MEASUREMENT METHOD FOR HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBs) IN BLOOD BY LC/MS/MS

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Abstract

We developed measurement method for hydroxylated chlorinated biphenyls concentrations in human blood samples using a LC/MS/MS with an electrospray ionization interface in negative ion and selective reaction monitoring mode. This developed method is effective to determine the concentrations of PCDDs, PCDFs, Co-PCBs and OH-PCBs at the same time without a derivatization of a sample. The major OH-PCB metabolites were 4-OH-CB187, 4-OH-CB146 and 4-OH-CB107 + 3-OH-CB118 in human blood samples.

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-CB107 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-CB107 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. We developed measurement method for OH-PCBs by LC/MS.[5] This method does not need a special treatment such as a derivatization of a sample. And, we developed an analytic method for measuring the concentrations of PCDDs, PCDFs and Co-PCBs in human blood samples.[6] We tried to modify this method for a determination of OH-PCBs in human blood samples.

The aim of this study to develop a measurement method for hydroxylated penta- through hepta- chlorinated biphenyls concentrations in blood samples using a LC/MS/MS with an electrospray ionization interface in negative ion and selective reaction monitoring mode.

Materials and Methods

Chemicals and reagents: OH-PCBs standards were purchased from Wellington Laboratories, Inc. (Canada). These OH-PCBs standards are listed in Table 1. Each 1 mg/L standard solution was prepared by dilution with acetonitrile. OH-[$^{13}C_{12}$]-PCBs, as internal standards, were also purchased from Wellington Laboratories, Inc. These 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).

Sample preparation: 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. 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 30mL of dichloromethane as the second fraction. The eluate was concentrated to near dryness with a multiple sample concentrator, and

transferred to an LC injection vial with 1mL of methanol.

LC/MS/MS Measurement: All LC/MS/MS analysis was performed using an Alliance 2695 series high-performance Liquid Chromatograph Separations Module (Waters, USA) equipped with Quattro micro API mass spectrometer (Waters Micromass, USA). An analytical column, CAPCELL PAK C18, 2.1 mm × 150 mm, 5 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 60:40 methanol / 0.1% 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.

Table 1. OH-PCBs standards

Compounds	Abbreviation	
4-OH-2,2',4',6,6'-PeCB	4'-OH-CB104	4H104
4-OH-2,3,3',4',5-PeCB	4-OH-CB107	4H107
4-OH-2',3,3',4',5-PeCB	4'-OH-CB108	4H108
3-OH-2,3',4,4',5-PeCB	3-OH-CB118	3H118
4-OH-2,2',3,3',4',5-HxCB	4'-OH-CB130	4H130
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
3-OH-2,2',3',4,4',5,5'-HpCB	3'-OH-CB180	3H180
4-OH-2,2',3,4',5,5',6-HpCB	4-OH-CB187	4H187

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

4-OH-2',3,4',5,5'-PeCB	4'-OH-CB120	M4H120
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

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

Flow Rate		0.2 mL/min.
Injection Volume		10 μL
Column Temperature		40 °C
Mobile Phase		0.1% Formic acid : Methanol = 40 : 60 → 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

Results and Discussion

Chromatograms of LC/MS/MS measurement: Figure 2, 3 and 4 illustrate the LC/MS/MS chromatograms of hydroxylated penta- through hepta- chlorinated biphenyls in SRM mode. The standard solution contains all OH-PCB congeners shown in Table 1 and 2. $[M-H]^-$ ions were observed from each OH-PCBs standard solutions in negative ion mode. Precursor ion and product ion were set with *m/z*: 340.87 → *m/z*: 340.87 and *m/z*: 352.91 → *m/z*: 352.91 for the native and ^{13}C-labelled, respectively. And other mass methods for the LC/MS/MS measurement were summarized in Table 4.

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

Figure 2 shows the three peaks were appeared on the channel of 340.87 → 340.87. 4H104 and 4H108 had each single peak but 4H107 and 3H118 could not be separated. Figure 3 shows the two peaks were appeared on the channel of 374.83 → 374.83. 4H146 had a single peak but 4H130 and 3H138 could not be separated. And,

4H187 had a single peak but 3H180 and 4H172 were not separated on the cannel of 408.79 → 408.79 in figure 4.

Sensitivity of LC/MS/MS measurement: Calibration curves were made by measuring a standard solution ranged between 0.1-2.0 ng/mL with 1.0 ng/mL of ^{13}C-labelled internal standard. When a standard was injected, 1.0pg of 4-OH-CB107, 3'-OH-CB138 and 4-OH-CB187 were detected with about S/N=3. These results indicate that pg-level of OH-PCBs can be detected using LC/MS/MS with an electrospray ionization interface in negative ion mode and selective reaction monitoring mode.

Analysis of OH-PCBs: The flow chart of the measurement method for OH-PCBs in human blood samples is shown in figure 1. This method is effective to determine the concentrations of PCDDs, PCDFs, Co-PCBs and OH-PCBs at the same time. Figure 5, 6 and 7 show the LC/MS/MS chromatograms of OH-PeCBs, OH-HxCBs and OH-HpCBs in 5g of human blood samples, respectively. The peaks of 4H107 + 3H118, 4H130 + 3H138, 4H146, 4H187 and 3H180 + 4H172 were detected. Some small other peaks could be observed but could not be identified. The major OH-PCB metabolite was 4-OH-CB187 (54 pg/g) followed by 4-OH-CB146 (32 pg/g) and 4-OH-CB107 + 3-OH-CB118 (6 pg/g). The recovery of the 13C-labelled internal standards was 60% overall when the concentrations of OH-PCBs in the human blood were measured by developed method.

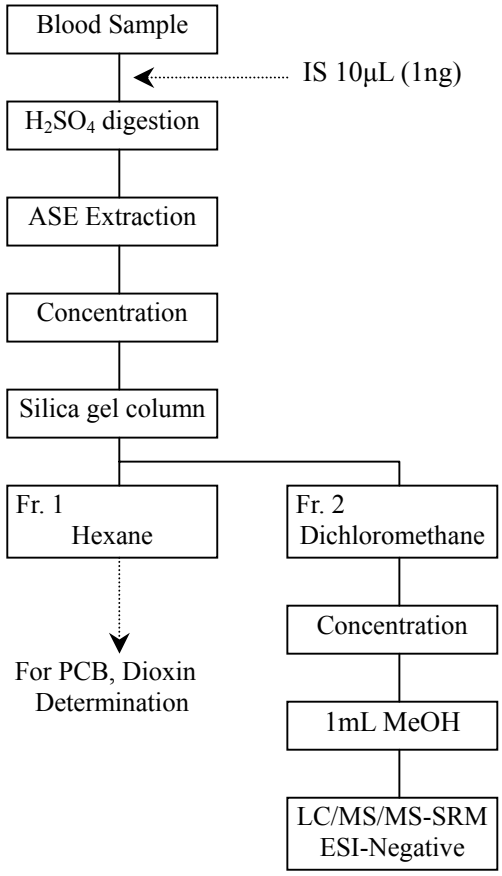

Figure 1. Flow chart of the measurement method for OH-PCBs in blood samples.

References

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3. Sakiyama T., Yamamoto A., Kakutani N., Fukuyama J. and Okumura T. *Organohalogen Comp* 2007; 69: 1380.
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5. Tobiishi K., Tsukatani H. *Organohalogen Comp* 2008; 70: 2309.
6. Todaka T., Hirakawa H., Tobiishi K., Iida T. *Fukuoka Igaku Zasshi* 2003; 94: 148.

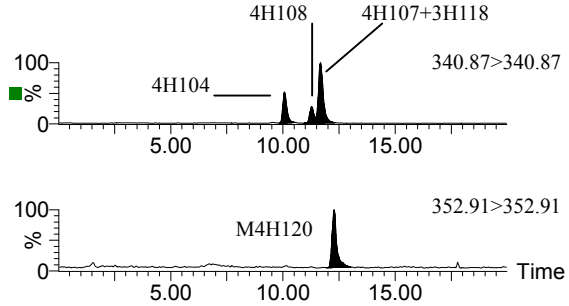

Figure 2. LC/MS/MS chromatograms of 1 ng/mL OH-PeCB standards

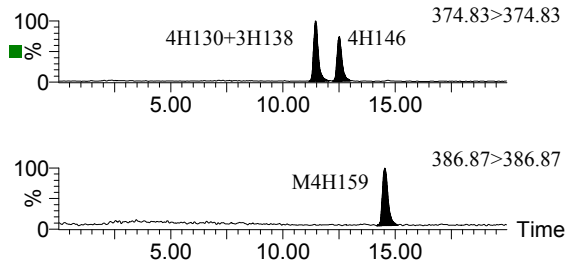

Figure 3. LC/MS/MS chromatograms of 1 ng/mL OH-HxCB standards

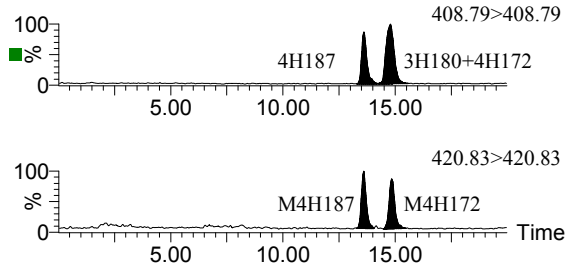

Figure 4. LC/MS/MS chromatograms of 1 ng/mL OH-HpCB standards

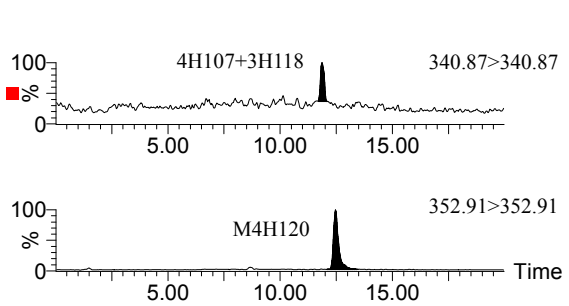

Figure 5. LC/MS/MS chromatograms of OH-PeCBs in human blood

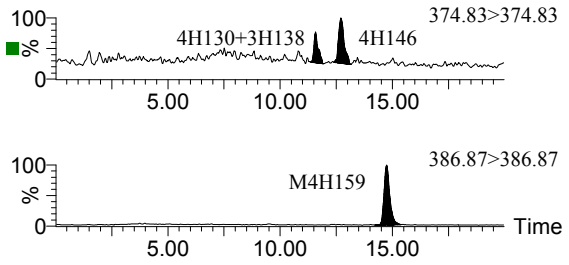

Figure 6. LC/MS/MS chromatograms of OH-HxCBs in human blood

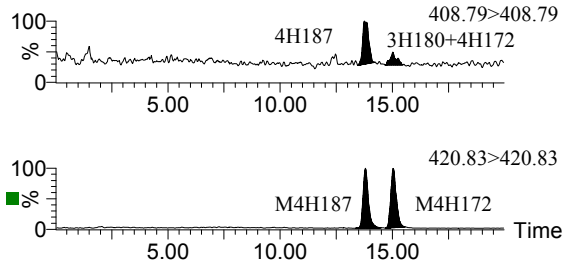

Figure 7. LC/MS/MS chromatograms of OH-HpCBs in human blood