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Determination of hydroxy metabolites of polychlorinated biphenyls in plasma and tissue by gas chromatography/mass spectrometry

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Abstract

This study examines a novel sample preparation method for the determination of 11 hydroxy metabolites of polychlorinated biphenyls (PCBs) in plasma and organ tissues, followed by gas chromatography with mass spectrometric detection (GC/MS). The clean-up method was optimized to eliminate the interference matter by using a silica column and 10 mL of *n*-hexane/dichloromethane (4:6, v/v) as an eluent. Solid-phase and solvent extraction procedures were used for the plasma and tissues samples, respectively. Compared to C_{18} and C_8 solid-phase, C_2 showed higher extraction efficiency with *n*-hexane as the eluent for plasma. The hydroxy-PCB extraction recoveries achieved with this combined extraction and clean-up procedure from plasma ranged from 87 to 117%, while those from tissues ranged from 82 to 111%. The linear detector responses for propyl derivatives of hydroxy-PCBs were obtained with the coefficients of determination varying from 0.992 to 0.998 in the concentration range of 0.1–20 ng mL^{-1}. The method detection limits ranged from 0.1 to 0.5 ng mL^{-1} in 1 mL of plasma and from 0.1 to 0.5 ng g^{-1} in 1 g of tissues. This procedure was successfully applied to the study of 3-OH-2,3′,4,4′,5-PeCB in rat plasma and liver samples after intraperitoneal injection (20 mg/kg) of 2,3′,4,4′,5-PeCB.

Keywords: Hydroxy-PCBs; GC/MS; SPE; Clean-up; Plasma; Tissue

1. Introduction

Polychlorinated biphenyls (PCBs) are an important class of well-known toxic substances that have been used for diverse commercial applications, such as hydraulic fluids, printing inks, or dielectric fluids for capacitors [1]. PCBs are lipophilic persistent substances which allows them to be distributed and circulated throughout the environment and in fat-rich tissues of the human body [2]. PCBs are recognized as causing numerous adverse effects on human reproduction and on enzyme induction in liver cell lines from trout, and as exhibiting dioxin-like activity [3–5].

The major metabolic pathway of PCBs proceeds via P450-mediated formation of arene oxide intermediates, with immediate formation of hydroxylated metabolites, mostly in the liver [6–9]. It has been reported that some metabolites of PCBs are tissue-persistent, and have been detected as residues in environmental and biological samples [10,11]. The most common technique for the analysis of PCBs is the gas chromatography (GC) with electron-capture detection (ECD) [12,13], which is sensitive for halogenated compounds. Despite of the high sensitivity of ECD, mass spectrometric detection is preferred to increase of selectivity and identify each PCB from the interfering compounds because of its ability to provide information on the structure and molecular mass of analytes [14,15].

A few studies have been reported on the analysis of hydroxy-PCBs from biological samples by GC/mass spectrometry (GC/MS) following derivatization. Haraguchi et al. [16] used soxhlet extraction or liquid–liquid extraction (LLE) to isolate the hydroxy-PCBs from biological samples following methylation with diazomethane. In a recent study, Sandala et al. identified the hydroxy-PCBs in whole blood with GC/ECD after methylation [17]. By other means, Wehler et al. deter-

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mined hydroxy metabolites of pentachlorobiphenyl (PeCB) by radioactivity measurement from mink [18].

It is generally accepted that sample preparation is the most important step in the whole analytical procedure. While LLE was traditionally used for the extraction of PCBs from biological samples, solid-phase extraction (SPE) has become widely accepted as an alternative extraction method in biological and environmental analysis [19,20]. Moreover, SPE is also very important for the clean-up of complex samples that requires the removal of interfering compounds from complex matrix to increase the selectivity of the entire analytical method [21,22].

Hydroxy-PCB congener residues have mainly been reported in blood matrices [23], but also in the plasma of male and female polar bears collected in 1997 from the Resolute Bay area of the Canadian Arctic [24]. Hydroxy-PCBs demonstrated some accumulative potential as they have been reported in Swedish human adipose and liver tissue, although at very low levels [25]. Sandala et al. identified 16 hydroxy-PCB congeners and one dihydroxy-PCB congener, and found very high total hydroxy-PCB concentrations ranging from 93.5 to 382.1 ng/g wet weight in the whole blood of the Greenland bears [17]. This evidence of widespread presence in biological samples supports the need for further research on a suitable analytical method of PCBs and their hydroxyl metabolites.

Coplanar PCBs have attracted great concern due to their persistence in the environment, highly bioaccumulative nature and adverse effects on wildlife and humans. We have previously reported analytical methods based on GC/MS for the quantification of hydroxy-PCBs in urine [26,27]. Therefore, in the present study on the metabolites of 2,3′,4,4′,5-PeCB, which is one of the coplanar PCBs after intraperitoneal rat injection, we compare the extraction recoveries of 11 hydroxy-PCBs from plasma and tissues and investigate the clean-up procedure for the elimination of proteins and fatty acids.

2. Experimental

2.1. Material

Hydroxy-PCBs were purchased from Accustandard Inc. (New Haven, CT, USA). The structures of hydroxy-PCBs are shown in Fig. 1. Several 1000 μg mL^{-1} of standard stock solutions were prepared by dissolving the appropriate amount of each hydroxy-PCB compound in *n*-hexane and stored at 4 °C in the dark. The derivatizing agent iodopropane was purchased from Aldrich (Milwaukee, WI, USA) and all solvents and acids were obtained from J.T. Baker (Philipsburg, NJ, USA). Solid-phase cartridges were purchased from Waters (Milford, MA, USA).

2.2. Apparatus

Analysis of hydroxy-PCBs was performed by using an Agilent 6890 Gas Chromatograph with Mass Selective Detector (Agilent 5973). The vacuum manifold used for SPE was purchased from Supelco (Bellefonte, Philadelphia, USA). The heating block used for the derivatization and the vortex mixer for the extraction were purchased from Thermolyne (Dubuque, Iowa, USA). The shaker used for the extraction from tissues was purchased from Edmund Buhler (Bodelshausen, Germany), the homogenizer from Janke & Kunkel Model Ultra-Turax T25 (Wilmington, NC, USA) and the centrifuge from Sorvall Model RT6000B (Asheville, NC, USA).

2.3. Sample preparation procedure

2.3.1. Extraction

One milliliter of plasma was placed in the test tube and 10 μL of the internal standard (ISTD; pyrene-d_{10}, 1 μg mL^{-1}) and 100 μL of 2 M perchloric acid were added. For the SPE, the Sep-Pak cartridges were placed on a vacuum extraction system and the adsorbents were activated and conditioned with 10 mL of methanol and 10 mL of deionized water. The samples were passed through the cartridge and washed with 10 mL of deionized water. After drying for 5 min at full pressure, the target compounds were eluted with 5 mL of *n*-hexane into the test tube, and evaporated to 1 mL on a rotary evaporator.

One gram of tissue was placed in the test tube and homogenized with saline (tissue/saline = 1:2, w/w). After 10 μL of the ISTD (pyrene-d_{10} I1 μg mL^{-1}) and 100 μL of 2 M perchloric acid were added, hydroxy-PCBs were extracted by vortex mixing for 10 min and shaking for 30 min with 10 mL of *n*-hexane. After centrifugation for 10 min at 3000 × *g*, the organic phase was transferred to another test tube and then evaporated to 1 mL on a rotary evaporator.

2.3.2. Clean-up

The silica column was placed on the vacuum extraction system and the adsorbents were activated and conditioned with 10 mL *n*-hexane. One milliliter of extraction solution was applied to the silica column and finally dried for 5 min at full pressure. The target compounds were eluted with 10 mL of *n*-hexane/dichloromethane (4:6, v/v) into the test tube, and the eluent was evaporated to dryness in a vacuum rotary evaporator.

2.3.3. Derivatization

The dried residue was dissolved in 150 μL of acetone and then 20 mg of potassium carbonate and 50 μL of iodopropane were added. This solution was heated for 30 min at 100 °C in a heating block. Two microliters of the solution was injected into the GC/MS after cooling to room temperature. The entire sample preparation procedure is summarized in Fig. 2.

2.4. GC/MS determination

The hydroxy-PCBs were analyzed by using an Agilent 6890 Gas Chromatograph with Mass Selective Detector (Agilent 5973). Chromatographic separation was carried out on a capillary column (25 m × 0.2 mm I.D. × 0.33 μm film thickness, coated with cross-linked 5% phenylmethylsiloxane) from Agilent. The ion source was operated in the electron impact ionization mode (EI; 70 eV, 180 °C). Full-scan mass spectra (*m/z* 40–700) were recorded for analyte identification. The temperatures of the injection port and transfer line were 250 and 280 °C, respectively. Samples were injected in the splitless mode

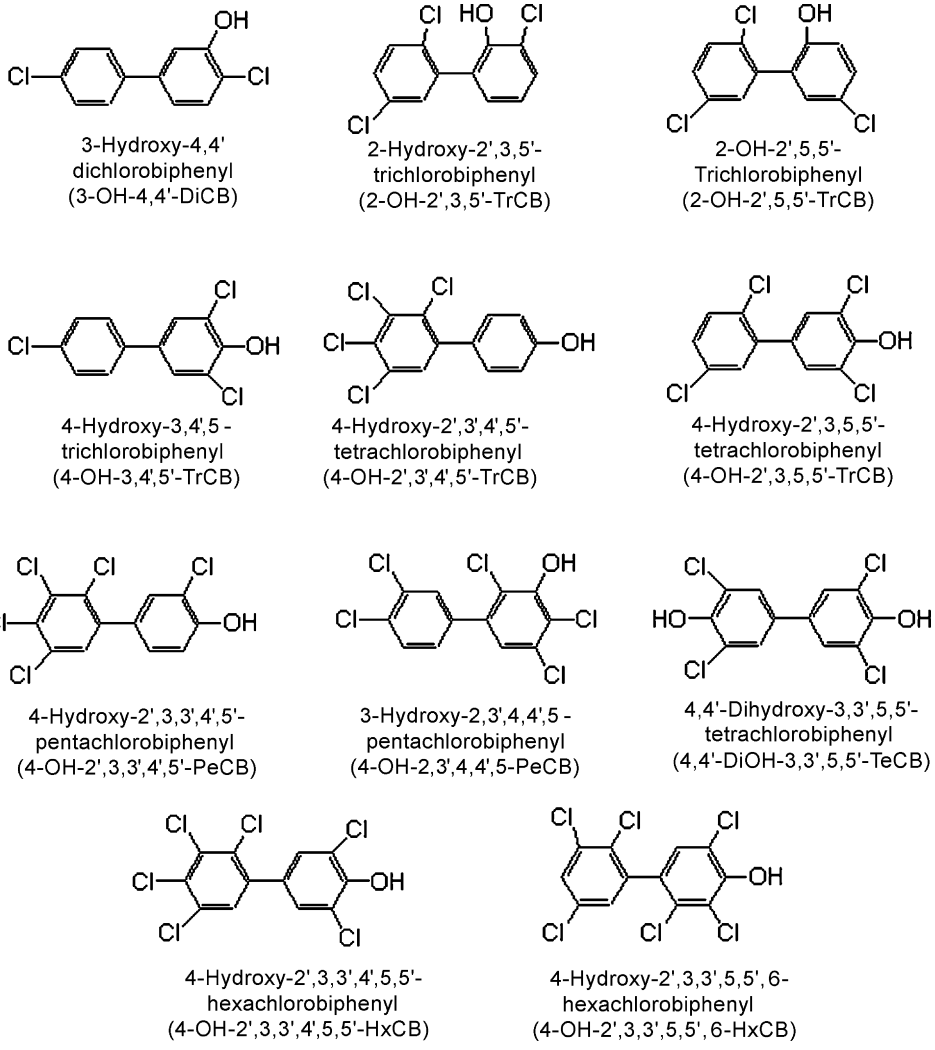

Fig. 1. Chemical structures of hydroxy-PCBs.

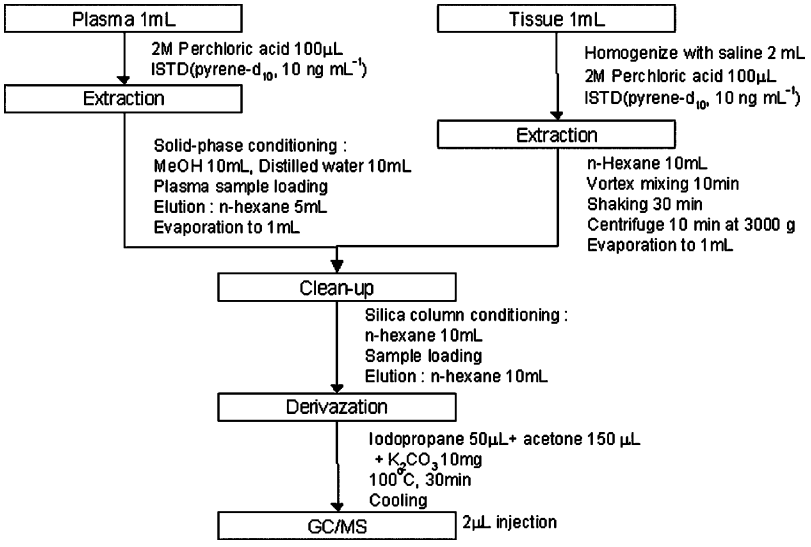

Fig. 2. Sample preparation procedure of hydroxy-PCBs in plasma.

Table 1
GC/MSD operating conditions for derivatives of hydroxy-PCBs

GC/MS: Agilent 6890 Gas Chromatograph/5973 Mass Selective Detector				
Column: Ultra-2 (Cross-linked 5% phenylmethylsilicone, 25 m × 0.2 mm I.D. × 0.33 μm film thickness)				
Carrier gas: He at 0.8 mL/min				
Injection mode: Splitless (Purge on at 0.75 min)				
Injection port temperature: 250 °C				
Transfer line temperature: 280 °C				
Oven temperature program:				
Initial temperature (°C)	Initial time (min)	Rate (°C/min)	Final temperature (°C)	Final time (min)
100	0	15.0	240	6.0
		15.0	300	3.0
Run time: 22.3 min				
SIM mode (solvent delay: 3.0 min)				
Group	Start time (min)	Selected ions, *m/z*		
1	3.0	(272, 274, 314, 228, 240, 280, 326)		
2	13.5	(326, 308, 306, 348, 342, 344, 382)		
3	17.5	(376, 378, 416, 342, 344, 384, 324, 322, 406)		

(purge delay time of 0.75 min). The GC/MS instrumental conditions for the analysis of hydroxy-PCBs are summarized in Table 1.

2.5. Calibration and detection limits

Calibration curves were established for seven points within the concentration range of 0.1–20 ng mL^{-1}, each containing 10 ng mL^{-1} of ISTD (pyrene-d_{10}). The ratio of the peak area for each compound to that of the ISTD was used for the quantification.

2.6. Animal treatment

Sprague–Dawley male rats weighing 260–300 g were used. By intraperitoneal injection, 2,3′,4,4′,5-PeCB (20 mg/kg body weight) dissolved in corn oil (20 mg/mL) was administered. Blood and liver samples were collected at 1, 2, 3, 5 and 7 days after administration. All samples were directly weighed and stored at −20 °C until analysis..

3. Result and discussion

3.1. GC separation and mass spectra

As the presence of hydroxy groups in PCB metabolites raises difficulties in using GC for trace analysis, derivatization is necessary to overcome the high reactivity of the hydroxy group in the injection port and GC column. Our previous studies demonstrated the superior sensitivity of the propylation procedure with iodopropane (at 100 °C for 30 min) for the derivatization of hydroxy-PCBs [23].

GC chromatograms obtained after the propylation of 11 compounds spiked in plasma are shown in Fig. 3b. All compounds were completely separated within 20 min and displayed a single peak with good peak shape. As shown in Fig. 4, the molecular ion $[M]^+$ was detected in each of propylated derivatives. Base peak ions for di-, tri-, tetra-, penta-, hexachlorobiphenyls and dihydroxytrichlorobiphenyls were at *m/z* 238, 272, 342, 324, 376 and 308, respectively. Three characteristic ions for each compound were chosen for GC/MS/SIM analysis and the highest abundant mass ion was used to quantitative the analysis.

3.2. Optimization of clean-up procedure

Prior to GC/MS analysis, a solid-phase extraction procedure was selected for sample clean-up in order to eliminate endogenous compounds that might interfere with the quantification. The analysis of propylated hydroxy-PCBs in rat plasma and tissues was first attempted by injection of extracts directly. However, the chromatographic peak shape and sensitivity were lost because of the protein and fatty acids. As the recoveries obtained without hydrolysis ranged from 0 to 80%, the clean-up procedures were examined to eliminate any interferences, such as protein and fatty acids. Before the extraction, protein precipitation was carried out with several acids. The comparison of acid treatment with non-hydrolysis indicated that the highest recoveries for the most compounds were obtained with 2 M perchloric acid (Table 2).

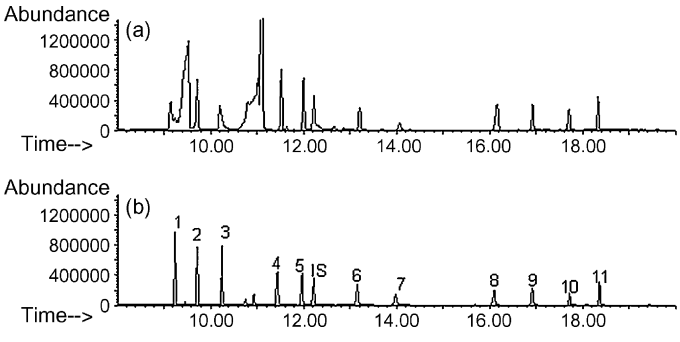

Fig. 3. GC/MS chromatograms of propylated hydroxy-PCBs from spiked plasma (a) and plasma extract spiked after silica clean-up (b) (peak identity: 1. 2-OH-2′,3,5′-TrCB; 2. 2-OH-2′,5,5′-TrCB; 3. 3-OH-4,4′-DiCB; 4. 4-OH-3,4′,5-TrCB; 5. 4-OH-2′,3,5,5′-TeCB; 6. 3-OH-2,3′,4,4′5-PeCB; 7. 4-OH-2′,3′,4′,5′-TeCB; 8. 4-OH-2′,3,3′,5,5′,6-HxCB; 9. 4-OH-2′,3,3′,4′,5′-PeCB; 10. 4-OH-2′,3,3′,4′,5,5′-HxCB; 11. 4,4′-diol-3,3′,5,5′-TeCB; IS. Pyrene-d_{10}).

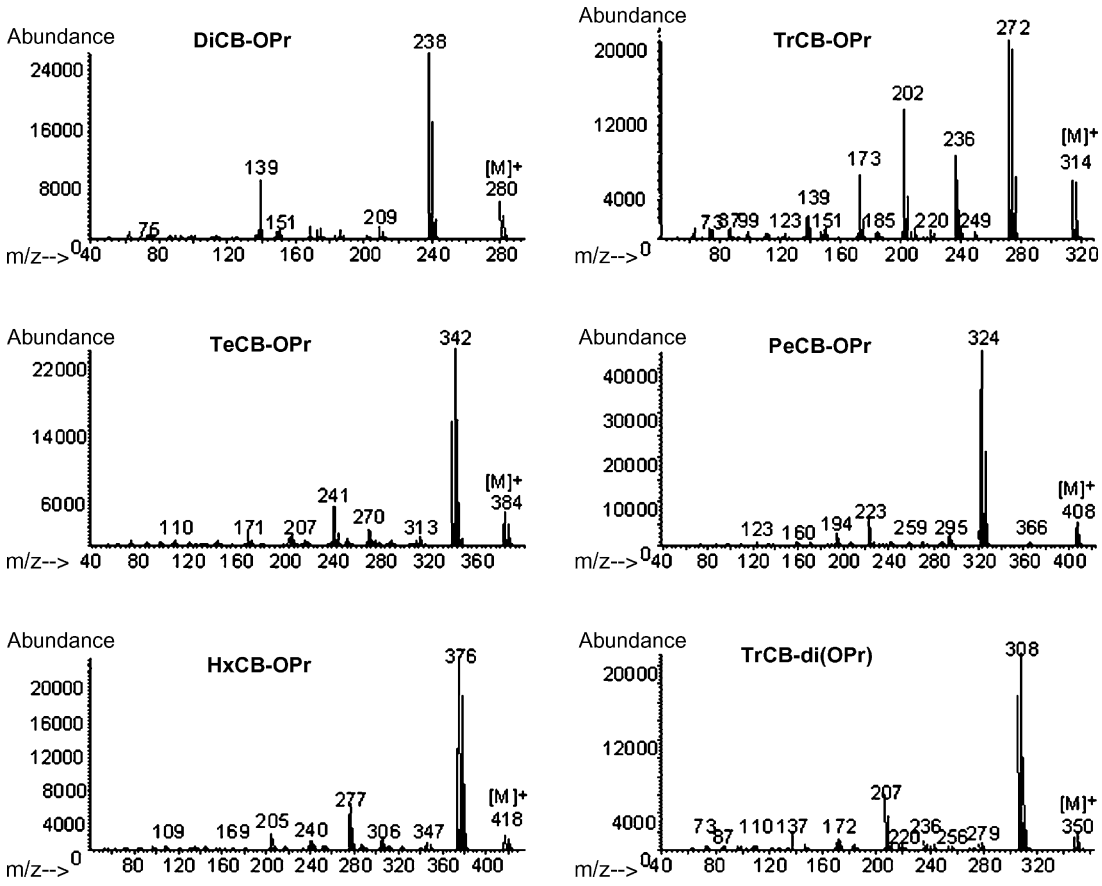

Fig. 4. Mass spectra for propyl derivatives of hydroxy-PCBs.

The chromatogram of a deproteinized spiked extract (with no additional clean-up) is presented in Fig. 3a. The large peaks obtained between 9 and 12 min prevented the quantification of target compounds under these separation conditions. These peaks were confirmed as propyl esters of hexadecanoic acid and octadecanoic acid. Therefore, another clean-up procedure for removal of these fatty acids was performed by using silica cartridge and the mixed eluents of *n*-hexane and dichloromethane. With the mixture of *n*-hexane and dichloromethane (DCM), we tested the recoveries of hydroxyl-PCBs and the effects of the clean-up at compositions of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 (v/v). The clean-up proceeded satisfactorily but the hydroxyl-PCBs were completely eluted over 40:60 (v/v) (Fig. 5a).

Next, result of the test for the mixed solvent volume indicated that an increase in the volume of eluent from 2 to 10 mL improved the recovery for each analyte (Fig. 5b). The chromatograms without the interfering peaks confirmed the

Table 2
Extraction recoveries of hydroxy-PCBs after acid hydrolysis

Hydroxy-PCBs	Extraction recoveries (%)				
	Without hydrolysis	HCl		Perchloric acid	
		1 M	6 M	1 M	2 M
3-OH-4,4′-DiCB	73.3	104.4	55.3	103.4	102.3
2-OH-2′,3,5′-TrCB	80.4	97.3	42.5	100.2	97.9
2-OH-2′,5,5′-TrCB	57.3	96.8	50.0	101.7	101.4
4-OH-3,4′,5-TrCB	11.7	109.0	50.6	103.7	103.9
4-OH-2′,3′,4′,5′-TeCB	76.4	88.0	36.2	86.8	95.8
4-OH-2′,3,5,5′-TeCB	1.1	96.8	35.1	99.3	100.4
4,4′-DiOH-3,3′,5,5′-TeCB	0.2	75.6	51.2	82.2	108.4
4-OH-2′,3,3′,4′,5′-PeCB	51.2	102.9	31.0	85.7	90.3
3-OH-2,3′,4,4′,5-PeCB	61.2	92.3	42.8	88.5	97.2
4-OH-2′,3,3′,4′,5,5′-HxCB	11.6	79.0	21.0	79.4	98.9
4-OH-2′,3,3′,5,5′,6-HxCB	23.1	93.2	25.2	91.0	93.3

Concentration = 10 ng mL^{-1}.

Table 3
Extraction recoveries of hydroxy-PCBs from plasma

Compounds	Extraction recoveries (%)					
	C_{18}		C_8		C_2	
	Mean	RSD	Mean	RSD	Mean	RSD
3-OH-4,4′-DiCB	96.6	7.6	87.1	5.5	104.8	8.1
2-OH-2′,3,5′-TrCB	117.1	12.4	103.2	5.3	115.4	4.7
2-OH-2′,5,5′-TrCB	105.7	13.8	104.6	7.4	113.1	11.2
4-OH-3,4′,5-TrCB	92.4	6.3	89.1	2.4	103.1	6.2
4-OH-2′,3′,4′,5′-TeCB	77.1	11.3	79.2	5.4	117.1	7.8
4-OH-2′,3,5,5′-TeCB	99.6	9.1	96.1	2.1	106.2	5.6
4,4′-DiOH-3,3′,5,5′-TeCB	79.7	7.1	48.1	11.4	869	1.9
4-OH-2′,3,3′,4′,5′-PeCB	82.6	8.1	79.4	2.7	105.5	6.1
3-OH-2,3′,4,4′,5-PeCB	81.3	5.6	87.9	4.8	98.5	3.7
4-OH-2′,3,3′,4′,5,5′-HxCB	68.4	12.2	67.1	5.4	97.7	5.4
4-OH-2′,3,3′,5,5′,6-HxCB	795	9.7	79.7	3.2	101.7	5.4

$n = 3$, concentration = 10 ng mL^{-1}.

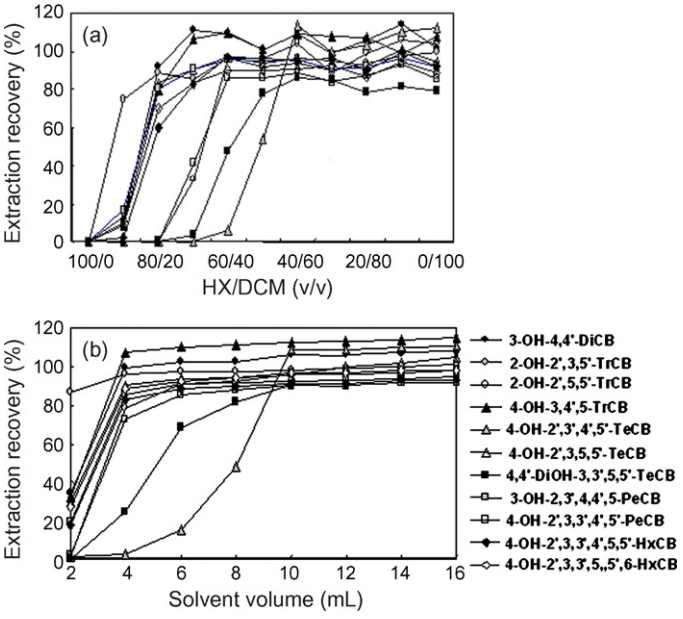

Fig. 5. Effect of clean-up by using silica column. (a) *n*-Hexane/dichloromethane fraction and (b) solvent volume.

excellent efficiency of the sample clean-up using the silica column (Fig. 3b).

The applied sample clean-up procedures proved to be very suitable for proteins and fatty acid-rich biological matrices, such as plasma and tissues. The possibility of simultaneously processing a large number of samples is an extra advantage of this approach.

3.3. Extraction recovery

The optimum conditions for the extraction of analytes from the spiked plasma were investigated with C_{18}, C_8 and C_2 solid-phases and with 5 mL of *n*-hexane as the eluent. Comparing the three different adsorbent materials, the highest recoveries for most of the hydroxy-PCBs were obtained with C_2. The extraction recoveries of analytes from the spiked plasma sample using the ISTD ranged from 86.9 to 117.1% for the C_2 solid-phase with a relative standard deviation (RSD) of 1.9–11.2% (Table 3).

Next, solvents for the extraction of hydroxy-PCBs from tissues were examined with *n*-hexane, *t*-butyl-methyl ether (MTBE) and ethyl acetate (EA). As shown in Table 4, the highest

Table 4
Extraction recoveries of hydroxy-PCBs from tissues

Compounds	Extraction recoveries (%)					
	n-Hexane		*t*-Butyl methyl ether		Ethyl acetate	
	Mean	RSD	Mean	RSD	Mean	RSD
3-OH-4,4′-DiCB	103.3	2.3	49.3	18.9	65.2	1.8
2-OH-2′,3,5′-TrCB	110.5	0.9	65.4	2.5	67.2	0.7
2-OH-2′,5,5′-TrCB	91.5	2.1	59.1	2.5	58.0	4.4
4-OH-3,4′,5-TrCB	107.0	1.5	64.9	17.7	69.7	2.8
4-OH-2′,3′,4′,5′-TeCB	99.1	4.2	62.7	11.4	49.9	6.0
4-OH-2′,3,5,5′-TeCB	105.8	2.5	64.4	12.7	62.8	4.1
4,4′-DiOH-3,3′,5,5′-TeCB	91.8	3.8	42.0	17.7	42.3	11.4
4-OH-2′,3,3′,4′,5′-PeCB	96.5	5.7	56.8	14.2	55.3	7.7
3-OH-2,3′,4,4′,5-PeCB	87.1	3.5	49.6	12.1	60.3	5.7
4-OH-2′,3,3′,4′,5,5′-HxCB	93.9	4.3	51.0	10.5	46.7	9.7
4-OH-2′,3,3′,5,5′,6-HxCB	87.2	4.1	47.9	14.1	44.2	6.5

$n = 3$, concentration = 10 ng g^{-1}.

Table 5
Calibration data and detection limits of hydroxy-PCBs in plasma and tissues

Compounds	Q. ion (*m/z*)	Calibration curve ($Y = aX + b$)			MDL[a]
		a	*b*	r^2	
Plasma					
3-OH-4,4′-DiCB	240	2.03	−0.00607	0.998	0.1
2-OH-2′,3,5′-TrCB	272	2.01	−0.00050	0.998	0.1
2-OH-2′,5,5′-TrCB	272	1.59	−0.00070	0.998	0.1
4-OH-3,4′,5-TrCB	272	2.60	−0.00064	0.998	0.5
4-OH-2′,3′,4′,5′-TeCB	306	1.00	−0.00037	0.993	0.1
4-OH-2′,3,5,5′-TeCB	306	2.32	−0.00030	0.998	0.2
4,4′-DiOH-3,3′,5,5′-TeCB	324	1.24	−0.00001	0.993	0.2
4-OH-2′,3,3′,4′,5′-PeCB	342	2.47	−0.00097	0.998	0.1
3-OH-2,3′,4,4′,5-PeCB	342	1.55	−0.00143	0.997	0.1
4-OH-2′,3,3′,4′,5,5′-HxCB	376	1.92	−0.00032	0.998	0.1
4-OH-2′,3,3′,5,5′,6-HxCB	376	2.93	−0.00023	0.998	0.1
Tissue					
3-OH-4,4′-DiCB	240	1.587	0.00833	0.992	0.2
2-OH-2′,3,5′-TrCB	272	1.278	0.00604	0.992	0.1
2-OH-2′,5,5′-TrCB	272	0.828	0.00522	0.993	0.1
4-OH-3,4′,5-TrCB	272	1.770	0.00451	0.998	0.2
4-OH-2′,3′,4′,5′-TeCB	306	0.702	0.00278	0.995	0.2
4-OH-2′,3,5,5′-TeCB	306	1.539	0.00275	0.997	0.5
4,4′-DiOH-3,3′,5,5′-TeCB	324	1.620	−0.000813	0.995	0.2
4-OH-2′,3,3′,4′,5′-PeCB	342	1.251	0.00718	0.995	0.2
3-OH-2,3′,4,4′,5-PeCB	342	1.152	0.00464	0.998	0.2
4-OH-2′,3,3′,4′,5,5′-HxCB	376	0.939	0.00133	0.998	0.1
4-OH-2′,3,3′,5,5′,6-HxCB	376	1.710	0.00352	0.996	0.1

[a] ng mL^{-1} for plasma, ng g^{-1} for tissue.

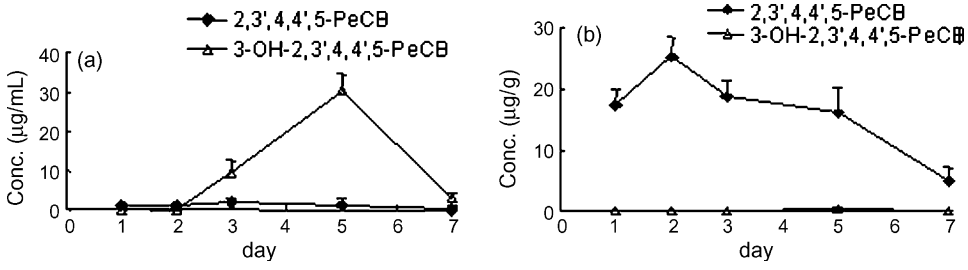

Fig. 6. Concentration–time profile of 2,3′,4,4′,5-PeCB in rats after intraperitoneal injection (20 mg/kg, $n = 3$). (a) Plasma and (b) liver.

recoveries for the hydroxy-PCBs were obtained with *n*-hexane (82.3–110.5% with an RSD of 0.9–5.7%).

3.4. Linearity and detection limits

The linearity of the calibration curve for each analyte in the plasma and tissue was determined by computing a regression line of the peak area ratio of the standard to ISTD on its concentration ratio using least-squares fit. As shown in Table 5, the linear relationships with the coefficients of determination (r^2) ranged from 0.992 to 0.998 and the method detection limits ranged from 0.1 to 0.5 ng mL^{-1} for 1 mL of plasma and 0.1 to 0.5 ng g^{-1} of tissues, respectively. The detection limits were evaluated by a minimum signal to noise ratio of 3.

3.5. Application

The present method was applied for the quantification of plasma and liver tissue concentrations of hydroxy metabolites of 2,3′,4,4′,5-PeCB after an intraperitoneal injection of 2,3′,4,4′,5-PeCB to male rats. Fig. 6 shows the plasma and liver concentration–time profiles of 2,3′,4,4′,5-PeCB and 3-OH-2,3′,4,4′,5-PeCB, which were two of the target compounds. The concentration of 2,3′,4,4′,5-PeCB in the rat plasma and liver ranged from 0.12 to 1.88 and 4.89 to 25.3 μg mL^{-1}, following the intraperitoneal injection of 20 mg/kg. The concentrations for

Table 6
Concentration of 2,3′,4,4′,5-PeCB and its metabolites in rat

	Concentration	
	2,3,4,4′,5-PeCB	3-OH-2,3′,4,4′,5-PeCB
Plasma (μg mL^{-1})	0.12–18.8	0.13–35.3
Liver (μg g^{-1})	4.89–25.3	0.06–0.41
Lung (μg g^{-1})	1.05–4.32	0.34–2.96
Kidney (μg g^{-1})	13.4–30.7	0.54–2.59
Stomach (μg g^{-1})	7.71–32.9	2.57–24.6

3-OH-2,3′,4,4′,5-PeCB in the plasma and liver ranged from 0.09 to 35.3 and 0.06 to 0.41 μg mL^{-1}, respectively (Table 6).

4. Conclusion

The proposed method which coupled extraction with a new clean-up procedure was found to be useful for the analysis of hydroxy-PCBs in plasma and tissue. Using a silica column with 10 mL of *n*-hexane/dichloromethane (4:6, v/v), fatty acids were effectively removed from the plasma. The extraction recoveries from plasma using C_2 solid-phase followed by elution with 5 mL of *n*-hexane ranged from 86.9 to 117.13%, with an RSD of 1.9–11.2%, while those from tissues ranged from 82 to 111%, with an RSD of 0.9–5.7%. This method enables the detection of hydroxy-PCBs in plasma at a trace level of 0.1 ng mL^{-1}. This procedure was successfully applied to the study of 3-OH-2,3′,4,4′,5-PeCB in rat plasma and liver samples after intraperitoneal injection (20 mg/kg) of 2,3′,4,4′,5-PeCB.

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