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https://doi.org/10.15017/19737

出版情報：福岡醫學雜誌. 102 (4), pp.153-158, 2011-04-25. 福岡医学会
バージョン：
権利関係：

Measurement Method for Hydroxylated Polychlorinated Biphenyls in the Blood of Yusho Patients by Liquid Chromatography-Electrospray Tandem Mass Spectrometry

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Abstract Hydroxylated polychlorinated biphenyls (OH-PCBs) are formed as major metabolites of PCBs by cytochrome P450 enzyme-mediated oxidation. It has been reported that their total concentration in serum samples of Yusho patients ranged from 390 to 1300 pg/g.
We developed a measurement method for OH-PCBs in blood samples by LC/MS/MS. This method is effective at determining the concentrations of PCDDs, PCDFs, Co-PCBs and OH-PCBs from the same sample without special treatment of the sample.
The concentration of OH-PCBs in the blood of Yusho patients was examined using this method. The major OH-PCB metabolites were 4-OH-CB187 (54-906 pg/g-wet), 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 OH-PCBs ranged from 95 to 1740 pg/g-wet.

Key words : Yusho, Blood, OH-PCB, LC/MS/MS

Introduction

Polychlorinated biphenyls (PCBs) are one of the persistent and bioaccumulative chemicals. Hydroxylated polychlorinated biphenyls (OH-PCBs) are well known as metabolites of PCBs formed by the cytochrome P450 enzyme-mediated oxidation of PCBs. Enomoto et al.[1)] investigated the concentrations of OH-PCBs in the Japanese human blood plasma reporting that the major congeners and levels were 4-OH-CB109 10-230 pg/g-wet, 4-OH-CB146 13-340 pg/g-wet and 4-OH-CB187 12-110 pg/g-wet. Linderholm et al.[2)] reported that the highest 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; further, that the total of 6 OH-PCB metabolites ranged between 390 and 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 methylation by trimethylsilyldiazomethane was an effective derivatization method. On the other hand, R.J. Letcher et al.[5)] 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, PCDFs and Co-PCBs in human blood samples[6]. Here, we modify this 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. This method is effective at determining the concentrations of PCDDs, PCDFs, Co-PCBs and OH-PCBs 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. (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

2. Sample preparation

The blood samples examined in this study were collected from 27 Yusho patients from whom informed consent was obtained. Each 5g blood sample was loaded into an 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 the extraction solvent for an accelerated solvent extractor. After the extract was evaporated to near dryness, it was dissolved in n-hexane and treated with sulfuric acid 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 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. 1.

3. 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, 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 with the flow rate 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. Detection was performed on a quadrupole analyzer operated in negative electrospray ionization

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

Flow Rate		0.2 mL/min.
Injection Volume		10 μL
Column Temperature		40 ℃
Mobile Phase		0.1% Formic acid : Methanol = 60 : 40 → 10 : 90 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	2.0 kV
Collision		Argon, 15 eV
Ionization		ESI-Negative

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

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

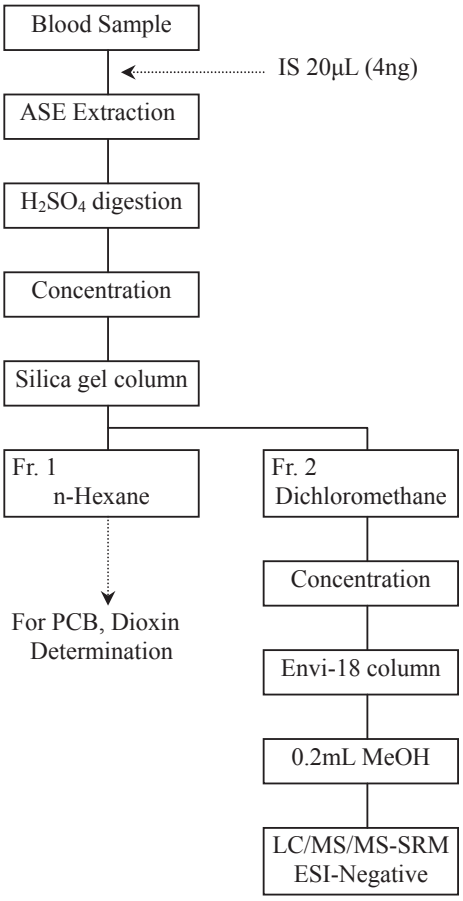

Fig. 1 Flow chart of the measurement method for OH-PCBs in blood samples

(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 2.0 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.

Results and Discussion

1. LC/MS/MS measurements

Fig. 2 shows the calibration curve of 4-OH-CB187, which ranged from 0.1 to 2.0 ng/mL. Fig. 3, 4 and 5 illustrate the LC/MS/MS chromatograms of hydroxylated penta- through hepta-chlorinated biphenyls in SRM mode. The standard solution contains all OH-PCB congeners as shown in Table 1 and 2. $[M-H]^-$ ions were

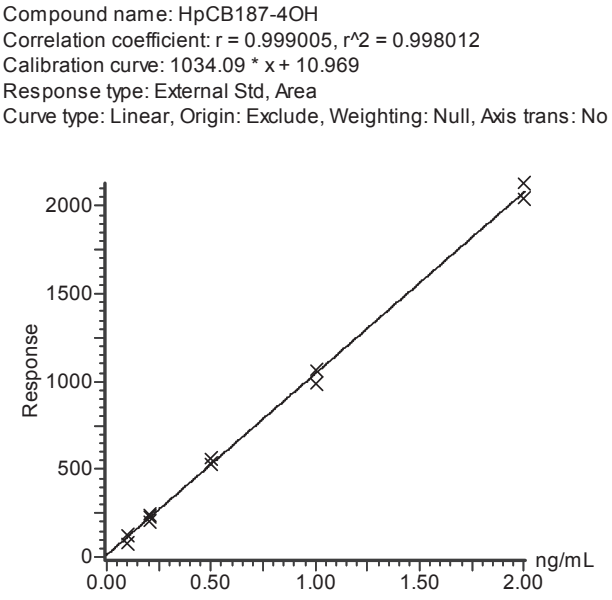

Fig. 2 Calibration curve of 4-OH-CB187 (0.1～2.0ng/mL)

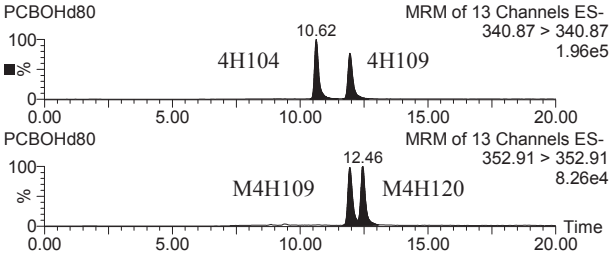

Fig. 3 LC/MS/MS chromatograms of 8 ng/mL OH-PeCB standards

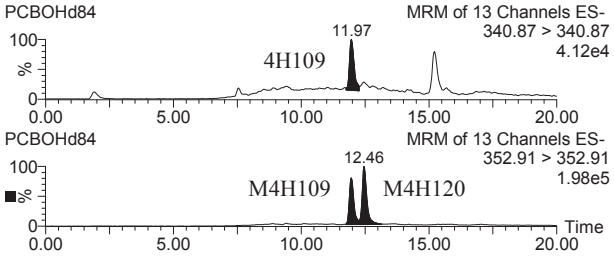

Fig. 6 LC/MS/MS chromatograms of OH-PeCBs in the blood of Yusho patient

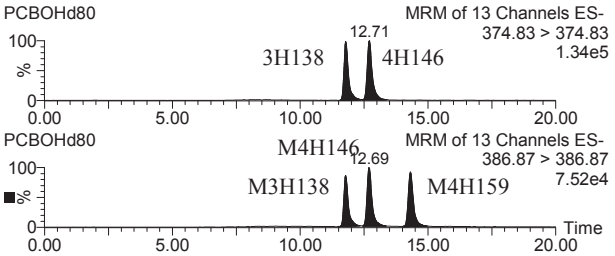

Fig. 4 LC/MS/MS chromatograms of 8 ng/mL OH-HxCB standards

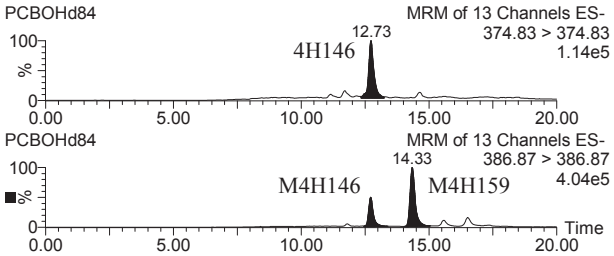

Fig. 7 LC/MS/MS chromatograms of OH-HxCBs in the blood of Yusho patient

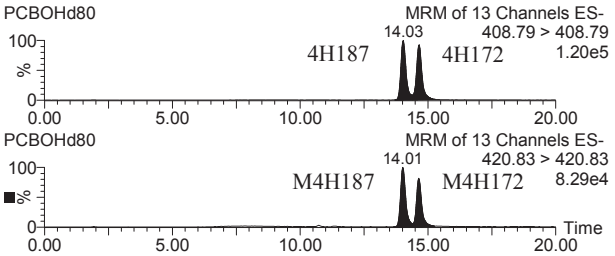

Fig. 5 LC/MS/MS chromatograms of 8 ng/mL OH-HpCB standards

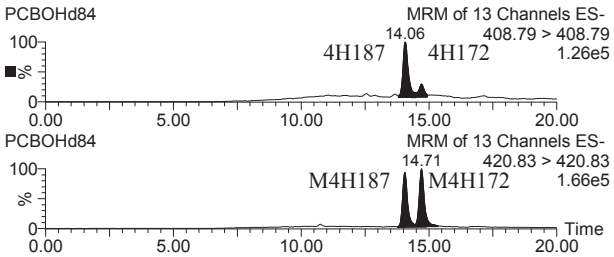

Fig. 8 LC/MS/MS chromatograms of OH-HpCBs in the blood of Yusho patient

observed from each OH-PCBs standard solution in the 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 ions, respectively. Other mass methods for the LC/MS/MS measurement are summarized in Table 4.

2. Analysis of OH-PCBs in blood samples

Fig. 6, 7 and 8 show the LC/MS/MS chromatograms of OH-PCBs in one of the blood samples collected. 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 27 Yusho patients are summarized in Table 5. The major OH-PCB metabolite (range) was 4-OH-CB187 (54-906 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 95 and 1740 pg/g-wet with a mean value of 687 pg/g-wet. These results were in good agreement with those reported by Linderholm et al.

In conclusion, we developed measurement method for OH-PCBs in blood by LC/MS/MS.

Table 5 Concentrations of OH-PCBs in blood of Yusho patients (pg/g-wet, n = 27)

Congeners	Mean	Median	Min.	Max.	SD	CV
4-OH-CB109	86	86	ND	229	55.0	0.642
4-OH-CB146 + 3-OH-CB153	215	211	32	527	101	0.470
4-OH-CB187	326	326	54	906	172	0.527
4'-OH-CB172	60	50	ND	143	39.9	0.661
Total OH-PCBs	687	602	95	1740	332	0.483

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

Developed method was effective at determining the concentrations of PCDDs, PCDFs, Co-PCBs and OH-PCBs from a single blood sample without special treatment.

Acknowledgements

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health Labour and Welfare, Japan.

References

1) Enomoto T, Nishino Y, Matsuda M, Kawano M and Wakimoto T : Hydroxylated polychlorinated biphenyl metabolites (HO-PCBs) in human blood plasma-Development of analytical methods and their residue in Japanese general population-J. Environ. Chem. 16 : 51-59, 2006.

2) Linderholm L, Masuda Y, Athanasiadou M and Bergman Å : PCB and PCB metabolites in serum from Yusho patients 37 years after accident. Organohalogen Comp. 69 : 2141-2144, 2007.

3) Sakiyama T, Yamamoto A, Kakutani N, Fukuyama J and Okumura T : Hydroxylated polychlorinated biphenyls (OH-PCBs) in the aquatic environment : Levels and congener profiles in sediments from Osaka, Japan. Organohalogen Comp. 69 : 1380-1383, 2007.

4) Matsumoto K, Iseki N, Kameda H, Okumura T, Kashima Y and Shiozaki T : The highly effective methylation method for hydroxylated polychlorinated biphenyls. Organohalogen Comp. 68 : 2442-2445, 2006.

5) R J Letcher, H X Li and S G Chu : Determination of hydroxylated polychlorinated biphenyls (HO-PCBs) in blood plasma by high-performance liquid chromatography-electrospray ionization-tandem quadrupole mass spectrometry. J. Anal. Toxicology 29 : 2005.

6) Todaka T, Hirakawa H, Tobiishi K and Iida T : New protocol of dioxin analysis in human blood. Fukuoka Igaku Zasshi 94 : 148-157, 2003.

(Received for publication March 23, 2011)

（和文抄録）

液体クロマトグラフ質量分析計による油症認定患者血液中の水酸化ポリ塩化ビフェニルの測定法

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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．OH-PCBsは体内でチトクロームP450酵素誘導によりPCBから生成され，油症認定患者の血清から390-1300pg/gの濃度で検出された報告がある．

OH-PCBsの測定法に関し，従来の分析法では複雑な前処理を必要としていたが，測定装置にLC/MS/MSを用いることで，前処理の簡略化を達成した．本法の利点は，従来から測定を行っているダイオキシン類分析用の血液試料から，OH-PCBsを分離することで，OH-PCBsの分析のために新たに血液試料を確保する必要が無いことである．

油症認定患者の血液を用いて，本法に基づいて分析した結果，主要なPCBの代謝物は，4-OH-CB187（54-906　pg/g-wet），4-OH-CB146　+　3-OH-CB153（32-527　pg/g-wet），4-OH-CB109（ND-229 pg/g-wet），4'-OH-CB172（ND-143 pg/g-wet）であり，その合計は95-1740 pg/g-wetであった．