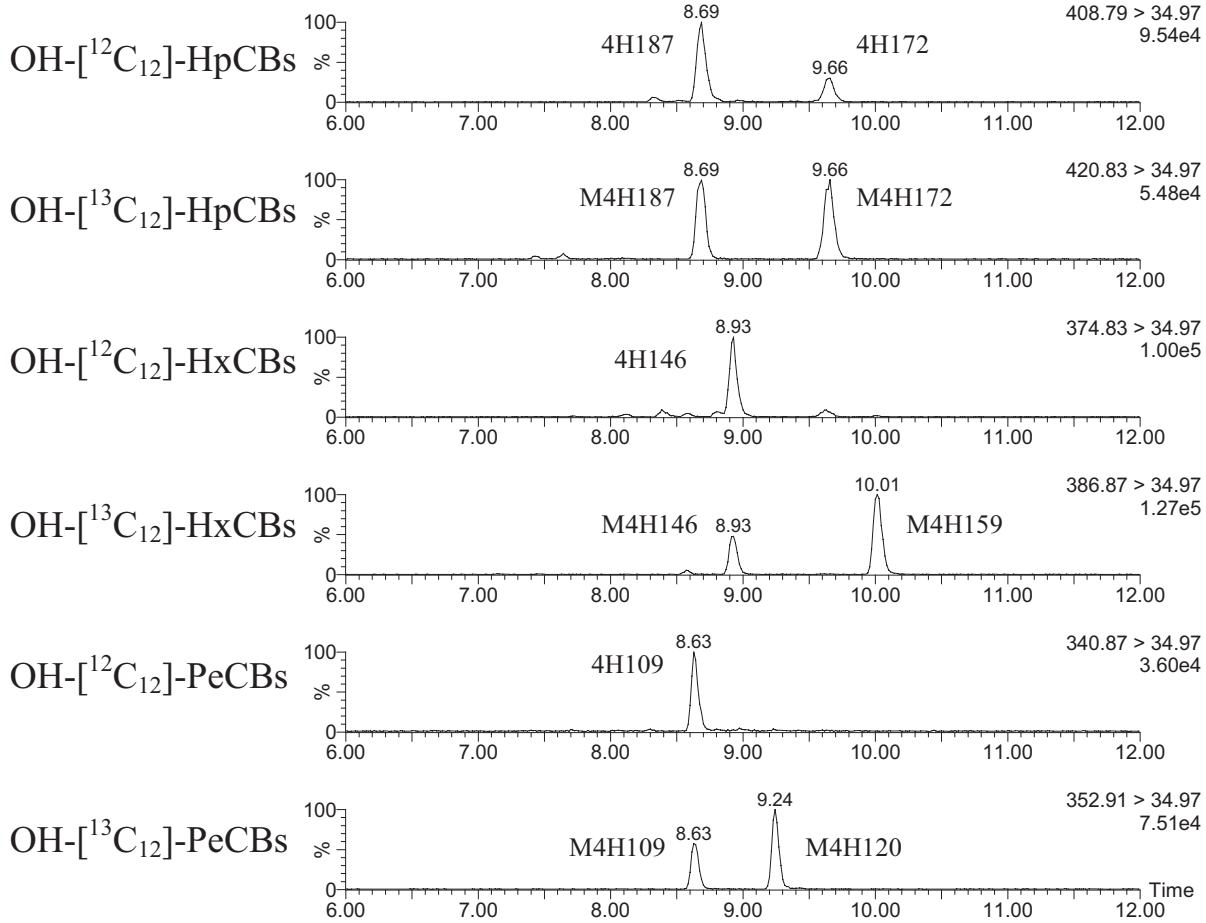

Fig. 11 LC/MS/MS chromatograms of OH-PCBs in the blood of Yusho patient

LC/MS/MS. Improved method was sensitive and effective at determining the concentrations of OH-PCBs, PCBs and dioxins from a single blood sample without special treatment.

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（和文抄録）

LC/MS/MS を用いた血液試料中の水酸化ポリ塩化ビフェニル（OH-PCBs）測定法の改良

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水酸化ポリ塩化ビフェニル（OH-PCBs）は，人体内における PCB の主要代謝物である．LC/MS/MS を用いた血液試料中の OH-PCBs 測定法の改良を行った．新しい粒径 2μm の LC カラムを採用し，LC/MS/MS 測定条件の最適化を行った．改良法では，5g の血液試料で従来法より感度が向上した．また，誘導体化のような試料の特別な処理も不要で，OH-PCB 類，PCB 類およびダイオキシン類の同一試料による同時定量も可能である．