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Optimization of congener-specific analysis of 40 polybrominated diphenyl ethers by gas chromatography/mass spectrometry

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The analysis by gas chromatography coupled with mass spectrometry (GC/MS) of 40 different congeners of polybrominated diphenyl ethers (PBDEs) containing 1–7 bromine atoms is described. Two different MS approaches were used, negative chemical ionization (NCI-MS) and electron ionization (EI-MS). Operating parameters such as electron energy and source temperature were optimized in order to obtain the maximum sensitivity in the EI-MS study. For NCI-MS analyses, the effects of the moderating gas (methane or ammonia), source temperature and system pressure were studied. The quality parameters of the two approaches tested were compared. NCI-MS gave detection limits between 30 fg and 1.72 pg, whereas EI-MS gave detection limits between 0.53 and 32.09 pg. The main advantage of EI-MS is that it provides better structural information. Moreover, the use of EI-MS allowed the use of an isotope dilution method for quantification, making the analysis more reliable at trace levels. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: polybrominated diphenyl ethers; flame retardant; gas chromatography/mass spectrometry; negative chemical ionization; electron ionization

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a wide variety of electrical appliances, including televisions and computers, building materials and textiles.¹ Structural similarity to other environmental chemicals with known toxic effects (PCBs, PBBs, dioxins) could indicate that PBDEs also could be harmful to health. The acute toxicity of PBDEs is low, but, there is concern regarding its long-term effects on the endocrine system.^{2,3} For this reason, there is a growing tendency to determine PBDEs in materials such as air,^{4,5} sediments,^{6,7} sludges,^{8,9} wildlife,^{10–12} human adipose tissue,^{13,14} human plasma¹⁵ and human milk.^{16,17}

In order to evaluate the global distribution, movement and fate of PBDEs in the environment, a sensitive, comprehensive and interference-free analytical method is required for their determination in complex environmental matrices. Several methods for the qualitative and quantitative analysis of PBDEs have been developed involving gas chromatography coupled with either negative chemical ionization (GC/NCI-MS)^{5,6,8,12} or electron ionization mass spectrometry (GC/EI-MS).^{18–21} Most of the analyses have concentrated

on only a few specific major PBDE congeners. However, a reliable method for the separation and ultra-trace quantification of individual congeners is required to determine the extent of environmental exposure, the risk associated with specific congeners and their fate in the environment.

Until recently, quantitative work has been performed by using technical PBDE products, e.g. Bromkal 70-5DE (BK70), owing to the lack of pure reference standards for most BDE congeners. The three major components in BK70 have been identified as BDE 47, BDE 99 and BDE 100,²² and only these congeners could be quantified. Since more than 30 BDE congeners now are available, it has become possible to measure each individual BDE. Moreover, the availability of some ¹³C-labeled standards allow the development of a methodology based on quantification by the isotopic dilution method. Some workers, such as Ryan and Patry²³ and Lebeuf and Trottier,²⁴ have used [¹³C]BDE surrogate standards. Previous reports had used other ¹³C- or ¹²C-labeled PCB and other organochlorine surrogates that, in general, give poorer precision and accuracy in the determination of the analytes.

Given the lack of a robust method for the simultaneous determination of 40 PBDEs, the purpose of this work was (i) the optimization of GC/NCI-MS analyses, (ii) the optimization of different parameters for GC/EI-MS, (iii) the characterization of each PBDE and (iv) a comparison between NCI and EI in their application to PBDE analyses. This paper reports the optimal analytical parameters of each

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approach. Special emphasis was focused on parameters such as sensitivity and selectivity.

EXPERIMENTAL

Chemicals

The Polybrominated Diphenyl Ether Analytical Standard Solution EO-4980 was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The components of this solution were three monoBDEs (BDE 1, 2 and 3), seven diBDEs (BDE 7, 8, 10, 11, 12, 13 and 15), eight triBDEs (BDE 17, 25, 28, 30, 32, 33, 35 and 37), six tetraBDEs (BDE 47, 49, 66, 71, 75 and 77), seven pentaBDEs (BDE 85, 99, 100, 105, 116, 119 and 126), six hexaBDEs (BDE 138, 140, 153, 154, 155 and 166) and three heptaBDEs (BDE 181, 183 and 190). Moreover, the mixture also contained five ^{13}C -labeled BDE congeners: two tetraBDEs (^{13}C BDE 47 and ^{13}C BDE 77) and three pentaBDEs (^{13}C BDE 99, ^{13}C BDE 100 and ^{13}C BDE 126). The concentrations of each compound ranged from $100\text{ pg }\mu\text{L}^{-1}$ for the mono congeners to $250\text{ pg }\mu\text{L}^{-1}$ for the hepta congeners. The decachlorinated biphenyl (PCB 209) and the commercial mixture Bromkal 70-5DE were purchased from Lab. Dr Ehrenstorfer (Augsburg, Germany).

Five different solutions were prepared in order to check the linearity of the method. These solutions contained the 40 PBDEs present in the EO-4980 mixture, at different concentrations ranging between 5 and $200\text{ pg }\mu\text{L}^{-1}$, with PCB 209 always at $100\text{ pg }\mu\text{L}^{-1}$.

Gas chromatography/mass spectrometry

GC/NCI-MS and GC/EI-MS analyses were performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 Network mass spectrometer. An HP-5ms capillary column ($30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness) containing 5% phenyl methyl siloxane (Model HP 19091S-433) was used with helium as the carrier gas at 10 psi. The temperature program was from 110°C (held for 1 min) to 180°C (held for 1 min) at 8°C min^{-1} , then from 180 to 240°C (held for 5 min) at 2°C min^{-1} , and then from 240 to 280°C (held for 6 min) at 2°C min^{-1} , using the splitless injection mode during 1 min.

GC/NCI-MS operating conditions

The GC/NCI-MS operating conditions were as follows: ion source temperature between 130 and 250°C , methane and ammonia as chemical ionization moderating gas at an ion source pressure between 1.2×10^{-4} and 2.7×10^{-4} Torr ($1\text{ Torr} = 133.3\text{ Pa}$). Initial experiments were carried out to optimize the NCI parameters such as chemical ionization moderating gas, source temperature and system pressure. All optimization experiments were carried out using the standard solution EO-4980 in the SIM mode. Two different reagent gases, methane and ammonia, were tested. The optimization of the source temperature was undertaken modifying its value from 130 to 200 and 250°C , and the optimization of the system pressure was carried out between 1.2×10^{-4} and 2.1×10^{-4} Torr (1.2×10^{-4} , 1.4×10^{-4} , 1.6×10^{-4} , 1.9×10^{-4} and 2.1×10^{-4} Torr) for NH_3 experiments, and between 1.3×10^{-4} and 2.7×10^{-4} Torr

(1.3×10^{-4} , 1.6×10^{-4} , 1.8×10^{-4} , 2.0×10^{-4} , 2.5×10^{-4} and 2.7×10^{-4} Torr) for CH_4 experiments.

GC/EI-MS operating conditions

The GC/EI-MS operating conditions were as follows: ion source temperature between 130 and 250°C , interface temperature 270°C and ionization energy between 30 and 70 eV. Initial experiments were conducted to optimize the EI parameters such as source temperature and electron energy. All optimization experiments were carried out using the commercial mixture Bromkal 70-5DE in the full-scan mode. The optimization of the source temperature was undertaken modifying its value from 130 to 200 and 250°C . The optimization of electron energy was carried out between 30 and 70 eV (30, 35, 45 and 70 eV).

Quantification

Three different methods for quantification were tested: external standard calibration, internal standard calibration using the PCB 209 as internal standard (PCB 209 is the most common compound reported as an internal standard for PBDE determinations in the literature) and the isotope dilution method. Quantification by the isotope dilution method is based on the use of five ^{13}C -labeled standards: ^{13}C tetraBDE 47, ^{13}C tetraBDE 77, ^{13}C pentaBDE 99, ^{13}C pentaBDE 100 and ^{13}C pentaBDE 126, and could only be used with GC/EI-MS.

RESULTS AND DISCUSSION

Mass spectra

NCI mass spectra

Mass spectra depend strongly on the type of ionization used. Two different reagent gases, methane and ammonia, were tested in the NCI system. For all the PBDEs studied, the NCI spectra were dominated by the mass fragment $[\text{Br}]^-$ (m/z 79, 81), and the molecular cluster was not observed or constituted a minority peak. These NCI spectra differed from the NCI spectra obtained for chlorine compounds such as PCBs, in which the molecular ion is the predominant peak.²⁵ As an example, Fig. 1 shows the mass spectra obtained for the hexaBDE 154 under methane-NCI and ammonia-NCI conditions. No significant differences were observed between the methane- and ammonia-NCI systems; both spectra showed the same peaks with similar relative intensities. Only some differences were observed between the isotopic ratio of specific clusters (e.g. m/z 329/331 and m/z 483/485) due to variation in the experimental conditions. The mass fragment $[\text{Br}]^-$ was the base peak and the molecular peak did not appear, but other fragments were observed at $<10\%$ of the $[\text{Br}]^-$ peak intensity. These fragments corresponded to the $[\text{HBr}_2]^-$ fragment (m/z 161) and fragments corresponding to the losses of one $[\text{M} - \text{HBr}]^-$ fragment, at m/z 562, two $[\text{M} - \text{HBr}_2]^-$ fragment at m/z 483, three $[\text{M} - \text{HBr}_3]^-$ fragment, at m/z 403 and four bromine atoms $[\text{M} - \text{HBr}_4]^-$ fragment, at m/z 329. The same structures were reported by Stemmler and Hites.²⁶ For the di-, tri- and tetra-BDEs, it was observed that the signal intensity of the $[\text{HBr}_2]^-$ fragment increased for the 2,4-substituted or

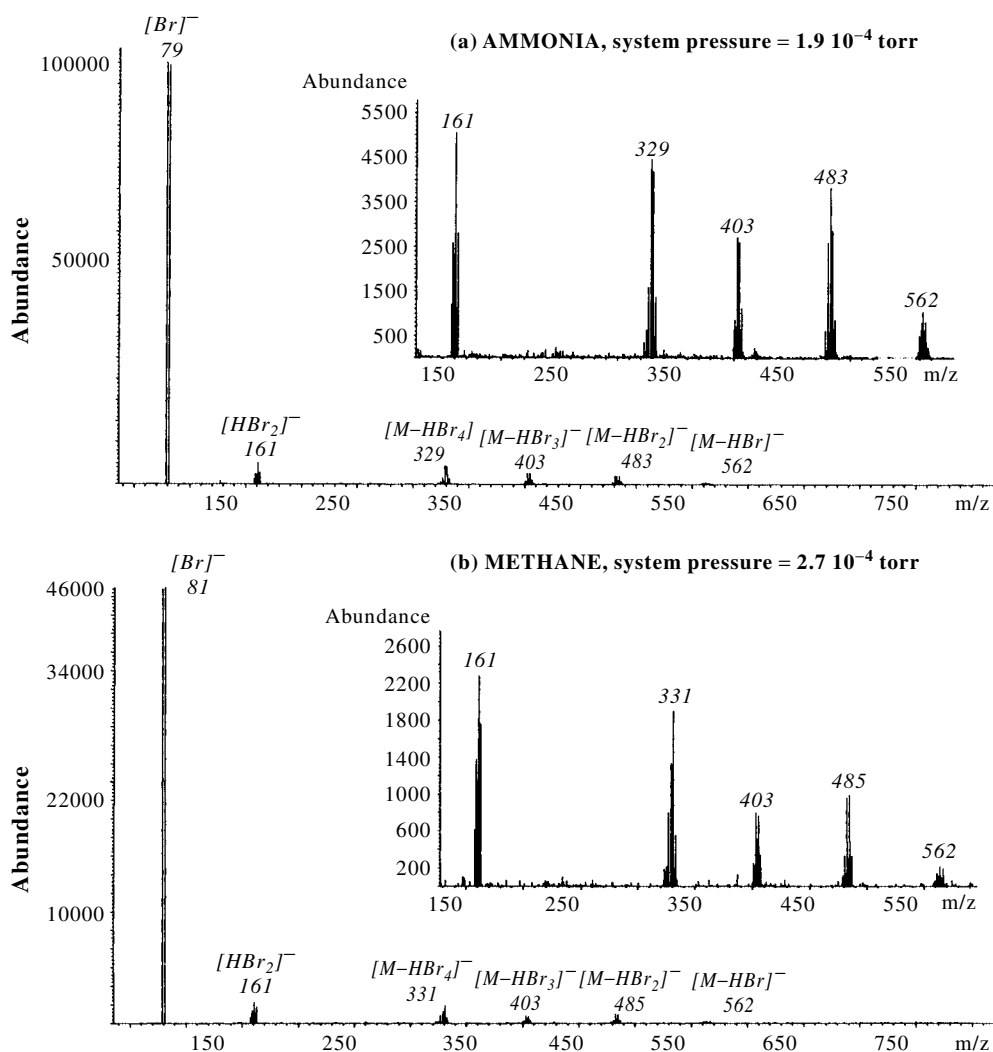


Figure 1. Negative chemical ionization mass spectra of hexaBDE 154 using (a) ammonia and (b) methane as moderating gas.

2,6-substituted isomers, and could represent more than 30% of the $[\text{Br}]^-$ peak intensity: 31% for BDE 10 (2,6), 65% for BDE 30 (2,4,6), 39% for BDE 32 (2, 4', 6), 42% for BDE 17 (2, 2', 4), 41% for BDE 25 (2, 3', 4), 36% for BDE 75 (2, 4, 4', 6), 41% for BDE 71 (2, 3', 4', 6), 53% for BDE 49 (2, 2', 4, 5') 43% for BDE 47 (2, 2', 4, 4') and 43% for BDE 66 (2, 3', 4, 4'). However, this fragment did not appear for BDE 11 (3, 3'), BDE 12 (3,4), BDE 13 (3, 4'), BDE 15 (4, 4') BDE 35 (3, 3', 4), BDE 37 (3, 4, 4') and BDE 77 (3, 3', 4, 4'). Moreover, small variations in $[\text{Br}]^-/[\text{M}]^-$ ratios with source temperature were observed.

GC/NCI-MS in the SIM mode was applied in order to enhance the sensitivity. The experiments were carried out monitoring the two most intense isotope peaks from the mass spectra corresponding to m/z 79 and 81 ($[\text{Br}]^-$), and additional fragment ions corresponding to $[\text{M}]^-$, $[\text{M} - \text{HBr}]^-$ or $[\text{M} - \text{HBr}_2]^-$. Chromatographic windows for each group of PBDE homologues (from mono- to hepta-BDEs) were defined. Each group eluted separately, with the exception of pentaBDEs and hexaBDEs, where hexaBDE 155 eluted before pentaBDE 105. For this reason, eight chromatographic windows were established, seven for each degree of bromination (from mono- to hepta-) and an additional one for the chromatographic region with elution

of penta + hexaBDEs. Table 1 lists the ions monitored for each group of PBDE homologues and the chromatographic windows. The most intense peak from the NCI spectra (m/z 79) was used for quantification purposes. Confirmation criteria for the detection and quantification of PBDEs should include the following: (a) all m/z monitored for a given analyte should maximize simultaneously $\pm 1\text{s}$, with signal-to-noise ratio ≥ 3 for each; and (b) the ratio between m/z 79 and 81 should be within 15% of the theoretical value of 1.03.

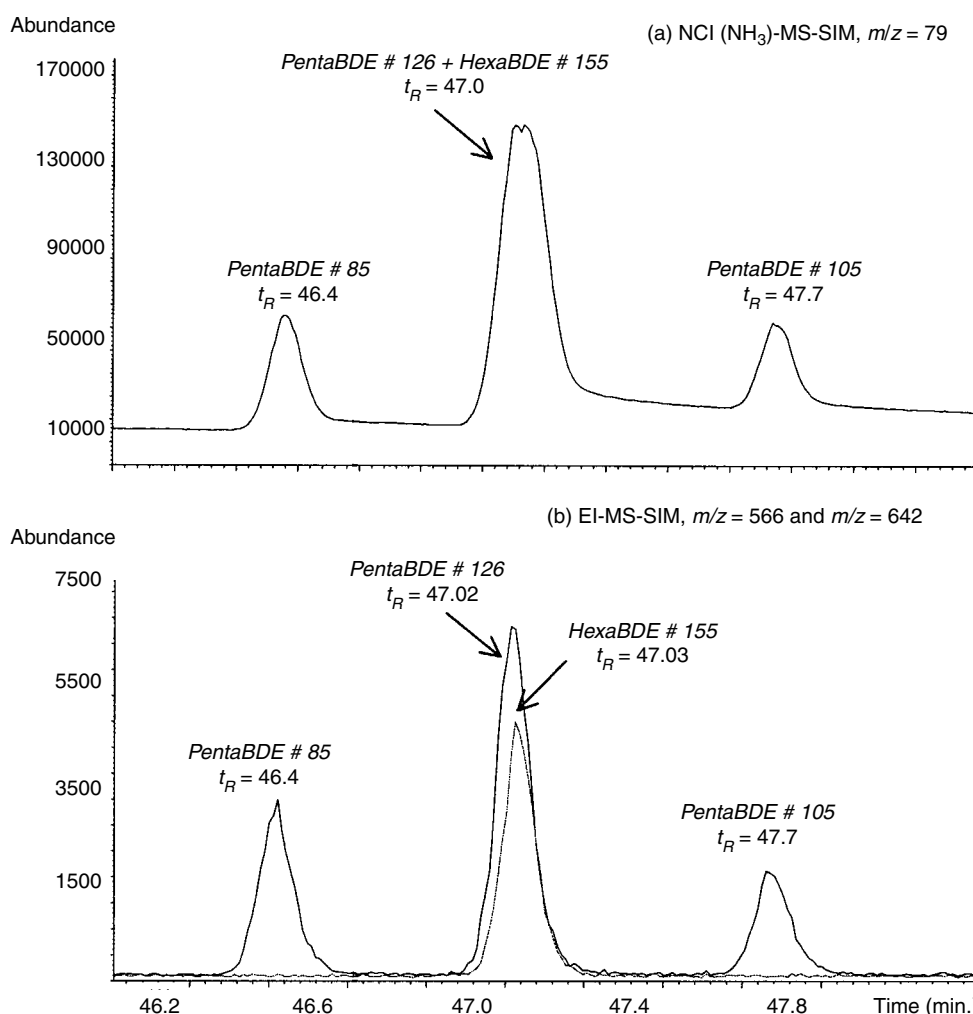
It should be pointed that under the conditions used, pentaBDE 126 co-eluted with hexaBDE 155. Since all the PBDE congener spectra were dominated by the same mass fragment $[\text{Br}]^-$ (m/z 79, 81), and these are the ions monitored in the NCI/MS-SIM experiments for quantitative purposes, the NCI system was not able to resolve this co-elution, and could not differentiate between the two compounds (Fig. 2).

EI mass spectra

Regarding the EI analyses, significant features of these spectra include the sequential losses of bromine atoms. Figure 3 shows the EI mass spectrum of pentaBDE 99: the most intense peak is at m/z 404, indicating the loss of two bromine atoms ($[\text{M} - \text{Br}_2]^+$), but the molecular ion at m/z 559

Table 1. Selected ions for the GC/NCI-MS-SIM experiments

| Compounds | Window (min) | Ions monitored (m/z) |
|-------------------|--------------|---|
| MonoBDEs | 4:00–13:00 | 79/81 [Br] [−] , 247[M] [−] |
| DiBDEs | 13:00–20:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 327[M] [−] |
| TriBDEs | 20:00–30:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 327[M − HBr] [−] |
| TetraBDEs | 30:00–39:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 327[M − HBr ₂] [−] |
| PentaBDEs | 39:00–45:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 405[M − HBr ₂] [−] |
| Penta- + hexaBDEs | 45:00–50:00 | 79/81 [Br] [−] , 643[M] [−] |
| DecaCB | | 498/500 [M] [−] |
| HexaBDEs | 50:00–58:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 483[M − HBr ₂] [−] |
| HeptaBDEs | 58:00–71:00 | 79/81 [Br] [−] , 161[HBr ₂] [−] , 563[M − HBr ₂] [−] |

**Figure 2.** Chromatograms of pentaBDE 126 and hexaBDE 155 obtained under (a) NCI-MS-SIM and (b) EI-MS-SIM conditions.

([M]⁺) also gives an intense peak. In general, for the mono- to tetra-BDE congeners, [M]⁺ is the predominant peak, whereas for the penta- to hepta-BDE congeners, [M − Br₂]⁺ is the most intense peak. Normally, the losses of two, four, etc., bromine atoms were more intense than the losses of one, three, etc. This behavior was also observed for the losses of chlorine atoms in organochlorine compounds.^{27–29} Variations in the ion abundances in the spectra of <10% were observed at different electron energies.

GC/EI-MS in the SIM mode was applied in order to enhanced the sensitivity. The experiments were carried out

monitoring the two most intense isotope peaks from the molecular ion region for each level of bromination, and also an additional abundant characteristic fragment ion corresponding to [M − Br₂]⁺. Chromatographic windows for each group of PBDE homologues (from mono- to hepta-BDEs) were defined. Table 2 lists the ions monitored for each group of PBDE homologues, the chromatographic windows and the ions used for quantification. The most intense peak from the molecular cluster was used for the quantification of the mono- to tetra-BDE congeners, whereas the fragment [M − Br₂]⁺ was selected for the quantification

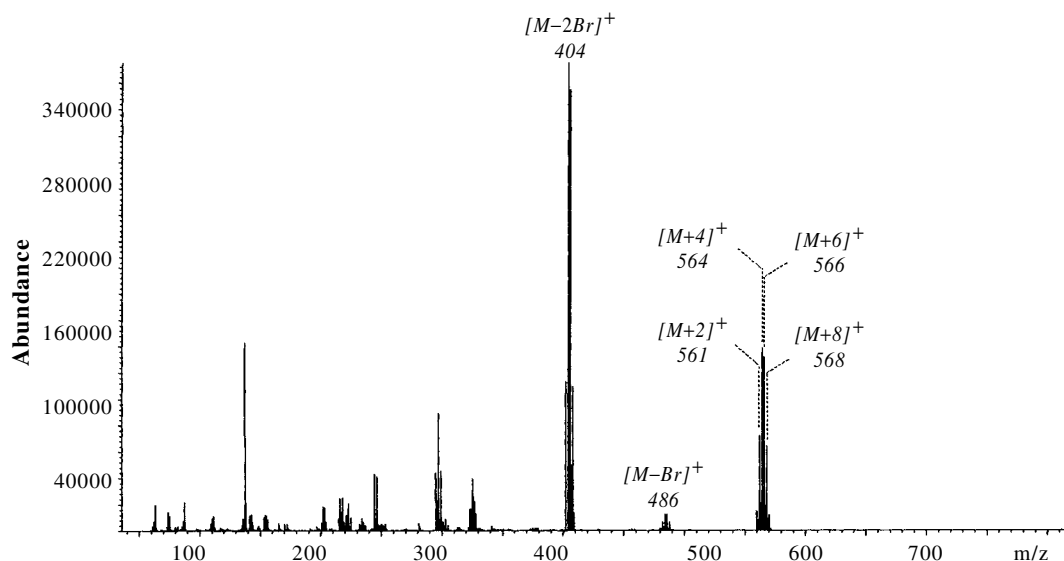


Figure 3. Electron ionization mass spectra of pentaBDE 99.

Table 2. Selected ions for the GC/EI-MS-SIM experiments

| Compounds | Window (min) | Ions monitored from molecular cluster (<i>m/z</i>) | | | | Additional ion monitored (<i>m/z</i>) |
|-----------------------------|--------------|--|---|-----|---|---|
| MonoBDEs | 4:00–13:00 | 248 ^a | [M] ⁺ | 250 | [M + 2] ⁺ | 169 [M – Br] ⁺ |
| DiBDEs | 13:00–20:00 | 326 | [M] ⁺ | 328 | [M + 2] ⁺ | 168 [M – Br ₂] ⁺ |
| TriBDEs | 20:00–30:00 | 406 | [M + 2] ⁺ | 408 | [M + 4] ⁺ | 246 [M – Br ₂] ⁺ |
| TetraBDEs | 30:00–39:00 | 484 | [M + 2] ⁺ | 486 | [M + 4] ⁺ | 326 [M – Br ₂] ⁺ |
| [¹³ C]TetraBDEs | | 496 | [¹³ C]-[M + 2] ⁺ | 498 | [¹³ C]-[M + 4] ⁺ | |
| PentaBDEs | 39:00–45:00 | 564 | [M + 4] ⁺ | 566 | [M + 6] ⁺ | 404 [M – Br ₂] ⁺ |
| [¹³ C]PentaBDEs | | 576 | [¹³ C]-[M + 4] ⁺ | 578 | [¹³ C]-[M + 6] ⁺ | |
| PentaBDEs | 45:00–50:00 | 564 | [M + 4] ⁺ | 566 | [M + 6] ⁺ | 404 [M – Br ₂] ⁺ |
| [¹³ C]PentaBDEs | | 576 | [¹³ C]-[M + 4] ⁺ | 578 | [¹³ C]-[M + 6] ⁺ | |
| HexaBDEs | | 642 | [M + 4] ⁺ | 644 | [M + 6] ⁺ | 484 [M – Br ₂] ⁺ |
| DecaCB | | 498 | [M + 4] ⁺ | 500 | [M + 6] ⁺ | |
| HexaBDEs | 50:00–58:00 | 642 | [M + 4] ⁺ | 644 | [M + 6] ⁺ | 484 [M – Br ₂] ⁺ |
| HeptaBDEs | 58:00–71:00 | 722 | [M + 6] ⁺ | 724 | [M + 8] ⁺ | 562 [M – Br ₂] ⁺ |

^a Ions selected for quantification are given in italics.

of penta- to hepta-BDE congeners. Confirmation criteria for the detection and quantification of PBDEs should include the following: (a) all *m/z* monitored for a given analyte should maximize simultaneously $\pm 1s$, with signal-to-noise ratio ≥ 3 for each; and (b) the ratio between the two ions of the [M]⁺ cluster should be within 15% of the theoretical value.

Regarding the co-elution of pentaBDE 126 with hexaBDE 155 observed in the NCI study, it should be pointed that the EI-MS-SIM mode allowed the separation of these two compounds by monitoring selected ions of each bromination group (Fig. 2). Therefore, the EI mode gives a better selectivity than the NCI mode. This is one of the main advantages of EI.

Optimization of analytical parameters

NCI(NH₃)-MS optimization

Different experiments were conducted to optimize the NCI(NH₃) parameters such as source temperature and

system pressure. The abundances of the ion monitored (*m/z* 79) vs system pressure and source temperature for each degree of bromination (from mono to hepta) are given in Fig. 4. Maximum abundances were obtained at 1.9×10^{-4} Torr and 250 °C for virtually all BDE congeners. Regarding the source temperature, 250 °C was optimal for all the congeners studied, with the exception of some diBDEs and triBDEs for which 200 °C gave better results. The abundance increase when working at 250 °C was 48% relative to 200 °C, whereas for some diBDEs and triBDEs the decreases were in the range 1–8%. Regarding the system pressure, the abundances increased with increase in system pressure up to 1.9×10^{-4} Torr. At system pressures $> 1.9 \times 10^{-4}$ Torr, the abundances decreased. This behavior was clearly observed for the mono-, di-, tri- and tetrabrominated congeners; however, for higher degrees of bromination (penta- to hepta-BDEs), the abundances remained virtually constant with system pressures $\geq 1.6 \times 10^{-4}$ Torr.

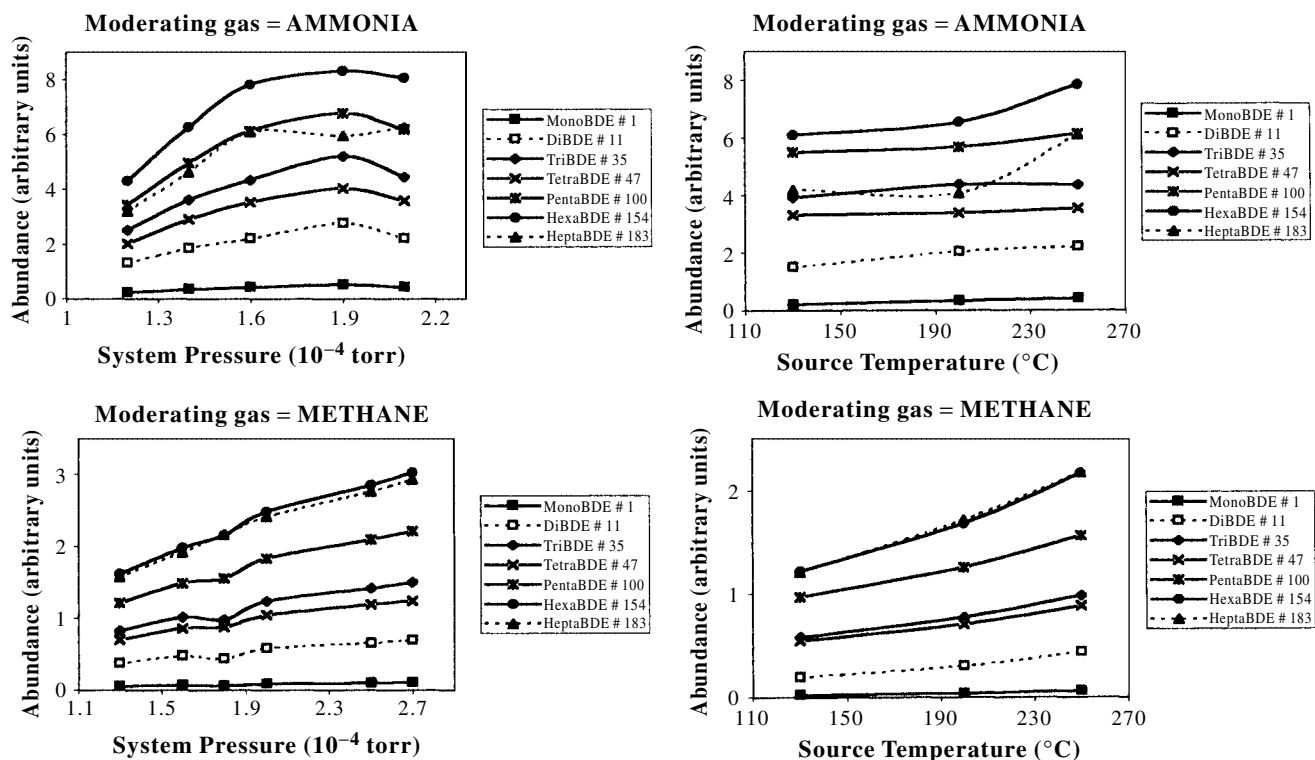


Figure 4. Variation of the abundance of m/z 79 for PBDEs vs system pressure and source temperature, using ammonia and methane as moderating gases.

NCI(CH_4)-MS optimization

Similar experiments were carried out to optimize the NCI(CH_4) parameters and the results are also given in Fig. 4. Maximum abundances were obtained at 2.7×10^{-4} Torr and 250°C for all BDE congeners. In this case, a source temperature of 250°C was optimal for all the congeners studied, without exceptions. The abundance increases when working at 250°C were between 15 and 45% relative to 200°C . Regarding the system pressure, the abundances increased with increase in system pressure: the abundance increases when working at 2.7×10^{-4} Torr were $\sim 50\%$ relative to 1.3×10^{-4} Torr.

EI-MS optimization

The optimization of the EI analyses was undertaken modifying the source temperature and the electron energy. The abundances of the TIC vs source temperature and electron energy for TeBDE 47 and pentaBDE 99 are given in Fig. 5. Maximum abundances of TIC were obtained at 250°C and 35 eV. Similar conditions were used to determine PCDDs and PCDFs by GC/EI-MS.³⁰

Quantitative analysis

In order to evaluate the three different approaches tested, different quality parameters such as linearity, intra- and inter-assay variation and sensitivity were studied and compared. The results are presented in Table 3.

Linearity

Calibration curves were determined for all 40 PBDEs by both EI-MS and NCI-MS. For the NCI-MS experiments, the linear

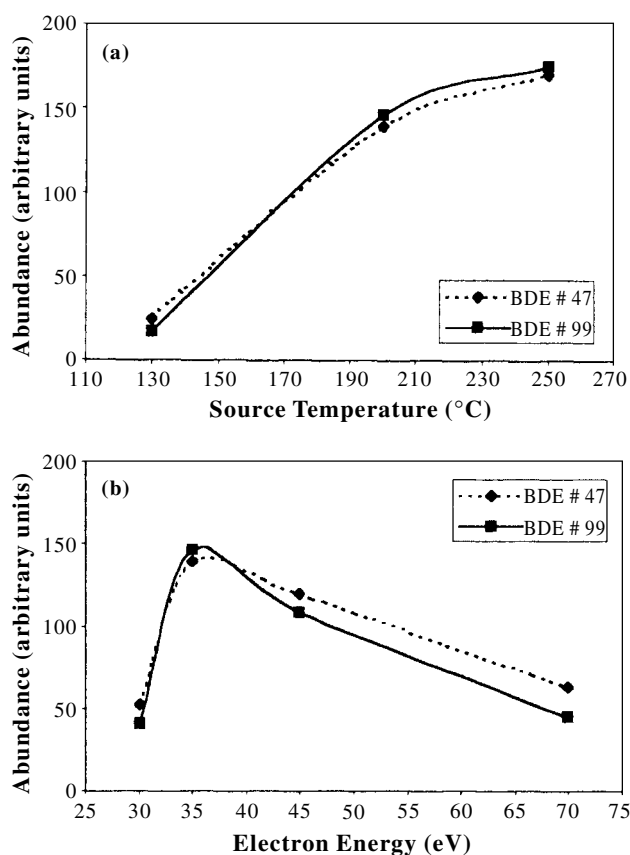


Figure 5. Variation of TIC abundance for tetraBDE 47 and pentaBDE 99 vs (a) source temperature and (b) electron energy.

Table 3. Comparison between the quality parameters obtained for the three ionization approaches tested: GC/NCI(NH₃)-MS, GC/NCI(CH₄)-MS and GC/EI-MS

| | Detection limit | | | Inter-assay variation (<i>n</i> = 4) (% C.V.) | | | | | | Intra-assay variation (<i>n</i> = 3) (% C.V.) | | | | | |
|------------------|-------------------------------|-------------------------------|------------|--|-----|-----------------------|-----|------|------|--|------|-----------------------|------|------|------|
| | NCI(NH ₃) (fg) | NCI(CH ₄) (fg) | EI (pg) | NCI(NH ₃) | | NCI(CH ₄) | | EI | | NCI(NH ₃) | | NCI(CH ₄) | | EI | |
| | | | | ES | IS | ES | IS | ES | IS | ES | IS | ES | IS | | |
| | | | | | | | | | | | | | | | |
| <i>MonoBDEs</i> | | | | | | | | | | | | | | | |
| BDE 1 | 572 | 528 | 0.86 | 2.4 | 6.4 | 4.8 | 4.3 | 2.9 | 3.4 | 6.4 | 7.8 | 3.9 | 8.1 | 2.5 | 12.8 |
| BDE 2 | 723 | 1056 | 0.61 | 2.6 | 5.8 | 8.2 | 7.9 | 3.5 | 4.4 | 8.2 | 10.2 | 4.6 | 12.1 | 0.7 | 13.8 |
| BDE 3 | 1716 | 1373 | 0.56 | 8.6 | 6.8 | 4.8 | 3.2 | 2.9 | 4.4 | 5.2 | 8.6 | 5.8 | 8.2 | 1.7 | 16.2 |
| <i>DiBDEs</i> | | | | | | | | | | | | | | | |
| BDE 10 | 51 | 64 | 1.02 | 2.6 | 4.5 | 2.0 | 1.7 | 6.9 | 4.5 | 3.4 | 6.5 | 5.6 | 5.3 | 6.4 | 7.8 |
| BDE 7 | 64 | 56 | 1.19 | 2.7 | 3.5 | 2.2 | 1.7 | 6.6 | 4.7 | 3.2 | 6.0 | 5.7 | 5.9 | 7.1 | 8.1 |
| BDE 11 | 99 | 106 | 0.95 | 3.8 | 3.0 | 1.9 | 1.4 | 7.2 | 4.4 | 3.4 | 6.0 | 5.0 | 6.2 | 10.1 | 4.5 |
| BDE 8 | 126 | 102 | 1.31 | 3.1 | 3.5 | 2.4 | 1.9 | 7.3 | 5.6 | 3.3 | 6.1 | 4.6 | 6.1 | 7.3 | 8.8 |
| BDE 12 + 13 | 154 | 108 | 0.53 | 4.1 | 2.7 | 2.2 | 1.6 | 6.7 | 5.5 | 3.1 | 6.0 | 5.5 | 6.4 | 7.9 | 7.2 |
| BDE 15 | 102 | 163 | 0.86 | 5.4 | 2.6 | 2.5 | 1.8 | 6.8 | 6.6 | 4.1 | 6.8 | 5.7 | 8.3 | 8.4 | 7.4 |
| <i>TriBDEs</i> | | | | | | | | | | | | | | | |
| BDE 30 | 33 | 89 | 1.62 | 3.2 | 4.5 | 0.9 | 1.8 | 6.1 | 2.9 | 2.3 | 5.1 | 5.5 | 4.5 | 18.9 | 6.4 |
| BDE 32 | 81 | 75 | 2.11 | 3.4 | 4.1 | 1.8 | 1.8 | 6.6 | 3.6 | 1.7 | 4.1 | 5.0 | 5.0 | 20.1 | 8.9 |
| BDE 17 | 79 | 86 | 1.72 | 2.6 | 2.4 | 1.2 | 1.6 | 7.2 | 4.5 | 2.3 | 4.4 | 5.1 | 5.1 | 19.8 | 8.5 |
| BDE 25 | 63 | 71 | 2.29 | 4.6 | 1.3 | 4.4 | 2.1 | 9.2 | 4.9 | 2.2 | 4.2 | 5.5 | 4.4 | 19.1 | 7.6 |
| BDE 28 + 33 | 89 | 91 | 0.69 | 4.5 | 2.4 | 2.3 | 1.1 | 6.6 | 3.3 | 3.0 | 3.3 | 5.5 | 5.2 | 21.3 | 9.3 |
| BDE 35 | 61 | 66 | 2.11 | 5.9 | 2.5 | 3.0 | 1.3 | 5.5 | 2.5 | 3.9 | 4.0 | 5.7 | 5.5 | 19.8 | 7.7 |
| BDE 37 | 89 | 92 | 1.83 | 7.4 | 3.4 | 2.8 | 1.0 | 7.0 | 3.0 | 3.6 | 4.3 | 6.0 | 5.5 | 22.0 | 9.6 |
| <i>TetraBDEs</i> | | | | | | | | | | | | | | | |
| BDE 75 | 69 | 60 | 1.53 | 3.8 | 0.7 | 2.2 | 0.8 | 6.5 | 3.1 | 3.8 | 2.7 | 5.7 | 3.4 | 23.2 | 10.9 |
| BDE 71 | 77 | 63 | 2.29 | 4.1 | 0.3 | 2.5 | 0.9 | 7.8 | 4.0 | 4.5 | 2.7 | 5.6 | 3.6 | 22.3 | 9.8 |
| BDE 49 | 51 | 64 | 2.75 | 4.3 | 0.4 | 2.6 | 1.0 | 7.0 | 2.9 | 3.4 | 3.1 | 5.5 | 3.6 | 21.4 | 8.8 |
| BDE 47 | 76 | 92 | 1.96 | 5.7 | 1.4 | 2.9 | 0.8 | 5.6 | 1.4 | 4.6 | 2.6 | 6.0 | 3.4 | 20.3 | 7.7 |
| BDE 66 | 79 | 89 | 3.43 | 7.2 | 2.9 | 2.9 | 0.7 | 9.7 | 6.1 | 5.6 | 4.0 | 6.1 | 3.5 | 28.1 | 16.3 |
| BDE 77 | 57 | 74 | 1.83 | 8.5 | 4.5 | 3.7 | 1.4 | 7.5 | 4.1 | 8.1 | 6.2 | 6.4 | 3.5 | 26.6 | 14.6 |
| <i>PentaBDEs</i> | | | | | | | | | | | | | | | |
| BDE 100 | 80 | 75 | 4.30 | 5.0 | 0.9 | 2.9 | 0.8 | 6.2 | 1.9 | 6.2 | 3.5 | 6.9 | 2.0 | 24.4 | 12.1 |
| BDE 119 | 68 | 69 | 3.91 | 6.3 | 2.1 | 2.9 | 0.7 | 7.3 | 3.3 | 7.1 | 4.1 | 6.8 | 2.1 | 27.5 | 15.7 |
| BDE 99 | 81 | 96 | 6.62 | 6.6 | 2.5 | 3.2 | 1.0 | 6.0 | 1.8 | 7.9 | 5.2 | 7.2 | 1.9 | 25.6 | 13.5 |
| BDE 116 | 115 | 80 | 8.60 | 6.1 | 1.9 | 3.3 | 1.0 | 10.8 | 6.6 | 6.0 | 4.1 | 7.2 | 2.4 | 29.0 | 17.3 |
| BDE 85 | 86 | 106 | 12.29 | 7.8 | 3.8 | 4.0 | 1.8 | 1.6 | 3.7 | 9.1 | 6.7 | 7.5 | 1.8 | 28.4 | 17.0 |
| BDE 126 | — | — | 17.20 | — | — | — | — | 11.5 | 9.9 | — | — | — | — | 29.4 | 19.9 |
| BDE 105 | 134 | 118 | 21.50 | 8.6 | 4.8 | 4.6 | 2.2 | 7.9 | 5.5 | 11.2 | 8.6 | 8.3 | 1.6 | 28.0 | 16.8 |
| <i>HexaBDEs</i> | | | | | | | | | | | | | | | |
| BDE 155 | — | — | 5.21 | — | — | — | — | 4.2 | 2.0 | — | — | — | — | 49.1 | 40.5 |
| BDE 154 | 61 | 99 | 2.55 | 5.3 | 1.3 | 3.4 | 1.1 | 6.4 | 2.1 | 8.9 | 5.8 | 8.3 | 0.8 | 11.0 | 4.8 |
| BDE 153 | 126 | 76 | 4.78 | 7.1 | 3.3 | 4.2 | 1.8 | 4.5 | 1.9 | 12.0 | 8.9 | 8.8 | 0.6 | 8.8 | 21.1 |
| BDE 140 | 54 | 62 | 3.96 | 7.4 | 3.7 | 4.1 | 1.7 | 5.2 | 3.7 | 12.6 | 9.3 | 8.6 | 0.2 | 34.6 | 23.5 |
| BDE 138 | 106 | 89 | 6.37 | 8.8 | 5.2 | 4.7 | 2.4 | 12.2 | 8.1 | 14.2 | 11.0 | 9.4 | 1.8 | 27.5 | 33.0 |
| BDE 166 | 168 | 158 | 8.19 | 8.3 | 4.6 | 4.8 | 2.5 | 8.5 | 9.5 | 12.1 | 9.2 | 9.6 | 1.9 | 26.1 | 15.0 |
| <i>HeptaBDEs</i> | | | | | | | | | | | | | | | |
| BDE 183 | 128 | 117 | 13.58 | 7.9 | 4.4 | 5.2 | 2.8 | 17.2 | 13.4 | 22.1 | 18.3 | 10.1 | 1.4 | 29.2 | 21.4 |
| BDE 181 | 197 | 177 | 29.42 | 11.4 | 8.0 | 6.2 | 3.8 | 3.0 | 6.9 | 30.2 | 26.2 | 12.4 | 4.2 | 17.4 | 13.1 |
| BDE 190 | 209 | 165 | 32.09 | 10.2 | 7.1 | 6.4 | 4.1 | 7.8 | 9.9 | 38.6 | 34.5 | 12.8 | 4.5 | 24.6 | 17.8 |

calibration range studied was from 5 to 200 pg, whereas for the EI-MS study the range was between 10 and 400 pg. In both cases, good correlations were obtained within the interval studied. However, better results were obtained for

the NCI mode with either CH₄ or NH₃, with correlation coefficients ranging between 0.9933 and 0.9999. When EI was used, the correlation coefficients ranged from 0.9556 to 0.9993.

Inter- and intra-assay variation

In order to evaluate the inter-assay variation of the methods used, four consecutive injections were performed under the optimum conditions for NCI and EI described in the previous section. The coefficients of variation (CVs) between the four values were calculated for all 40 PBDE congeners. The two quantitative methods proposed previously were applied, one based on an external standard and the other based on the use of an internal standard (PCB 209). Regarding the NCI system, the CV values were always <10%, indicating good inter-assay variation, and the experiments carried out with CH₄ gave CVs slightly better than those obtained with NH₃ as moderating gas. When the external standard method was applied, the CVs increased with increase in the degree of bromination (2–4% for the low-brominated BDEs and 6–10% for the high-brominated BDEs). However, when the internal standard method was used, the CVs were slightly better, always <8%, and remained more constant over all the range of bromination studied. Regarding the EI system, the CVs were higher than those obtained in the NCI study, ranging between 3 and 17% for the external standard method and between 1 and 13% for the internal standard method. As occurred in NCI, in the EI study the CVs increased increase in the degree of bromination, but this situation was observed for the two methods of quantification.

Three injections were carried out on three different days to establish the intra-assay variation of the methods. The same quantitative analyses as used for the inter-assay variation study were applied. As expected, the CVs obtained for the intra-assay variation were higher than those obtained for the inter-assay variation. Concerning the NCI system, more significant differences were observed between the two moderating gases tested. The CVs ranged between 2 and 39% with the NH₃ system and between 4 and 13% with the CH₄ system. In both cases, the CV values increased with increase in the degree of bromination. Regarding the experiments carried out with the EI mode, a poor intra-assay variation was observed. For most of the 40 PBDEs tested, the CVs exceeded 20%. This situation was improved with the use of an internal standard method, but in most cases the CVs remained >15%. The large variation of the intra-assay variation using the internal standard method was mainly observed for mono-, di- and tri-BDEs with both NCI and EI, because the internal standard PCB 209 elutes in the same window as the penta- and hexa-BDEs (see Table 2), which supposes a different response factor from the lower brominated BDEs.

Since some isotopically labeled PBDE standards (uniformly ¹³C labeled) have become readily available, use of these standards is preferred over external or internal calibration techniques. This quantitative method was also tested in the EI system, and the inter- and intra-assay variations were greatly enhanced with respect those obtained previously. As an example, the relative response factors (RRFs) for the tetra- and penta-BDEs were calculated with respect to [¹³C]tetraBDE 47 and [¹³C]pentaBDE 99. The inter-assay variation of these RRFs was between 1 and 5% for the tetra-BDEs and between 1 and 10% for the penta-BDEs. Regarding the intra-assay variation, the ranges were from 3 to 7% and

from 4 to 20% for the tetra- and penta-BDEs respectively. The CV values obtained with this isotope dilution method were <10%, indicating good inter-assay variation, and below <20%, indicating good intra-assay variation.

Sensitivity

The limit of detection (LOD), defined as the minimum amount of analyte which produces a peak with an acceptable bromine isotope ratio and with a signal-to-noise ratio of 3, was calculated for each compound. Better LODs were obtained in the GC/NCI-MS experiments: NCI-MS gave LODs between 30 fg and 1.72 pg, whereas EI-MS gave LODs between 0.53 and 32.09 pg. Regarding the NCI system, the LODs were similar from the di- to hepta-BDEs; however, the sensitivity decreased considerably for the mono-BDEs. The LODs obtained for the two reagent gases tested were similar, and only slight differences were observed. For the EI mode, the LODs increased with increase in the degree of bromination.

CONCLUSIONS

The specific analysis of 40 PBDE congeners was optimized by three different approaches: GC/NCI(NH₃)-MS, GC/NCI(CH₄)-MS and GC/EI-MS. The mass spectra, analytical parameters and quality parameters of the these approaches were compared. No structural information on the degree of bromination was obtained by NCI. The mass spectra of all PBDEs were dominated by the molecular ion [Br]⁺ and did not show any molecular ion. However, EI provided better structural information, giving the molecular ions and the sequential losses of bromine atoms.

The optimization of analytical parameters showed that the best working conditions were system pressure 1.9×10^{-4} Torr and source temperature 250 °C for NCI(NH₃), system pressure 2.7×10^{-4} Torr and source temperature 250 °C for NCI(CH₄) and electron energy 35 eV and source temperature 250 °C for EI.

The study of the quality parameters showed good inter- and intra-assay variations for the NCI mode, and slight differences were observed between NCI(NH₃) and NCI(CH₄). Regarding the EI mode, the inter- and intra-assay variations were greatly improved with the use of an isotope dilution method. Regarding the sensitivity, NCI showed LODs than EI: NCI gave ~15 times higher responses than EI for PBDEs.

The methods proposed are recommended for the detection of PBDEs in environmental samples, given the low detection limits obtained and high robustness. Future work will involve the characterization of minor PBDEs in environmental samples.

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