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Liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish

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ABSTRACT

A comprehensive, sensitive and high-throughput liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry (LC–APPI-MS/MS) method has been developed for analysis of 36 halogenated flame retardants (HFRs). Under the optimized LC conditions, all of the HFRs eluted from the LC column within 14 min, while maintaining good chromatographic separation for the isomers. Introduction of the pre-heated dopant to the APPI source decreased the background noise fivefold, which enhanced sensitivity. An empirical equation was proposed to describe the relation between the ion intensity and dopant flow. The excellent on-column instrument detection limits averaged 4.7 pg, which was similar to the sensitivity offered by gas chromatography–high-resolution mass spectrometry (GC–HRMS). This method was used to analyze a series of fish samples. Good agreement was found between the results for PBDEs from LC–APPI-MS/MS and GC–HRMS.

1. Introduction

Strict fire regulations require industrial materials and consumer products to have fire retarding properties to minimize fire damage and fire-/smoke-related deaths. Therefore, hundreds of different chemicals have been developed and are used globally for this purpose. Brominated (39%) and chlorinated (23%) flame retardants were produced in the largest amounts [1]. Many of these halogenated flame retardants (HFRs) are persistent and bioaccumulative, and exhibit some toxicity and/or endocrine disrupting properties [2]. These chemicals have been detected in environmental matrices including air, water, sludge, sediment and biota. One of the most common and widely used classes of HFRs is the polybrominated diphenylethers (PBDEs), which have been detected routinely in humans and are ubiquitous in the environment [3]. Therefore, a good analytical method for the determination of these flame retardants is important to protect our ecosystems and human health.

Halogenated flame retardants have been analyzed mostly by gas chromatography–mass spectrometry (GC–MS) [4,5]. GC–MS, especially GC–high-resolution MS (GC–HRMS), offers high resolution and good sensitivity for the determination of these flame retardants. However, thermal decomposition of some compounds was observed due to the high temperature in the GC injection port [6]. This influenced quantification and required more frequent cleaning of the liner and cutting the capillary column. Isomerization is another issue which can result in problems quantifying the individual isomers. This problem has been reported in the case of hexabromocyclododecane (HBCD) [7].

An alternative approach to GC–MS is to utilize liquid chromatography with tandem mass spectrometry (LC–MS/MS). Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) are three commonly used atmospheric pressure ionization (API) techniques employed to couple LC–MS. So far, all three LC–MS ionization sources have been investigated for the purpose of halogenated flame retardant analyses. ESI has a limited overall applicability for analysis of HFRs because this API technique can efficiently ionize only HBCDs and 3,3′,5,5′-tetrabromobisphenol A (TBBP-A) [8–10]. Until recently, the technique of APCI has only been applied for analysis of TBBP-A and HBCDs [11–15]. In the literature, APPI is the preferred ionization method for the determination of PBDEs [14,16–18].

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Table 1
Information of halogenated flame retardants and ion(s) in APPI source.

Compound	Abbreviation	Chemical formula	Ion(s) in source (relative intensity)
Allyl 2,4,6-tribromophenyl ether	ATE	$C_9H_7Br_3O$	$[M-Br+O]^-$ (100%)
1,2-Bis(2,4,6-tribromophenoxy) ethane	BTBPE	$C_{14}H_8Br_6O_2$	$C_6Br_3H_2O^-$ (100%), $[M-HBr+O_2]^-$ (4%)
2,2′,4-Tribromodiphenyl ether	BDE-17	$C_{12}H_7Br_3O$	$[M-Br+O]^-$ (100%)
2-Bromoallyl 2,4,6-tribromophenyl ether	BATE	$C_9H_6Br_4O$	$[M-Br+O]^-$ (100%), $C_6Br_3H_2O^-$ (43%), $[M-HBr+O_2]^-$ (33%)
2,2′,4,4′-Tetrabromodiphenyl ether	BDE-47	$C_{12}H_6Br_4O$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (55%), $[M-HBr-Br+O]^-$ (28%), $[M-H_2+Br]^-$ (14%), $[M+O_2]^-$ (4%)
2,3′,4,4′-Tetrabromodiphenyl ether	BDE-66		
2,3′,4′,6-Tetrabromodiphenyl ether	BDE-71		
3,3′,4,4′-Tetrabromodiphenyl ether	BDE-77		
Pentabromoethylbenzene	PBEB	$C_8H_5Br_5$	$[M-Br+O]^-$ (100%)
2,3-Dibromopropyl 2,4,6-tribromophenyl ether	DPTE	$C_9H_7Br_5O$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (51%)
2-Ethylhexyl-2,3,4,5-tetrabromobenzoate	EHTeBB	$C_{15}H_{18}Br_4O_2$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (2%)
Hexabromobenzene	HBB	C_6Br_6	$[M-Br+O]^-$ (100%)
2,3,4,7,8-Pentabromodibenzofuran	BDF-23478	$C_{12}H_3Br_5O$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (14%), $[M-H+O]^-$ (2%), $[M+O_2]^-$ (2%)
2,2′,4,4′,6-Pentabromodiphenyl ether	BDE-100	$C_{12}H_5Br_5O$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (16%), $[M-HBr-Br+O]^-$ (7%)
2,2′,4,4′,5-Pentabromodiphenyl ether	BDE-99		
3,3′,4,4′,5-Pentabromodiphenyl ether	BDE-126		
3,3′,5,5′-Tetrabromobisphenol A	TBBP-A	$C_{15}H_{12}Br_4O_2$	$[M-H]^-$ (100%), $[M-HBr-H+O]^-$ (8%), $[M+2OH-Br]^-$ (2%)
2,2′,4,4′,5,5′-Hexabromobiphenyl	BB-153	$C_{12}H_4Br_6$	$[M-Br+O]^-$ (100%)
Hexachlorocyclopentadienyl-dibromocyclooctane	HCDBCO	$C_{13}H_{12}Br_2Cl_6$	$[M+O_2]^-$ (100%)
2,2′,3,4,4′,5′-Hexabromodiphenyl ether	BDE-138	$C_{12}H_4Br_6O$	$[M-Br+O]^-$ (100%), $[M-HBr+O_2]^-$ (26%), $[M-HBr-Br+O]^-$ (18%)
2,2′,4,4′,5,6′-Hexabromodiphenyl ether	BDE-154		
2,2′,4,4′,5,5′-Hexabromodiphenyl ether	BDE-153		
Anti-dechlorane plus	a-DP	$C_{18}H_{12}Cl_{12}$	$[M-Cl+O]^-$ (100%), $[M-3Cl-2H+O]^-$ (100%), $[M-H]^-$ (100%), $[M-H-4Cl+2O_2]^-$ (62%), $[M+O_2]^-$ (51%)
syn-Dechlorane plus	s-DP	$C_{18}H_{12}Cl_{12}$	
α-Hexabromocyclododecane	α-HBCD	$C_{12}H_{18}Br_6$	$[M-H]^-$ (100%), $[M+O_2]^-$ (25%), $[M-3H-2Br+2O_2]^-$ (8%), $[M-3Br+3O_2]$ (7%), $[M-Br+O_2]^-$ (4%)
β-Hexabromocyclododecane	β-HBCD		
γ-Hexabromocyclododecane	γ-HBCD		
Bis(2-ethyl-1-hexyl)tetrabromophthalate	BEHTBP	$C_{24}H_{34}Br_4O_4$	$[M-Br+O]^-$ (100%), $[M-C_9H_{16}-Br-O]^-$ (12%)
2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	BDE-183	$C_{12}H_3Br_7O$	$[M-Br+O]^-$ (100%), $[M-HBr-Br+O]^-$ (11%), $[M-Br+O_2]^-$ (6%)
2,2′,3,3′,4,4′,6,6′-Octabromodiphenyl ether	BDE-197	$C_{12}H_2Br_8O$	$[M-Br+O]^-$ (100%), $[M-Br]^-$ (12%), $[M-2Br+H+O]^-$ (7%), $[M-HBr+O_2]^-$ (5%), $[M-H]^-$ (5%)
2,3,3′,4,4′,5,5′,6-Octabromodiphenyl ether	BDE-205		$[M-Br+O]^-$ (100%), $[M-HBr-Br+O]^-$ (7%), $[M-Br+O_2]^-$ (7%), $[M-Br_2+O_2]^-$ (1%)
Octabromotrimethylphenylindane	OBIND	$C_{18}H_{12}Br_8$	$[M-Br+O]^-$ (100%), $[M-HBr+O]^-$ (12%), $[M-3H-Br+O_2]^-$ (9%), $[M-2Br+2O]^-$ (3%)
2,2′,3,3′,4,4′,5,5′,6-Nonabromodiphenyl ether	BDE-206	$C_{12}HBr_9O$	$[M-Br+O]^-$ (100%), $[M-2Br+H+O]^-$ (53%), $[M-Br]^-$ (14%), $[M-Br+O_2]^-$ (6%), $[M-H]^-$ (2%)
2,2′,3,3′,4,5,5′,6,6′-Nonabromo-4′-chlorodiphenyl ether	4PC-BDE208	$C_{12}Br_9ClO$	$C_6Br_5O^-$ (100%), $C_6Br_4ClO^-$ (89%), $[M-Br+O]^-$ (26%), $[M-Br]^-$ (2%)
2,2′,3,3′,4,4′,5,5′,6,6′-Decabromodiphenyl ether	BDE-209	$C_{12}Br_{10}O$	$C_6Br_5O^-$ (100%), $[M-Br+O]^-$ (35%)
Decabromodiphenylethane	DBDPE	$C_{14}H_4Br_{10}$	$[M-Br+O]^-$ (100%)

The objective of this work was to develop a comprehensive, sensitive and high-throughput LC–APPI-MS/MS method for the determination of 36 HFRs and to overcome the limitations of previous studies in terms of applicability to a larger number of flame retardants, to investigate the relation of the APPI ion intensity and the dopant flow, and to apply this approach to analysis of fish samples.

2. Experimental

2.1. Chemicals and supplies

Individual standards or partial mixtures listed in Table 1 were supplied by Wellington Laboratories (Guelph, Canada). Information on the chemical structures of these compounds as well as the standard purity (>98%) can be found on the Wellington Laboratories (www.well-labs.com) website. HPLC grade methanol, isopropanol (IPA), water, acetone, toluene, and ethylether were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Chlorobenzene, dimethyl sulfoxide (DMSO), and anisole with the highest purity were obtained from Sigma–Aldrich (Oakville, ON, Canada). Hexabromobenzene-$^{13}C_6$ (HBB-$^{13}C_6$) was obtained from Cambridge Isotope Labs (Andover, MA, USA). Individual standard solutions or mixtures (50 mg/L) in either toluene or IPA/toluene (9:1, v/v) were stored at 0–4 °C in the dark. Decabromodiphenylethane (DBDPE) was kept at room temperature in toluene due to its low solubility in this solvent. Working solutions were prepared by a series of successive 10-fold dilutions for calibration and sample preparation. The final standards or samples were made in IPA/toluene (9:1, v/v) prior to injection for instrumental analysis. For small-volume analysis, a glass insert with a volume of 200 μL was placed in a 2-mL vial.

2.2. Sample preparation

Twenty-two fish samples were collected from the five Great Lakes as well as two additional lakes in Ontario, Canada. Details of the fish sample preparation including extraction and cleanup were previously reported [5,19,20]. Briefly, isotope labeled standards, BDE-47-$^{13}C_{12}$ (2.0 ng), BDE-99-$^{13}C_{12}$ (2.0 ng), BDE-153-$^{13}C_{12}$ (2.0 ng), BDE-154-$^{13}C_{12}$ (2.0 ng), BDE-183-$^{13}C_{12}$ (4.0 ng), and BDE-209-$^{13}C_{12}$ (10.0 ng) (Wellington Laboratories, Guelph,

Canada), were added to each sample prior to any extraction to obtain recoveries as determined by GC–HRMS. Automated pressurized liquid extraction (Fluid Management Systems, Watertown, MA) was applied to approximately 5 g samples. Biota extracts were cleaned using an automated sample preparation system (Fluid Management Systems, Watertown, MA). An acid silica column was initially employed for cleanup to remove bulk chemical interferences. Then, carbon columns were used to split the sample into two fractions. The forward fraction (forward elution from carbon) included non-planar compounds (HFRs and ortho-substituted polychlorinated biphenyls). The reverse fraction (reverse elution from carbon) contained the planar compounds (dioxins/furans, nonortho-polychlorinated biphenyls and polychlorinated naphthalenes). Each final extract from the forward fraction was brought to dryness via nitrogen evaporation. The individual residue was redissolved using 100 μL IPA/toluene (9:1, v/v) containing HBB-$^{13}C_6$ (10.0 ng) as the instrumental internal standard prior to LC–APPI-MS/MS analysis.

2.3. Ion intensity and dopant flow

To establish the relationship between the ion intensity and the dopant flow, flow injection analysis was performed using an Agilent 1200 XL LC system and API-4000 QTRAP triple quadrapole mass spectrometer (Applied Biosystems-MDS Sciex, Concord, ON, Canada) with an atmospheric pressure photoionization (APPI) source. Methanol at a flow rate of 200 μL/min was used to deliver the analyte from the injector to the APPI source. Source parameters were set as follows: curtain gas (CUR) 20 psi, collision associated dissociation gas (CAD) 12 psi, ion transfer voltage (IS) −850 V, temperature (TEM) 300 °C, nebulizer gas (GS1) 55 psi, and turbo gas (GS2) 40 psi. A Model 22 digital syringe pump (Harvard Apparatus, Holliston, MA, USA) was utilized to deliver the dopant (acetone or toluene). Two experiments were carried out. The first experiment was performed by injecting a fixed amount of compound BDE-209 at different dopant flow rates. BDE-209 (2.0 ng) in methanol was automatically injected with a dopant flow rate of 5, 8, 10, 12, 15, 18, 20, 25, 30, 35, or 40 μL/min. The second experiment was conducted by injecting 1.0, 1.6, 2.0, 2.4, 3.0, 3.6, or 4.0 ng BDE-209 in methanol. A constant flow of both 10 and 40 μL/min was used for both acetone and toluene as the dopant.

2.4. LC–MS/MS

The determination of target analytes was performed using a liquid chromatograph–tandem mass spectrometer system (LC–MS/MS) consisting of an Agilent 1200 XL Series LC coupled to an ABS API-4000 QTRAP MS/MS with an APPI interface. The Agilent LC system was modified from standard configuration to low delay volume mode.

A Restek Ultra II C18 column (100 mm × 2.1 mm, 2.2 μm) was used for the chromatographic separation. Mobile phases consisted of (A) methanol/water (85:15) and (B) methanol. LC separation was carried out at 25 °C with a linear gradient elution from 100% A to 100% B over a 6 min period, followed by an isocratic hold for 8 min at 100% B. The column was equilibrated for 4 min between runs. The flow rate of the mobile phase was 400 μL/min for the first 10 min and 500 μL/min for the last 4 min. A thorough-screening of individual dopants and combinations of dopants with the ionization potential less than 10 eV was performed in this study. These included (1) acetone, (2) toluene (3) chlorobenzene, (4) toluene:acetone (1:1), (5) toluene:anisole (99.5:0.5), (6) acetone:anisole (99.5:0.5), (7) DMSO, and (8) ethylether. Acetone was finally selected as the best dopant for APPI with a flow rate of 80 μL/min, as delivered by an Agilent isocratic pump. The dopant introduction to the APPI source with/without pre-heating was also compared. In the case of pre-heating, acetone and toluene were heated to 55 °C and 100 °C, respectively, in the oven chamber of the Agilent 1200 LC system before either of them was introduced to the APPI source. For the final applications, pre-heated acetone was employed. The injection volume of a standard or a sample solution was 2 μL.

Negative APPI (−APPI) was used in this work. For both selected reaction monitoring (SRM) determination and sample analysis, the optimum source-dependent parameters were held constant for all analytes and were set as follows: CUR 15 psi, CAD 12 psi, IS −850 V, TEM 300 °C, GS1 55 psi, and GS2 40 psi. Compound-dependent SRM settings were determined using the Compound Optimization program in Analyst 1.5 software (Applied Biosystems). Each target analyte, at a concentration of 1.0 mg/L in methanol, was injected automatically into the source. Methanol was used as the carrier medium at a flow rate at 400 μL/min, with acetone as the dopant at 80 μL/min. The most abundant fragment from the collision cell was selected as the product ion.

For LC–MS/MS analysis, scheduled SRM (sSRM) was used with 20 s of SRM detection window and 1 s of target scan time. Scheduled SRM is defined as a SRM with the amount of time for detection that surrounds the retention time for each transition. Mass spectrometer response, sensitivity and linearity were monitored before and after each set of experimental samples by injecting 2 μL of a series of standards (1–500 μg/L) prepared in IPA/toluene (9:1, v/v). Analyst version 1.5 software (Applied Biosystems) was used to control all components of the system and also for data collection and analysis.

2.5. GC–HRMS

An isotope dilution GC–high-resolution mass spectrometry (GC–HRMS) method developed previously, was also utilized for the determination of PBDEs in these fish samples [20] for data comparison. Briefly, the GC–HRMS technique used an Agilent Technologies 6890 Plus (Wilmington, DE) GC interfaced to a VG Autospec-Ultima NT HRMS (Waters, Manchester, UK) in EI positive with an electron energy of 40 eV using isotope dilution. Splitless injection was used with a direct injection sleeve: 1.5 mm i.d. (Supelco). The chromatographic separation was carried out on a DB-5HT 15 m × 0.25 mm × 0.10 μm (J&W Scientific, Folsom, CA). The GC–HRMS system was tuned to greater that 10,000 RP (10% valley definition). The GC conditions were: 110 °C hold for 1 min, ramp to 200 °C at 40.0 °C/min, ramp to 330 °C at 10 °C/min and hold for 5.5 min. The carrier gas was He with a constant flow rate of 1.0 mL/min.

3. Results and discussion

3.1. LC separation

The main challenge in the development of this method was the separation of isomeric chemicals (α,β,γ-HBCDs and a,s-DPs) as well as isobaric compounds (BDE-47/BDE-66/BDE-71/BDE-77, BDE-100/BDE-99/BDE-126, BDE-138/BDE-154/BDE-153, and BDE-197/BDE-205) which exhibited the same SRM transitions. The first step to develop the LC method was to select a suitable column. To achieve high-sensitivity and high-throughput, a column with ∼2 μm particle size was chosen as recommended in the literature [21]. Compared to other columns with larger diameter packing (i.e., 3 or 5 μm particle size), it was found that the column with ∼2 μm particle size produced narrower peaks, which improved both sensitivity and resolution. This observation agreed with other reports in the literature [21]. A screening of a variety of column phases was completed in order to determine the

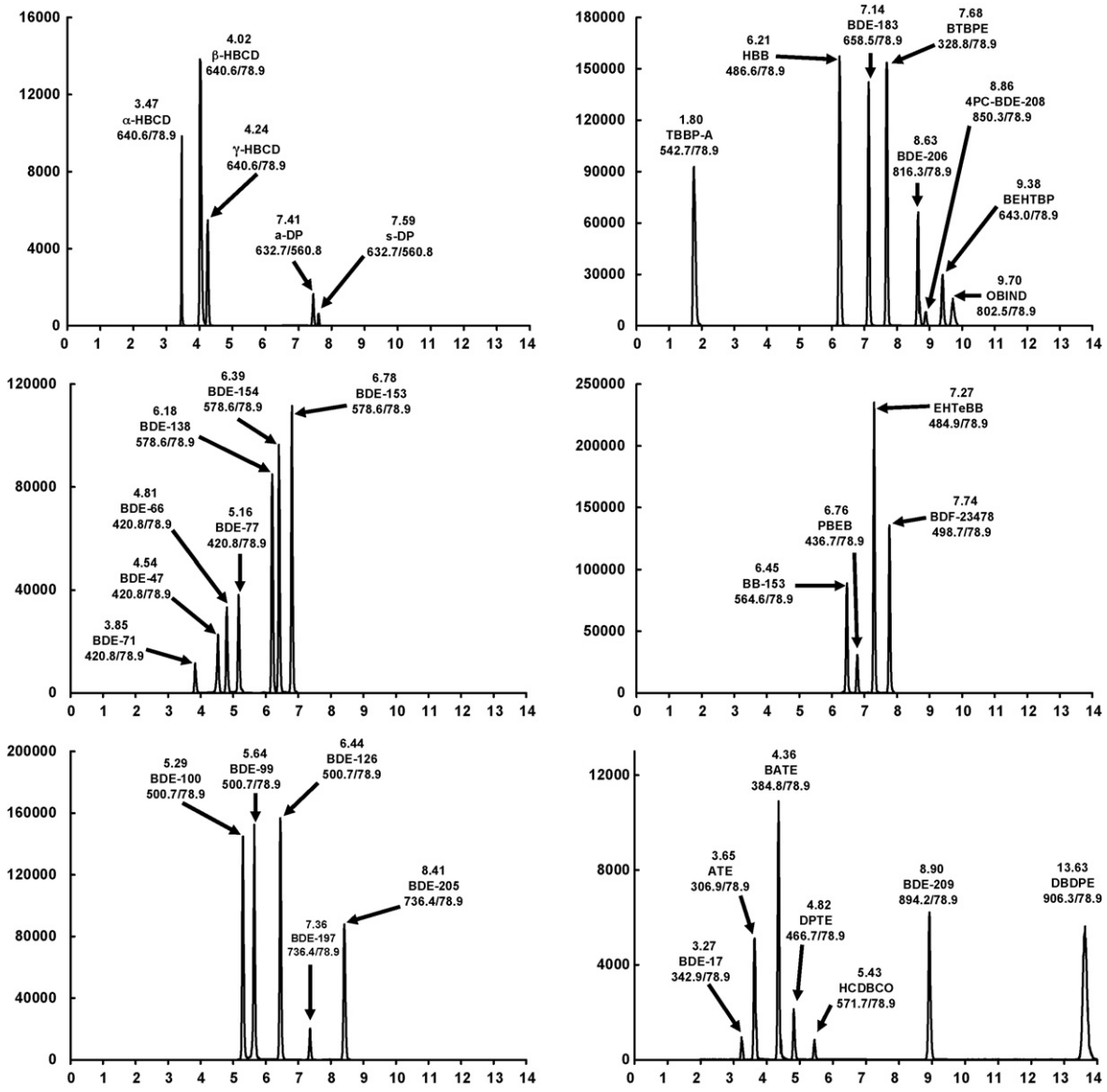

Fig. 1. Reconstructed MRM chromatograms obtained from 400 pg on-column injection with the peak intensity (cps) of *y*-axis and retention time (min) of *x*-axis. Mobile phases consisted of (A) methanol/water (85:15) and (B) methanol. A linear gradient elution was performed from 100% A to 100% B during 6 min, followed by an isocratic hold for 8 min at 100% B. The flow rate of the mobile phase was 400 μL/min for the first 10 min and 500 μL/min for the last 4 min.

most suitable column type. In general, the Ultra II C18 column yielded the most favorable separations compared to the biphenyl, Pinnacle DB aqueous-C18, and Ultra II Aqueous-C18 stationary phases.

Subsequently, an appropriate mobile phase composition needed to be established. After two separate gradient elution trials using water/methanol and water/acetonitrile, the combination of water/methanol was found to be superior, especially for DPs and PBDEs with the same SRM transitions. Column temperature was also considered during the LC method development stage. It was found that increased column temperature only slightly shortened the overall retention time by approximately 0.03 min/°C. Thus, a higher column temperature was not necessary to increase throughput. The effect of increasing temperature on the resolution depended on the specific compounds. Resolution was increased in some cases and reduced in others. There was no obvious correlation to this effect [22]. In this work, with an increased temperature, resolution decreased, which was consistent with other researchers' observations [23]. Moreover, an elevated column temperature compromises column lifetime. Therefore, LC separations were performed at ambient temperature (25 °C).

In addition to the parameters mentioned above, the LC flow rate was also considered for optimization. To achieve the best separation, a flow rate of 400 μL/min was employed. Increasing the flow rate slightly worsened overall separations, which suggested that the flow rate of 400 μL/min resulted in the minimum theoretical plate height. The feasibility of isocratic elution was also investigated. Isocratic elution could not be applied to these compounds listed in Table 1. An isocratic elution caused early-eluting peaks to overlap or resulted in late-eluting compounds with unacceptably long retention times. For gradient elution, the gradient time was examined. Although better separation was achieved with a longer gradient time, the cycle time between injections also became longer. Therefore, to balance peak resolution and the cycle times, the gradient time was set to 6 min which resulted in the last compound being eluted before 14 min. The optimized LC conditions allowed 18 min per run, including 14 min of column separation and 4 min for column conditioning. The first column of Fig. 1 presents LC

separations with groups of isomeric compounds that have the same SRM transitions. Baseline separation was achieved within all of the isomeric groups. The second column of Fig. 1 shows reconstructed ion chromatograms of compounds with the different SRM transitions. Retention time and SRM transition for individual analyte can be found next to the compound name in Fig. 1 as well.

The solubility of HFRs was another concern in this study because of their varying physical and chemical properties. IPA/toluene (9:1, v/v) was used as the injection solution because this solution balanced both compatibility with LC mobile phases and solubility of these hydrophobic compounds, especially DBDPE that does not dissolve well in methanol. Since the injection solution was different from the mobile phase, the injection volume was investigated. An injection volume of 2 μL or less generated good chromatographic peak shapes for all the compounds. Peak fronting of TBBP-A, the most hydrophilic compound, was observed with an injection of more than 2 μL.

3.2. Ion intensity and dopant flow

APPI is a relatively new atmospheric pressure ionization technique compared to ESI and APCI, and an interesting topic for LC–MS and LC–MS/MS research. The number of papers related to APPI has rapidly increased since the first publications of LC–APPI-MS in 2000 [24,25]. Several reviews have been published recently [26–29]. A dopant is required to assist APPI in most cases, and especially for reversed-phase LC. APPI chemical reactions are complicated and ion intensity is affected by different parameters. When LC–APPI-MS/MS is applied to routine analyses, little variation in LC flow rate and eluent compositions is possible due to the requirement of LC separation. However, the flow rate of a dopant is independent of LC separation. Thus, it is necessary to understand how the ion intensity and the dopant flow are related to each other. A simple relationship between ion intensity and dopant flow rate has not been developed [30]. According to the data obtained in this study, as well as the profiles found in the literature [30], the following empirical equation should describe the relation between the ion intensity and the dopant flow:

$$I = I_0[1 - \exp(af)] \quad (1)$$

where I is the ion intensity at the dopant flow, f, I_0 is the maximal ion intensity, and a is the flow constant, which is related to the composition of LC eluents, LC flow rate, the APPI source parameters as well as the geometric source design. (*Note*: the value of the flow constant, a, must be a negative value)

An example of the results obtained using Eq. (1) is shown in Fig. 2(a), which is similar to profiles found in the literature. Eq. (1) indicates that a higher dopant flow gives a higher value of I, which agrees with the literature [31]. Although I_0 is never reached, the value of I asymptotically approaches the value I_0. Fig. 2(a) indicates that the value of I approaches I_0 at a dopant flow of 40 μL/min, when the mobile phase flow was set at 200 μL/min. Thus, a dopant flow rate of 40 μL/min was used to produce I_0 when acetone or toluene was employed as the dopant. The experimental data in this work demonstrated that further dopant flow increases had virtually no effect on ion intensity. This study showed that the dopant flow rate, at 20% of LC flow rate, generated the maximal ion intensity, which agreed with the recommendation of the manufacturer and others observations [17].

Fig. 2(a) is referred to an intensity-flow profile, which is a non-linear function. The best approach to validate Eq. (1) is to utilize linear approaches. By rearranging Eq. (1), the following equations are obtained:

$$\ln\left(1 - \frac{I}{I_0}\right) = af \quad (2)$$

$$I_0 = \frac{1}{1 - \exp(af)} I \quad (3)$$

When LC–APPI-MS/MS is applied for routine analyses, the LC eluent compositions and LC flow rate are fixed by the requirements for separation. Moreover, APPI source-dependent parameters such as temperature, nebulizer gas flow and ion transfer voltage are constant during LC runs to minimize any variations for batch analyses. Under these conditions, the flow constant, a, can be considered to be a constant. From Eq. (2), a plot of $\ln(1 - (I/I_0))$ versus f yields a straight line with a slope of a. The profile from Eq. (2) is called the flow profile. The same principle can be applied to Eq. (3). When the flow constant (a) and dopant flow (f) are constant, a plot of I_0 versus I should produce a straight line with a slope of $1/(1 - \exp(af))$. The profile from Eq. (3) can be referred to the intensity profile. Excellent linearity was observed in the examples of Fig. 2(b) (flow profile) and (c) (intensity profile). When toluene was utilized as the dopant, excellent linearity with $R^2 > 0.994$ was observed as well, which suggests that Eq. (1) should describe the relation between the ion intensity and the dopant flow. It was interesting to find that the a value decreased as the dopant boiling point increased.

3.3. APPI optimization

To obtain the highest ionization efficiency, it is essential to find the best dopant for APPI method development. The dopants or dopant combinations (1)–(6) listed in Section 2.4 generated similar ionization efficiencies. The last two dopants, DMSO and ethylether, gave around 6- and 100-fold lower intensity, respectively. Therefore, DMSO and ethylether were not considered for further method development. An approach using dopant combinations was not used for the next investigation due to technical simplicity. A single dopant was not found to provide the highest intensities for all the analytes listed in Table 1. In comparison of acetone with toluene as the dopant reagent, toluene provided around 10% higher ion intensity for these HFRs that were less hydrophobic, and acetone offered about 10% stronger signal for these analytes that were more hydrophobic.

The dopant introduction to the APPI source was also studied. It was found that dopant pre-heating was essential to lower background noise. The boiling points of acetone and toluene are 56.5 °C and 110.6 °C, respectively. The background noise with pre-heating to 55 °C for acetone decreased fivefold compared to that without pre-heating. This phenomenon was also observed when toluene was pre-heated to 100 °C before it entered the APPI source. A similar baseline height was observed for both acetone and toluene by the pre-heating approach. However, without pre-heating, toluene generated a higher background noise than acetone. A reasonable explanation for this observation is that the pre-heated dopant consumes less desolvation energy in the APPI source. Acetone was selected as the ideal dopant for the next experiments in terms of similar ionization efficiency, lowest boiling point, and lower toxicity compared to toluene or chlorobenzene.

While optimizing the APPI source conditions, it was observed that temperature was the variable with the greatest effect on the sensitivity of the flame retardants examined. No single temperature or other common source parameter resulted in maximum sensitivity for all of the analytes. Also, it was not practical to use a temperature gradient program in the APPI source. Therefore, optimized BDE-47 APPI parameters were employed in this work for two reasons: (1) most chemicals in this study belong to PBDEs; (2) the LC retention time of BDE-47 was approximately in the middle of the LC run, so it balanced both early- and late-eluting compounds.

More than one precursor ion was generated in the APPI source for most flame retardants as shown in Table 1. This indicates the complexity of photoionization as well as the varying physi-

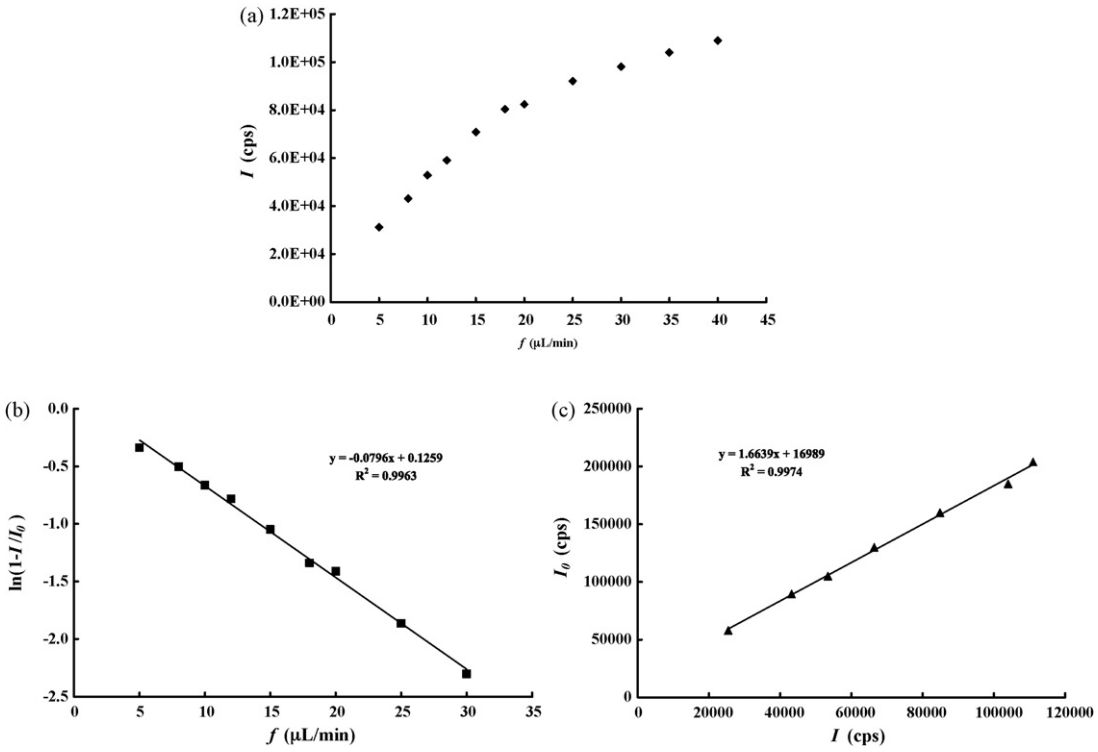

Fig. 2. Relation between the ion intensity and the dopant flow. (a) shows relation between ion intensity and dopant flow; (b) illustrates the linear relation between $\ln(1-(I/I_0))$ and f; and (c) presents the linear relation between I_0 and I. (Acetone was used as the dopant and BDE-209 as the target compound.)

Table 2
MRM transitions and optimization parameters* for the MRM detection during LC–APPI-MS/MS analysis.

Chemical	1st MRM	2nd MRM	DP	EP	CE	CXP
ATE	306.9/78.9	306.9/80.9	−30	−4	−50	−11
BTBPE	328.8/78.9	328.8/80.9	−70	−15	−70	−12
BDE-17	342.9/78.9	342.9/80.9	−40	−6	−40	−10
BATE	384.8/78.9	384.8/80.9	−30	−4	−40	−11
BDE-47	420.8/78.9	420.8/80.9	−30	−6	−40	−12
BDE-66			−50	−4	−80	−11
BDE-71			−80	−3	−80	−11
BDE-77			−70	−7	−70	−11
PBEB	436.7/78.9	436.7/80.9	−70	−10	−70	−12
DPTE	466.7/78.9	466.7/80.9	−30	−5	−40	−12
EHTeBB	484.9/78.9	484.9/80.9	−80	−5	−110	−13
HBB	486.6/78.9	486.6/80.9	−75	−10	−90	−12
BDF-23478	498.7/78.9	498.7/80.9	−85	−8	−110	−10
BDE-100	500.7/78.9	500.7/80.9	−60	−5	−90	−7
BDE-99			−60	−5	−90	−7
BDE-126			−100	−6	−90	−9
TBBP-A	542.7/78.9	542.7/80.9	−100	−12	−90	−12
BB-153	564.6/78.9	562.6/80.9	−60	−8	−110	−9
HCDBCO	571.7/78.9	571.7/80.9	−35	−10	−45	−13
BDE-138	578.6/78.9	578.6/80.9	−100	−5	−80	−10
BDE-154			−50	−5	−100	−13
BDE-153			−50	−5	−100	−13
a-DP, s-DP	632.7/560.8	634.7/560.8	−40	−7	−15	−9
α-HBCD, β-HBCD, γ-HBCD	640.6/78.9	640.6/80.9	−35	−4	−40	−12
BEHTBP	643.0/78.9	643/80.9	−130	−12	−100	−12
BDE-183	658.5/78.9	658.5/80.9	−50	−12	−110	−12
BDE-197	736.4/78.9	736.4/80.9	−70	−5	−100	−10
BDE-205			−60	−9	−110	−11
OBIND	802.5/78.9	802.5/80.9	−70	−12	−100	−11
BDE-206	816.3/78.9	816.3/80.9	−60	−7	−120	−11
4PC-BDE208	850.3/78.9	850.3/80.9	−60	−9	−125	−12
BDE-209	894.2/78.9	894.2/80.9	−70	−6	−125	−12
DBDPE	906.3/78.9	906.3/80.9	−85	−10	−130	−11

DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Table 3
On-column instrument detection limits (IDL), Limits of quantitation (LOQs), number of samples above LOQ, and concentrations of analytes detected in fish samples.

Chemical	On-column IDL (pg)	LOQ (ng/g)	#Above LOQ (n = 22)	Conc. range (mean) (ng/g)
ATE	4	0.037	0	<LOQ
BTBPE	0.5	0.011	0	<LOQ
BDE-17	40	3.20	0	<LOQ
BATE	2	0.32	0	<LOQ
BDE-47	4	0.049	22	0.66–740 (110)
BDE-66	1	0.016	19	0.030–3.4 (0.76)
BDE-71	4	0.036	1	0.089
BDE-77	2	0.011	1	0.047
PBEB	2	0.018	0	<LOQ
DPTE	20	0.11	0	<LOQ
EHTeBB	1	0.0061	7	0.011–0.041 (0.029)
HBB	0.5	0.0045	0	<LOQ
BDF-23478	0.5	0.0060	0	<LOQ
BDE-100	0.5	0.0063	22	0.049–29 (5.9)
BDE-99	1	0.0045	22	0.22–49 (9.5)
BDE-126	0.5	0.0027	2	0.030–0.073 (0.052)
TBBP-A	0.5	0.012	0	< LOQ
BB-153	1	0.036	20	0.041–2.93 (0.60)
HCDBCO	20	0.39	0	<LOQ
BDE-138	1	0.0062	1	0.039
BDE-154	0.5	0.020	20	0.090–12 (3.3)
BDE-153	0.5	0.0031	22	0.023–6.7 (1.8)
a-DP	4	0.11	0	<LOQ
s-DP	20	0.37	0	<LOQ
α-HBCD	10	0.048	18	0.32–11 (3.4)
β-HBCD	4	0.051	2	0.11–0.38 (0.24)
γ-HBCD	10	0.19	8	0.27–2.2 (0.67)
BEHTBP	0.5	0.042	4	0.044–0.078 (0.060)
BDE-183	0.5	0.0041	22	0.0047–0.19 (0.064)
BDE-197	2	0.054	18	0.056–0.38 (0.16)
BDE-205	0.5	0.0098	0	<LOQ
OBIND	1	0.0088	0	<LOQ
BDE-206	0.5	0.0096	22	0.041–1.1 (0.20)
4PC-BDE208	2	0.082	0	<LOQ
BDE-209	4	0.086	22	0.74–15 (4.5)
DBDPE	4	0.020	0	<LOQ

cal and chemical properties, differing structures, and the thermal liability of each chemical. Three categories of precursor ions were summarized in the APPI source: (1) displacement products, e.g. $[M-Br+O]^-$ and $[M-HBr-Br+O_2]^-$; (2) elimination products, e.g. $[M-H]^-$; and (3) association product, $[M+O_2]^-$. The dominant precursor ion observed for the HFRs was $[M-Br+O]^-$. After the optimal APPI conditions were finalized, the compound-dependent MS/MS transitions and the optimized parameters for detection and quantification were determined, which are presented in Table 2.

3.4. Linearity and detection limits

The highest concentration of standards for the analytes used in this work was 500 ng/mL. Excellent linearity was observed for each analyte in this study. Table 3 shows on-column instrument detection limits (IDLs), which were obtained from the optimized LC–APPI-MS/MS conditions. The on-column IDLs were defined as the injected amount of a target analyte that offered three times the signal to noise ratio. The range of IDLs was from 0.5 to 40 pg with an average of 4.7 pg. Twelve of the 36 HFRs reached the IDLs of 0.5 pg. The on-column IDLs represent an improvement for these chemicals analyzed by LC–APPI-MS/MS [17]. In comparison with GC–HRMS, the LC–APPI-MS/MS approach provided similar sensitivity for the chemicals listed in Table 1. LC–APPI-MS/MS offered lower on-column IDLs for 36% of all the analytes, while GC–HRMS gave better sensitivity for 44% of them [5,20]. For 19% of the compounds studied, results were either unavailable by GC–HRMS, or there were ionization problems using the GC–MS technique (TBBP-A and HBCDs). There are two advantages associated with LC–APPI-MS/MS over GC–HRMS or GC–MS. Firstly, LC–APPI-MS/MS is a more comprehensive approach and covers all of the analytes including TBBP-A and HBCDs. Secondly, LC–APPI-MS/MS provides lower on-column IDLs for flame retardants with higher molecular weights. For example, BDE-209 is thermally unstable and decomposes in the hot GC injection port, which leads to a higher on-column IDL. The same phenomenon was observed for the non-BDE flame retardants in this study. In addition, to determine the higher brominated diphenyl ethers by GC–MS, column switching had to be conducted, which was an inconvenient technique [32].

3.5. Application to real samples

The results of the screening analysis carried out on real sample matrices (fish) are summarized in Table 3. Limits of quantitation (LOQs) were equal to 10 times the standard deviation of repetitive measurements on a blank [33]. For most analytes, the LOQs were below 1 ng/g for fish sample. A second SRM transition for each analyte listed in Table 2 was utilized for confirmatory purposes on the basis of necessity and in consideration of complex real sample matrices and potential interferences. An isotope dilution GC–HRMS method was used for comparison of PBDEs data in these samples [20]. Good correlations for analysis of PBDEs were achieved between the concentrations obtained by GC–HRMS and LC–APPI-MS/MS, which are illustrated in Fig. 3, confirming the efficiency of LC–APPI-MS/MS.

There are several advantages associated with the LC–APPI-MS/MS technique. Firstly, it is comprehensive because this method covers 36 HFRs of varying physical and chemical properties. Previously only a few flame retardants have been analyzed by LC–APPI-MS/MS [17]. Moreover, GC–MS has not been applied for the determination of TBBP-A and HBCDs. Secondly, this developed

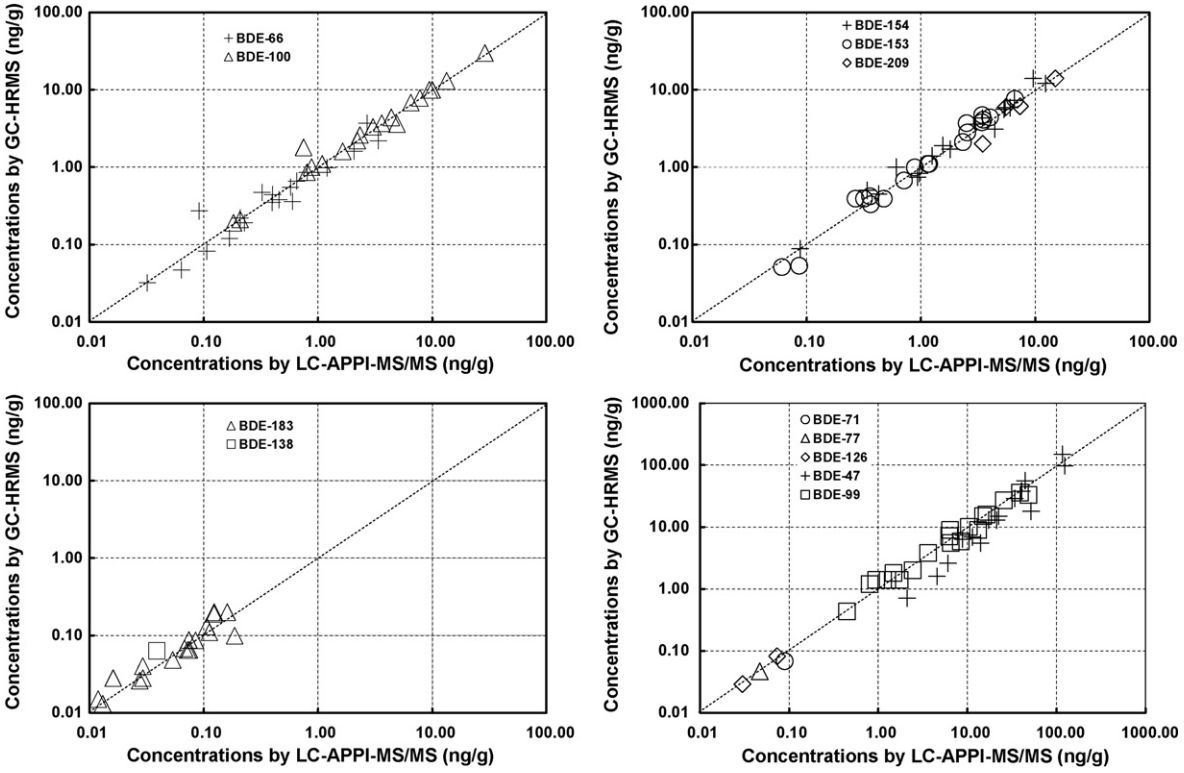

Fig. 3. Correlations of concentrations determined by GC–HRMS and LC–APPI-MS/MS for several PBDEs in fish.

method is sensitive by comparison with analysis of HFRs. The on-column IDLs in this work are lower than other literature reported values by LC–APPI-MS/MS [17]. This method offers a similar sensitivity to GC–HRMS for the determination of HFRs [5,20]. Thirdly, it is a high-throughput approach because of the combination of scheduled SRM with the shorter LC run times. Scheduled SRM provides enough points across the chromatographic peak to give better peak detection. In comparison with the reported retention time of BDE-209, this method improved instrumental sample throughput twofold or more [14,17]. Several factors contributed to these advantages: (1) ~2 μm packing material in the LC column, (2) optimized LC conditions, (3) dopant introduction by pre-heating, (4) wide range of APPI application, and (5) scheduled SRM.

4. Conclusions

In this work, a comprehensive, sensitive and high-throughput liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry (LC–APPI-MS/MS) method has been developed to analyze 36 HFRs. An empirical equation was proposed to describe the relation between the ion intensity and the dopant flow rate. The ~2 μm packing material and the optimized LC conditions allowed excellent separation for isomeric compounds with the same SRM transitions. The wide application range of APPI covered numerous HFRs with varying physical and chemical properties. The utility of pre-heated dopant decreased the level of background noise fivefold, which enhanced sensitivity. The employment of scheduled SRMs made high-throughput analysis possible. The excellent on-column IDLs by LC–APPI-MS/MS, which averaged 4.7 pg, were similar to those achieved by GC–HRMS and better than the values previously reported. Analyses of environmental samples (fish) generated comparable results for PBDEs by GC–HRMS, indicating that this method is a viable alternative approach for the determination of PBDEs and possibly other HFRs in environmental samples.

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