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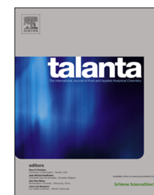

Simultaneous detection of multiple hydroxylated polychlorinated biphenyls from a complex tissue matrix using gas chromatography/isotope dilution mass spectrometry

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ABSTRACT

In this study, we developed a comprehensive, highly sensitive, and robust method for determining 53 congeners of three to eight chlorinated OH-PCBs in liver and brain samples by using isotope dilution gas chromatography (GC) coupled with electron capture negative ionization mass spectrometry (ECNI-MS). These results were compared with those from GC coupled with electron ionization high-resolution mass spectrometry (EI-HRMS). Clean-up procedures for analysis of OH-PCBs homologs in liver and brain samples involve a pretreatment step consisting of acetonitrile partition and 5% hydrated silica-gel chromatography before derivatization. Recovery rates of tri- and tetra-chlorinated OH-PCBs in the acetonitrile partition method followed by the 5% hydrated silica-gel column (82% and 91%) were higher than conventional sulfuric acid treatment (2.0% and 3.5%). The method detection limits of OH-PCBs for each matrix obtained by GC/ECNI-MS and GC/EI-HRMS were 0.58–2.6 pg g^{-1} and 0.36–1.6 pg g^{-1} wet wt, respectively. Recovery rates of OH-PCB congeners in spike tests using sample matrices (10 and 50 pg) were 64.7–117% (CV: 4.7–14%) and 70.4–120% (CV: 2.3–12%), respectively. This analytical method may enable the simultaneous detection of various OH-PCBs from complex tissue matrices. Furthermore, this method allows more comprehensive assessment of the biological effects of OH-PCB exposure on critical organs.

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1. Introduction

Polychlorinated biphenyls (PCBs) are a class of chlorinated hydrocarbons. They have a diverse range of applications in various materials, such as electrical equipment, paint, and carbon paper. The production and use of PCBs was restricted or banned globally due to their persistent, bioaccumulative, and toxic properties [1].

PCBs are formed by oxidative metabolism by the cytochrome P450 (CYP450) mono-oxygenases enzyme system in the liver [2]. The metabolism of PCBs results in the formation of a relatively large number of hydroxylated PCB congeners (OH-PCBs), which might be associated with disrupted thyroid homeostasis and neurodevelopmental deficits [3], [4–6]. Theoretically, there are 837 mono-hydroxylated PCB congeners with a substitution of 1–9 chlorine atoms, and each congener could have a specific toxicological effect [7], [8]. It has been proposed that the mechanism involved in the disruption of thyroid hormone (TH) homeostasis is the competitive binding of OH-PCBs with transthyretin (TTR), the TH transport protein, in blood [3,9]. It has been demonstrated that the binding affinity of OH-PCBs to TTR is much stronger than that of the parent PCBs [10]. TH plays critical roles in the development of the central nervous system and brain functions [11]. A recent study using reporter gene assays demonstrated that extremely low doses of OH-PCBs (i.e., 10^{-10} Mol) suppress the 3,5,5′-triiodothyronine (T3)-induced transcriptional activation of the TH receptor [12]. These studies indicate that the brain, which is commonly the target tissue for OH-PCBs, and the liver which plays a major role in the metabolism of PCBs, are suitable for understanding the toxicity and kinetics of OH-PCBs.

OH-PCB concentrations and distributions in human serum and wildlife blood have previously been investigated [13–20]. Although OH-PCBs have been detected in the blood of several wildlife species such as marine mammals and birds [15–18,21], detailed information regarding OH-PCBs levels in the animal brain and liver are still scarce. In addition, these studies on wildlife investigated only a small number of OH-PCB congeners (mainly penta- to octa-chlorinated OH-PCB congeners) or were limited to global data as opposed to

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individual congener information. Recently, species-specific accumulation data, such as data of tri- to penta-chlorinated OH-PCBs in the blood of dolphins [18], octa-chlorinated OH-PCBs in polar bear (*Ursus maritimus*) plasma [22], and hepta- to octa-chlorinated OH-PCBs in terrestrial mammal blood [20] have been reported. These studies suggested that the levels and profiles of OH-PCBs in animal blood vary by species and that several animals may be at a risk from these metabolites including congeners which are not found in human are present. However, because of difficulties in detecting low-chlorinated (e.g., 3–4 chlorine atoms) OH-PCBs in biological tissues, comprehensive investigations on the levels of various OH-PCB homologs in the brain and liver are still limited [23,24], In fact, because tri- and tetra-chlorinated OH-PCBs are unstable and characterized by low recovery rates, conventional pretreatment procedures (e.g., sulfuric acid treatment or multilayer silica-gel column) [15,23,24] cannot be used for their analysis. Thus, to analyze lower-chlorinated OH-PCBs, mild pretreatment methods before derivatization are required. In this study, we tested an acetonitrile partition method coupled to a deactivated silica-gel column as pretreatment before derivatization and compared this new method with conventional sulfuric acid treatment.

To perform this analysis, a high degree of separation of OH-PCBs from the complex environmental matrix is required and the chosen method must have high selectivity, sensitivity, and precision. To meet these requirements, many previous environmental OH-PCBs studies utilized a gas chromatography (GC)-electron capture detector (ECD) [25]. Other studies used GC/electron ionization high-resolution mass spectrometry (GC/EI-HRMS) [18,26] GC/electron capture negative ionization mass spectrometry (GC/ECNI-MS) [5,15,27] or liquid chromatography/mass spectrometry (LC/MS) [26,28]. Although LC/MS is characterized by high sensitivity and selectivity, its resolution might not be adequate to separate many OH-PCB congeners. Although GC-ECD is a sensitive technique and is easy to handle, a complete purification of the samples prior to the chromatographic separation of individual substances is needed because the internal standards cannot be spiked. Although GC/HRMS can provide highly sensitive and selectively, the instrumentation is too expensive for conducting routine or high-throughput analyses and its operation requires specialized technical skills. In contrast, GC/ECNI-MS is a sensitive and selective instrument particularly suitable for the analysis of halogenated compounds. The ECNI mode allows the use of $^{13}C_{12}$-labeled internal standards for a more precise determination of the target compounds. So far, it has been used for the analysis of numerous contaminants, including OH-PCBs, in various environmental matrices [5,15,27]. Nevertheless, the effectiveness of GC/ECNI-MS for the analysis of tri- to tetra-chlorinated OH-PCBs has not been clarified yet and analytical methods using GC/ECNI-MS and the GC/EI-HRMS were not compared for complex biological samples.

In this study, we developed a comprehensive, highly sensitive, and robust method for determining OH-PCBs, including various homologs (3–8 chlorine atoms), in liver and brain samples by using isotope dilution GC/ECNI-MS and/or GC/EI-HRMS. The current method incorporates several modifications and improvements for a more sensitive and selective analysis of a wide range of OH-PCB homologs in complex biological tissues.

2. Materials and methods

2.1. Chemicals and reagents

Fifty-three OH-PCBs (tri- to octa-, methoxylated derivatives: MeO-PCBs) isomers were used for identification and quantification. Thirteen compounds were synthesized by thermal diazo-coupling between a chlorophenol and a chloroaniline diazonium salt [29,30], 8 compounds were obtained from AccuStandard, Inc. (New Haven, CT), and 31 from Wellington Laboratories Inc. (Guelph, ON, Canada) (Table S1).

Dichloromethane (DCM), *n*-hexane, methanol, ethanol, methyl *tertiary*-butyl ether (MTBE), decane, and silica gel (Wako-gel S1) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trimethylsilyldiazomethane (TMSDM) for derivatization was supplied by Tokyo Chemical Industry (Tokyo, Japan). Anhydrous sodium sulfate (purity > 99%) and acetone were obtained from Nacalai Tesque (Kyoto, Japan). Silica gel was baked at 130 °C for 3 h prior to use. Five percent hydrated silica gel (5% H_2O deactivated) was prepared by slowly adding an appropriate amount of Milli-Q water (Millipore Corp., Bedford, MA) to activated silica at room temperature.

2.2. Sample collection

Brain samples of finless porpoise (*Neophocaena phocaenoides*) carcasses stranded or caught during 2005–2010 along the Japanese coast were transported and stored at −25 °C at local universities and aquariums until biometric measurements and dissections were conducted [18]. Liver samples of Baikal seals (*Phoca sibirica*) were collected from Lake Baikal in 2005 [31]. Permission was obtained from the Lake Baikal Basin Committee for Protection, Reproduction of Fish Resources and Fishing Control (known by its Russian acronym BAIKALRYBVOD) under the annual seal culling quota. The animals were shot and immediately dissected. The brain and liver samples were stored frozen in the environmental specimen bank (*es*-BANK) of Ehime University, Matsuyama, Japan, at −25 °C [32].

2.3. Sample preparation

The OH-PCB extraction procedure used in this study is similar to that described in a previous report [18]. $^{13}C_{12}$-labeled OH-PCBs (1 ng of each 4′OH-CB29, 4′OH-CB61, 4′OH-CB79, 4OH-CB107, 4′OH-CB120, 4′OH-CB159, 4′OH-CB172, and 4OH-CB187) were spiked as surrogate internal standards. The liver and brain samples (2.5 g) were denatured with 3 mL of 6 M hydrochloric acid. After adding 2-propanol (9 mL), the target compounds were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane by a homogenizer (11,000 rpm, 10 min) (POLYTRON PT 2100: Kinematica, Luzernerstrasse, Switzerland). After centrifugation, the organic phases were combined and washed with 5% NaCl prepared in hexane-washed water. The resulting organic phase was evaporated by a rotary evaporator and re-dissolved in hexane. Potassium hydroxide (KOH; 1 M) in 50% ethanol/water (20 mL) was added and the solution was shaken to ensure mixing. The partition step of the neutral and phenolic fractions (alkaline phases) was repeated and the alkaline phases were combined.

In the next step, the KOH solution phase containing OH-PCBs was acidified (pH 2) with sulfuric acid. Then, OH-PCBs were extracted twice with 50% MTBE/hexane (60 mL). The phases were separated, and the organic phase was combined and evaporated by a rotary evaporator. A mixture of acetonitrile:hexane (1:1 v/v; 10 mL) was later added and shaken. Again, the partition step of the neutral and phenolic fractions was repeated, and the alkaline phases were combined. To the acetonitrile solution phase containing OH-PCBs was added 100 mL of hexane-washed water, and the solution was acidified to pH 2 with sulfuric acid. Then, OH-PCBs were again extracted twice with 50% MTBE/hexane (60 mL). Subsequently, the phases were separated, and the organic phase was combined and evaporated by a rotary evaporator.

The solvent-evaporated residue was dissolved in hexane and passed through a glass column packed with 3 g hydrated silica gel (Wako-gel S-1, 5% H_2O deactivated). The OH-PCBs fraction was eluted with 50% DCM/hexane (100 mL), concentrated, and dissolved in hexane (1 mL). Each treated analyte in hexane was

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