Journal of Chromatography B, 879 (2011) 1086–1090

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Analysis of hydroxylated polybrominated diphenyl ethers in rat plasma by using ultra performance liquid chromatography–tandem mass spectrometry

Yongquan Lai[a], Xueguo Chen[a], Michael Hon-Wah Lam[b], Zongwei Cai[a,*]

[a] Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Hong Kong SAR, China
[b] Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 3 January 2011
Accepted 10 March 2011
Available online 21 March 2011

Keywords:
Hydroxylated polybrominated diphenyl ethers
Ultra performance liquid chromatography
Selected reaction monitoring
Pharmacokinetic study

ABSTRACT

Ultra performance liquid chromatography (UPLC) provides improved resolution, speed and sensitivity compared to conventional high performance liquid chromatography (HPLC). In this study, a robust UPLC–ESI–MS/MS method was developed for the rapid determination of nine hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in rat plasma. Under the optimized conditions, the OH-PBDE congeners were eluted within 7.0 min. The limits of quantification defined at the signal-to-noise ratio of 10 were 0.17–2.78 ng/mL in rat plasma. The method provided good linearity for the calibration curves with recoveries of 93.3–114.0% and repeatability with relative standard deviation (RSD) of 0.6–5.8% for intra-day and 3.2–10.4% for inter-day measurements. The developed method was applied for supporting the pharmacokinetics investigation of 6-OH-BDE-47 in two groups of Sprague–Dawley rats that received, respectively a single dose of 0.60 mg/kg (high dose) and 0.15 mg/kg (low dose) by intravenous injection. The results showed that plasma levels of 6-OH-BDE-47 declined bi-exponentially with elimination half-life of 71.7 and 85.6 min for lower and higher dose group, respectively. The obtained results of short elimination half-life suggested that 6-OH-BDE-47 might not accumulate significantly in rat.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) were widely used in commercial and industrial products such as textile, rubbers, high-impact polystyrenes, polyamides and electronic equipments for the purpose of fire prevention [1,2]. The general chemical formula of PBDEs is $C_{12}H_nBr_{(10-n)}O$ and the number of possible congeners is 209. PBDEs have been of particular concern as ubiquitous contamination in both biotic and abiotic environment [3–6]. The extensive use of PBDEs and their lipophilicity and persistency properties have led to the increasing bioaccumulation and retention in both animals and humans [7–11]. The potential toxic effects of PBDEs involved biological endpoints including thyroid hormone disruption, neurodevelopment deficits and cancer [12].

Hydroxylated PBDEs (OH-PBDEs) are a relative new group of phenolic compounds, which have attracted particular interests due to their similar chemical structure to PBDEs and potential toxicities. While OH-PBDEs have been detected from both anthropogenic and natural origins [13–16], the compounds were also identified as metabolites of PBDEs in rat, mice and fish [17–23] as well as human body [24–27]. Among the reported OH-PBDE congeners, 6-OH-BDE-47 was detected with the highest level [15,27]. This might be due to the fact that BDE-47 was one of the major components in commercial penta-BDE products and thus was the predominant congener found in environment [3]. On the other hand, OH-PBDEs could also be formed from naturally produced organohalogens by marine organisms such as sponges [28,29], tunicates [30,31] and algae [14]. However, very limited data of OH-PBDEs have been reported in abiotic environmental samples. Several OH-PBDEs were determined in surface water, rainwater and sewage, which were probably produced through the reaction of PBDEs and atmospheric OH radicals [16,32].

Although OH-PBDEs would be less persistent compared to their precursor PBDEs, they have more severe toxic effects as metabolites [22]. Thyroxine-like and estrogen-like OH-PBDEs have been reported to bind to transthyretin and thyroid hormone receptor *in vitro*, and to be estrogen receptor ERα and ERβ agonists [33–35]. Results obtained from *in vitro* endocrine studies on the interactions with estrogen and thyroid hormone receptor systems showed that hydroxylated metabolites were more potent than the parent compounds [34,36–38]. 6-OH-BDE-47 was also confirmed to have considerably higher neurotoxic potential to affect exocytosis and calcium homeostasis in PC12 cells compared to BDE-47 [39].

Gas chromatography coupled to mass spectrometry (GC–MS) was widely applied for the determination of xenobiotic contaminants, including PBDEs, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. However, GC–MS is not suitable for the direct analysis of xenobiotic metabolites, including hydrox-

* Corresponding author. Tel.: +852 34117070; fax: +852 34117348.
E-mail address: zwcai@hkbu.edu.hk (Z. Cai).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.03.024

ylated derivatives, glucuronic acid or glutathione conjugates due to their high polarity [40]. LC–MS has rapidly become an important analytical technique for broad analytes in various matrices without chemical derivatization [32]. A LC–MS/MS method was developed for the determination of triclosan in waste and surface waters at parts-per-billion (ppb) levels, where 2′-OH-2,4,4′-tribromodiphenyl ether (2′-OH-BDE-28) was used as internal standard. During the analysis of triclosan, two OH-triBDEs were also detected in the water samples [32]. In addition, a LC–MS method was developed and applied for the simultaneous determination of eight OH-PBDEs in four different environmental matrices [40]. Most recently, Kato et al. developed a LC–APCI–MS method for the determination of OH-PBDEs and methoxylated PBDEs (MeO-PBDEs) in marine biota [41]. Under the optimized conditions, the reported limits of quantification ranged from 0.10 to 0.25 ng/g lipid for the phenolic analytes in tiger shark and bull shark liver samples.

Compared to the conventional high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC) provides improved resolution, speed and sensitivity. In this study, a robust method of UPLC–ESI–MS/MS was developed and applied for the direct analysis of nine OH-PBDEs in rat plasma. The developed method was applied for supporting a pharmacokinetic study of 6-OH-BDE-47. This is the first report on pharmacokinetic study of OH-PBDEs, to the best of our knowledge.

2. Material and methods

2.1. Chemicals

The OH-PBDEs were received from Dr. Michael H.W. Lam (Department of Biology and Chemistry, City University of Hong Kong), including 3′-OH-2,4-dibromodiphenyl ether (3′-OH-BDE-7), 6′-OH-2,2′,4-tribromodiphenyl ether (6′-OH-BDE-17), 4′-OH-2,2′,4-tribromodiphenyl ether (4′-OH-BDE-17), 2′-OH-2,4,4′-tribromodiphenyl ether (2′-OH-BDE-28), 4′-OH-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-49), 6-OH-2,2′,4, 4′-tetrabromodiphenyl ether (6-OH-BDE-47), 2′-OH-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE-68), 6-OH-2,2′,3,4,4′-pentabromodiphenyl ether (6-OH-BDE-85) and 6-OH-2,2′,3,4,4′,5-hexabromodiphenyl ether (6-OH-BDE-137). Stock solutions containing all nine OH-PBDEs were prepared in acetonitrile at three different concentrations of 1.0 mg/mL, 10.0 μg/mL and 0.1 μg/mL. 3,5-Dibromophenol (3,5-DBP) was purchased from Sigma and used as internal standard. 3,5-DBP solution was prepared in acetonitrile at concentration of 10.0 μg/mL. Acetonitrile was supplied by Tedia Company in ABSOLV grade. Dimethyl sulfoxide (DMSO) in analytical grade was purchased from AJAX Chemicals. Water was purified by employing a Milli-Q Reagent Water System (Millipore, Billerica, USA).

2.2. Sample extraction

100 μL plasma was transferred to a plastic tube followed by adding known amounts of OH-PBDE congeners and internal standard. After 400 μL acetonitrile was added, the sample was vortex mixed and centrifuged at 14,000 g for 20 min. The procedure was repeated two times and the collected supernatants were combined and concentrated to near dryness using a gentle flow of nitrogen gas at room temperature. The dry residue was reconstituted in 100 μL methanol and the sample was centrifuged at 14,000 g for 20 min prior to the UPLC–MS analysis.

2.3. Animal experiment

Six male Sprague–Dawley rats (about 12 weeks old) were purchased from Laboratory Animal Services Center, the Chinese University of Hong Kong. The animal experiments were conducted according to the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals. The procedures were approved by the department of health, the government of the Hong Kong special administrative region, China. The rats were divided into two groups ($n = 3$ each) randomly and kept in ambient temperature and humidity controlled room under a 12 h/12 h light/dark cycle with free access to food and water. The dosing solution containing 6-OH-BDE-47 was prepared in DMSO at concentration of 1.50 mg/mL. Two groups of rats received respectively a single dose of 0.60 mg/kg (high dose) and 0.15 mg/kg (low dose) by intravenous injection through tail vein. Blood sample (approximate 100 μL) was collected from each rat as control through tail vein before the dose. Serial blood samples were obtained after the dose. Sodium heparin was used as anticoagulant. Blood samples were centrifuged at 5000 × *g* for 10 min and the collected plasma samples were prepared after the internal standard was added with the procedure described in the previous section and analysed by UPLC–ESI–MS/MS.

2.4. UPLC–ESI–MS/MS analysis

Separations of the OH-PBDEs and internal standard were performed on an UPLC (Waters ACQUITY UPLC system, Waters Corporation, Milford, MA) with a reversed phase Ethylene Bridged Hybrid C_{18} column (1.7 μm, 2.1 mm × 50 mm). The mobile phase composition used in the chromatographic separation was optimized by binary mixtures of water (A) and acetonitrile (B). Because the OH-PBDEs vary widely in substituted bromine numbers and positions at two phenyl rings, a gradient elution program was developed to obtain completely separation. The elution program started with an initial composition of 70:30 water/acetonitrile (v/v) and increased linearly to 5:95 water/acetonitrile (v/v) in 6.0 min, at which point it was held at 95% acetonitrile for 1.0 min before being returned to initial condition. The column was re-equilibrated for another 3.0 min. The flow rate of the mobile phase throughout the elution was 0.4 mL/min. A volume of 10 μL of each sample was injected into the UPLC system.

The UPLC was coupled to a Waters ACQUITY TQ Detector equipped with electrospray ionization source (Waters Corporation, Milford). OH-PBDEs were detected by using ESI–MS/MS in negative ion mode by monitoring their precursor–product transition ions in selected reaction monitoring mode (SRM). The product ions of $[Br]^-$ and $[C_6H_3Br_2O]^-$ were selected for the analysis. For the quantitative analysis, the transition ion pair consisting the deprotonated molecular ion $[OH\text{-}PBDE\text{-}H]^-$ and product ion $[Br]^-$ was chosen (Table 1) because its intensity was higher than that of the ion pair $[OH\text{-}PBDE\text{-}H]^- > [C_6H_3Br_2O]^-$. The SRM transition ion $[OH\text{-}PBDE\text{-}H]^- > [C_6H_3Br_2O]^-$ was used as confirmation ion. MS parameters were optimized by using 6-OH-BDE-47 standard solution at concentration of 20 ng/mL by adjusting capillary, cone and extractor voltages, ESI source and desolvation gas temperatures, cone gas and the desolvation gas flows. The optimized parameters were summarized in Table 1 and described below: the capillary voltage was 2500 V; the cone and collision voltages were 30 and 40 V, respectively; the dwell time was 0.05 s and the extractor voltage was 2.5 V; temperatures of the negative ESI source and desolvation gas were 118 and 500 °C, respectively; the cone gas and the desolvation gas flows were 40 and 650 L/h, respectively. Instrument operation and data acquisition were processed by using the Waters MassLynx V4.1 SCN562 software package. Nitrogen (99.99% purity) for the ESI–MS was provided by the on-line nitrogen generation system.

2.5. Method validation and pharmacokinetics data analysis

A series of rat plasma samples spiked with OH-PBDE congeners and 3,5-DBP were analysed to determine the linear calibration

Table 1
MS/MS parameters for the quantification of OH-PBDE congeners.

Compound	Precursor ion Q1 (*m/z*)	Product ion Q3 (*m/z*)	Cone voltage (V)	Collision energy (V)
3,5-DBP	250.9	78.9	30	40
3′-OH-BDE-07	342.9	78.9	30	40
6′-OH-BDE-17	420.8	78.9	30	40
4′-OH-BDE-17	420.8	78.9	30	40
2′-OH-BDE-28	420.8	78.9	30	40
4′-OH-BDE-49	500.7	78.9	30	40
6-OH-BDE-47	500.7	78.9	30	40
2′-OH-BDE-68	500.7	78.9	30	40
6-OH-BDE-85	578.6	78.9	30	40
6-OH-BDE-137	658.5	78.9	30	40

ranges. Limit of quantification (LOQ) of each compound was calculated at a signal-to-noise ratio of 10. To evaluate the precision of the method, the intra-day variations were assessed with six consecutive injections of the spiked samples and inter-day variations were determined by measuring the spiked samples with six injections on three consecutive days. Method recovery was determined based on the comparison of the results form the analysis of spiked samples with those from the standard solution containing the same amount of the target analytes.

Matrix effects were examined to evaluate effects of coextracted contaminants from the plasma samples for the analysis of OH-PBDEs. The potential spectral interference caused by coextracted contaminants was evaluated with the analysis of blank plasma sample by monitoring the SRM ions. In addition, triplicate samples with OH-PBDEs added in the blank plasma sample extract were analysed and compared with the corresponding OH-PBDEs standard solution in order to evaluate the possible alternation of signal response resulted from matrix effects.

Calculation of pharmacokinetic parameters was carried out by non-compartmental assessment of data using the computer program PK solutions 2.0. The maximum plasma concentration (C_{max}) was determined from the plasma concentration–time plot with extrapolation time to zero. The area under the plasma concentration–time curve from time zero to the time of the last sample (AUC_{0-t}) was calculated by the linear trapezoidal method. The elimination rate constant (k_e) was determined from the terminal portion of the concentration–time curve, and the corresponding elimination half-life ($t_{1/2}$) was then calculated as follows: $t_{1/2} = 0.693/k_e$. Systemic clearance (CLs) is based on observed data points (AUC_{0-t}).

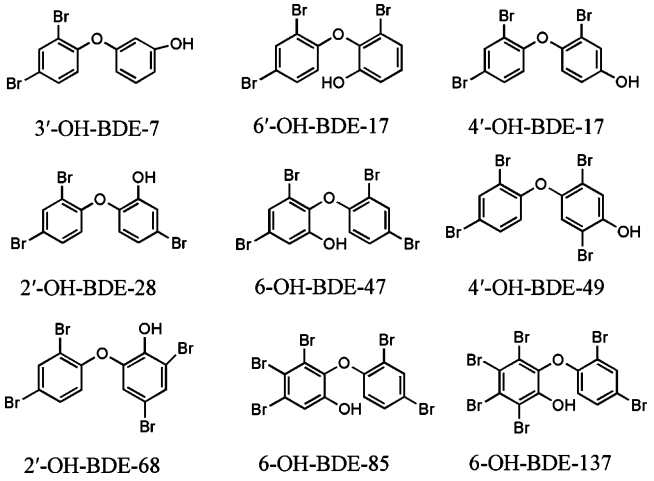

Fig. 1. Chemical structures of the studied OH-PBDE congeners. The full names of OH-PBDE congeners are given in Section 2 according to the IUPAC nomenclature used for PCBs.

3. Results and discussion

3.1. Optimization of UPLC and MS parameters

PBDEs have become world wide contaminants due to their potential toxicities and pervasiveness in the environment and human body. OH-PBDEs are a relative new group of phenolic compounds which have attracted particular interests. The hydroxylated metabolites would be less persistent and more water-soluble compared to their precursor PBDEs. Even though the formation of OH-PBDEs was considered to facilitate the excretion of PBDEs through P450-dependent phase I metabolism, hydroxylated metabolites also resulted in the bioactivity of the precursor PBDEs because of the more potential toxicity related to OH-PBDEs. Therefore, it is important to develop a fast, sensitive and selective method for the analysis of OH-PBDE metabolites at relative low concentration in biological samples.

In the current study, nine OH-PBDE congeners with the number of bromine atoms ranging from 2 to 6 were selected for the method development using UPLC separation. These OH-PBDE congeners included 3′-OH-BDE-7, three OH-triBDE isomers (6′-OH-BDE-17, 4′-OH-BDE-17, 2′-OH-BDE-28), three OH-tetraBDE isomers (4′-OH-BDE-49, 6-OH-BDE-47, 2′-OH-BDE-68), 6-OH-BDE-85, and 6-OH-BDE-137 (Fig. 1). Standard solution containing all testing congeners at concentration of 20 ng/mL and the internal standard at 100 ng/mL was prepared and used for the optimization of UPLC conditions. The gradient solvent elution program was optimized with an initial composition of 70:30 water/acetonitrile (v/v). Under the optimized conditions, all OH-PBDE congeners and 3,5-DBP were separated completely within 7.0 min (Fig. 2).

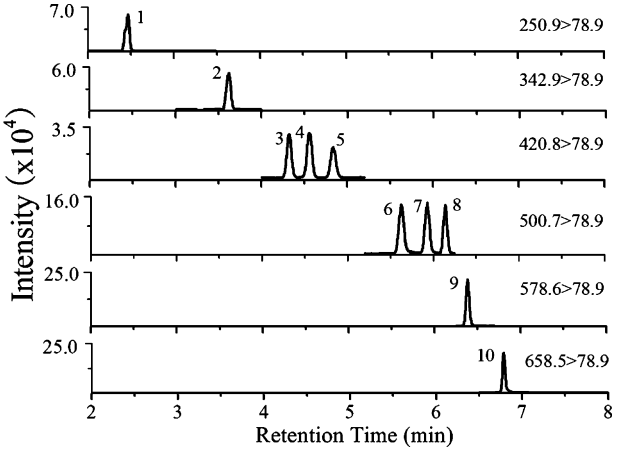

Fig. 2. SRM chromatograms from the UPLC–ESI–MS/MS analysis of OH-PBDEs at 20 ng/mL and the internal standard at 100 ng/mL. (1) 3,5-DBP, (2) 3′-OH-BDE-7, (3) 6′-OH-BDE-17, (4) 4′-OH-BDE-17, (5) 2′-OH-BDE-28, (6) 4′-OH-BDE-49, (7) 6-OH-BDE-47, (8) 2′-OH-BDE-68, (9) 6-OH-BDE-85, and (10) 6-OH-BDE-137.

Table 2
Results of method validation for the determination of the OH-PBDEs.

Compound	Retention time (min)	LOQ ng/mL ($n=3$)	Recovery ($n=3$)		Linearity ng/mL (R^2)	Intra day RSD ($n=6$)		Inter day RSD ($n=6$)	
			10 ng/mL	100 ng/mL		10 ng/mL	100 ng/mL	10 ng/mL	100 ng/mL
3′-OH-BDE-07	3.63	1.72 ± 0.55	98.7 ± 7.1	93.3 ± 4.4	4–400 (0.9950)	4.6	4.5	8.5	6.9
6′-OH-BDE-17	4.24	2.13 ± 0.62	94.8 ± 3.7	106.0 ± 1.6	4–400 (0.9949)	3.7	3.1	7.4	10.4
4′-OH-BDE-17	4.47	1.57 ± 0.10	102.3 ± 2.4	105.97 ± 4.25	4–400 (0.9983)	0.6	2.0	3.5	6.6
2′-OH-BDE-28	4.72	2.78 ± 0.33	105.4 ± 6.1	104.7 ± 3.9	4–400 (0.9988)	3.9	5.8	8.4	7.7
4′-OH-BDE-49	5.62	1.71 ± 0.35	94.4 ± 3.9	99.4 ± 6.9	4–400 (0.9978)	1.1	2.3	7.4	4.9
6-OH-BDE-47	5.93	1.38 ± 0.18	100.9 ± 6.6	97.7 ± 7.8	4–400 (0.9766)	2.0	0.6	3.5	6.6
2′-OH-BDE-68	6.14	1.22 ± 0.30	105.1 ± 9.0	114.0 ± 3.3	4–400 (0.9972)	1.9	1.9	3.2	3.8
6-OH-BDE-85	6.40	0.49 ± 0.12	98.6 ± 10.1	113.4 ± 5.0	2–200 (0.9987)	4.8	4.9	6.6	6.8
6-OH-BDE-137	6.80	0.17 ± 0.04	109.6 ± 10.9	113.3 ± 5.4	2–200 (0.9753)	2.4	2.5	4.8	6.6

Previous studies indicated conventional HPLC lacked efficient separation of OH-PBDE congeners because of peak overlapping and long analysis time. For example, severely overlapping peaks were observed from the separation of OH-triBDE isomers as well as OH-tetraBDE isomers [40]. Three OH-tetraBDE isomers were separated with retention time up to 25 min [42]. Thus, UPLC provided improved resolution and short analysis time compared to the conventional HPLC.

SRM in negative mode with six functions was applied for the analysis of 3,5-DBP, 3′-OH-BDE-7, OH-triBDE isomers, OH-tetraBDE isomers, 6-OH-BDE-85 and 6-OH-BDE-137. Analysis time for each function was optimized according to retention time of the OH-PBDEs to eliminate cross-over between function channels and to reduce the background noise. The ESI–MS/MS parameters were optimized, including capillary, collision energy and cone voltages, ESI source and desolvation gas temperatures, cone gas and the desolvation gas flows. Collision energy and the cone voltages were found to be the most influential parameters for signal intensity.

3.2. Method validation using rat plasma

To evaluate the performance of the UPLC–ESI–MS/MS method, recovery, calibration linear range, sensitivity, inter-day and intra-day variations were investigated. Good method recoveries were achieved in the range from 93.3 to 114.0% at the spiked levels of 10.0 and 100.0 ng/mL for the tested compounds (Table 2). Calibration curve was obtained with internal standard method. Linear calibration ranges from 5.0 to 400.0 ng/mL for 3′-OH-BDE-7, OH-triBDEs and OH-tetraBDEs, and from 2.0 to 200.0 ng/mL for 6-OH-BDE-85 and 6-OH-BDE-137 in plasma were achieved with the correlation coefficients (R^2) from 0.9753 to 0.9988. The repeatability was examined by assessing the inter-day and intra-day deviations. The obtained results indicated that intra-day deviations represented by relative standard deviation (RSD) were in the range of 0.6–5.8%. The RSD for inter-day deviations ranged from 3.2 to 10.4%. LOQ defined as the minimum concentration with signal to noise ratio of 10 ranged from 0.17 to 2.78 ng/mL for the analysis of the OH-PBDEs. Higher sensitivity was achieved from the detection of 6-OH-BDE-85 and 6-OH-BDE-137, which was consistent with results in previous report [40]. The achieved better LC–MS response of the more acidic congeners 6-OH-BDE-85 and 6-OH-BDE-137 might be due to the stabilization of the deprotonated molecular ions.

Matrix effects with contaminants coextracted from the plasma matrix might alter the signal response and cause the spectral interference for the analysis of OH-PBDEs. Results from the analysis of the rat blank plasma sample under the same UPLC–MS/MS conditions indicated that the matrix background did not cause interfering problem for the SRM analysis of the targeted OH-PBDEs and internal standard. The analytical data of the blank plasma sample extract spiked with 10 ng/mL of OH-PBDEs were compared with those of standard solution at the same OH-PBDEs concentration in order to evaluate the alteration of signal response caused by coextracted contaminants. The obtained results from triplicate analysis showed the alteration of OH-PBDEs signal responses in the range from 94.0% to 117.0%, indicating that the interference caused by coextracted contaminants from the plasma matrix was insignificant for the quantification of OH-PBDEs in rat plasma.

3.3. Method application for supporting pharmacokinetic study

Recently, PBDEs have received increasingly concerns due to their ubiquitous present in both biotic and abiotic environment, as well as their potential toxicity. Although OH-PBDEs are much less persistent compared to their parent PBDEs, they showed greater biological effects. OH-PBDE metabolites and their biological effects have been examined through *in vitro* endocrine studies, with the major focus on 6-OH-BDE-47. The results indicated that hydroxylated metabolites of PBDEs were more potent than their parent compounds [34,36–38]. The effect of 6-OH-BDE-47 on exocytosis and calcium homeostasis in PC12 cells further confirmed that the bioactivation by oxidative metabolism might add considerably to the neurotoxic potential of PBDEs [39]. Pharmacokinetic study is often applied for the evaluation of biological or toxic effects of drug or toxicant. Understanding of the kinetics of not only PBDE but also OH-PBDE congeners is essential for assessing the risk of these contaminants. However, no report has been published on the pharmacokinetic study of OH-PBDEs so far, to the best of our knowledge.

The developed analytical method was applied for supporting a preliminary pharmacokinetic study of 6-OH-BDE-47 that was intravenously dosed to male Sprague–Dawley rats. The concentration of 6-OH-BDE-47 was determined by using UPLC–ESI–MS/MS with the established calibration curve. When the concentration of 6-OH-BDE-47 was over the highest calibration point, the sample was diluted with acetonitrile and reanalysed. The obtained plasma concentrations from 3 rats were averaged for each sample collection point. Fig. 3 shows the plasma 6-OH-BDE-47 concentrations from 5 min to 180 min after the intravenous administration at 0.15 and 0.60 mg/kg. The obtained pharmacokinetic parameters of 6-OH-BDE-47 are summarized in Table 3. Plasma levels of 6-OH-BDE-47 declined bi-exponentially for both high and low doses, with initial distribution of 1.6 and 3.3 min, and elimination half-life of 71.7 and 85.6 min, respectively. No significant difference in these half-lives as a function of the dosing levels was observed. There were also no significant differences in the systemic clearance of 6-OH-BDE-47 as a function of the dosing levels. It was reported that BDE-47 has terminal half life of 664 days in humans, 31 days in rats and approximately 30–40 days in mice [43]. The results obtained from the present study indicated that 6-OH-BDE-47 was much less persistent compared to its parent compound BDE-47. Although 6-OH-BDE-47 was unlikely to accumulate significantly in the rats given the short elimination half-life, it is important to further investigate differential pharmacokinetic parameters associated with other OH-PBDE congeners.

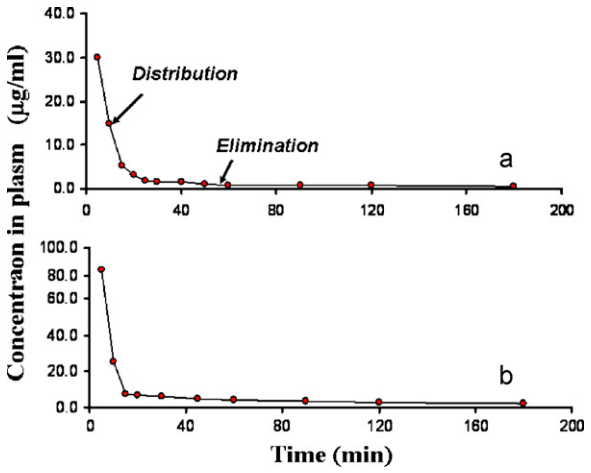

Fig. 3. Mean plasma concentration-time profile of 6-OH-BDE-47 after intravenous administration at 0.15 mg/kg (a) and 0.60 mg/kg (b).

Table 3
Pharmacokinetics data of 6-HO-BDE-47 after the intravenous doses at 0.15 mg/kg and 0.60 mg/kg.

	Dose (mg/kg)	
	0.15	0.60
C_{max} (μg/mL)[a]	90.6	775.5
$t_{1/2}$, λ1 (min)[b]	3.3	1.6
$t_{1/2}$, λ2 (min)[c]	71.7	85.6
AUC (μg × min/mL)[d]	627.5	3049.5
Cls (mL/min)[e]	0.239	0.197

[a] Plasma concentration extrapolated to time zero.
[b] Initial distribution half-life.
[c] Terminal elimination half-life.
[d] Area under the plasma concentration–time curve.
[e] Systemic clearance.

4. Conclusion

A UPLC–ESI–MS/MS method was developed for the determination of OH-PBDE congeners in rat plasma. Advantages of the established method included relative short analysis time, improved chromatographic resolution and the simple sample preparation procedure. The developed method with good recovery, accuracy and precision was applied to support pharmacokinetic study of 6-OH-BDE-47 in male Sprague–Dawley rats after the intravenous administrations. The obtained results showed the short elimination half-life of 6-OH-BDE-47, indicating that the compound was unlikely to accumulate significantly in the rats. It should be pointed out, however, that different OH-PBDE congeners might have different pharmacokinetic parameters. The application of the developed method could be extended for supporting the pharmacokinetic study of other OH-PBDE congeners. Moreover, the method might also be applied for the determination of hydroxylated metabolites of PBDEs in both *in vitro* and *in vivo* metabolism studies.

Acknowledgements

The authors thank the special post-graduate studentship program of "Persistent Toxic Substances" from Research Grant Council, University Grants Committee of Hong Kong SAR. Financial support of Collaborative Research Fund from the Research Grant Council (HKBU1/CRF08) and the collaborative fund from National Sciences Foundation of China (NSFC20621703) is acknowledged.

References

[1] M. Alaee, P. Arias, A. Sjodin, A. Bergman, Environ. Int. 29 (2003) 683.
[2] A. Sandholm, B.M. Emanuelsson, E.K. Wehler, Xenobiotica 33 (2003) 1149.
[3] R.A. Hites, Environ. Sci. Technol. 38 (2004) 945.
[4] S.S. Streets, S.A. Henderson, A.D. Stoner, D.L. Carlson, M.F. Simcik, D.L. Swackhamer, Environ. Sci. Technol. 40 (2006) 7263.
[5] W.L. Song, A. Li, J.C. Ford, N.C. Sturchio, K.J. Rockne, D.R. Buckley, W.J. Mills, Environ. Sci. Technol. 39 (2005) 3474.
[6] L.Y. Zhu, R.A. Hites, Environ. Sci. Technol. 38 (2004) 2779.
[7] G. Lindstrom, H. Wingfors, M. Dam, B. von Bavel, Arch. Environ. Contam. Toxicol. 36 (1999) 355.
[8] C.A. de Wit, Chemosphere 46 (2002) 583.
[9] K. Noren, D. Meironyte, Chemosphere 40 (2000) 1111.
[10] J.K. Huwe, M. Lorentzsen, K. Thuresson, A. Bergman, Chemosphere 46 (2002) 635.
[11] A. Sjodin, D.G. Patterson, A. Bergman, Environ. Int. 29 (2003) 829.
[12] T.A. McDonald, Chemosphere 46 (2002) 745.
[13] G. Marsh, M. Athanasiadou, A. Bergman, L. Asplund, Environ. Sci. Technol. 38 (2004) 10.
[14] A. Malmvarn, G. Marsh, L. Kautsky, M. Athanasiadou, A. Bergman, L. Asplund, Environ. Sci. Technol. 39 (2005) 2990.
[15] K. Valters, H.X. Li, M. Alaee, I. D'Sa, G. Marsh, A. Bergman, R.J. Letcher, Environ. Sci. Technol. 39 (2005) 5612.
[16] D. Ueno, C. Darling, M. Alaee, G. Pacepavicius, C. Teixeira, L. Campbell, R.J. Letcher, A. Bergman, G. Marsh, D. Muir, Environ. Sci. Technol. 42 (2008) 1657.
[17] S. Burreau, D. Broman, U. Orn, Chemosphere 40 (2000) 977.
[18] H. Hakk, G. Larsen, E. Klasson-Wehler, Xenobiotica 32 (2002) 369.
[19] A. Morck, H. Hakk, U. Orn, E.K. Wehler, Drug Metab. Dispos. 31 (2003) 900.
[20] H. Hakk, J. Huwe, M. Low, D. Rutherford, G. Larsen, Xenobiotica 36 (2006) 79.
[21] U. Orn, E. Klasson-Wehler, Xenobiotica 28 (1998) 199.
[22] X.H. Qiu, M. Mercado-Feliciano, R.M. Bigsby, R.A. Hites, Environ. Health Perspect. 115 (2007) 1052.
[23] T. Malmberg, M. Athanasiadou, G. Marsh, I. Brandt, A. Bergmant, Environ. Sci. Technol. 39 (2005) 5342.
[24] L. Hovander, T. Malmberg, M. Athanasiadou, L. Athanassiadis, S. Rahm, A. Bergman, E.K. Wehler, Arch. Environ. Contam. Toxicol. 42 (2002) 105.
[25] X.H. Qiu, R.M. Bigsby, R.A. Hites, Environ. Health Perspect. 117 (2009) 93.
[26] M. Athanasiadou, S.N. Cuadra, G. Marsh, A. Bergman, K. Jakobsson, Environ. Health Perspect. 116 (2008) 400.
[27] Y. Wan, K. Choi, S. Kim, K. Ji, H. Chang, S. Wiseman, P.D. Jones, J.S. Khim, S. Park, J. Park, M.H.W. Lam, J.P. Giesy, Environ. Sci. Technol. 44 (2010) 5233.
[28] B.F. Bowden, L. Towerzey, P.C. Junk, Aust. J. Chem. 53 (2000) 299.
[29] X.O. Fu, E.J. Schmitz, M. Govindan, S.A. Abbas, K.M. Hanson, P.A. Horton, P. Crews, Lloydia 58 (1995) 1384.
[30] X. Fu, M.B. Hossain, F.J. Schmitz, D. vanderHelm, J. Org. Chem. 62 (1997) 3810.
[31] R.W. Schumacher, B.S. Davidson, Tetrahedron 51 (1995) 10125.
[32] W.Y. Hua, E.R. Bennett, R.J. Letcher, Environ. Int. 31 (2005) 621.
[33] L.J. Chen, E.H. Lebetkin, J.M. Sanders, L.T. Burka, Xenobiotica 36 (2006) 515.
[34] I.A.T.M. Meerts, R.J. Letcher, S. Hoving, G. Marsh, A. Bergman, J.G. Lemmen, B. van der Burg, A. Brouwer, Environ. Health Perspect. 109 (2001) 399.
[35] I.A.T.M. Meerts, J.J. van Zanden, E.A.C. Luijks, I. van Leeuwen-Bol, G. Marsh, E. Jakobsson, A. Bergman, A. Brouwer, Toxicol. Sci. 56 (2000) 95.
[36] R.F. Canton, J.T. Sanderson, S. Nijmeijer, A. Bergman, R.J. Letcher, M. van den Berg, Toxicol. Appl. Pharmacol. 216 (2006) 274.
[37] R.F. Canton, J.T. Sanderson, R.J. Letcher, A. Bergman, M. van den Berg, Toxicol. Sci. 88 (2005) 447.
[38] M. Harju, T. Hamers, J.H. Kamstra, E. Sonneveld, J.P. Boon, M. Tysklind, P.L. Andersson, Environ. Toxicol. Chem. 26 (2007) 816.
[39] M.M.L. Dingemans, A. de Groot, R.G.D.M. van Kleef, A. Bergman, M. van den Berg, H.P.M. Vijverberg, R.H.S. Westerink, Environ. Health Perspect. 116 (2008) 637.
[40] S. Mas, O. Jauregui, F. Rubio, A. de Juan, R. Tauler, S. Lacorte, J. Mass Spectrom. 42 (2007) 890.
[41] Y. Kato, S. Okada, K. Atobe, T. Endo, F. Matsubara, T. Oguma, K. Haraguchi, Anal. Chem. 81 (2009) 5942.
[42] S.J. Lupton, B.P. McGarrigle, J.R. Olson, T.D. Wood, D.S. Aga, Rapid Commun. Mass Spectrom. 24 (2010) 2227.
[43] D.F. Staskal, J.J. Diliberto, M.J. DeVito, L.S. Birnbaum, Toxicol. Sci. 83 (2005) 215.