DEVELOPMENT OF LOW LEVEL HYDROXYLATED POLYCHLORINATED BIPHENYL (OH-PCBs) ANALYTICAL METHOD IN HUMAN BLOOD WITH UPLC/Q-TOf MS

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Abstract

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, OH-PCBs was made metoxy-derivatization and analyzed with GC-MS. It was made derivatization, and this has been analyzed in GC-HRMS (high resolution MS). Derivatization GC-HRMS method has some issues that complicated preparations are needed and metoxy metabolized PCBs and metoxy-derivatization OH-PCBs cannot be differentiated.

An analytical method is developed to measure mono- to hepta-chlorinated OH-PCBs in human blood without derivatization and elution order for 51 isomers and congeners of OH-PCBs were determined with UPLC/Q-Tof MS. Signal to Noise (S/N) of 0.5ppb 4-OH-CB-54 obtained with UPLC/QTof MS is more than 10, and major 6 components of OH-PCB 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) can be separated with UPLC and total analytical time of injection cycle is shortened for 20 minutes.

Introduction

Oxidative metabolism of PCBs by cytochrome P450 monooxygenases enzymatic system in the human body forms hydroxylated polychlorinated biphenyls (OH-PCBs) and thyroid hormone homeostasis make disturb by OH-PCBs (Morse et al.,1995, 1996; Darnerud et al., 1996; Sinjari and Darnerud, 1998;Meerts et al., 2002). The competition of OH-PCBs and thyroxine (T4) to transthyretin (TTR), a carrier protein of T4, in the blood is the mechanism involved in the disturbance of TH homeostasis. In fact, some competitive binding assays demonstrated that the binding of para-substituted high chlorinated OH-PCB congeners with chlorine atoms on each of adjacent meta-positions to TTR was distinctly high and the binding affinity of several OH-PCB isomers

was stronger than that of T4 (Lans et al., 1993; Cheek et al., 1999; Meerts et al., 2002). Such para-substituted OH-PCBs have high retention in the blood and a few OH-PCB isomers have longer half-life than parent compounds (Sinjari et al., 1998; Oberg et al.,2002). Studies of OH-PCBs in human blood have shown the prevalence of these metabolites (Sandau et al., 2000; Sjodin et al., 2000;Fangstrom et al., 2002; Hovander et al., 2002; Sandanger et al.,2004). OH-PCBs have also been detected in umbilical cord blood,suggesting transfer of these metabolites across the placenta to the fetus (Sandau et al., 2002; Soechitram et al., 2004). Sandau et al. (2002) found a significant negative correlation between concentrationsof free T4 and the sum of OH-PCBs and pentachlorophenol(PCP) in umbilical cord blood and suggested that these chlorinated phenolic compounds are possibly altering TH status in newborns. It has been shown from a competitive binding assay that PCP also bind to human TTR and the binding affinity was about twice that of T4 (Van den Berg, 1990). On almost studies, quantitative analysis of OH-PCBs in human blood was generally used GC-HRMS. The present study aimed at developing an analytical method to measure mono- to hepta-chlorinated OH-PCBs in human blood without derivatization with UPLC/Q-Tof MS. Moreover, major 6 components of OH-PCB 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) can be separated with UPLC and total analytical time of injection cycle is shortened for 20 minutes.

Materials and Methods

OH-PCBs Standard Solution:

Standard solution of OH-PCBs were purchased from Accustandard Inc. (New Haven, CT) and Wellington Laboratories Inc. (Guelph,ON, Canada) and each solution was diluted to 10ppm in acetone. Major 6 components of OH-PCBs in human blood were shown in Fig.1.

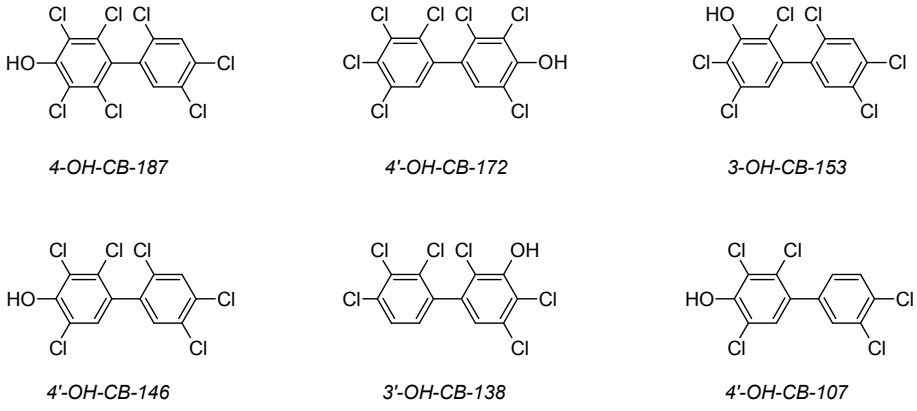

Fig.1 Major 6 components of OH-PCB in human blood

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 QTof MS) with a resolving power of more than 10000 (Table 1).

Table 1 Analytical conditions of UPLC/Q-Tof MS

UPLC

Column Oven (℃)		60		
Column	ACQUITY UPLC BEH C18 2.1mmID x 150mm x1.7um			
Solvent A		5mM AcONH4 in water		
Solvent B		THF/ACN(v/v:1/4)		
Gradient Table				
	Time(min.)	Flow Rate	Solvent A	Solvent B
1	Initial	0.50	75.0	25.0
2	17.0	0.50	25.0	75.0
3	18.0	0.50	1.0	99.0
4	18.5	0.50	75.0	25.0

Xevo QTof MS

Capillary (kV)	1.5
Sampling Cone	40
Source Temperature (℃)	120
Desolvation Temperature (℃)	600
Cone Gas Flow (L/Hr)	20
Desolvation Gas Flow (L/Hr)	800

Results and Discussion

Analytical method with UPLC/HRMS is developed to measure mono- to hepta-chlorinated OH-PCBs in human blood without derivatization and elution order for 51 isomers and congeners of OH-PCBs were determined (Table 2). Signal to Noise (S/N) of 0.5ppb 4-OH-CB-54 obtained with UPLC/QTof MS is more than 10, and major 6 components of OH-PCB 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) can be separated with UPLC (Fig.2) and total analytical time of injection cycle is shortened for 20 minutes.

Table2. Elution Order of 51 OH-PCBs

tR min.	MoCB-OH m/z 203.0264
6.56	4-OH-CB-1
6.71	4OH-CB-2
6.98	4'-OH-CB-3
7.41	6-OH-CB-2
tR min.	DiCB-OH m/z 236.9874
7.66	2'-OH-CB-5
7.67	4-OH-CB-14
7.87	2'-OH-CB-9
7.96	3'-OH-CB-9
8.05	4'-OH-CB-9
8.96	2'-OH-CB-12
tR min.	TrCB-OH m/z 270.9484
9.11	4'-OH-CB-18
9.37	3'-OH-CB-30
9.62	4'-OH-CB-30
9.63	4'-OH-CB-26
9.82	6'-OH-CB-26
tR min.	TeCB-OH m/z 306.9066
8.3	3-OH-CB-54
9.07	4-OH-CB-54
9.52	5'-OH-CB-51
9.87	4-OH-CB-72
10.31	3'-OH-CB-65
10.51	4'-OH-CB-65
10.62	4'-OH-CB-50
10.67	2'-OH-CB-65
11.03	4'-OH-CB-69
11.07	6'-OH-CB-69
11.14	2'-OH-CB-61
11.27	3'-OH-CB-61
11.46	4'-OH-CB-61
tR min.	PeCB-OH m/z 340.8676
8.95	4'-OH-CB-109
9.51	3-OH-CB-118
11.20	4'-OH-CB-121
11.20	4'-OH-CB-107
11.46	4'-OH-CB-93
11.77	6'-OH-CB-101
11.79	4'-OH-CB-112
11.83	6'-OH-CB-112
11.85	4'-OH-CB-104
12.34	4'-OH-CB-101
12.37	3'-OH-CB-101
12.51	4'-OH-CB-86
12.81	6'-OH-CB-106
12.84	4'-OH-CB-106
tR min.	HxCB-OH m/z 374.8285
9.19	4-OH-CB-130
9.49	4'-OH-CB146
9.65	3-OH-CB-138
11.65	4'-OH-CB-165
12.51	4'-OH-CB-159
tR min.	HpCB-OH m/z 408.7896
8.42	4-OH-CB-187
8.88	5-OH-CB-183
10.36	4'-OH-CB-172
10.73	3'-OH-CB-180

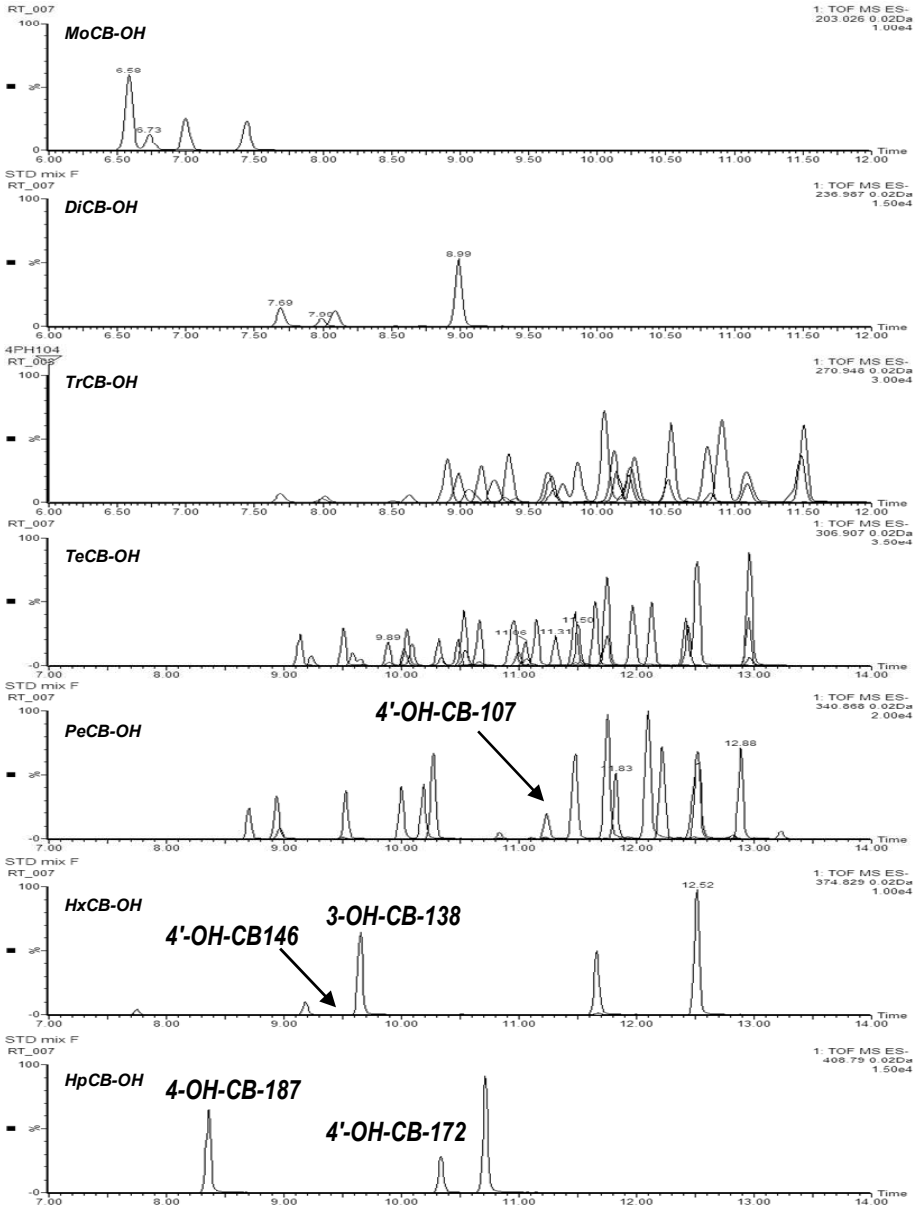

Fig.2 Selected ion chromatogram of OH-PCB (Mass resolution : >10000)

References

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