RESEARCH ARTICLE

Comprehensive Two-Dimensional Separation of Hydroxylated Polybrominated Diphenyl Ethers by Ultra-Performance Liquid Chromatography Coupled with Ion Mobility-Mass Spectrometry

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Abstract

A comprehensive two-dimensional system coupling ultra-performance liquid chromatography (UPLC) and ion mobility-mass spectrometry (IM-MS) has been applied for the separation and analysis of hydroxylated polybrominated diphenyl ethers (OH-PBDEs). A complex mixture containing 23 OH-PBDE congeners ranging from hydroxylated monobromodiphenyl ether (OH-monoBDE) to hydroxylated octabromodiphenyl ether (OH-octaBDE) was satisfactorily separated within 16 min of analysis time. The first-dimensional reversed-phase UPLC was performed on a sub-2 μm BEH C_{18} chromatographic column using acetonitrile-water gradient elution program with a flow rate ramp. It enabled excellent chromatographic separation for both between-class and within-class OH-PBDEs based on their differences in hydrophobicity. Following the pre-ionization resolution in the first dimension, the second-dimensional IM-MS employed a hybrid electrospray quadrupole ion mobility time-of-flight mass spectrometer and added an extra post-ionization separation for between-class OH-PBDE congeners on account of their relative mobility disparity during a very short period of 8.80 ms. The orthogonality of the developed two-dimensional system was evaluated with the correlation coefficient of 0.9665 and peak spreading angle of 14.87° . The peak capacity of the system was calculated to be approximately 2 and 15 times higher than that of the two dimensions used alone, respectively. The two-dimensional separation plane also contributed to the removal of background interference ions and the enhanced confidence in the characterization of OH-PBDEs of interest.

Key words: Hydroxylated polybrominated diphenyl ethers, Ultra-performance liquid chromatography, Ion mobility-mass spectrometry, Two-dimensional separation

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Introduction

Brominated flame retardants (BFRs) are a diverse group of industrial chemicals widely used in a vast amount of consumer products for the purpose of fire prevention and resistance, which typically include hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), polybrominated

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biphenyls (PBBs), and polybrominated diphenyl ethers (PBDEs) [1, 2]. Among them, PBDEs are an important class of additive BFRs, produced by brominating diphenyl ethers in the presence of a catalyst, and are mainly applied to furniture foam, textile coatings, consumer electronics, wire and cable insulation, plastics for personal computers and small appliances, etc. [3]. The extensive use of the products containing BFRs like PBDEs has resulted in the release of these compounds to the environment during usage and disposal, and poses serious environmental and human risks. With the increasing concern about the potential hazard of PBDEs, as their structural analogues, hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been shown to bioaccumulate in abiotic environments, including surface water [4], precipitation [4] and sediment [5, 6], and organisms [6–11]. Laboratory studies support the emergence of certain OH-PBDEs as the resulting metabolites in rodents (rats or mice) [12, 13], fish [14], chickens [15], and humans [16] after exposure to parent PBDEs, whereas several OH-PBDE congeners have been reported to be isolated and structurally identified as natural products in marine sponges [17, 18], ascidians [19], tunicates [20], algae [21], and mussels [21]. Thus, the occurrence of OH-PBDEs may be originated from the uptake and metabolism of anthropogenic PBDEs and/or the accumulation of naturally existing compounds [22]. OH-PBDEs differ in biological effects compared with their precursor PBDEs, and there are indications that the exposure to OH-PBDEs may cause thyroid hormone transport and metabolism disorder [23, 24], neurotoxicity [25], cytotoxicity [26], and endocrine disruption [27, 28].

In order to evaluate the environmental distribution of OH-PBDEs, a number of papers have described analytical methods for the determination of OH-PBDEs, primarily focusing on gas chromatography (GC) coupled with electron capture detection (ECD) [7], low-resolution mass spectrometry (LRMS) [6–8, 21] operating in electron ionization (EI), chemical ionization (CI), or electron capture negative ionization (ECNI) mode, and sometimes high-resolution mass spectrometry (HRMS) [4, 6, 9]. However, owing to OH-PBDEs' low volatility, a derivatization procedure via methylation or acetylation reaction is inevitably required, which may lead to several disadvantages: (1) low yield and loss of analytes arising from the time-consuming derivatization protocol; (2) handling of highly toxic and carcinogenic derivatizing reagents (e.g., diazomethane); and (3) possible systematic quantitative errors due to differences in reaction kinetics for individual OH-PBDE congeners and structural isomers during derivatization.

Alternatively, mass spectrometry hyphenated with electrospray ionization (ESI), pneumatically assisted ion-spray ionization (ISP), or atmospheric pressure chemical ionization (APCI) interface has become preferred detection scheme following liquid chromatography (LC) for the analysis of OH-PBDEs [5, 29, 30]. The LC-based methodology has exhibited superiority in fulfilling direct determination of OH-PBDEs without tedious derivatization, making it a better choice than the GC-based approach. Nevertheless, only a very limited number of OH-PBDE congeners, confined to certain

hydroxylated dibromodiphenyl ethers (OH-diBDEs), hydroxylated tribromodiphenyl ethers (OH-triBDEs), and hydroxylated tetrabromodiphenyl ethers (OH-tetraBDEs), were investigated in the aforementioned literatures. As hydroxylated substituents for PBDEs, OH-PBDEs theoretically cover 209 possible congeners, with a variation in bromine units as well as in structural isomerism. Given that their properties, toxicities, environmental occurrence, and fate may depend on structural details, a sensitive and specific analytical method is needed for their analysis. The challenge lies in the great complexity of the wide-ranging OH-PBDEs, making the separation and analysis difficult.

A two-dimensional separation is generally considered to be advantageous over its one-dimensional counterpart for resolving complex mixtures because of extended peak capacity and higher resolution. It is recommended that the two dimensions be connected in an on-line manner, referred to as a comprehensive two-dimensional separation, rather than in an off-line or heart-cut manner, in order to approach the ideal multiplicative total of peak capacity. To date, a broad range of separation techniques such as LC, GC, capillary electrophoresis (CE), and supercritical fluid chromatography (SFC) have been mutually interfaced to construct comprehensive two-dimensional separation systems [31–37]. Currently, LC in conjunction with ion mobility separation and MS measurement is becoming increasingly popular for complex mixture analysis, due to complementary separation steps and elevated peak capacity. The applications include the analysis for rat urinary metabonome [38], human plasma proteome [39], peptide mixtures [40, 41], pharmaceutical formulation [42], etc. The principle for ion mobility-mass spectrometry (IM-MS) has been described in detail elsewhere [43]. Briefly, IM-MS is a gas-phase electrophoretic technique that allows ionized target molecules to be separated on the basis of their mobility in the presence of an inert carrier gas and under the influence of a weak electric field. The system incorporating ion mobility separation in tandem with LC and MS enables the initial separation of components by LC, then transfers and disperses the ions generated in the ion source according to their differing mobility, and finally records their mass-to-charge ratio (m/z) by a time-of-flight (TOF) mass analyzer.

The introduction of ultra-performance liquid chromatography (UPLC), harnessing chromatographic potential of sub-2 μm packing particles, leads to significant improvements in terms of resolution, analysis speed, and detection sensitivity in comparison with traditional LC [44, 45]. The advent of UPLC also reduces consumption of mobile phases and cost of analysis, and provides a great deal of green chemistry benefits [46]. A wide diversity of commercial stationary phases offers various options in selectivity for the analytes. Presumably, the hyphenation of UPLC as a replacement for conventional LC and IM-MS will perform even better for the separation and analysis of complex mixtures.

In this study, we have engaged in developing an effective two-dimensional tool combining UPLC and IM-MS (UPLC×IM-MS) for the analysis of 23 OH-PBDE homologues

ranging from hydroxylated monobromodiphenyl ether (OH-monoBDE) to hydroxylated octabromodiphenyl ether (OH-octaBDE). The aim of the present work is to exploit the unique strength in the separation and characterization of OH-PBDEs by virtue of the developed comprehensive UPLC×IM-MS system.

Experimental

Materials

Authentic reference standards: (1) 2'-hydroxy-4-monobromodiphenyl ether (abbreviated name 2'-OH-BDE-3), (2) 3'-hydroxy-2,4-dibromodiphenyl ether (3'-OH-BDE-7), (3) 4'-hydroxy-2,2',4-tribromodiphenyl ether (4'-OH-BDE-17), (4) 3'-hydroxy-2,4,4'-tribromodiphenyl ether (3'-OH-BDE-28), (5) 2'-hydroxy-2,4,4'-tribromodiphenyl ether (2'-OH-BDE-28), (6) 4-hydroxy-2,2',3,4'-tetrabromodiphenyl ether (4-OH-BDE-42), (7) 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (3-OH-BDE-47), (8) 4'-hydroxy-2,2',4,5'-tetrabromodiphenyl ether (4'-OH-BDE-49), (9) 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47), (10) 2'-hydroxy-2,3',4,5'-tetrabromodiphenyl ether (2'-OH-BDE-68), (11) 3-hydroxy-2,2',4,4',6-pentabromodiphenyl ether (3-OH-BDE-100), (12) 6-hydroxy-2,2',3,3',4-pentabromodiphenyl ether (6-OH-BDE-82), (13) 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether (4-OH-BDE-90), (14) 4'-hydroxy-2,2',4,5',5'-pentabromodiphenyl ether (4'-OH-BDE-101), (15) 5'-hydroxy-2,2',4,4',5-pentabromodiphenyl ether (5'-OH-BDE-99), (16) 6'-hydroxy-2,2',4,4',5-pentabromodiphenyl ether (6'-OH-BDE-99), (17) 6-hydroxy-2,2',3,4,4',5-hexabromodiphenyl ether (6-OH-BDE-137), (18) 3-hydroxy-2,2',4,4',5',6-hexabromodiphenyl ether (3'-OH-BDE-154), (19) 6-hydroxy-2,3,3',4,4',5'-hexabromodiphenyl ether (6-OH-BDE-157), (20) 4-hydroxy-2,2',3,4',5,6,6'-heptabromodiphenyl ether (4-OH-BDE-188), (21) 6-hydroxy-2,2',3,4,4',5,5'-heptabromodiphenyl ether (6-OH-BDE-180), (22) 6-hydroxy-2,2',3,4,4',5,6'-heptabromodiphenyl ether (6-OH-BDE-182), and (23) 4'-hydroxy-2,2',3,3',4,5',6,6'-octabromodiphenyl ether (4'-OH-BDE-201), all dissolved in acetonitrile with the concentration of 10 or 50 µg/mL, were purchased from AccuStandard (New Haven, CT, USA). The nomenclature for OH-PBDEs in this paper followed the U.S. Environmental Protection Agency (EPA) numbering approach to simplify comparison with corresponding PBDE congeners [47]. Chemical structures of the OH-PBDE congeners involved in this study are shown in Scheme 1. Acetonitrile of LC-MS grade (J.T.Baker, Phillipsburg, NJ, USA) and Milli-Q ultrapure water (Millipore, Bedford, MA, USA) were employed as mobile phase constituents for UPLC separations.

Instrumentation

The UPLC separation was performed on an ACQUITY UPLC system (Waters, Milford, MA, USA) equipped with a binary solvent manager, a sample manager and a column heater. A Waters ACQUITY UPLC BEH C₁₈ analytical column (150 mm×2.1 mm, 1.7 µm), preceded by a Waters VanGuard

pre-column (5 mm×2.1 mm, 1.7 µm) of the same packing materials was used. The chromatographic elution was conducted with binary mobile phase gradient consisting of water (A) and acetonitrile (B). Initial gradient conditions were set to 63% B at the flow rate of 0.3 mL/min before incorporating a linear gradient increasing to 68% B over 2 min and held for 3 min. At 9 min, the gradient was programmed to 75% B. The gradient was then increased to 95% B and the flow rate was ramped to 0.5 mL/min over 6 min. At 16 min, the gradient was returned to the initial conditions. The column temperature was maintained at 35 °C. The samples were kept at 10 °C in the thermostated sample manager and a sampling volume of 10 µL was injected for each run. The strong and weak needle wash solvents were 200 µL of 100% B and 600 µL of 63% B respectively.

The entire column eluent from UPLC was directed into a SYNAPT HDMS hybrid quadrupole ion mobility orthogonal acceleration time-of-flight mass spectrometer (Waters, Manchester, UK). The instrument was operated in electrospray negative-ion mode, enabling ionization of OH-PBDEs with a capillary voltage of 3.0 kV and a nitrogen desolvation gas flow rate of 400 L/h at 350 °C. A sampling cone voltage of 30 V and an extraction cone voltage of 4.0 V were set for the experiment. The quadrupole mass filter was run in the wide-band pass-through mode. The OH-PBDE ions were then accumulated into a trap ion guide region at the pressure of 0.03 mbar with argon trap collision energy set at 6.0 V and nitrogen trap gas flow rate of 1.5 mL/min. The ions were subsequently pulsed into an ion mobility drift cell over a period of 100 µs to undertake an ion mobility-based separation at 0.62 mbar. Each individual mobility experiment was 8.80 ms long with a 300 m/s traveling wave amplitude at 8.0 V and a nitrogen mobility gas at the flow rate of 28 mL/min. The ions passing through the drift cell were then continually released to a transfer ion guide region with argon transfer collision energy set at 4.0 V. Finally, the mobility-separated ions were conveyed to the reflection TOF mass analyzer operating in V-Optics mode whilst maintaining the temporal separation. Data were recorded over the range of *m/z* 50 to 1000 Da, with the repeated 1.0-s scan time and 0.02-s inter-scan interval. The TOF mass analyzer was externally calibrated with a sodium formate solution. All analyses were acquired using an independent LockSpray reference sprayer infusing 200 ng/mL deprotonated leucine enkephalin solution (*m/z* 554.2615 Da) at a flow rate of 5 µL/min to verify mass accuracy. Instrumentation control, data acquisition, and mining were carried out by MassLynx ver. 4.1 and DriftScope ver. 2.0 software supplied with the instrument (Waters, Manchester, UK).

Results and Discussion

First-Dimensional Separation of OH-PBDEs by UPLC

In this study, eight groups of OH-PBDE analogues individually substituted with one to eight bromine units were subject to the UPLC×IM-MS analysis. Additionally, there are sub-series of

Scheme 1. Chemical structures of the 23 OH-PBDE compounds studied

isomeric species within certain OH-PBDE groups, specifically, three OH-tribDE isomers, five OH-tetraBDE isomers, six isomers within hydroxylated pentabromodiphenyl ethers (OH-pentaBDEs), three isomers within hydroxylated hexabromodiphenyl ethers (OH-hexaBDEs), and three isomers within hydroxylated heptabromodiphenyl ethers (OH-heptaBDEs). The isomers distinguish from each other by the bromine substitution sites as well as the placement of single hydroxyl group, i.e., the location in the *ortho*-, *meta*-, or *para*-position relative to the diphenyl ether bridge. The compositional complexity of the analytes therefore poses a significant challenge for achieving both between-class and within-class separation for numerous OH-PBDE homologues and isomers.

In an attempt to explore the separation efficiency of sub-2 μm particles, UPLC was configured in the instrumentation for the first dimension of separation in the present study. To achieve optimal band spacing for multicomponent OH-PBDEs, a systematic study of the parameters used to optimize chromatographic separation, such as stationary phase type, mobile phase composition, flow rate, and column temperature, was carried out. In view of the fact that the chemical nature of column packing materials may have a major effect on OH-PBDEs' chromatographic behavior, a wide diversity of commercially available UPLC columns based on high-pressure bearing ethylene bridged

hybrid (BEH) particle substrate, such as BEH C_{18} , C_8 , Shield RP18, Phenyl, HILIC, and Amide, and high strength silica (HSS) particle substrate, such as HSS C_{18} , C_{18} SB, and T3 were investigated during the method development. The columns vary in functionalities, choice of covalently bonded silane reactions, completeness of bonding, presence or absence of endcapping, support surface area, pore size, carbon concentration, etc., and thereby exhibiting substantial differences in selectivity for the separation of OH-PBDEs. Table 1 summarizes various UPLC columns investigated.

In addition to the characteristics of column packing, selectivity may result from mobile phase composition as well. For this purpose, an integrated survey combining optimization of chromatographic columns plus mobile phase composition was conducted. Three water-miscible organic solvents commonly used in reversed-phase chromatography, i.e., acetonitrile, methanol, and tetrahydrofuran, were separately paired with water as the mobile phase for assessing the selectivity of serial OH-PBDEs on different columns (all with the dimension of 50 mm $\times$ 2.1 mm). The target OH-PBDE congeners have a wide range of octanol/water partition coefficients $\log K_{ow}$ from 3.62 to 9.85, calculated by an open source Estimation Program Interface (EPI) Suite ver. 4.0 software, indicating considerable disparity in hydrophobicity. To acquire satisfactory chromatographic resolution for OH-

Table 1. Summary of Different Types of UPLC Columns Used in the Study

Column	Bonding features	Particle size (μm)	Pore size (Å)	Surface Area (m ² /g)	Carbon load (%)
BEH C ₁₈	Trifunctional C ₁₈ , fully endcapped, bonded to BEH particle substrate	1.7	130	185	18
BEH C ₈	Trifunctional C ₈ , fully endcapped, bonded to BEH particle substrate	1.7	130	185	13
BEH Shield RP18	Monofunctional C ₁₈ with polar group, fully endcapped, bonded to BEH particle substrate	1.7	130	185	17
BEH Phenyl	Trifunctional C ₆ phenyl, fully endcapped, bonded to BEH particle substrate	1.7	130	185	15
BEH HILIC	Unbonded BEH particle substrate	1.7	130	185	-
BEH Amide	Trifunctional amide, bonded to BEH particle substrate	1.7	130	185	12
HSS C ₁₈	High coverage trifunctional C ₁₈ , fully endcapped, bonded to HSS particle substrate	1.8	100	230	15
HSS C ₁₈ SB	Intermediate coverage trifunctional C ₁₈ , no endcapping, bonded to HSS particle substrate	1.8	100	230	8
HSS T3	T3 (C ₁₈) bonding and endcapping, bonded to HSS particle substrate	1.8	100	230	11

PBDE homologues in a reasonable period of time, a gradient elution program was applied. For each particular type of column, the optimum initial solvent strength and gradient elution condition were carefully adjusted to obtain utmost retention and separation under the circumstance. Since ESI ionization occurs in solution state, mobile phase composition may affect ionization efficiency and detection sensitivity of the solutes. For this reason, both chromatographic performance and mass spectrometric sensitivity were taking into account to determine the optimal protocol. Experimental results demonstrated that selectivity was quite different from column to column and even for the same column when matched with different mobile phases. BEH HILIC and Amide columns were not effective in resolving OH-PBDEs and the compounds all emerged into one coeluting peak. BEH C₁₈ column together with acetonitrile-water mobile phase gave maximum resolution and sensitivity for the mixture of OH-PBDE compounds by visual inspection. UPLC also favors acetonitrile as the organic part of mobile phase in terms of lower viscosity, which is beneficial to alleviating column backpressure caused by sub-2 μm packing particles. Afterwards, the OH-PBDEs mixture was eluted on the BEH C₁₈ column with mobile phases consisting of acetonitrile paired with various buffer solutions adjusted to varying pH values within appropriate buffer range: aqueous 5 mM ammonium formate (adjusted to pH value of 2.5, 3.0, and 3.5 with formic acid, respectively), aqueous 5 mM ammonium acetate (adjusted to pH at 4.0, 4.5, 5.0, 5.5, and 6.5 with acetic acid, respectively), aqueous 5 mM ammonium bicarbonate (adjusted to pH at 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, and 11.0 using ammonia, respectively). The obtained chromatograms revealed that the addition of buffer solution lead to serious deterioration in chromatographic peak shape for some OH-PBDE congeners in comparison with those without buffer. Given the optimum stationary phase type and mobile phase composition, BEH C₁₈ columns with the same inner diameter and particle size, but differing lengths (50, 100, and 150 mm) were further compared. It was observed that OH-PBDEs, especially within-class structural isomers, were better resolved on the column of 150 mm, with the average resolution of 1.35, which was 1.67 times higher than that of 50 mm and 1.15 times higher than that of 100 mm.

The influence of mobile phase flow rate within the range from 0.2 to 0.6 mL/min on the separation of OH-PBDEs was evaluated. The optimal resolution of adjacent isomers within OH-triBDEs, OH-tetraBDEs, OH-pentaBDEs, and OH-hexaBDEs was achieved at the flow rate of 0.3 mL/min, with the mean resolution being 0.41, 1.24, 0.99, and 1.16, respectively. One remark is that the elevation of flow rate preferably benefits the separation of OH-heptaBDEs isomers, and the best resolution was measured to be 0.60 at the flow rate of 0.4 mL/min. Hence, a flow rate ramp was utilized during the last phase of gradient elution to improve the resolution for OH-heptaBDEs isomers. Although a change in temperature is less effective as a means of altering selectivity in most cases, the band spacing for OH-hexaBDE structural isomers changed slightly when column temperature was varied from 30 °C to 55 °C in our study, amongst which 35 °C presented an optimum average resolution of 1.16 for OH-hexaBDE isomers.

After all the optimization experiments described above, the 23 OH-PBDEs were chromatographically separated by reversed-phase UPLC based on their differences in hydrophobicity. The total ion chromatogram of OH-PBDEs by the first-dimensional UPLC separation is shown in Figure 1a, with each OH-PBDE peak labeled with the same authentic reference standard number as described in the [Materials](#) section. The level of bromination played a dominating role in the separation of OH-PBDE homologues. OH-monoBDE with the lowest hydrophobicity was eluted earliest in the chromatogram with the retention factor *k'* of 0.84, while OH-octaBDE with the highest hydrophobicity was eluted towards the end with the retention factor *k'* of 7.68. The vast majority of within-class structural isomers of OH-PBDEs were well resolved according to slight difference in hydrophobicity, except that only minor overlaps between two pairs of isomers (4'-OH-BDE-17 and 3'-OH-BDE-28, both from OH-triBDEs, with the resolution of 0.25; 4'-OH-BDE-101 and 5'-OH-BDE-99, both from OH-pentaBDEs, with the resolution of 0.10) and between two congeners (2'-OH-BDE-68 from OH-tetraBDEs and 3-OH-BDE-100 from OH-pentaBDEs, with the resolution of 0.25) appeared. The retention times and resolutions for the OH-PBDEs are shown in Table 2.

Figure 1. (a) Total ion chromatogram for OH-PBDE mixture solution at a concentration of 5 $\mu\text{g/mL}$ by UPLC separation, analyzed on a BEH C_{18} column (150 mm $\times$ 2.1 mm, 1.7 μm) preceded by a VanGuard BEH C_{18} pre-column (5 mm $\times$ 2.1 mm, 1.7 μm); mobile phase: acetonitrile-water gradient elution with a flow rate ramp program; column temperature: 35 $^{\circ}\text{C}$. (b) Ion mobility chromatogram for directly infused OH-PBDE mixture solution at a concentration of 5 $\mu\text{g/mL}$ by ion mobility separation. OH-PBDEs ions were generated by ESI operating in negative mode and then subjected to an ion mobility separation with nitrogen mobility gas flow rate of 28 mL/min, mobility wave velocity of 300 m/s and mobility wave height of 8.0 V. The mass spectra were collected by a TOF mass analyzer running in V-Optics mode from m/z 50 to 1000 Da, with a scan time of 1.0 s and an inter-scan interval of 0.02 s

Second-Dimensional Separation of OH-PBDEs by IM-MS

Following the first-dimensional pre-ionization resolution of OH-PBDEs afforded by UPLC, the whole chromatographic eluting OH-PBDE species underwent further second-dimensional post-ionization ion mobility separation in an on-line manner without diminishing the fidelity of the first dimension of separation. Initial studies aiming at optimizing a series of MS parameters and ion mobility separation conditions were performed by infusing the OH-PBDE mixture solution into the ESI ion source of the spectrometer using an integrated syringe pump. IM-MS gas flow rate

demonstrated great impact on ion mobility separation of OH-PBDEs. By varying the IM-MS gas flow rate from 5 to 40 mL/min to examine its effect on the ion mobility data acquired, a flow rate of 28 mL/min appropriately allowed all OH-PBDE ions to traverse across the ion mobility drift tube during one pulse cycle. The optimum velocity and height of traveling wave were 300 m/s and 8.0 V, although variations in the wave velocity and height showed less impact on OH-PBDEs separation compared with the IM-MS gas flow rate in this experiment. The ion mobility chromatogram is shown in Figure 1b.

The OH-PBDE ions passed through the ion mobility gas with drift time being dependent on their mobility values, in

this case, mainly on the basis of the degree of halogen substitution for diphenyl ether skeleton. The OH-PBDE compounds from OH-monoBDE to OH-octaBDE were broadly fractionated according to their collision cross-section disparity in gas phase. The rapid ion mobility separation was carried out during a very short period of 8.80 ms with the mean peak width at half height of 1.125 ms for OH-PBDEs. A 0.57-ms average ion mobility drift time difference was observed for between-class OH-PBDEs from OH-monoBDE to OH-octaBDE. The resolution for between-class OH-PBDEs by the second-dimensional ion mobility separation ranges from 0.10 (between OH-heptaBDE and OH-octaBDE) to 0.57 (between OH-monoBDE and OH-diBDE). A significant feature of the ion mobility separation for OH-PBDEs is that the associated millisecond ion mobility timescale (8.80 ms) is intermediate between the UPLC elution time (16 min) and the microsecond detection time (45 μ s/scan) required for TOF-MS. The ion mobility separation can therefore occur within one UPLC chromatographic peak with the mean peak width at half height of 13.2 s, much narrower than that of conventional LC, while effectively feed components into TOF-MS very rapidly. The drift time and resolution for the OH-PBDEs are shown in Table 2.

The nested data set derived from the UPLC×IM-MS separation are demonstrated by three correlated visualized plots, i.e., drift time versus retention time plot (data summed over all m/z values), m/z versus retention time plot (data summed over all drift times) and m/z versus drift time plot (data summed over all retention times). The band color reflects the signal intensity of the ions, with white representing the highest intensity and black the lowest

intensity. As displayed in the drift time versus retention time plot (Figure 2), UPLC realized the first-dimensional separation for OH-PBDE congeners along the axis of retention time based on the differences in hydrophobicity, and IM-MS then achieved the second-dimensional separation along the axis of drift time based on the differences in ion mobility. Roughly, eight sections corresponding to each class of OH-PBDE homologues were observed in the drift time versus retention time plot. Each individual OH-PBDE peak visually located in the plot was characterized and labeled with corresponding reference number. OH-monoBDE with the smallest collision cross-sectional area drifted most rapidly through the mobility region, emerging in the lower left-hand part of the plot. OH-octaBDE moved towards the end due to the largest collision cross-sectional area, showing up in the upper right-hand part. Within-class OH-PBDE structural isomers also showed subtle differences in drifting trajectories. Maintaining the effects of separation on OH-PBDEs obtained by the first-dimensional UPLC, IM-MS added a further separation for between-class OH-PBDEs. On the other hand, with regard to the separation of within-class OH-PBDE isomers, IM-MS showed a weaker separation capability and with less variables to be optimized in comparison with UPLC. For the several pairs of structural isomers with limited resolution by UPLC, IM-MS can hardly improve. Despite the relatively poor resolving power presumably due to the quite similar cross-sectional areas for OH-PBDE structural isomers, with the addition of a second-dimensional ion mobility separation, the signals of OH-PBDE ions of interest can be precisely extracted from the complete data set for further analysis by the DriftScope software tool. Many of the ions with low

Table 2. Molecular Weights, $\log K_{ow}$, Retention Times, Drift Times and Resolutions of OH-PBDE Congeners

OH-PBDE species	Compound	M_w	$\log K_{ow}$	Retention time (min)	Resolution by UPLC	Drift time (ms)	Resolution by IM-MS
OH-monoBDE	2'-OH-BDE-3	263.9786	3.62	2.82	-	3.26	-
OH-diBDE	3'-OH-BDE-7	341.8891	4.51	3.65	2.56	4.22	0.97
OH-triBDE	4'-OH-BDE-17	419.7996	5.40	4.62	2.73	4.40	0.45
	3'-OH-BDE-28			4.71	0.25	4.71	
	2'-OH-BDE-28			4.91	0.56	5.02	
OH-tetraBDE	4-OH-BDE-42	497.7101	6.29	5.84	2.33	5.24	0.53
	3-OH-BDE-47			6.26	1.01	5.28	
	4'-OH-BDE-49			6.68	0.97	5.37	
	6-OH-BDE-47			7.20	1.31	5.32	
	2'-OH-BDE-68			7.77	1.68	5.32	
OH-pentaBDE	3-OH-BDE-100	575.6206	7.18	7.67	0.25	5.72	0.47
	6-OH-BDE-82			8.41	1.64	5.81	
	4-OH-BDE-90			8.75	0.72	6.03	
	4'-OH-BDE-101			9.23	1.16	6.03	
	5'-OH-BDE-99			9.26	0.10	5.76	
	6'-OH-BDE-99			9.70	1.33	5.76	
OH-hexaBDE	6-OH-BDE-137	653.5311	8.07	10.55	2.57	6.38	0.49
	3'-OH-BDE-154			10.80	0.74	6.38	
	6-OH-BDE-157			11.36	1.57	6.51	
OH-heptaBDE	4-OH-BDE-188	731.4417	8.96	11.95	1.74	6.91	0.16
	6-OH-BDE-180			12.10	0.49	6.60	
	6-OH-BDE-182			12.32	0.71	6.95	
OH-octaBDE	4'-OH-BDE-201	809.3522	9.85	13.28	3.18	7.22	0.16

Figure 2. The drift time versus retention time plot of OH-PBDEs

molecular weight less than 200 Da normally traversed fastest with the drift time between 1.9 and 2.3 ms. The OH-PBDE analytes can be discriminated from the aforementioned background interference ions, aiding in their interpretation and reducing the complexity of the data. A marked reduction in undesirable background noise is observed in Figure 3b.

The background-subtracted spectrum became quite apparent above the interfering substances. The mass spectrum was simplified for easier data interpretation and yielded improved signal-to-noise ratio, which was not available by LC-MS alone (Figure 3a). For instance, the characteristic ion peaks corresponding to OH-monoBDE are clearly displayed

Figure 3. (a) Mass spectrum summed throughout the experiment without ion mobility separation (upper spectrum); and (b) Corresponding background-subtracted mass spectrum with ion mobility separation (lower spectrum)

Figure 4. The m/z versus drift time plot of OH-PBDEs

in Figure 3b, but almost not visible in Figure 3a. The quantitative enhancement in the signal-to-noise ratio was measured for certain representative OH-PBDE compounds based on the inclusion of ion mobility: the signal-to-noise ratio for 2'-OH-BDE-3 increased from 25.78 to 71.93, 2.79 times higher; 4-OH-BDE-42 increased from 16.73 to 62.57, 3.74 times higher; 6-OH-BDE-157 increased from 12.12 to 52.48, 4.33 times higher. Furthermore, confidence in the identification and assignment of OH-PBDEs is enhanced by the presence of the ion of the correct m/z at the expected ion mobility drift time and UPLC retention time associated with an OH-PBDE congener. A fast scanning TOF-MS enabled individual OH-PBDEs to be well identified by means of the dominant deprotonated pseudo-molecular ion peak obtained in the negative ESI mode. The presence of different numbers of bromines in OH-PBDEs was manifested by characteristic isotopic clusters of peaks in the mass spectra (Figure 3). The observed isotope cluster pattern as well as their relative abundance ratios matched well with the theoretical spectra simulated by an “isotope modeling” computing module embedded in the MassLynx software.

A well-defined set of spots in the shape of a flight of steps vertically scattered in regular equidistance are observed in the m/z versus retention time plot (see [Supplementary Material](#), which can be found in the electronic version of this article, Figure S1). Each step labeled in white ellipse represents a group of OH-PBDE congeners, which are evenly spaced in the mass distance of a bromine unit. The bottom stair stands for OH-monoBDE, while the top stair for OH-octaBDE. Individual stair may be classified into horizontally adjacent

subsections of well-separated within-class structural isomers. Some coeluting interferences with the same retention time as OH-PBDEs are observed in the plot. The ion mobility separation may offer the advantage in an extra dimensional separation for the target OH-PBDEs from the background interferences and realize the simplification of complex data. The information-rich m/z versus retention time plot also confirmed that OH-PBDEs were eluted in the order of increasing bromine units by UPLC of first dimension. Figure 4 reveals the relationship between m/z and drift time. Different bands corresponding to various classes of OH-PBDE homologues (in white ellipse) display ordered arrangements at different heights. Different classes of OH-PBDE homologues exhibit differing drifting velocities. The OH-PBDE ions with less bromines and lower mass carry higher mobility, thus, traverse faster across the drift region. The OH-PBDE analytes are also shown to be effectively isolated from the early-traveling components with the drift time between 1.76 and 2.64 ms, leading to a reduction in background noise.

Table 3. The Calculated Correlation Coefficient and Peak Spreading Angle

	Correlation coefficients (r)			Peak spreading angle (β , deg)		
	UPLC	IM-MS	M_w	UPLC	IM-MS	M_w
UPLC	1.0000	0.9665	0.9780	0	14.87	12.05
IM-MS	0.9665	1.0000	0.9842	14.87	0	10.19
M_w	0.9780	0.9842	1.0000	12.05	10.19	0

Evaluation on Orthogonality and Practical Peak Capacity of the UPLC × IM-MS System

Orthogonality and peak capacity are interrelated parameters for estimating difference in selectivity or resolving power of a comprehensive two-dimensional system. Normally, orthogonality is dependent not only on the separation mechanism but also on the properties of the solutes and the separation conditions. According to the literature [48], factor analysis was utilized to quantitatively estimate the degree of UPLC×IM-MS separation orthogonality. Correlation coefficient (r) is a frequently used parameter to evaluate the orthogonality between the two dimensions. A perfect retention correlation is obtained when $r=1$, and truly orthogonal separation is achieved when $r=0$. Most two-dimensional separations lie between perfect correlation and perfect orthogonality. In our study, the r -value was calculated based on a correlation matrix incorporating the retention time and drift time of OH-PBDEs in the two dimensions, as well as their molecular weight (Table 2). The peak spreading angle (β) between the retention axes in the orthogonal retention space was computed as the arc cosine value of r . A value of β close to 90° indicates a nearly orthogonal separation. The calculated correlation coefficients and peak spreading angles are summarized in Table 3, which reveal the similarity in retention behavior of OH-PBDE congeners between UPLC and IM-MS on the defined operational conditions. OH-monoBDE with the lowest hydrophobicity was eluted first in the first-dimensional UPLC, and also drifted fastest across the second-dimensional IM-MS due to the lowest mobility. On the other hand, OH-octaBDE with the highest hydrophobicity and mobility undertook the longest retention in both dimensions. Because both retention vectors are strongly correlated with the extent of bromination of OH-PBDEs, i.e., their molecular weight, the UPLC×IM-MS separation is close to perfect correlation under the experimental conditions (see Supplementary Material, Figure S2).

The measured peak capacities for UPLC of the first dimension and IM-MS of the second dimension are 65 and 9, respectively, computed from the ratio of total retention time to mean peak width at half height in each dimension. Hence, the theoretical peak capacity for the UPLC×IM-MS plane is 585. Based on the peak spreading angle of 14.87° , the experimentally derived peak capacity is calculated to be approximately 135. The actual peak capacity obtained from the comprehensive two-dimensional separation is, respectively, 2 and 15 times higher than those from the two dimensions used alone.

Conclusions

In this study, the developed UPLC×IM-MS system incorporating UPLC and IM-MS has demonstrated unique selectivity and improved peak capacity for the analysis of OH-PBDE compounds by making sufficient use of the two-dimensional separation plane. The first-dimensional UPLC,

as a pre-ionization separation technique, takes advantage of great separation efficiency originated from the sub-2 μm BEH particles and realizes excellent chromatographic resolution for both between-class and within-class OH-PBDE congeners. IM-MS, as a second-dimensional post-ionization separation technique, provides an additional rapid orthogonal means of separation for between-class OH-PBDEs according to their relative mobility. By virtue of the combination of UPLC and IM-MS, comprehensive isolation of 23 OH-PBDE congeners based on both hydrophobicity difference and mobility disparity has been achieved, with each separation dimension selective to a particular sample attribute. A fast scanning TOF-MS enables enough information to be acquired and recorded during the whole procedure. The UPLC×IM-MS system proves to be a feasible tool to well resolve OH-PBDE homologues, reduce mass spectral congestion and aid in the identification and characterization of OH-PBDEs.

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