STUDY ON MEASUREMENT METHOD FOR HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBs) BY LC/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-HO-CB107 10-230 pg/g, 4-HO-CB146 13-340 pg/g and 4-HO-CB187 12-110 pg/g. Sakiyama et al.[2] reported that OH-PCBs were derivatized with dimethyl sulfate, and the methoxylated PCBs were determined using HRGC/HRMS. Matsumoto et al.[3] reported that effective derivatization method was trimethylsilyldiazomethane methylation. LC/MS method does not need a special treatment such as a derivatization of a sample.

In this paper, we demonstrate a method to detect OH-PCBs using LC/MS with an electrospray ionization interface in negative ion and selective ion monitoring mode.

Materials and Methods

Chemicals and reagents: OH-PCBs standards were purchased from Wellington Laboratories, Inc. (Canada). These OH-PCBs standards are listed in Table 1. Each 1 mg/L standard solution was prepared by dilution with acetonitrile. Acetonitrile, formic acid and ultra pure water of LC/MS grade were purchased from Wako Pure Chemical Industries (Japan). The 0.1% ammonia solution was obtained from 250 times dilution of 25% ammonia water with ultra pure water.

LC/MS Measurement: All LC/MS analysis was performed using an Alliance 2695 series high-performance Liquid Chromatograph Separations Module (Waters, USA) equipped with Quattro micro API mass spectrometer (Waters Micromass, USA). Two types of XBridge C18 column were used under an isocratic solvent condition. Short-type (2.1 mm × 50 mm, 5 µm) column was used for the determination of LC/MS conditions. Long-type (2.1 mm × 150 mm, 3.5 µm) was for separation of OH-PCBs congeners. An XBridge C18 column can be operated under the pH range from 1 to 12. Two types

Table 1. OH-PCBs standards

4-OH-2,2’,6,6’-TeCB	4-HO-CB54
4-OH-2,2’,4’,6,6’-PeCB	4’-HO-CB104
4-OH-2,3,3’,4’,5-PeCB	4-HO-CB107
4-OH-2’,3,3’,4’,5-PeCB	4’-HO-CB108
3-OH-2,3’,4,4’,5-PeCB	3-HO-CB118
4-OH-2,2’,3,3’,4’,5-HxCB	4’-HO-CB130
3-OH-2,2’,3’,4,4’,5-HxCB	3’-HO-CB138
4-OH-2,2’,3,4’,5,5’-HxCB	4-HO-CB146
4-OH-2,2’,3,3’,4’,5,5’-HpCB	4’-HO-CB172
3-OH-2,2’,3’,4,4’,5,5’-HpCB	3’-HO-CB180
4-OH-2,2’,3,4’,5,5’,6-HpCB	4-HO-CB187

Table 2. Analytical conditions for the LC/MS measurement

Flow Rate		0.2 mL/min.
Injection Volume		10 µL
Column Temperature		40 °C
Mobile Phase		0.1% Formic acid : Acetonitrile = 20 : 80
		0.1% Ammonia : Acetonitrile = 20 : 80
Temperature;	Source	120 °C
	Desolvation	350 °C
Gas Flow;	Cone	Nitrogen, 50 L/hr
	Desolvation	Nitrogen, 600 L/hr
Voltage;	Cone	40 V
	Capillary	4.0kV
Ionization		ESI-Negative

of mobile phase were prepared. The acidic mobile phase was 80:20 acetonitrile / 0.1% formic acid in water. The basic was 80:20 acetonitrile / 0.1% ammonia water. And other analytical conditions for the LC/MS measurement were summarized in Table 2.

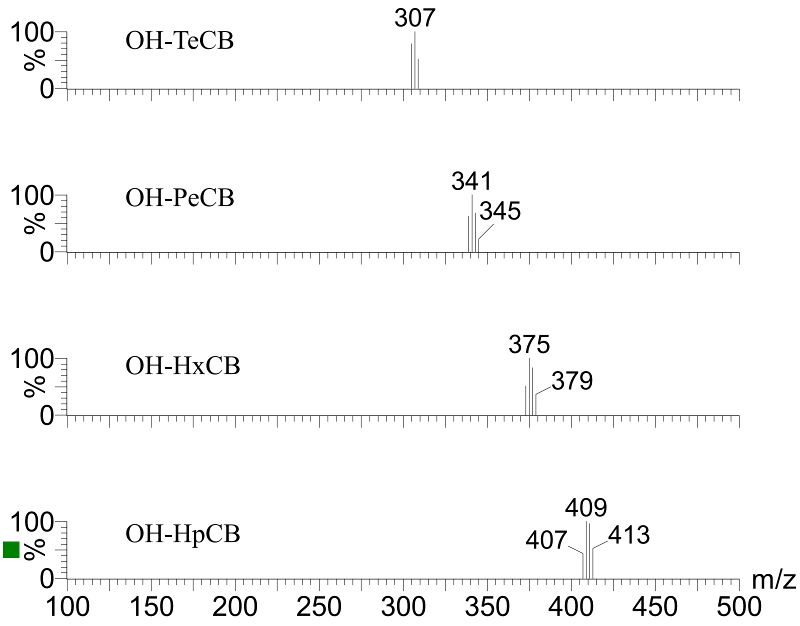

Figure 1. LC/MS spectra of 1 mg/L OH-PCBs in scan mode

Results and Discussion

LC/MS measurement at scan mode: Figure 1 illustrates the LC/MS spectra of hydroxylated tetra- through hepta- chlorinated biphenyls in scan mode. $[M-H]^-$ ions were observed from each OH-PCBs standard solutions in negative ion mode. Ions were intense at m/z 307 and 305 in OH-TeCB measurement. These ions are derived from chlorine isotope. The calculated monoisotopic mass are 306.91 and 304.91, respectively. Ions of m/z 341 in OH-PeCB, m/z 375 in OH-HxCB and m/z 409 in OH-HpCB were intense, respectively.

Mobile phase: Figure 2 shows the relationship between the cone voltage and the peak height of 4-HO-CB107 using the different mobile phase. The peak height when ammonia was used as the mobile phase was higher than

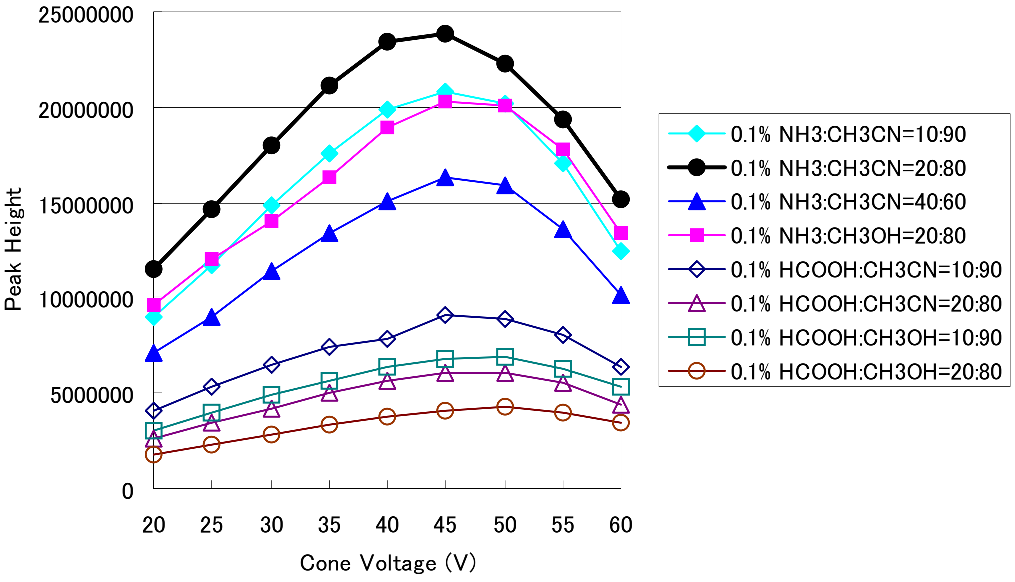

Figure 2. Relationship between cone voltage and peak height of 4-HO-CB107 using the different mobile phases

when formic acid was used. And, the peak height when acetonitrile was used as the mobile phase was higher than when methanol was used. It was found that the most sensitive mobile phase was 80:20 of acetonitrile / 0.1% ammonia solution. In the acidic mobile phase, 90:10 of acetonitrile / 0.1% formic acid solution was the most sensitive. The sensitive cone voltage was 45 V using any mobile phase.

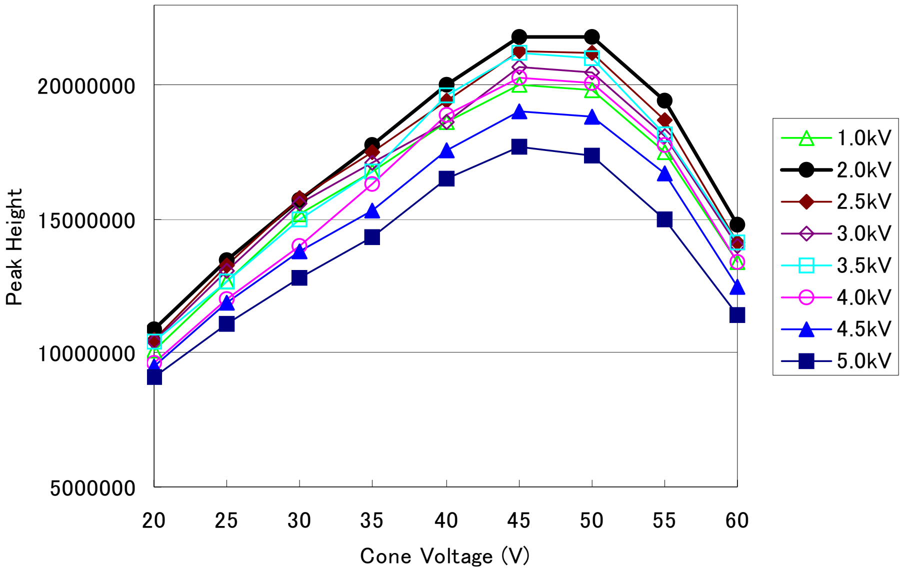

Figure 3. Relationship between cone voltage and peak height of 4-HO-CB107 under the different capillary voltages

Optimizations of the cone and capillary voltage: Figure 3 shows the relationship between the cone voltage and the peak height of 4-HO-CB107 under the different capillary voltages in the range from 1.0 to 5.0 kV. The mobile phase was 80:20 of methanol / 0.1% ammonia solution. It was found that the most sensitive capillary voltage was 2.0 kV. The cone voltage was 45 - 50 V at that time.

Comparison of chromatograms at the different mobile phases: Figure 4 shows the LC/MS chromatograms of 1 mg/L OH-PCBs standards at the basic mobile phase (Acetonitrile / 0.1% ammonia = 80 / 20). Figure 5 shows the LC/MS chromatograms at the acidic mobile phase (Acetonitrile / 0.1% formic acid = 80 / 20). All peaks were detected within about 2 minutes in the chromatograms at the basic mobile phase. It was suspected that OH-PCBs were not retained by an XBridge C18 column at the basic mobile phase. On the other hand, hydroxylated tetra- through heptachlorinated biphenyls were detected on each retention time at the acidic mobile phase. But, it was not enough to separate each isomer. This comparison demonstrates that the acidic mobile phase is useful to measure the hydroxylated polychlorinated biphenyls though low sensitivity against the basic mobile phase.

Sensitivity of LC/MS measurement: When a standard was injected using the acidic mobile phase, 10pg of 4-HO-CB107 was detected with S/N=10 and 4-HO-CB146 was detected with S/N=6. These results indicate that pg-level of OH-PCBs can detect using LC/MS with an electrospray ionization interface in negative ion mode and selective ion monitoring mode.

References

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2. Sakiyama T., Yamamoto A., Kakutani N., Fukuyama J. and Okumura T. *Organohalogen Comp* 2007; 69: 1380.
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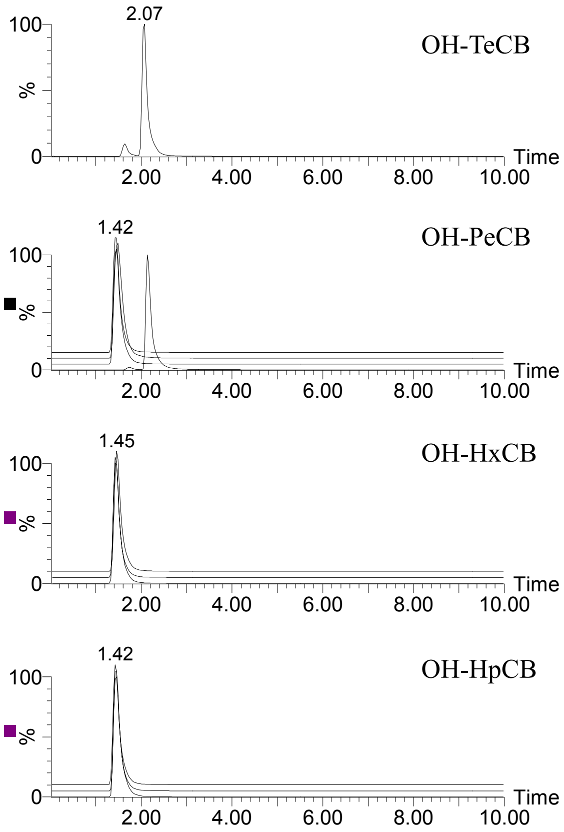

Figure 4. LC/MS chromatograms of 1 mg/L OH-PCBs at the basic mobile phase

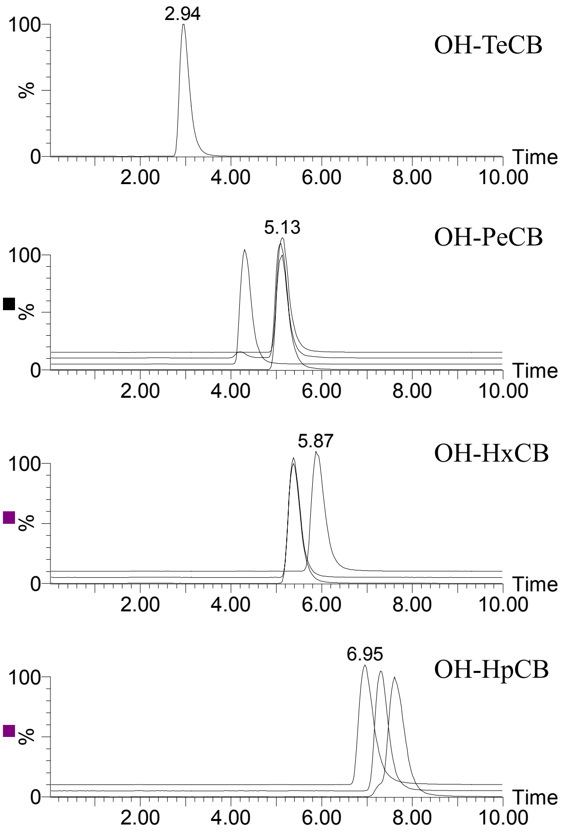

Figure 5. LC/MS chromatograms of 1 mg/L OH-PCBs at the acidic mobile phase