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COMPARED ANALYTICAL BEHAVIOR (SEPARATION AND MASS SPECTROMETRIC DETECTION) OF POLYBROMINATED DIPHENYLETHERS AND TETRABROMOBISPHENOL A

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Introduction

The impact of brominated flame retardants on the environment and their potential risk for animals is a present time concern for the scientific community. Numerous studies relating analytical methods for tetrabromobisphenol A (TBBP-A) and polybrominated diphenylethers (PBDEs) have been developed for a few years ago, mainly based on GC-ECD[1], GC-NCI-MS[2] or GC-EI-HRMS[3]. Recently, tandem mass spectrometry has been used to analyze tri to heptaBDEs in human adipose tissue[4]. Despite a few number of PBDE congeners are commercially available, debromination[5] and degradation reactions[6] can lead to relatively high number of compounds to be identified. This study was devoted to the comparison of different analytical techniques based on mass spectrometry (LC-MS/MS, GC-MS/MS) for the measurement of main brominated flame retardant, with the further objective to be applied to their potential degradation products and/or metabolites.

Materials and Methods

MSTFA reagent was provided by Fluka (Buchs, Switzerland) *n*-Nonane solvent was provided by Sigma (Steinheim, Germany). Methanol and acetic acid (respectively HPLC and analytical grade) were provided by Solvents Documentation Syntheses (SDS, Peypin, France). Standards purchased from Cambridge Isotope Laboratories were monoBDEs (IUPAC numbers 1, 2, 3), diBDEs (7, 8, 10, 11, 12, 13, 15), triBDEs (17, 25, 28, 30, 32, 33, 35, 37), tetraBDEs (47, 49, 66, 71, 75, 77), pentaBDEs (85, 99, 100, 116, 118, 119, 126), hexaBDEs (138, 153, 154, 155, 166), heptaBDEs (181, 183, 190), decaBDE (209) and TBBP-A (^{13}C-labelled or native). Other congeners, obtained by photolytic degradation, were octaBDEs (numbers not determined) and nonaBDEs (206, 207, 208).

For LC-MS/MS optimization, an Alliance® 2690 HPLC pump with quaternary gradient system and automatic injector was used (Waters, Milford, MA, USA). Reversed phase liquid chromatography was realized on octadecyl grafted silica stationary phase Nucleosil® C_{18}AB (50 x 2.1 mm, 5 mm + guard column 10 x 2.1 mm) from Macherey-Nagel (Düren, Germany). Elution solvents were methanol (A) and water containing 0.5% (v/v) acetic acid (B). Mobile phase composition (A:B ; v/v) was 50:50 at 0 min, 95:5 from 10 to 15 min, and 50:50 from 17 to 22 min. Flow rate was 0.3 mL.min^{-1} and injected volume was 10 µL. Mass spectrometric data were acquired in negative ESI mode and in MRM mode, using a Quattro LC triple quadrupole (Micromass, Manchester, UK). The assessment of LC-APPI-MS was achieved using a Hypercard porous graphitic carbon LC column with hexane/dichloromethane solvent systems at a flow rate of

0.3 mL.min^{-1}. An LCQ DecaXP quadrupole ion trap (ThermoFinnigan, Les Ulis, France) fitted with the ThermoFinnigan APPI source was used for APPI-MS experiments.

For GC-MS/MS optimization, an HP 6890 gas chromatograph (Palo Alto, CA, USA) was used. Before injection, TBBP-A was derivatized in *n*-nonane/MSTFA (50:50, v/v) (ambient temperature, 15 min), leading to the diTMS derivative. Volumes of 1 to 2 µL were injected in the splitless GC injector (280°C, purge splitless 1 min). The gas chromatograph was fitted with a capillary column, 15 m x 0.25 mm id. x 0.25 µm film thickness, coated with a diphenyl(5%)-dimethylpolysiloxane(95%) stationary phase (DB-5MS). Helium was used as carrier gas at constant pressure of 82.74 kPa. The temperature program for PBDEs was from 130°C (held for 2 min) to 320°C (held for 8.5 min) at 20°C.min^{-1}, and the one for TBBP-A was from 110°C (held for 2 min) to 320°C (held for 8.5 min) at 30°C.min^{-1}. Mass spectrometric data were acquired on a VG Quattro II (Micromass, Manchester, UK) triple quadrupole mass spectrometer, in positive electronic impact and in MRM acquisition mode. The electronic beam energy was set at 70 eV.

Results and Discussion

Polybrominated diphenylethers (PBDEs)

PBDEs were mainly studied by GC-MS/MS. According to literature, the chromatographic conditions did not allowed the separation of BDE-8/11 and BDE-28/33. $[M]^{+\bullet}$ and $[M\text{-}Br_2]^{+\bullet}$ were the most abundant ions produced in the source. The fragmentation of these ions led to the sequential losses of bromine atoms, and possible subsequent loss of CO. More than ten transitions for each congener were monitored. The loss of two bromine atoms from the molecular ion correspond to the most sensitive transition. The observed LODs varied from 0.5 to 12.5 pg for mono to heptaBDEs (Table 1), in accordance to other studies[4]. Moreover, a discrimination about the different congeners inside homologue groups can be achieved on the basis of the diagnostic ion ratios. Total ion current chromatograms for PBDEs are showed in Figure 1.

In LC-MS/MS, ESI and APCI were found to be not suited for the analysis of PBDEs. On the other hand, preliminary experiments achieved using atmospheric pressure photo-ionization (APPI) gave promising results. Using hexane/dichloromethane as the solvent system, $[M]^{+\bullet}$ ions were observed as the molecular species generated in the APPI source, and $[M\text{-}Br_2]^{+\bullet}$ ions were the main fragment ions obtained under collisional excitation in MS/MS experiments achieved into an ion trap device.

Table 1 : Diagnostic MRM transitions and limits of detection (LOD) for homologue groups of PBDEs in GC-(EI+)-MS/MS. nd : not determined.

Homologue groups	First MRM transition	Second MRM transition	LOD (pg)
MonoBDEs	$[M]^{+\bullet}>[M\text{-}Br\text{-}CO]^{+}$	$[M]^{+\bullet}>[M\text{-}Br]^{+}$	1.1 to 1.5
DiBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_2\text{-}CO]^{+\bullet}$	0.7 to 2.0
TriBDEs	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_3\text{-}CO]^{+}$	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	0.5 to 2.5
TetraBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_4\text{-}CO]^{+\bullet}$	1.7 to 4.3
PentaBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_5\text{-}CO]^{+}$	1.4 to 7.5
HexaBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_5\text{-}CO]^{+}$	2.2 to 6.0
HeptaBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_5\text{-}CO]^{+}$	2.9 to 12.5
OctaBDEs	$[M]^{+\bullet}>[M\text{-}Br_2]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_5\text{-}CO]^{+}$	nd
NonaBDEs	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_5\text{-}CO]^{+}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_3\text{-}CO]^{+}$	nd
DecaBDE	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_4]^{+\bullet}$	$[M\text{-}Br_2]^{+\bullet}>[M\text{-}Br_3]^{+}$	nd

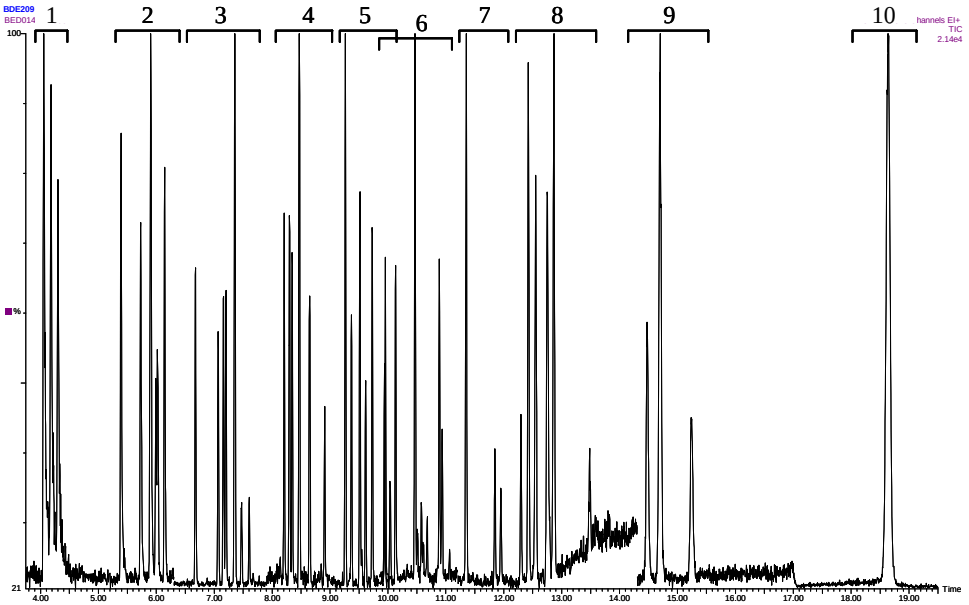

Figure 1 : GC-(EI+)-MS/MS total ion current chromatograms for PBDEs. 1 to 10 : mono to decaBDE homologue groups.

Tetrabromobisphenol A (TBBP-A)

In LC-MS/MS, fragmentation of the $[M-H]^-$ ion produced in ESI-, led to about ten product ions. The ionization potential (50 V) and the collision energies (Table 2) were optimized for each transition. The most sensitive ones allowed to identify TBBP-A according to the European Directive 2002/657/CE criteria with an estimated LOD at 150 pg.

In GC-MS/MS, fragmentation of the $[M]^{+\bullet}$ or $[M-CH_3]^+$ ions led also to about ten product ions. Collision energy for each transition was optimized. Chosen specific transitions (Table 2) permitted to achieve a LOD of 2,5 pg of TBBP-A.

Table 2 : Fragmentation parameters for analysis of TBBP-A by LC-(ESI-)-MS/MS, GC-(EI+)-MS/MS, and percentages of the base pic.

LC-(ESI-)-MS/MS MRM Transition	Collision energy (eV)	% of the base pic	GC-(EI+)-MS/MS MRM Transition	Collision energy (eV)	% of the base pic
$[M-H]^-$>$[Br]^-$	55	100	$[M]^{+\bullet}$>$[M-CH_3]^+$	20	100
$[M-H]^-$>$[M-H-HBr-CH_3]^-$	40	91	$[M-CH_3]^+$>$[M-C_9H_{11}OBr_2Si]^+$	20	44
$[M-H]^-$>$[M-H-HBr-CH_3-CO]^-$	50	69	$[M-CH_3]^+$>$[M-C_{10}H_{15}OBr_2Si]^+$	30	10
$[M-H]^-$>$[M-2H-C_6H_3OBr_2]^-$	40	38	$[M-CH_3]^+$>$[M-C_{10}H_{14}OBr_3Si]^+$	40	9

A comparison between ion chromatograms obtained by LC-(ESI-)-MS/MS and GC-(EI+)-MS/MS can be seen Figure 2. Advantages of GC-MS/MS are an efficient resolution that allow a good separation from potential interference signals from matrixes, and 50 fold better sensitivity compared to the LC-MS/MS. By LC-MS/MS analysis, no derivatization stage is needed and the advantage could be direct analysis of potential metabolites of TBBP-A and/or PBDEs.

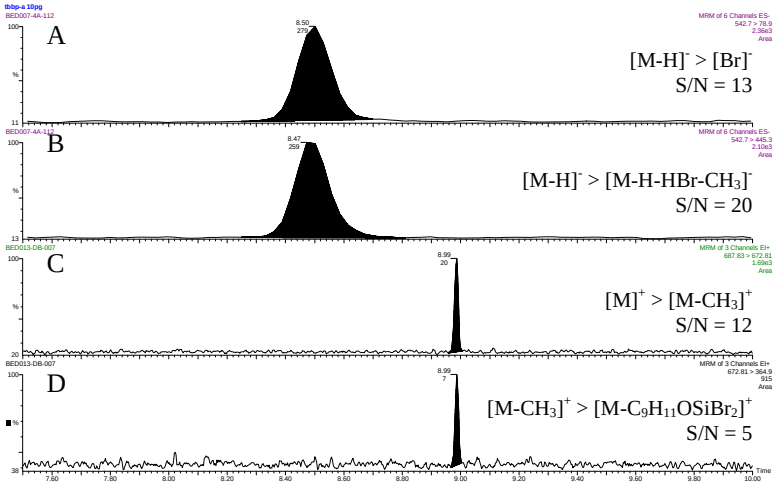

Figure 2 : LC-(ESI-)-MS/MS (AB, 1 ng) and GC-(EI+)-MS/MS (CD, 10 pg) MRM diagnostic ion chromatograms for TBBP-A.

Conclusion

Tandem mass spectrometry with triple quadrupole proved to be a good analytical choice for the analysis of TBBP-A and PBDEs at low concentration levels in biological matrices, especially when associated with the MRM acquisition mode that presents advantages in term of unambiguous identification and quantification. In term of separation, the resolution power of GC appeared clearly better than LC, for TBBP-A but almost for the separation of the different PBDE congeners. In term of ionization, APPI should constitute the ultimate choice for the analysis of PBDEs. Work is now in progress for determining the sensitivity of this technique.

References

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