MEASURMENT OF ACCUMULATION OF HYDROXYLATED POLYCHLORINATED BIPHENYL (OH-PCBs) IN HUMAN URINE AND BLOOD

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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-HRMS. Complicated preparations are needed for analyzing with GC-HRMS, beside that identifying methoxy metabolized PCBs and methoxy-derivatization OH-PCBs is difficult.

Our previous study determined elution order for 51 congeners of OH-PCB without derivatization and developed 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 4'-OH-CB-165 which is difficult to be separated from 3-OH-CB-153 with GC-HRMS, then applied analytical method to biological sample of human urine with UPLC/QTof MS.

The present study aimed at measuring of OH-PCBs in human urine and blood sample taken from same person , evaluating accumulation of each OH-PCB isomer and congener and comprehending dominant metabolized PCBs in each human urine and blood.

Materials and Methods

OH-PCBs Standard Solution:

Six major compounds standard solution of OH-PCBs in human blood and 4'-OH-CB-165 (see Fig.1) were purchased from Wellington Laboratories Inc. (Guelph,ON, Canada) and each solution was diluted in acetone.

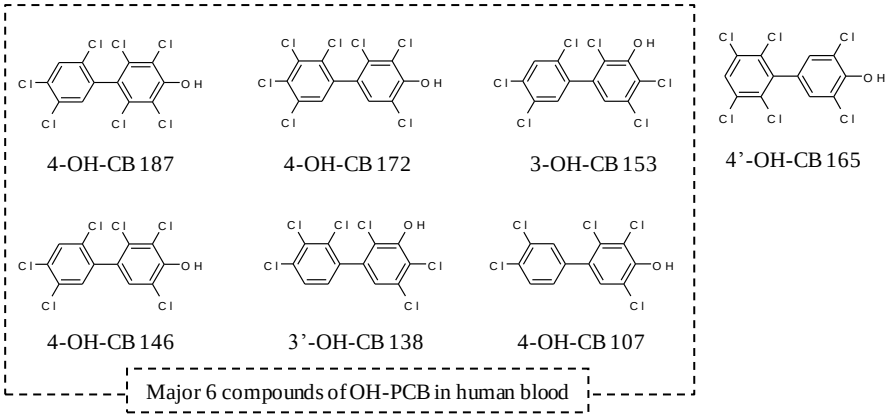


Fig.1 Major 6 compounds of OH-PCB in human blood and 4'-OH-CB-165

LC–TOF 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 22500(Table 1)

Table 1 Analytical conditions of UPLC/Q-Tof MS

UPLC	
Instrument:	ACQUITY UPLC
Column:	BEH C18 2.1ID X 150 mm, 1.7um
Flow rate:	0.5 mL/min.
Column heater:	60 degree centigrade
Mobile Phese A:	5mM CH3COONH4 aq.
Mobile Phese B:	THF/CH3CN (v/v: 1/4)

Gradient :

Time	%A	%B
Initial	75	25
17 min.	25	75
18 min.	1	99
18.5 min.	75	25
Total Run Time:	20 min	

MS	
Instrument:	Xevo G2 Q-TOF
Ionization mode:	ESI negative
Capillary:	1.5 kV
Sampling Cone:	40 V
Source Temp:	120 degree centigrade
Desolvation Temp:	600 degree centigrade
Cone Gas Flow:	20 L/hr.
Desolvation Gas Flow:	800 L/hr.
Resolving Power:	<22,500
Selected Ion:	
Penta-chloro/OH-CB	*m/z* =340.8675
Hexa-chloro/OH-CB	*m/z* =374.8286
Hepta-chloro/OH-CB	*m/z* =408.7896

Results and Discussion

m/z giving the highest intensity in each spectrum of compound was chosen for quantitative analysis. Developed method was able to separate 7 OH-PCBs without derivatization(Fig. 2).

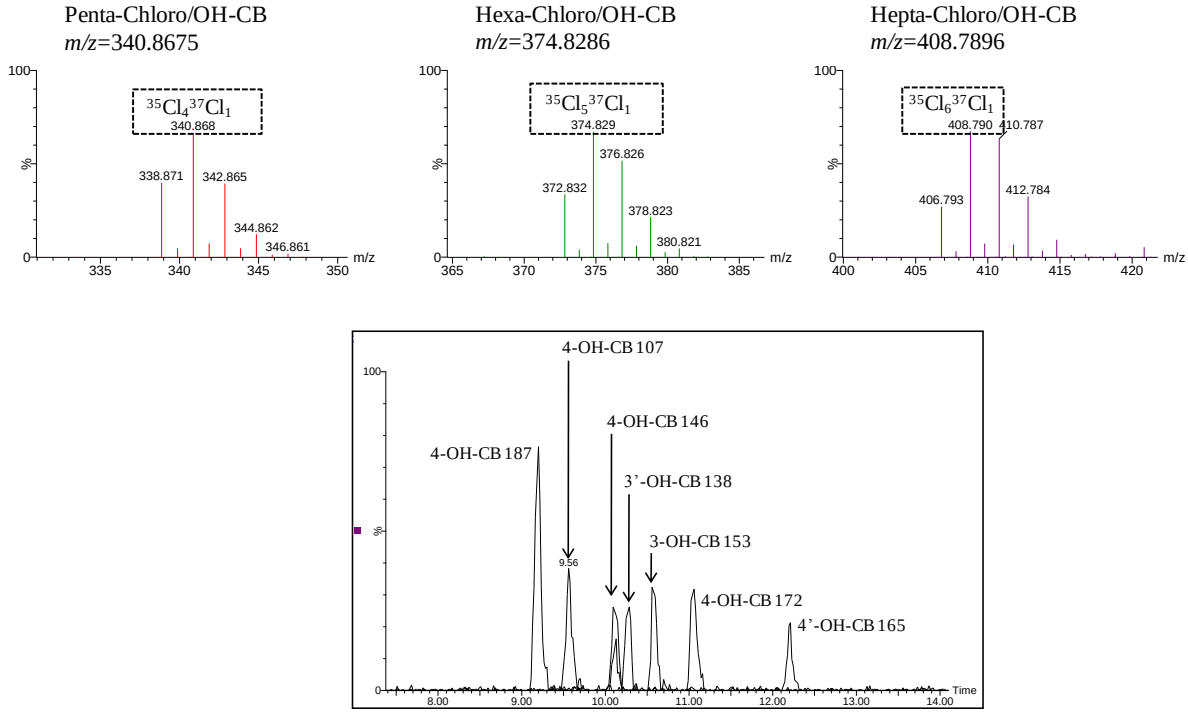
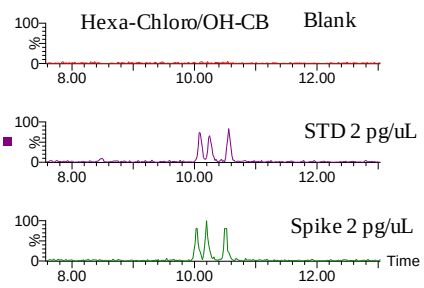


Fig2. MS spectra and separation of OH-PCBs

Before analyzing human sample, matrix effect was measured with blank and spiked urine sample at the concentration of 2pg/uL. Yield was more than 85%, matrix effect is less than -15 for each compounds(Table 2).

Table 2 Matrix effect of OH-PCBs in human urine.

Compounds	Urine blank	Urine Spike	yield
4-OH-CB 187	N.D.	2.00	100
4-OH-CB 107	N.D.	1.90	95
4-OH-CB 146	N.D.	1.70	85
3'-OH-CB 138	N.D.	1.90	95
3-OH-CB 153	N.D.	1.80	90
4-OH-CB 172	N.D.	2.00	100



This method applied for human biological samples. Concentration and order in urine and blood sample taken from same person are shown in Table 3 and 4. Concentration of OH-PCB in blood is much higher than that in urine, however pattern of detected OH-PCBs and order are different from each other.

This might indicate that each isomer and congener has individual characteristic to metabolic and transport pathway then pattern of detected OH-PCBs and order are different between blood and urine.

Table 3 Concentration of OH-PCBs in human urine and blood.

	Concentration (pg/mL)						
Sample	4-OH-CB 187	4-OH-CB 107	4-OH-CB 146	3‘-OH-CB 138	3-OH-CB 153	4-OH-CB 172	4’-OH-CB 165
Urine	nd	4.7	nd	5.3	6.0	nd	nd
Blood	125	225	298	200	nd	100	nd

Table 4 Concentration order of OH-PCBs in human urine and blood

Urine	3-OH-CB	>	3‘-OH-CB	>	4-OH-CB	>	4-OH-CB
Blood	4-OH-CB	>	4-OH-CB	>	3‘-OH-CB		

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