DETERMINATION OF HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBs) IN THE BLOOD OF YUSHO PATIENTS BY LC/MS/MS

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Introduction

Polychlorinated biphenyls (PCBs) are one of the persistent and bioaccumulative chemicals. The hydroxylated polychlorinated biphenyls (OH-PCBs) are well known as metabolites of polychlorinated biphenyls. They are formed by metabolism of PCBs by the cytochrome P450 enzyme-mediated oxidation. Enomoto et al.[1] reported the concentrations of OH-PCBs in the Japanese human blood plasma. Their major congeners and levels were 4-OH-CB109 10-230 pg/g, 4-OH-CB146 13-340 pg/g and 4-OH-CB187 12-110 pg/g. Linderholm et al.[2] reported the major OH-PCB metabolite in serum samples from 9 Yusho patients was 4-OH-CB187 followed by 4-OH-CB146, 4-OH-CB109 and 4'-OH-CB120. And the total of 6 OH-PCB metabolites ranged between 390-1300 pg/g serum with a mean value of 780 pg/g serum.

Sakiyama et al.[3] reported that OH-PCBs were derivatized with dimethyl sulfate, and the methoxylated PCBs were determined using HRGC/HRMS. Matsumoto et al.[4] reported that effective derivatization method was trimethylsilyldiazomethane methylation. On the other hand, R.J. Letcher et al.[5] determined the concentrations of OH-PCBs in the plasma of the Canadian polar bear by using LC/MS/MS technique. And, we developed the measurement method for OH-PCBs by LC/MS.[6] These methods do not need a special treatment such as a derivatization of a sample.

We previously developed an analytical method for measuring the concentrations of PCDDs, PCDFs and Co-PCBs in human blood samples.[7] Then, we modified this method for a determination of OH-PCBs in human blood samples using a LC/MS/MS with an electrospray ionization interface in negative ion and selective reaction monitoring mode.[8,9]

The aim of this study is to analyze the blood samples from Yusho patients for OH-PCBs using LC/MS/MS technique.

Materials and methods

Chemicals and reagents: OH-PCBs standards were purchased from Wellington Laboratories, Inc. (ON, Canada) and Cambridge Isotope Laboratories, Inc. (MA, 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. Acetonitrile, methanol, formic acid and ultra pure water of LC/MS grade were purchased from Wako Pure Chemical Industries (Japan). A cartridge of ENVI-18 (500mg / 6mL tube) was purchased from Sigma-Aldrich, Inc. (MO, US).

Table 1. OH-PCBs standards

Compounds	Abbreviations	
4-OH-2,2',4',6,6'-PeCB	4'-OH-CB104	4H104
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

Sample preparation: The blood samples examined in this study were collected from 49 Yusho patients from whom informed consent was obtained. Each 5g blood sample was loaded into the extraction cell filled with Isolute. After freeze-drying, OH-[$^{13}C_{12}$]-PCBs, [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs and [$^{13}C_{12}$]-Co-PCBs were added as internal standards. Acetone : n-hexane (1:4, v/v) was used as extraction solvent of an accelerated solvent extractor. After the extract was evaporated to near dryness, it was dissolved in n-hexane and treated with sulfuric acid for an overnight. The separated hexane layer was applied to a silver nitrate / silica gel column. The first fraction containing PCDDs, PCDFs and Co-PCBs was eluted with 15mL of n-hexane. OH-PCBs was 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. The flow chart of this method for OH-PCBs in human blood samples is shown in Figure 1.

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

Flow Rate		0.2 mL/min.
Injection Volume		10 µL
Column Temperature		50 °C
Mobile Phase		10mM Formic acid : Methanol = 60 : 40 → 10 : 90 linear gradient
Temperature;	Source	120 °C
	Desolvation	350 °C
Gas Flow;	Cone	Nitrogen, 50 L/hr
	Desolvation	Nitrogen, 600 L/hr
Voltage;	Cone	40 V
	Capillary	2.0kV
Collision		Argon, 10kV
Ionization		ESI-Negative

Table 4. Mass method for the LC/MS/MS measurement

Compounds		Precursor ion → product ion m/z
OH- PeCB	^{12}C	340.87 → 340.87
	^{13}C	352.91 → 352.91
OH- HxCB	^{12}C	374.83 → 374.83
	^{13}C	386.87 → 386.87
OH- HpCB	^{12}C	408.79 → 408.79
	^{13}C	420.83 → 420.83

LC/MS/MS Measurement: All LC/MS/MS analysis was performed using an Alliance 2695 series high-performance Liquid Chromatograph Separations Module (Waters, US) equipped with Quattro micro API mass spectrometer (Waters Micromass, US). An analytical column, CAPCELL PAK C18 MG III, 2.1 mm × 150 mm, 3 µm (SHISEIDO, Japan) was used under a linear gradient solvent condition and the flow rate was set at 0.2mL/min. The initial mobile phase was 40:60 methanol / 10mM formic acid in ultra pure water. The injection volume was 10 µL. The 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 cone and desolvation gas. Potential applied onto the capillary was 2.0kV. Cone potential and collision energy were optimized for each molecule. In the collision cell, argon was used as a collision gas. And other analytical conditions for the LC/MS/MS measurement were summarized in Table 3 and 4.

Results and Discussion

Analysis of OH-PCBs in the blood of Yusho patients: 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. Concentrations of OH-PCBs in the blood of the 49 Yusho patients are summarized in Table 5. The major OH-PCB metabolite (range) was 4-OH-CB187 (20-906 pg/g-wet) followed by 4-OH-CB146 + 3-OH-CB153 (32-527 pg/g-wet), 4-OH-CB109 (ND-229 pg/g-wet) and 4'-OH-CB172 (ND-143 pg/g-wet). The total of 4 OH-PCBs ranged between 53 and 1740 pg/g-wet with a mean value of 514 pg/g-wet. Linderholm et al. reported that the total OH-PCBs ranged between 390 and 1300 pg/g serum with a mean value of 780 pg/g serum. And, the major OH-PCB metabolite was 4-OH-CB187 followed by 4-OH-CB146 and 4-OH-CB109. These results were in good agreement.

Table 5. Concentrations of OH-PCBs in blood of Yusho patients (pg/g-wet, n=49)

Congeners	Mean	Median	Min.	Max.	SD	CV
4-OH-CB109	67	56	ND	229	48.1	0.715
4-OH-CB146 + 3-OH-CB153	170	160	32	527	99.4	0.585
4-OH-CB187	233	206	20	906	178	0.765
4'-OH-CB172	45	38	ND	143	38.2	0.853
Total OH-PCBs	514	461	53	1740	336	0.652

ND: Not detected, SD: Standard deviation, CV: Coefficient of variation

In conclusion, we analyzed the blood samples from Yusho patients for OH-PCBs using LC/MS/MS technique. The total OH-PCBs ranged from 53 to 1740 pg/g-wet.

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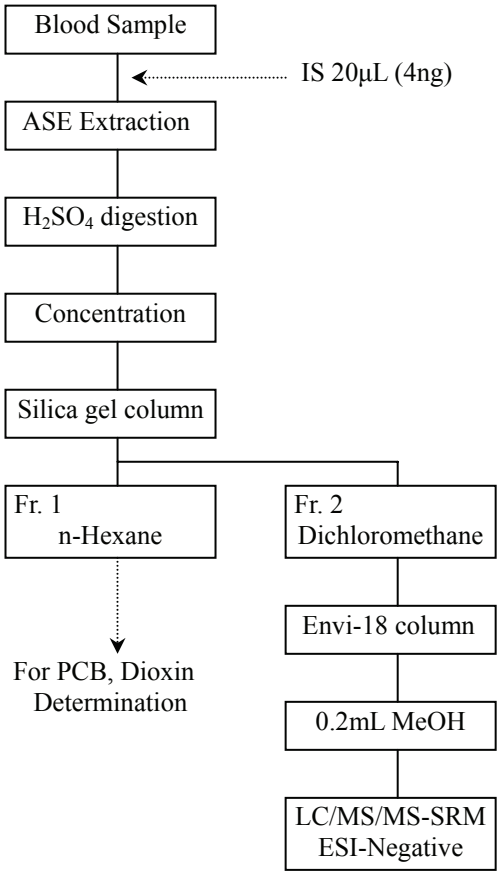

Figure 1. Flow chart of the measurement method for OH-PCBs in blood samples.