Development of Atmospheric Pressure Chemical Ionization technique for the determination of Halogenated Flame Retardants

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Abstract

Until recently, the technique of atmospheric pressure chemical ionization (APCI) had limited applicability to halogenated flame retardant (HFR) analysis. In this study, a comprehensive liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS) method has been developed to analyze 26 HFRs. The conditions for LC, APCI and MS/MS were optimized to allow fast and sensitive analysis and the APCI mechanism was investigated. Excellent limits of detection (0.41-4.9 ng/mL) and linearity (R^2 = 0.995-0.999) were observed. The method developed has been applied to real environmental sample matrices for screening purposes, with concentrations determined by external calibration agreeing with data obtained via another method.

Introduction

The global use of flame retardants has resulted in their detection in a multitude of environmental matrices including air, sediment and biota. Many of these chemicals are persistent, bioaccumulative, and exhibit some toxicity and endocrine disrupting behavior. In light of these facts, the development of a simple, comprehensive and robust analytical method is of key importance. Halogenated flame retardants (HFRs) have typically been analyzed using gas chromatography mass spectrometry (GC-MS)[1]. However, the high injection port temperatures can result in degradation of desired the compounds. For compounds such as hexbromocyclododecane (HBCD), isomerization is also a problem when it is necessary to quantify the individual isomers[2]. For these reasons, liquid chromatography tandem mass spectrometry (LC-MS/MS) has been developed to analyze flame retardants. The ionization methods examined include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). Although ESI efficiently ionizes all of the HBCD isomers and 3,3',5,5'-tetrabromobisphenol A (TBBP-A)[3], attempts at using this ionization method for the detection of other HFRs have failed. Therefore, its overall applicability is limited. In the literature, APPI is the preferred ionization method for the determination of polybrominated diphenyl ethers (PBDEs)[4] and APCI has been applied for analysis of TBBP-A and HBCDs[5]. However, the limited availability of APPI in most analytical laboratories has made this mode of ionization less appealing. The objective of this study was to develop a comprehensive LC-APCI-MS/MS method for HFR analysis and to overcome the limitations of previous studies in terms of applicability to a larger number of flame retardants. The mechanism of ionization and application of this method for screening real sample matrices (sludge and mussel samples) were also investigated.

Materials and Methods

Individual standards listed in Table 1 were supplied by Wellington Laboratories (Guelph, Canada). Details of the sample preparation of the sludge and mussel samples analyzed, including extraction and cleanup, have been previously reported[1]. Briefly, for the sludge samples, 0.3-1.0 g dried sludge was Soxhlet-extracted. Following extraction, cleanup involved a multi-layer silica chromatographic column. For biota (mussel) samples, automated pressurized liquid extraction (PLE) was applied to 2.5-5.5 g samples. Biota cleanup employed silica and carbon media using Teflon columns. Each final extract was concentrated to dryness. Sample residues were dissolved in 100 μL methanol methanol was used to dissolve the individual residues prior to LC-APCI-MS/MS analysis. Instrumental analysis was performed using a LC-MS/MS, consisting of an unmodified Agilent 1200 XL Series

LC coupled with an API-4000 QTRAP triple quadrupole mass spectrometer (Applied Biosystems-MDS Sciex, Concord, Canada). An Ultra Aqueous C18 column (100 mm × 4.6 mm, 3 μm, Restek, Bellefonte, USA) was used for the chromatographic separations. Mobile phases consisted of (A) acetonitrile/water (2:1) and (B) methanol. Three separate chromatographic runs were performed using the following methods: Run 1 employed a flow rate of 1.0 mL/min with an initial mobile phase of 0% B, followed by a 2 min ramp to 100% B, a 5 min hold, then a return to initial condition at 7.1 min, followed by a 7 min equilibration; Run 2 utilized a flow rate of 1.25 mL/min with a 100% B isocratic method; and Run 3 used the same gradient elution as Run 1, but with a 4 min hold. A 5 μL injection volume was used for the experimental samples. The mass spectrometer was operated in negative APCI (-APCI) multiple reaction monitoring (MRM) mode. The optimum MRM settings for each compound were determined using a Model 22 digital syringe pump (Harvard Apparatus, Holliston, Massachusetts, USA) by infusing a 1 mg/L methanol solution into the source at 10 μL/min.

Results and discussion

LC method development

A thorough evaluation of a variety of column phases showed that the Ultra Aqueous C18 column exhibited the best selectivity. Subsequently, an appropriate mobile phase composition needed was established. The main challenge for LC method development was the separation of isomers (α,β,γ-HBCDs and anti,syn-DPs) as well as isobaric compounds which exhibit the same MRM transitions (BDE-99/BDE-100, and BDE-154/BDE-153). It was found that β-HBCD and γ-HBCD co-eluted when the water/methanol combination was used as the mobile phase. However, this combination provided good separation for a-DP/s-DP, BDE-99/BDE-100, and BDE-154/BDE-153. Alternatively, the water/acetonitrile combination offered good separation for β-HBCD and γ-HBCD, but caused peak overlap of a-DP/s-DP, BDE-99/BDE-100, and BDE-154/BDE-153. This separation issue was resolved by using a combination of acetonitrile/water (2/1) as mobile phase A, utilized at the start of the LC run, and methanol as mobile phase B. Optimization of this eluent combination resulted in the gradient conditions of Run 1 and 3. The use of only acetonitrile and water at the beginning of the run allowed the separation of β- and γ-HBCD isomers, which eluted as single and discrete peaks. After the first 2 min, methanol was used to separate a-DP/s-DP, BDE-99/BDE-100, and BDE-154/BDE-153. Near baseline separation was observed for these individual pairs with the same MRM transitions. Run 3 was developed in order to achieve a good separation for ATE, BATE, and DPTE, which share the same MRM transition.

APCI mechanism

Determining conditions that were suitable for all of the target compounds was a challenge because of a broad range of physical and chemical properties with varying polarity. Table 1 presents the different ions produced from the flame retardants listed in the APCI source. The variation and complexity observed with respect to the products of ionization is due to the varying physical and chemical properties, differing structures, and the thermal lability of each chemical. Three possible ionization mechanisms are summarized: (1) displacement reactions, $M + O_2^{\bullet-} \rightarrow [M - R + O]^-$, or $M + O_2^{\bullet-} \rightarrow [M - R + O_2]^-$, where R = Br, [Br + HBr], or Cl; (2) elimination reactions, $M + O_2^{\bullet-} \rightarrow [M - R]^-$, where R = H, or part of a molecule; (3) association reaction, $M + O_2^{\bullet-} \rightarrow [M + O_2]^{\bullet-}$. This summary is based on experimental observations. The corona discharge needle produces a current to ionize air and generates primary ions such as $O_2^{\bullet-}$ ion[6]. The radical ions produced in the plasma function as reactants to generate different ions in the APCI source. The displacement reactions were the most dominant pathways observed in this work. The main precursor ion for the flamed retardants listed in Table 1 is $[M-Br+O]^-$. An analogous product ion was observed for DP with chlorine loss and oxygen capture to form $[M-Cl+O]^-$. When no current was applied to the corona discharge needle, no ion current was observed. This would indicate that the high temperature associated with the APCI thermal desolvation procedure does not initiate ionization. The displacement reaction product, $[M-HBr+O_2]^-$, was observed for BDE-47 and OBIND. This observation suggests that APCI ionization results from attack of the compound by a radical anion. Another observed displacement reaction product is $[M-HBr-Br+O]^-$, which suggests that the compound is thermally labile, and also indicates that an ortho-effect may be present. In most cases, $[M-HBr-Br+O]^-$ was found for the PBDE molecules that have ortho-Br atoms. This observation agrees with the literature report that ortho-(C-Br) bond are longer and weaker than those of both the meta- and the para- (C-Br) bonds[7]. Elimination reactions were

also often observed for several of the flame retardants investigated in the APCI source. Specifically, compounds with relatively high polarity such as TBBP-A and HBCD favoured proton elimination. This also suggests that both TBBP-A and HBCD have relatively high gas-phase acidities, which facilitates proton loss. Proton elimination was also observed during the ionization of the DP isomers although the ion intensity of $[M-H]^-$ cluster was lower than that of two displacement reaction product ions. The elimination of a part or functional moiety of a molecule was often observed for those compounds with a relatively high molecular weight such as BDE-209, BEHTBP, 4PC-BDE208, and BTBPE. This is consistent with higher molecular weight flame retardants having less thermal stability. The observation of association reactions during ionization in the APCI was an interesting finding in this study. $[M+O_2]^-$ was generated in the APCI source from several of the HFRs including HCDBCO, HBCD, and DP. In fact, $[M+O_2]^-$ was the sole precursor ion for HCDBCO. The molecules which exhibited this reactive behavior possess a structural similarity, specifically; they have two bromine or chlorine atoms neighboring each other on the aliphatic ring. This kind of molecular structure might favor formation an oxygen adduct, likely, a six atom ring (two carbons, two bromines or chlorines and two oxygens) and the resonance structure, which could allow the precursor ion to be stable in the APCI source. Oxygen addition further suggests that oxygen ions could initiate all of the reactions in the APCI source.

APCI parameter optimization

While optimizing the APCI source conditions, it was observed that temperature was the variable with the greatest effect on the sensitivity of the flame retardants examined. Three LC runs, each focusing on different analytes, were carried out with varied source temperature to optimize the sensitivity of the individual target analytes, in addition to the LC requirements for separation. Table 2 lists the optimized APCI parameters for each LC run. Run 1 separated the isomers of HBCD and DP as well as some of the BDEs and Run 2 narrowed the peaks of the more nonpolar compounds. Run 3 employed a higher APCI probe temperature to increase sensitivities of four flame retardants, TBBP-A, allyl 2,4,6-tribromophenyl ether (ATE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE) and 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE). It is possible that the hydroxyl group presenting in TBBP-A forms hydrogen bonds with methanol, and a higher solvation energy is expected. Therefore, a higher source temperature allows for a more complete desolvation of this analyte. For ATE, BATE, and DPTE, the higher APCI source temperature favors the cleavage of the oxygen and carbon bond to form precursor ions. It was also observed that the composition of the LC eluent present with the analyte in the APCI source influenced the ionization efficiency. The eluent containing only methanol generated the highest sensitivity for most of the HFRs compared to acetonitrile, acetonitrile-modified, or methanol-modified mobile phases. This can be explained by the higher volatility of methanol compared to that of acetonitrile and water. Apart from the two APCI parameters outlined above, other source parameters did not significantly affect the sensitivity of the analytes. The highest corona discharge current, -5 μA, was found to give the best sensitivity for most flame retardants discussed here. This developed method offered good limits of detection (LODs: 0.41 - 4.9 ng/mL, equivalent to 2 – 25 pg injected) and linearity ranging from 20 ng/mL to 500 ng/mL (R^2 = 0.995-0.999) for all of the HFRs analyzed.

Screening analysis for real samples

The results of the screening analysis carried out on real sample matrices (municipal wastewater treatment plant sludge and mussel biomonitors) are summarized in Figure 1. Only the HFRs with detections above instrumental detection limits are listed. Since the real sample matrices were very complex and contained many potential interferences, a second MRM transition for each analyte was utilized for confirmatory purposes. In most cases, higher concentrations of these flame retardants were observed in the sludge samples than in the mussel samples, as illustrated in Figure 1. An isotope dilution GC-high resolution mass spectrometry (GC-HRMS) method was also utilized for the determination of PBDEs in these samples[1]. The data obtained by this external calibration LC-APCI-MS/MS approach agrees quite well with those from GC-HRMS, ranging in difference by factors of 0.3-2.9. Based on these results, this LC-APCI-MS/MS approach may be a viable alternative for the analysis of the flame retardants listed in Table 1, which display varying physical and chemical properties. For this reason, it is quite possible that this method could be successfully applied to other flame retardants as well.

Acknowledgements
We thank Wellington Laboratories for providing the flame retardant standards and Restek for supplying LC columns.

Table 1: Halogenated flame retardants ion(s) in APCI source for LC-APCI-MS/MS analysis

Compound	Abbreviation	Ion(s) in source *
hexabromocyclododecane	HBCD	$[M-H]^-$, $[M+O_2]^-$
2,2',4,4'- tetrabromodiphenyl ether	BDE-47	$[M-Br+O]^-$, $[M-HBr+O_2]^-$
2,2',4,4',5-pentabromodiphenyl ether	BDE-99	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2,2',4,4',6-pentabromodiphenyl ether	BDE-100	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2,2',4,4',5,6'-hexabromodiphenyl ether	BDE-154	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2,2',4,4',5,5'-hexabromobiphenyl	BB-153	$[M-Br+O]^-$
dechlorane plus	DP	$[M-Cl+O]^-$, $[M+O_2]^-$, $[M-H]^-$
hexabromobenzene	HBB	$[M-Br+O]^-$
pentabromoethylbenzene	PBEB	$[M-Br+O]^-$
hexachlorocyclopentadienyl-dibromocyclooctane	HCDBCO	$[M+O_2]^-$
2,2',3,4,4',5',6-heptabromodiphenyl ether	BDE-183	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2-ethylhexyl-2,3,4,5-tetrabromobenzoate	EHTeBB	$[M-Br+O]^-$
1,2-bis (2,4,6-tribromophenoxy) ethane	BTBPE	$C_6Br_3H_2O^-$
2,3,3',4,4',5,5',6-octabromodiphenyl ether	BDE-205	$[M-Br+O]^-$, $[M-HBr-Br+O]^-$
2,2',3,3',4,5,5',6,6'-nonabromo-4'-chlorodiphenyl ether	4PC-BDE208	$C_6Br_5O^-$, $C_6Br_4ClO^-$, $[M-Br+O]^-$
2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether	BDE-209	$[M-Br+O]^-$, $C_6Br_5O^-$
Bis(2-ethyl-1-hexyl)tetrabromophthalate	BEHTBP	$[M-Br+O]^-$, $[M-C_8H_{17}+H-Br]^-$
octabromotrimethylphenylindane	OBIND	$[M-Br+O]^-$, $[M-HBr+O_2]^-$, $[M-HBr-Br+O]^-$
3,3',5,5'-tetrabromobisphenol A	TBBP-A	$[M-H]^-$, $[M-Br_2]^-$
allyl 2,4,6-tribromophenyl ether	ATE	$C_6Br_3H_2O^-$
2-bromoallyl 2,4,6-tribromophenyl ether	BATE	$C_6Br_3H_2O^-$, C_3H_6Br
2,3-dibromopropyl 2,4,6-tribromophenyl ether	DPTE	$C_6Br_3H_2O^-$

* Order with decreasing intensity if there was more than one ion generated in the APCI source.

Table 2: APCI source conditions for three separated runs*

Mobile phase run	Compound	CUR	NC	TEMP	GS1	CAD
1	α-HBCD, β-HBCD, γ-HBCD, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BB-153, a-DP, s-DP, HBB, PBEB, HCDBCO	10	-5	400	90	12
2	BDE-183, EHTeBB, BTBPE, BDE-205, 4PC-BDE208, BDE-209, BEHTBP, OBIND	10	-5	300	70	12
3	TBBP-A, ATE, BATE, DPTE	25	-5	675	30	12

*CUR, curtain gas; NC, nebulizer current; TEMP, source temperature; GS1, nebulizer gas; CAD, collision associated dissociation

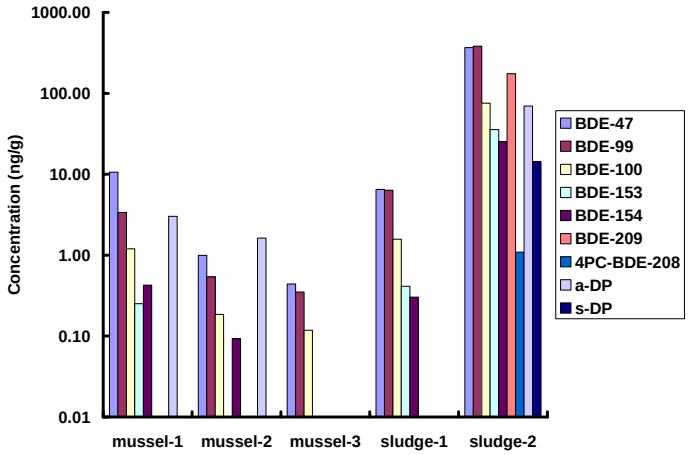

Figure 3: concentrations of halogenated flame retardants in environmental sample matrices

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