EVALUATION OF THE IONIZATION AND FRAGMENTATION BEHAVIOUR OF BROMINATED FLAME RETARDANTS UNDER GAS CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-TANDEM MASS SPECTROMETRY

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Introduction

Brominated flame retardants (BFRs) are used in a variety of products, such as housing and wiring of TV sets, computers and mobile phones, in electrical kitchen appliances, textiles, building materials and many plastic products, to reduce their flammability. Thus, exposure to BFRs may occur in many situations in daily life. Due to the fact that most BFRs are persistent, bioaccumulative and toxic or neurotoxic and can be potentially dangerous for the public and environmental health, the European Union (EU) has taken actions as regards to the use and applications of BFRs and it banned Penta-BDE and Octa-BDE mixtures in 2004 and Deca-BDE in electric and electronic products in 2009. In addition, limits of 0.1% by mass have been set for Penta-BDE and Octa-BDE in products placed on the market. As a result of bans, the use of novel BFRs, such as decabromodiphenyl ethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), used in replacement of Deca-BDE and Octa-BDE mixtures, respectively, and the use of organophosphorus flame retardants are increasing.
The analysis of these compounds relies on the use of methods that permit their unequivocal identification in environmental and biological matrices. Nowadays, gas chromatography (GC) coupled to mass spectrometry (MS) operating in both electron ionization (EI) and electron capture negative ionization (ECNI) modes using quadrupole, triple quadrupole or ion trap analyzers are widely used for the analysis of PBDEs[1].
In this study, GC coupled to triple quadrupole mass spectrometer with the new and soft atmospheric pressure chemical ionization source (GC-(APCI)MS/MS) has been explored for the determination of PBDEs, BTBPE and DBDPE in complex matrices. Favoring the formation of a highly abundant (quasi) molecular ion in the source has been the main goal because of the specificity added when choosing the molecular ion or protonated molecule as a precursor ion in tandem MS experiments. SRM transitions from different precursor to product ions have been evaluated at different collision energies in order to select the most intense and, if possible, to avoid interferents coming from other BFRs. Analytical parameters of the method such as linearity, repeatability and LODs have been studied.
This new soft ionization APCI source has been satisfactorily applied before for GC-amenable compounds such as pesticides, PAHs, PCBs and, very recently, dioxins and dioxin-like PCBs[2,3].

Materials and methods

A total of 15 PBDE congeners (numbers 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, and 209), plus two novel BFRs (BTBPE and DBDPE) (Wellington Laboratories, Ontario, Canada) were determined. Sample extraction and purification procedures are previously described in the literature[4]. Samples used for the evaluation of the applicability of the developed method gather samples belong to the "Interlaboratory Comparison on Dioxins in Food" organized by the Norwegian Institute of Public Health and some certified reference materials.
Data were acquired using a GC system (Agilent 7890A, Palo Alto, CA, USA) equipped with an autosampler (Agilent 7693) and coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), operating in APCI mode. Helium was used as carrier gas in constant flow mode (4 mL min^{-1}). The interface temperature was set to 340 ºC. The rest of chromatographic and mass spectrometric conditions were taken from previous works[2,5].

Results and discussion

Ionization and fragmentation behavior under EI and CI. Background knowledge.

In EI, the major ions formed from PBDEs are normally the $[M]^{+\bullet}$ and $[M\text{-}Br_2]^{+\bullet}$. Abundances of $[M]^{+\bullet}$ and $[M\text{-}Br_2]^{+\bullet}$ decrease with increasing bromine substituents being this effect more severe in the case of BDE 209 (**Figure 1A, bottom**). GC-(EI)qMS has not been used for the PBDE routine analysis, because of its relatively low sensitivity, especially for higher brominated BDE congeners ($Br_{7\text{-}10}$). It also shows interferences from other chemicals (especially PCBs). GC-(EI)HRMS has better sensitivity for the higher brominated congeners and could eliminate interferences between chlorinated and brominated compounds. However it requires higher maintenance costs and high level of experience.
The benefits of GC-(ECNI)qMS for the PBDE analysis are especially efficient ionization and higher sensitivity together with the capability of removing chlorinated interferences. However ECNI mass spectra are mainly dominated by the two ions of bromine (*m/z* 79 and 81) which difficult discrimination of PBDE congeners from different homolog groups, and among any bromo-compounds.
In GC-(EI)MS/MS experiments with QqQ and ITD, *m/z* ions coming from $[M]^{+\bullet}$ and $[M\text{-}Br_2]^{+\bullet}$ isotopic clusters are normally selected as precursor ions and consequently interferences from PCBs can be eliminated. However, co-eluting congeners with higher bromine content could still rise to isobaric precursor and product ions, which would contribute false signals to the MS/MS channel monitored for a specifically targeted PBDE.

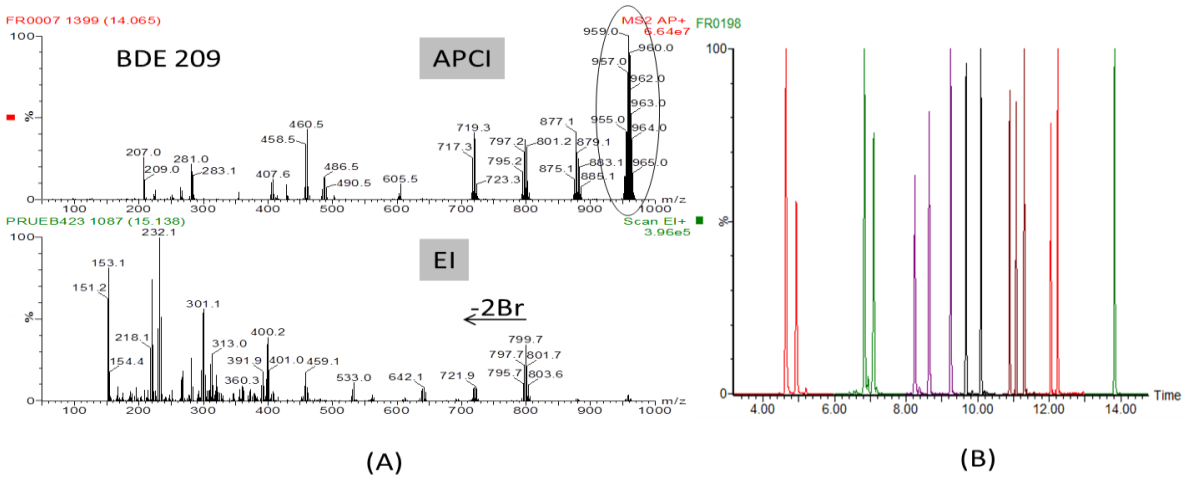

Figure 1. (A) APCI (up) and EI (bottom) spectrum of BDE 209. (B) GC-(APCI)MS/MS overlay SRM chromatograms for the lowest calibration level injected (1 ng mL^{-1} for $Br_{3\text{-}8}$ BDE and 5 ng mL^{-1} for BDE 209)

Ionization and fragmentation behavior of PBDEs, BTBPE and DBDPE in GC-(APCI)MS(/MS).

The "soft" ionization behavior of the new interface was tested using PBDEs, BTBPE and DBDPE standards in solvent. Two mechanisms of ionization are commonly observed under this APCI source: i) charge transfer in which the nitrogen plasma created by the corona discharge needle promotes the formation of $M^{+\bullet}$ and ii) proton transfer, where the presence of water vapur traces in the source favors the formation of the $[M+H]^+$ ion.
Under APCI, all the PBDEs studied showed a mixture of two isotopic patterns corresponding to $M^{+\bullet}$ and $[M+H]^+$ as base peak of the spectrum. **Figure 1A (up)** shows the APCI spectrum of BDE 209 where the isotopic cluster of $M^{+\bullet}/[M+H]^+$ can be observed as base peak of the spectrum. In the case of the $Br_{3\text{-}4}$ BDEs, the intensity of $[M+H]^+$ was higher than $M^{+\bullet}$. In contrast, for the $Br_{5\text{-}10}$ BDEs, the intensity of $M^{+\bullet}$ was a little higher than $[M+H]^+$ and increased with increasing bromine substituents. APCI mass spectrum of BTBPE also showed a mixture of two isotopic patterns corresponding to $M^{+\bullet}$ and $[M+H]^+$ (although in this case not as base peak). In the case of the mass spectrum of DBDPE, the base peak was a fragment corresponding to the ion $C_8H_4Br_5^{+\bullet}$ and neither $M^{+\bullet}$ nor $[M+H]^+$ was observed.

Cone voltage values between 5 and 50 V were tested in order to select the optimum value for each compound. No significant differences on in-source fragmentation pattern were observed, although voltages higher than 40 V generally led to a loss of abundance of the molecular ion and/or protonated molecule. For each compound, the optimized cone voltage that gave the highest intensity for the quasi-molecular ion (typically 20 V) was selected for further experiments.

Finally, the fragmentation of the PBDEs, BTBPE and DBDPE in the collision cell was studied. The molecular ion $[M]^{+\bullet}$, $[M+2]^{+\bullet}$ and $[M+4]^{+\bullet}$ were selected as precursor ions for the $Br_{3\text{-}4}$ BDEs; $[M+2]^{+\bullet}$, $[M+4]^{+\bullet}$ and $[M+6]^{+\bullet}$ for the $Br_{5\text{-}6}$ BDEs; $[M+4]^{+\bullet}$, $[M+6]^{+\bullet}$ and $[M+8]^{+\bullet}$ for the $Br_{7\text{-}10}$ BDEs. Fragmentation was performed at collision energies in the range 5-60 eV. The losses of Br_2 and Br_2+CO were the most abundant and common to all PBDEs studied. Moreover, the loss of Br_4 was observed for the $Br_{5\text{-}10}$ BDEs, Br_4+CO for $Br_{4\text{-}5}$ BDEs, and Br_5+CO for $Br_{5\text{-}6}$ BDEs. BTBPE showed the losses of $C_6H_2Br_3O$, $C_6H_2Br_4O$, and $C_6H_2Br_4O_2$ from the $[M+2]^{+\bullet}$, $[M+4]^{+\bullet}$ and $[M+6]^{+\bullet}$. DBDPE showed the losses of CH_2, Br, Br_2, and Br_3 from the fragment ion selected $C_8H_4Br_5^{+\bullet}$ ($[F+2]^{+\bullet}$, $[F+4]^{+\bullet}$ and $[F+6]^{+\bullet}$).

Ionization and fragmentation behavior of PBDEs in GC-(APCI)MS(/MS) adding water as modifier.

As explained above, all PBDEs showed the presence of $[M+H]^+$ and $M^{+\bullet}$ on APCI spectra. This fact encouraged us to introduce water as a modifier to promote the formation of the protonated molecule. Water was added on purpose as modifier and the presence/absence and/or improvement on the signal of the protonated molecule was evaluated.
The use of water as modifier favored the formation of the $[M+H]^+$ and the $M^{+\bullet}$ disappeared in most cases, increasing the proportion of $[M+H]^+$. These occurred for all the PBDEs studied except for the $Br_{7\text{-}10}$ BDEs and BTBPE for which a low percentage of $M^{+\bullet}$ still appeared. Protonation degree was improved for the BTBPE and $Br_{7\text{-}10}$ BDEs adding aqueous 1% HCOOH as modifier. The spectrum of DBDPE remained the same as without water.
Next, the PBDEs fragmentation in the collision cell was studied, by the selection of the protonated molecule as the precursor ion. The quasi-molecular ion $[M+H]^+$, $[M+H+2]^+$, and $[M+H+4]^+$ were selected as precursor ions for the $Br_{3\text{-}4}$ BDEs; $[M+H+2]^+$, $[M+H+4]^+$, and $[M+H+6]^+$ for the $Br_{5\text{-}6}$ BDEs; and $[M+H+2]^+$, $[M+H+4]^+$, and $[M+H+6]^+$ for the $Br_{7\text{-}10}$ BDEs. Again, fragmentation was performed at collision energies in the range 5-60 eV. The losses of Br and Br_3 were the most abundant and common to all the PBDEs studied. Then, losses of $CHBr_3O$ were observed for the Br_3 BDEs and CBr_4O for $Br_{4\text{-}5}$ BDEs. BTBPE showed the losses of $C_6H_2Br_3O$, $C_6H_2Br_3O$+Br, $C_6H_2Br_3O$+COBr, and $C_6H_2Br_3O$+Br+COBr from the $[M+H+2]^+$, $[M+H+4]^+$, and $[M+H+6]^+$. DBDPE showed the losses of CH_2, Br, Br_2, and Br_3 from the fragment ion selected $C_8H_4Br_5^{+\bullet}$ ($[F+2]^{+\bullet}$, $[F+4]^{+\bullet}$, and $[F+6]^{+\bullet}$).

Sensitivity and repeatability.

Sensitivity of the PBDEs, BTBPE and DBDPE was evaluated under the different conditions optimized. The sensitivity of the $Br_{3\text{-}4}$ BDEs increased 5 times when adding water as modifier while for the Br_5 BDEs, BTBPE and DBDPE remained the same and for $_{Br6\text{-}10}$ BDEs studied a 2-fold decrease was observed. When adding HCOOH 1%, the sensitivity remained almost the same as with only water.
The repeatability of response (n=10 at 25 ng mL^{-1}) was also studied for the different conditions. Results showed that the low reproducibility of response when working under charge transfer conditions (RSDs 13-25%) clearly improved under proton-transfer conditions (RSDs 7-14%) and became even better when the protonation was improved adding HCOOH 1% (RSDs 3-10%). This shows that, under charge transfer conditions a mixture of both molecular ion and protonated molecule occurred for some compounds and this formation was not stable and reproducible.

Specificity of the SRM transitions.

Despite the soft ionization character of APCI source, some in-source fragments are observed in the full scan spectra of the PBDEs. These are mainly losses of bromine atoms which also correspond to precursor ions of SRM transitions with lower brominated degree. This fact could led to a loose of specificity of the selected transitions in case of coelution of PBDEs with different bromine degree, and PBDEs with high bromine degree could interfere with the lower bromine degree ones. However, this fact only occurs when the experiments are performed under charge transfer conditions using the SRM transitions optimized that use $M^{+\bullet}$ as precursor ion. On the contrary, if precursor ions are $[M+H]^+$, this coincidence does not occur.

The addition of HCOOH 1% as modifier in the source for favoring the protonation was selected for further experiments due to the better repeatability and specificity of the SRM method with transitions selecting $[M+H]^+$ as precursor ion.

Linearity and LODs of selected optimized methodology.

Linearity of relative response of analytes was established by analyzing standards solutions, in triplicate, in the range of 1 – 500 ng mL^{-1} (for Br_{3-8} BDEs and BTBPE), 5 – 250 ng mL^{-1} (for BDE 209) and 10 – 5000 ng mL^{-1} (for DBDPE). Under proton transfer conditions, the correlation coefficients (r) were higher than 0.99, with residuals lower than 20% for all the compounds. LODs obtained were among 10-50 fg, being always lower than those previously reported in the literature using other ionizations sources[4,6-8]. The improvement in sensitivity obtained is more crucial for the highly brominated BDEs. Sensitivity of the method can be appreciated in **Figure 1B** (1 ng mL^{-1} for Br_{3-8} BDE and 5 ng mL^{-1} for BDE 209).

The developed method solves many of the disadvantages regarding the other techniques mentioned above. On the one hand, it solves the typical interferences coming from PCBs (GC-(EI)qMS) and from co-eluting PBDE congeners with higher bromine content (that may occurs in GC-(EI)MS/MS experiments with QqQ and ITD and also in GC-(ECNI)qMS). Moreover, it also shows high sensitivity for all the PBDEs, especially for the higher brominated PBDE congeners (even better than that obtained with GC-(ECNI)qMS and GC-(EI)HRMS). Finally, this technique requires lower maintenance costs and experience than GC-HRMS.

The developed method was finally applied to the analysis of the endogenous PBDE levels in samples belong to the "Interlaboratory Comparison on Dioxins in Food" organized by the Norwegian Institute of Public Health and some certified reference materials. As an example, cream sample was selected since it was contaminated with low levels of PBDEs (from 0.55 to 45 pg g^{-1} fresh weight). The concentrations obtained using the developed methodology were all within the range ($|z\text{-score}| < 2$) for all the congeners detected. It is important to note that only a small number of participants in the inter-laboratory comparison exercises are reporting PBDEs, compared with those reporting PCBs and PCDD/Fs. This fact, together with the high variability of the reported data (for some analytes close to 100%), illustrates the present difficulties in performing determinations of PBDE congeners in biological and food samples, and demonstrates the need for fast, accurate, selective, sensitive, and low cost methods, such as those proposed in this study. Unfortunately, the novel BFRs are still not included as target compounds in this type of inter-laboratory exercises.

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