# IMPROVEMENT OF THE MEASUREMENT METHOD FOR HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBs) IN BLOOD USING 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-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. 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 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]

The aim of this study is to develop the sensitive method for the determination of OH-PCBs using an additional sample clean-up.

## 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). Cartridges of ENVI-Carb C (100mg / 1mL tube) and ENVI-18 (500mg / 6mL tube) were 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:** 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 transferred to an LC injection vial with 0.5mL of methanol.

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 = 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 |

**Additional sample clean-up method:** Each 0.5mL of methanol solution was evaporated to near dryness, it was dissolved in 2mL methanol or acetonitrile and loaded onto an ENVI-Carb C cartridge or an ENVI-18 cartridge. The eluate was concentrated under the nitrogen flow and transferred to an LC injection vial with 0.1mL 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, US) equipped with Quattro micro API mass spectrometer (Waters Micromass, US). An analytical column, CAPCELL PAK C18 MG III, 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 40:60 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.

## 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.

**Additional sample clean-up method:** An Envi-18 cartridge contains silica gel based material that has octadecyl bonding and an Envi-Carb C cartridge does graphitized non-porous carbon. They are useful for additional sample clean-up because of easy handling and unique interaction with chemical substances. Table 5 shows that recoveries of the OH-PCBs standards eluted with methanol and acetonitrile from Envi-18 and

Table 5. Recoveries of the OH-PCB standards eluted with methanol or acetonitrile from cartridges. (%)

| | Envi-18 | | | | Envi-Carb C | | | |
|---|---|---|---|---|---|---|---|---|
| | Methanol | | Acetonitrile | | Methanol | | Acetonitrile | |
| | 0-2mL | 2-4mL | 0-2mL | 2-4mL | 0-2mL | 2-4mL | 0-2mL | 2-4mL |
| 4'-OH-CB104 | 33 | 60 | 39 | 62 | 95 | 10 | 101 | 3 |
| 4-OH-CB109 | 29 | 64 | 30 | 65 | 79 | 27 | 93 | 10 |
| 3'-OH-CB138 | 32 | 62 | 37 | 67 | 86 | 17 | 106 | 3 |
| 4-OH-CB146 | 29 | 63 | 29 | 66 | 76 | 26 | 103 | 6 |
| 4'-OH-CB172 | 23 | 79 | 20 | 65 | 70 | 37 | 97 | 11 |
| 4-OH-CB187 | 27 | 64 | 18 | 57 | 93 | 15 | 103 | 3 |

Envi-Carb C cartridges. In the Envi-18 cartridge, OH-PCBs can be eluted with both 4mL methanol and 4mL acetonirile. And, in the Envi-Carb C cartridge, they can be eluted with both 4mL methanol and 2mL acetonitrile.

**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. In order to get sensitivity each LC/MS sample solution need to concentrate into 0.1mL by using additional sample clean-up method. Figure 5, 6 and 7 show the LC/MS/MS chromatograms of OH-PCBs in 5g of human blood samples. The peaks of 4H109, 3H138, 4H146, 4H187 and 4H172 were detected. The major OH-PCB metabolite was 4-OH-CB146 (38 pg/g) followed by 4-OH-CB187 (37 pg/g), 4-OH-CB109 (20 pg/g) and 3'-OH-CB138 (18 pg/g).

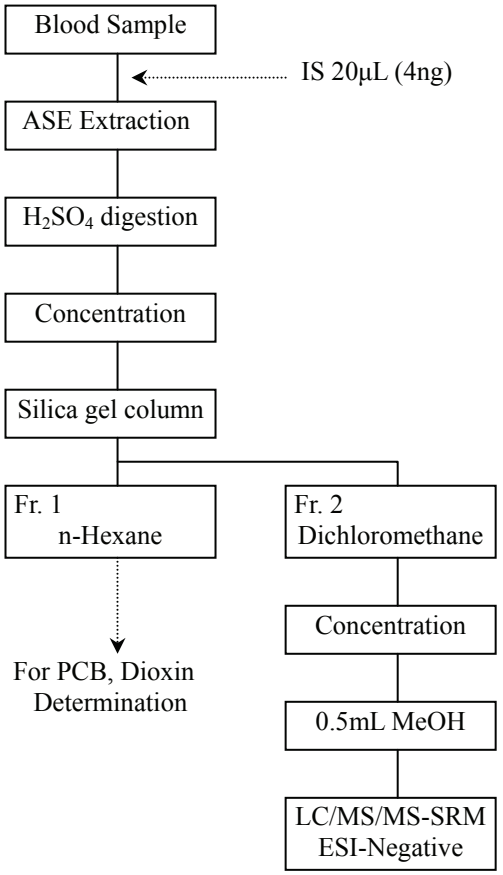


Figure 1. Flow chart of the measurement method for OH-PCBs in blood samples.

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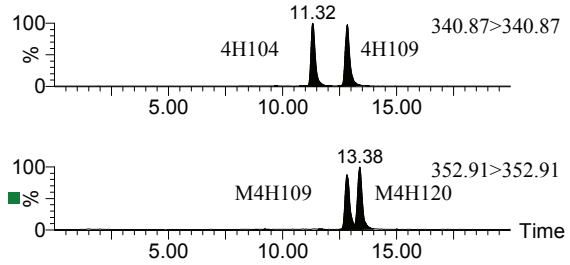


Figure 2. LC/MS/MS chromatograms of 8 ng/mL OH-PeCB standards

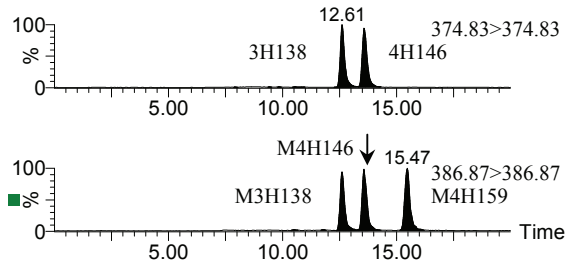


Figure 3. LC/MS/MS chromatograms of 8 ng/mL OH-HxCB standards

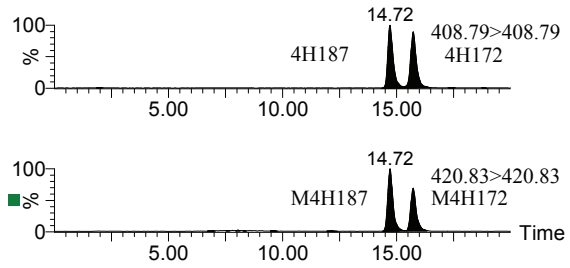


Figure 4. LC/MS/MS chromatograms of 8 ng/mL OH-HpCB standards

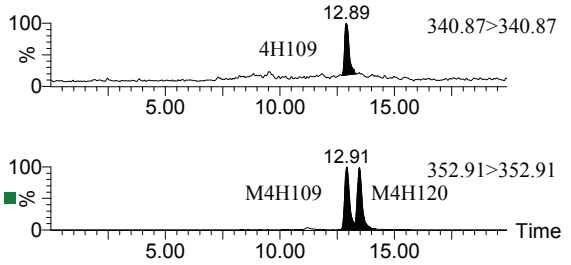


Figure 5. LC/MS/MS chromatograms of OH-PeCBs in human blood

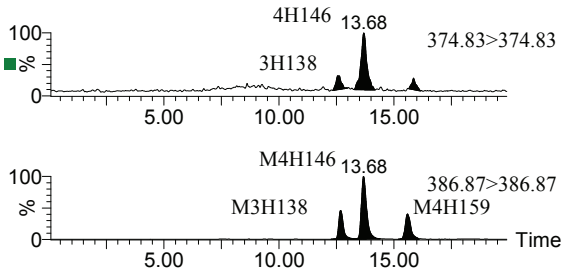


Figure 6. LC/MS/MS chromatograms of OH-HxCBs in human blood

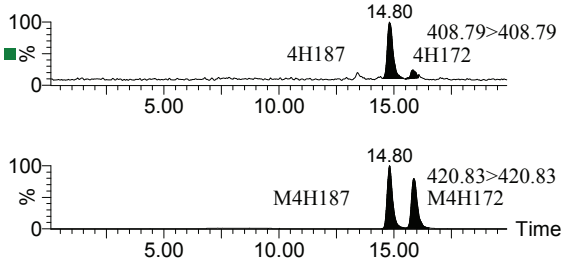


Figure 7. LC/MS/MS chromatograms of OH-HpCBs in human blood