DEVELOPMENT OF HYDROXYLATED POLYCHLORINATED BIPHENYL (OH-PCBs) ANALYTICAL METHOD IN HUMAN URINE WITH UPLC/Q-TOF MS

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Introduction

Polychlorinated biphenyls (PCBs) are known as environmental contaminants that may cause abnormal effect in various organs and some studies determined the residue levels and patterns of PCBs and metabolized PCBs (hydroxylated PCB : OH–PCBs) congeners in human blood. Hydroxyl group of OH-PCBs has high acidity, therefore OH-PCBs was made metoxy-derivatization and analyzed with GC-MS.

Derivatization GC-HRMS method has some issues that complicated preparations are needed and separation of methoxy metabolized PCBs and methoxy-derivatization OH-PCBs is difficult.

Our previous study determined elution order for 51 congeners of OH-PCB without derivatization with UPLC/QTof MS. The present study aimed at developing an analytical method for quantity to separate mixture of 6 major OH-PCBs in human blood (4'-OH-CB-107, 3-OH-CB-138, 4'-OH-CB-146, 3-OH-CB-153, 4'-OH-CB-172 and 4-OH-CB-187) and applying analytical method to biological sample of human urine.

An analytical method is developed to measure 6 major OH-PCBs in human blood without derivatization and total analytical time is within 20 minutes.

Materials and Methods

OH-PCBs Standard Solution:

Six major compounds standard solution of OH-PCBs (see Fig.1) were purchased from Wellington Laboratories Inc. (Guelph,ON, Canada) and each solution was diluted in acetone.

LC–MS/MS analysis:

Identification and quantification were performed using ultra performance liquid chromatography (UPLC: Waters Acquity UPLC system) and a high-resolution q-tof mass spectrometer (Xevo G2 QTof MS) with a resolving power of more than 20000(Table 1)

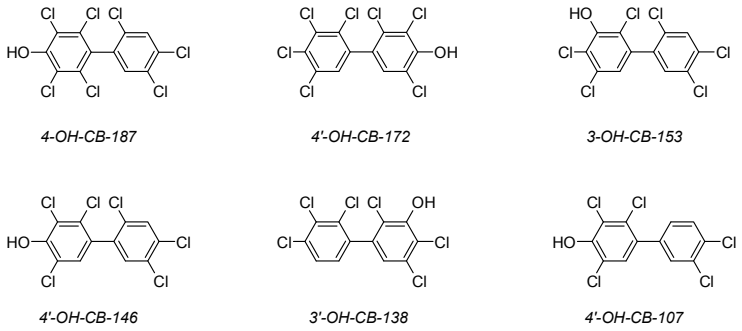

Fig.1 Major 6 components of OH-PCB in human blood

Table 1 Analytical conditions of UPLC/Q-Tof MS

UPLC			***MS***	
Column:	BEH C18 2.1ID X 150 mm, 1.7μm		ESI	negative
Flow rate	0.5 mL/min.		Capillary (kV)	1.5
Column heater	60℃		Sampling cone	40
			Source temp.	120℃
Gradient	5mM CH3COONH4 aq	THF/CH3CN (v/v: 1/4)	Desolvation temp.	600℃
Initial	75	25	Cone Gas Flow	20 L/hr.
17 min.	25	75	Desolvation Gas Flow	800 L/hr.
18 min.	1	99		
18.5 min.	75	25		

Results and Discussion

Analytical method with UPLC/Q-TOF MS is developed to separate mixture of 6 major OH-PCBs in human blood without derivatization. Figure 2 show the separation of OH-PCBs.This method is performable to separate into Six OH-PCBs. Moreover, this method enabled the separation of difficult 3-OH-153 and 4'-OH-165 in GC/HRMS with methoxy-derivatization.

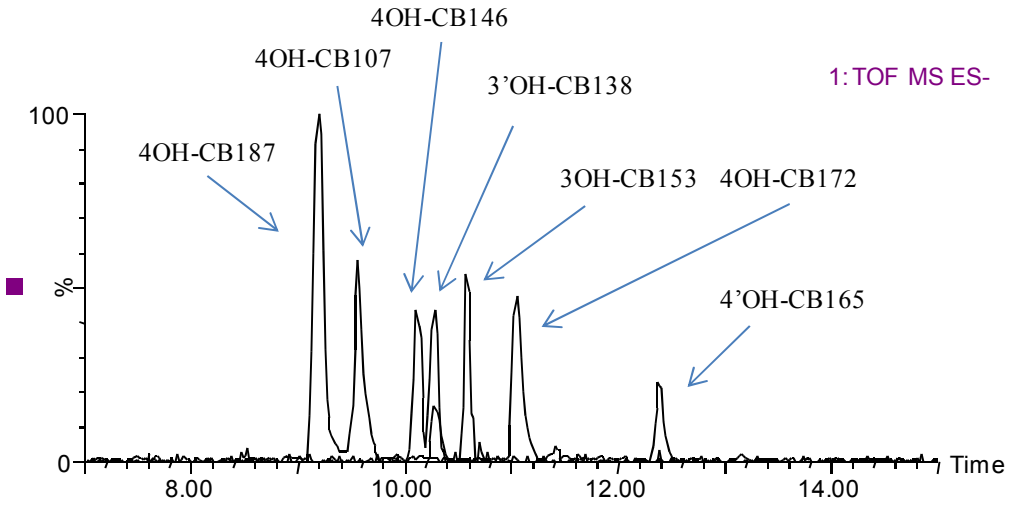

Fig2. Separation of OH-PCBs

Linearity of calibration curves at the concentration range of 0.5 to 50 ppb of 3-OH CB-138 and 4'-OH CB-153 that were detected in human urine sample is higher than 0.99 of R^2 (see Fig.3).

Compound name: 3-OH CB-H138
Correlation coefficient: r = 0.999553, r^2 = 0.999107
Calibration curve: 51.2173 * x + -16.7275
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis

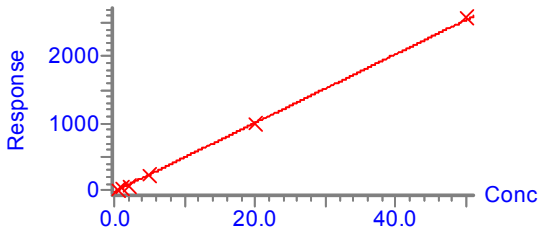

Fig3. Calibration curve of 3'OH-CB138

Compound name: 4'-OH CB-153
Correlation coefficient: r = 0.997974, r^2 = 0.995952
Calibration curve: 57.0245 * x + -7.55719
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis

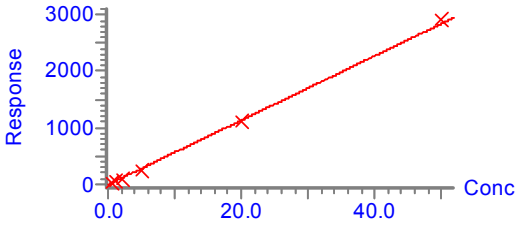

Fig.4 Calibration curve of 3OH-CB153

This method applied for human urine samples and . three OH-PCBs(4OH-CB107,3'OH-CB138 and 3OH-CB153) were detected.

Table 2 Concentration of OH-PCBs in human urine.

Compounds / Samples	Urine 1	Urine 2	Urea blank
4OH-CB187	N.D.	N.D.	N.D.
4OH-CB107	4.7 pg/mL	4.5 pg/mL	N.D.
4OH-CB146	N.D.	N.D.	N.D.
3'OH-CB138	5.3 pg/mL	5.5 pg/mL	N.D.
3OH-CB153	6.0 pg/mL	9.5 pg/mL	N.D.
4OH-CB172	N.D.	N.D.	N.D.

The ratios of detected 3 OH-PCBs are show in fig.5. The order of concentration is 4OH-CB107 < 3'OH-CB138 < 3OH-CB153 in both samples.

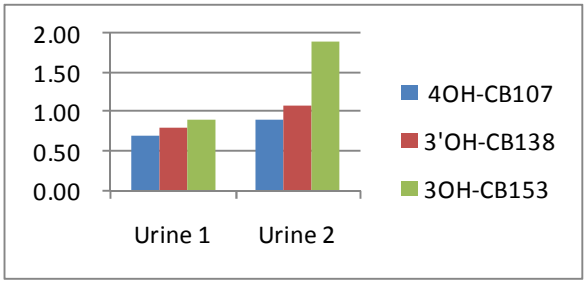

Fig.5 Ratio of detected OH-PCBs in human urine

Acknowledgements:

This research was partly supported by Grants-in-Aid for Scientific Research(B) (No. 21310027) from the Ministry of Education,Culture,Sports,Science and Technology, Japan and the Waste Management Research Grant (No. K22037) from the Ministry of the Environment, Japan.

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