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# Fast determination of hydroxylated polychlorinated biphenyls in human plasma by online solid phase extraction coupled to liquid chromatography-tandem mass spectrometry

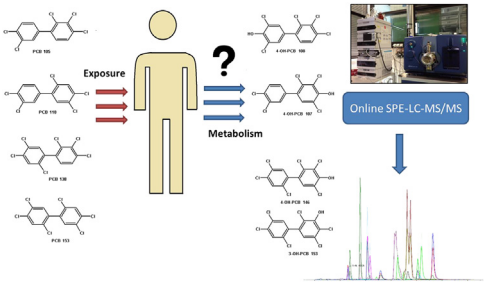
Natalia Quinete [a, *], Thomas Kraus [a], Vladimir N. Belov [b], Christina Aretz [a], André Esser [a], Thomas Schettgen [a]

[a] Institute for Occupational and Social Medicine, Medical Faculty, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany
[b] Max Planck Institute for Biophysical Chemistry, Facility for Synthetic Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

## HIGHLIGHTS

- We developed an online SPE-LC-MS/MS method for OH-PCB determination in human plasma.
- The method required only 100 μL of plasma and minimal sample preparation steps.
- 3OH-PCB 28 has been synthesized and identified for the first time in plasma.
- Penta-through hepta-chlorinated OH-PCBs were the predominant congeners.
- Notable presence of unknown OH-PCB congeners in several plasma samples.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Hydroxylated polychlorinated biphenyls (OH-PCBs) have been shown to be strongly retained in human blood causing endocrine-related toxicity, particularly on the thyroid system. Traditionally, analytical methods for the determination of OH-PCBs require labor-intensive and long-time consuming sample preparation with several extraction, evaporation and cleanup procedures steps and, in some cases, derivatization prior to the analysis by gas or liquid chromatography-mass spectrometry (GC–MS or LC-MS). The present study developed and validated a novel, sensitive and high throughput online solid phase extraction (SPE) method coupled to LC-tandem mass spectrometry (MS/MS) for the separation and quantitation of relevant congeners of OH-PCBs in human plasma. The developed method presented limits of quantification (LOQ) ranging from 0.02 to 0.5 ng $mL^{-1}$ and extraction recoveries from 71 to 134% for all congeners, requiring small amount of sample (only 100 μL) and minimal sample preparation. In order to evaluate the applicability of the method, preliminary tests (N = 93) were conducted in plasma from individuals occupationally exposed to very high levels of PCBs in a German cohort. Penta-through hepta-chlorinated OH-PCBs were the predominant congeners in human plasma with concentrations up to 44.5 ng $mL^{-1}$, while lower chlorinated OH-PCBs were occasionally detected. In addition, a new PCB 28

* Corresponding author.
*E-mail addresses:* nsoaresquine@ukaachen.de, nataliaquinete@yahoo.com.br (N. Quinete).

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metabolite has been synthesized and identified for the first time in human plasma and associations between OH-PCBs and their parent compounds in the studied cohort were also assessed.


## 1. Introduction

Over the last decade attention has been drawn to polychlorinated biphenyls (PCBs) metabolites and their potential adverse effects in humans and wildlife. The mechanism of PCB to hydroxylated polychlorinated biphenyls (OH-PCBs) metabolism generally involves the insertion of a OH-group on a biphenyl via arene oxide intermediate formation, possibly followed by 1,2 shift (NIH shift) [1,2] or via direct insertion of the hydroxyl group [3]. Despite the fact that most phenolic compounds are readily conjugated and excreted, several OH-PCBs have been found to be retained in human and wildlife blood [4–8] and due to their structural similarity to natural hormones have been shown endocrine-related toxicity, being capable of interacting and disrupting the thyroid hormone status in humans [9–11]. Approximately 40 different congeners have been identified in human plasma [12,13], which are predominantly penta-through heptachlorinated OH-PCBs (4-OH-CB187, 4-OH-CB107, 4-OH-CB146, 3-OH-CB153 and 3-OH-CB138) [12,14,15]. These congeners are dominant in a number of studies, however, which is most commonly occurring varies between studies and depends mainly on environmental factors and metabolism capability [5,16].

Traditional extraction and cleanup methods such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) for the analysis of contaminants in blood (plasma or serum) have been established for a long time [17,18], and, more recently, improvements have been made in order to also include phenolic organohalogens. As recently reviewed [19], methods reported in the literature required in general extensive sample preparation, which include a series of time consuming and low throughput liquid–liquid extraction and back extractions, evaporations, several steps of cleanup and, in some cases, derivatization prior to analysis by gas or liquid chromatography-mass spectrometry (GC–MS or LC-MS), highlighting the need for a fast, straightforward and reliable analytical method for the determination of these PCB metabolites.

Gas chromatography based methods are traditionally the most common methods for the determination of OH-PCBs and related halogenated phenolic compounds in blood matrices [20]. However as the presence of hydroxy groups in PCB metabolites raises difficulties in using GC for trace analysis, prior derivatization is mandatory to overcome the low sensitivity and high reactivity of the OH-PCBs in the injection port and GC column [21], which can result in either losses due to excessive sample handling or increased background interferences due to co-products of the derivatization [22,23].

Although LC-MS methods would represent a viable alternative for the qualitative and quantitative determination of OH-PCBs, very few LC-MS-based studies have been reported for the determination of OH-PCB separation in blood samples [23–25] and most of them still require extensive sample preparation procedures.

The present study aimed to develop and validate a novel, sensitive, selective and robust online SPE-LC-MS/MS method for the separation and quantitative determination of relevant congeners of OH-PCBs in human plasma, including the lower chlorinated congeners (mono to tri OH-PCBs). The applicability of the method was evaluated by the preliminary analysis of human plasma (N = 93) from individuals occupationally exposed to very high levels of PCBs in a German cohort as part of the biomonitoring program HELPcB (Health Effects in High-Level Exposure to polychlorinated biphenyls) [26].

## 2. Experimental section

### 2.1. Chemicals

Certified standards of the low chlorinated OH-PCB congeners (4-OH-CB3, 4-OH-CB9, 4-OH-CB15, 4-OH-CB18) were obtained from Chem Service Inc (West Chester, PA, USA). 3-OH-CB28 and its labelled internal standard ($^{13}$C-3-OH-CB28) were custom synthetized in the course of this study (V. N. Belov). Tetra-chlorinated OH-PCB (4-OH-CB61 and 4-OH-CB76) were obtained from Ultra Scientific (North Kingstown, RI, USA) and LGC Standards (Wesel, Germany), respectively. Pentachlorinated 4-OHCB101 and 3-OHCB101 were obtained from Accustandard (New Haven, CT, USA). Other pentachlorinated through heptachlorinated OH-PCB standards (4-OH-CB107, 4-OH-CB108, 3-OH-CB118, 4-OH-CB130, 3-OH-CB138, 4-OH-CB146, 3-OH-CB153, 4-OH-CB172, 3OH-CB180 and 4-OH-CB187) and labelled internal standards ($^{13}$C-4-OH-CB61, $^{13}$C-4-OH-CB172 and $^{13}$C-4-OH-CB187) were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada), while other labelled internal standards ($^{13}$C-4-OH-CB76, $^{13}$C-4-OH-CB107 and $^{13}$C- 4-OH-CB146) were purchased from Cambridge Isotope Laboratories Inc (Andover, MA, USA). Stock and working solutions were prepared with ultra-gradient HPLC grade methanol from JT Baker (Deventer, Netherlands) and stored at −20 °C. HPLC water gradient grade for chromatography (Lichrosolv) and ammonium acetate (Fractorpur) for analysis were purchased from Merck (Darmstadt, Germany). β-Glucuronidase/Arylsulfatase enzymatic solution was purchased from Roche (10127698001, 10 mL, Mannheim, Germany). IUPAC full chemical name, short names/abbreviations, chemical structures and molecular mass are presented in Table S1.

### 2.2. Study cohort

After high internal exposures of PCBs were discovered in workers in a transformer recycling company in Germany, the HELPcB program was created as a comprehensive medical surveillance plan for early detection of possible adverse health effects caused by occupational exposure to PCB, including not only workers of the transformer recycling company, but also their relatives and workers of surrounding companies [27]. In the present study, a preliminary assessment of OH-PCB metabolites was conducted in plasma samples from 93 participants with high occupational or environmental PCB exposure. This collective comprehends 85 males and 8 females, with an age range of 4–84 years (mean age: 42 ± 15 years). Among them, 55 individuals are smokers and 33, non-smokers. Previously, as part of HELPcB, PCB plasma levels have been measured in this same collective with concentrations ranging from 0.005 to 178 ng mL$^{-1}$, where PCB 28 contributed to the highest concentration observed in this exposed cohort [27].

### 2.3. Plasma collection and sample preparation

Blood samples from the PCB-exposed cohort were obtained