

Determination of polybrominated diphenyl ethers (PBDEs) using liquid chromatography coupled to negative ionisation atmospheric pressure photoionisation tandem mass spectrometry (LC-NI-APPI-MS/MS): validation and application to house dust.

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Abstract

Fourteen tetra- to deca- PBDE congeners were separated on a C₁₈ reversed phase LC column. PBDEs 47, 85, 99, 100, 153, 154, 183, 196, 197, 203, 206, 207, 208 and 209 were eluted using a gradient of methanol: water: toluene mobile phase system at a flow rate of 0.5 ml min⁻¹. ¹³C-BDE-47, ¹³C-BDE-99, ¹³C-BDE-153, BDE-128 and ¹³C-BDE-209 were used as internal standards while ¹³C-BDE-100 was used as a syringe standard. Separated analytes were ionised using an APPI source equipped with a 10 eV krypton lamp and operated in negative ion mode. [M-Br+O]⁻ ions were monitored as precursor ions for all studied BDEs except for BDE-208, BDE-209 and ¹³C-BDE-209 which produced higher intensity at the [C₆Br₅O]⁻ ion cluster. [Br]⁻ ions were monitored as fragment ions for all target compounds. Linear five point calibration curves (r²=0.996 to 0.998) were constructed for each of the studied PBDEs in range 20-5000 pg μl⁻¹. Method detection limits ranged from 12.3 to 29.8 pg. The method was applied for determination of PBDEs in SRM 2585 and favourable results were obtained. Unlike GC methods; no thermal degradation is encountered in the analysis of higher brominated PBDEs. In addition, this method allows the use of ¹³C-labelled internal standards which can compensate for any instrumental fluctuations and/or any matrix-related ion suppression or enhancement that can occur in the ion source.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of brominated compounds used widely as flame retardants. They are produced commercially in 3 main formulations: Penta (mainly BDEs 47 and 99 - 38-49% each, alongside other tri- to hepta-BDEs), Octa (hexa- to deca-BDEs mixture- the exact congener composition varies between the two principal formulations marketed) and Deca (92-97% decabromodiphenyl ether, plus various nona-(principally) and octa-BDEs)¹. The main uses for these commercial formulations are: the Penta-product to flame retard polyurethane foams in carpets, vehicle interiors, furniture and bedding, as well as printed circuit boards and microprocessor packaging in computers; the Octa-formulation (along with other flame retardants) to treat thermoplastics such as high impact polystyrene (HIPS) and acrylonitrile-butadiene-styrene copolymers (ABS); and the Deca-product in plastic housings for electrical goods like TVs and computers, as well as textiles². Several studies have discussed the levels of PBDEs in both biotic and abiotic matrices^{3, 4, 5}. Moreover, the extraction, chromatographic separation, and MS detection of PBDEs has recently been reviewed^{6, 7}. However, a relative lack of sensitivity is observed with GC-EI/MS analysis of higher brominated PBDEs (more than 6 Br). Therefore, electron capture negative ionization (ECNI), based on monitoring *m/z* 79 and 81 bromide ions, is the most widely used method for the analysis of higher PBDEs (hepta to deca congeners)⁶. This method does not provide mass discrimination between native and ¹³C-labelled isomers, and hence prevents the use of the latter as internal standards except for ¹³C-BDE-209 which produces the [C₆Br₅O]⁻ ions in abundance⁸. The GC-ECNI/MS analysis of BDE-209 is known to be difficult due to thermal degradation problems, which necessitates shorter columns with higher temperature limits and higher phase ratios to minimise on-column degradation. Additionally, optimized injection techniques (i.e., on-column injection, pressure pulses, split vent timing) are required to minimise the time spent in the heated injector zone to prevent thermal decomposition^{6, 7}. The thermal degradation problems encountered with GC analysis are usually avoided using LC-based methods. Recently, atmospheric pressure photoionisation (APPI) has appeared as a very soft ionisation technique, allowing the analysis of hydrophobic compounds using LC-MS/MS. Riu et al.⁹ reported the use of APPI ion source in NI mode to give "promising" results in the analysis of tetra-deca BDEs using LC-MS/MS (quadrupole ion trap). No such results were obtained using ESI or APCI sources. However, no quantitative data were provided. Recently, Lagalante and Oswald¹⁰ reported on the use of LC-APPI-MS/MS for determination of 8 PBDEs (octa and nona-BDEs not included) in dust using an external standard method (i.e. no internal standard used).

In light of the above, the aims of this study are: (1) to develop and validate a new analytical method using LC-NI-APPI-MS/MS for determination of 14 PBDEs in one run using ^{13}C -labelled isotopes as internal standards; (2) to apply the developed method for determination of PBDEs in indoor dust samples; (3) to investigate the debromination of BDE-209 in the studied dust samples and (4) to compare the results of the developed method to those obtained using a well established GC-ECNI-MS method¹¹.

Materials and Methods

LC-APPI-MS/MS analysis: Target PBDES were separated using a dual pump Shimadzu LC-20AB Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with SIL-20A auto sampler, a DGU-20A3 vacuum degasser and a Varian Pursuit XRS3 (Varian, Inc., Palo Alto, CA, USA) C₁₈ reversed phase analytical column (250 mm × 4.6 mm i.d., 3 μm particle size). A mobile phase program based upon (a) 85:10:5 methanol/toluene/water and (b) 1:1 methanol/water at a flow rate of 500 μL min⁻¹ was applied for elution of the target compound; starting at 85% (a) then increased linearly to 100% (a) over 30 min then held for 25 min. The column was equilibrated with 85% (a) for 5 min between runs. Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an APPI ion source operated in negative ion mode. Toluene was used as a doping agent introduced to a separate dopant port of the APPI source using a dedicated isocratic HPLC pump (Jasco PU-2800, Easton, MD, USA) at 12% of the flow rate of the mobile phase. Direct infusion of the target compounds into the MS/MS system was performed using a built-in Harvard syringe pump at a flow rate of 10 μl/min. The infusion experiments served for the tuning and adjusting the source and the compound-specific parameters during method development.

Dust sampling: Dust samples were collected between September 2006 and June 2007 from Homes (n=9), offices (n=8) and cars (n=8) in Birmingham, UK. Further details of sampling methodology, extraction and clean-up of dust samples can be found elsewhere¹².

GC-ECNI-MS analysis: This was conducted in the University of Antwerp using a well-defined methodology. Further details can be found elsewhere¹¹.

Results and Discussion

Optimization of APPI parameters: Toluene was used as the doping agent in preference to acetone, due to its more favorable ionisation energy and lower proton affinity that combine to facilitate better proton transfer reaction with the analytes. Maximum sensitivity was obtained upon introduction of dopant to the APPI source at 12% of the mobile phase flow rate. Methanol in the mobile phase produced higher sensitivity than acetonitrile. However, the use of mobile phase modifiers (e.g ammonium acetate 1-10 mM) had no significant effect on the intensity of the produced peaks. The optimised source parameters are given in table 1.

Compound-specific parameters: Stable $[\text{M}-\text{Br}+\text{O}]^-$ ions were formed for all the studied PBDEs via a substitution reaction between $[\text{M}-\text{Br}]^-$ ions and O_2 present in the ambient air of the APPI source or produced by thermal decomposition of oxygen-containing solvent molecules. The $[\text{M}-\text{Br}+\text{O}]^-$ ions were monitored as precursor (qualifier) ions at Q1 for all the studied compounds except for BDE-208 BDE-209 and ^{13}C -BDE-209 where a higher intensity ion cluster was observed for $[\text{C}_6\text{Br}_5\text{O}]^-$ ions which were thus selected as precursor ions for these 3 compounds. The $[\text{M}-\text{Br}+\text{O}]^-$ ions formed can fragment to produce $[\text{M}'-\text{HBr}]^-$ and/or $[\text{M}'-\text{Br}_2]^-$ ions with varying intensities depending on the pattern of Bromine substitution on the 2 phenyl rings of the studied BDE congener. However, maximum sensitivity was obtained when using a high collision energy to obtain $[\text{Br}]^-$ ions which were thus monitored as fragment (quantifier) ions at Q3 for all target compounds (table 2).

The collision energy (CE) and declustering potential (DP) were optimized for each target compound to produce maximum sensitivity (table 2). Linear five point calibration curves ($r^2=0.996$ to 0.998) were constructed for each of the studied PBDE congeners in the concentration range 20-5000 pg μl⁻¹. Inter- and intra-day variability were assessed via replicate injections of the same standard on the same day (n=3) and different days (n=3), and found to be minimal (RSD<10%). Method limits of detection (LODs) ranged from 12.3 to 29.8 pg per target compound on column (table 2). Target PBDEs were well-resolved on a retention time basis (figure 1) and from the ^{13}C -labelled analogues on MRM basis (e.g. BDE-99 was monitored at MRM 500.8→78.8 while ^{13}C -BDE-99 was monitored at 512.4→78.8). No interference between the native PBDEs and their ^{13}C -labelled analogues was observed even at a 100:1 concentration ratio.

Validation: The developed method was validated by applying it to the determination of PBDEs in NIST SRM 2585 (Organics in House Dust). SRM 2585 has certified values for 10 of the 14 PBDEs studied. The results obtained compared favourably to the certified values (figure 2) with internal

standard recoveries ranging from 71-87% and RSD < 10% for all target BDEs indicating good accuracy and precision of the developed method.

Comparison with GC-ECNI/MS: The developed LC-APPI-MS/MS method was further validated by analyzing 25 indoor dust samples and comparing the results to those obtained from a well-established GC-ECNI/MS method. While paired t-test and F-test revealed no statistically significant differences between the results, Pearson correlation coefficient (r) and slope values of the linear correlation between the 2 sets of results approaching 1 for all the studied congeners indicate excellent agreement between the results obtained from both techniques. (table 3).

Assessment of BDE-209 debromination: Since on-column thermal degradation of higher BDEs was not encountered, the developed method proved useful for investigating the debromination of BDE-209 in indoor dust. A representative example is given in figure 3 of a car dust sample where BDE-209 debromination is evident from:

- the ratio of BDE-208/BDE-209 = 1.46%. This ratio does not exceed 0.08% in the commercial formulations¹.
- detection of octa BDE-202. This has never been identified in any commercial BDE formulation and was recently reported to arise from the photodegradation of BDE-209 in house dust¹³.
- 197/201 = 1.72 %. This ratio is 28-35% in the commercial formulations and was recently reported by Stapleton et al.¹³ to decrease as a result of photodebromination of BDE-209 in house dust resulting in the production of BDE-201.

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Tables

Table 1: Optimised APPI source parameters

Parameter	Value
Curtain gas	25 psi
Collision (CAD) gas	High
Ion transfer voltage (IS)	1250 V
APPI temperature	400 °C
Probe nebuliser gas	60 psi
Auxillary gas	30 psi

Table 2: Optimised compound-specific parameters, linearity and LODs of target compounds.

BDE #	Precursor (m/z)	Fragment (m/z)	DP (V)	CE (V)	Linearity (0.02-5 ng/µl)	LOD (pg)
47	420.8	78.8	-12	-55	$r^2 = 0.997$	29.8
85	500.8	78.8	-8	-60	$r^2 = 0.996$	26.1
99	500.8	78.8	-11	-60	$r^2 = 0.996$	12.3
100	500.8	78.8	-14	-63	$r^2 = 0.998$	15.2
153	578.8	78.8	-8	-65	$r^2 = 0.996$	18.3
154	578.8	78.8	-8	-65	$r^2 = 0.997$	20.1
183	658.6	78.8	-12	-61	$r^2 = 0.998$	16.2
196	738.6	78.8	-14	-71	$r^2 = 0.996$	15.7
197	738.6	78.8	-12	-71	$r^2 = 0.997$	14.9
203	738.6	78.8	-15	-71	$r^2 = 0.997$	14.2
206	816.6	78.8	-11	-75	$r^2 = 0.996$	13.6
207	816.6	78.8	-12	-69	$r^2 = 0.996$	13.3
208	486.6	78.8	-10	-71	$r^2 = 0.997$	13.8
209	486.6	78.8	-8	-75	$r^2 = 0.996$	12.7

Table 3: Statistical comparison between concentrations of PBDEs in dust samples (n=25) obtained by GC-ECNI-MS and LC-APPI-MS/MS.

Statistical parameter*	BDE 183	BDE 197	BDE 203	BDE 196	BDE 209
t-test** paired for means	1.85	1.54	1.96	1.95	0.44
F-test*** for variance	0.41	0.43	0.38	0.39	0.26
Pearson correlation (r)	0.998	0.999	0.997	0.998	0.999
Slope GC vs LC	1.03	1.03	1.06	1.05	0.99

* Confidence level set at 95%

** t-critical = 2.06

*** F-critical = 0.52

Figures

Figure 1: Separation of target PBDEs on RP-C₁₈ column (250 x 4.6 mm; 3µm).

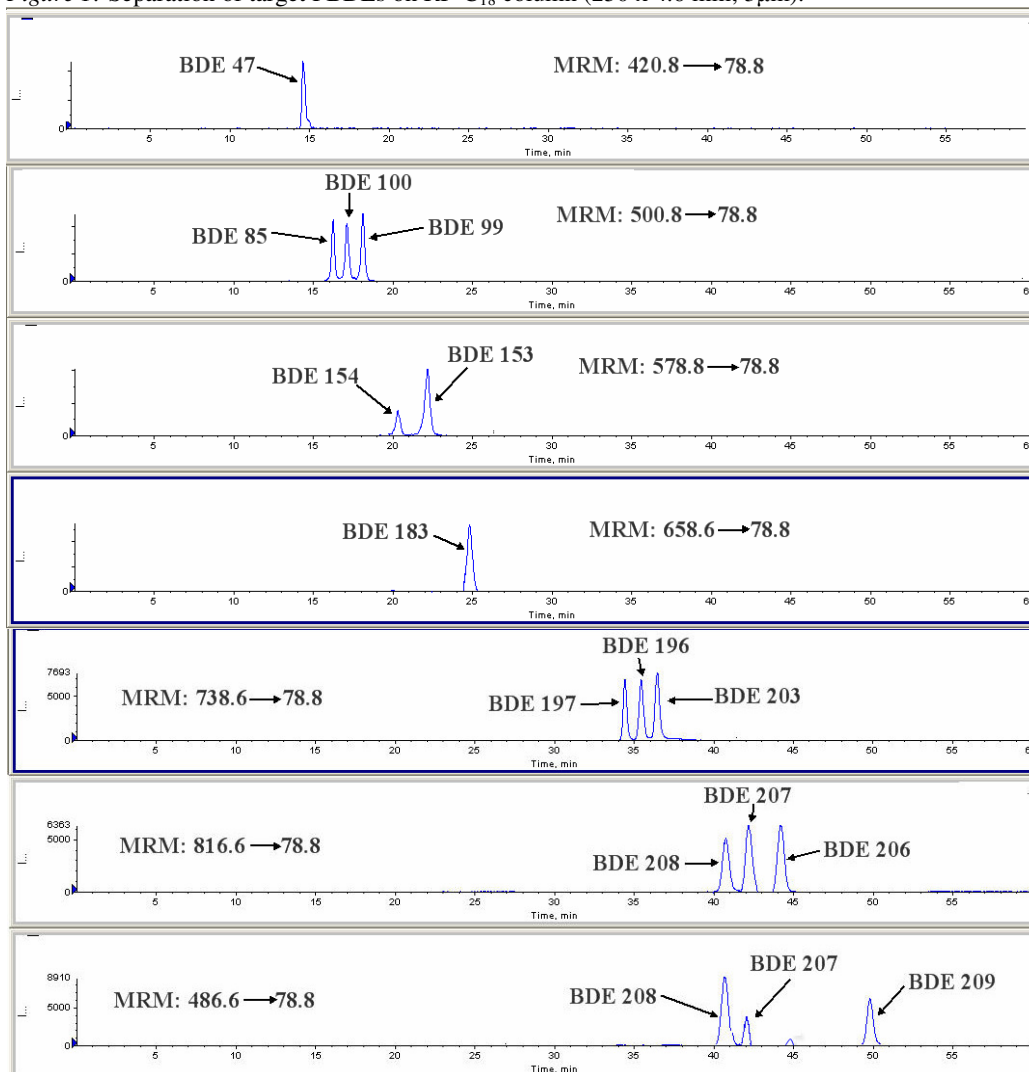


Figure 2: Comparison of certified PBDE concentrations (ng/g) in SRM2585 with the average values (n=5) obtained in this study.

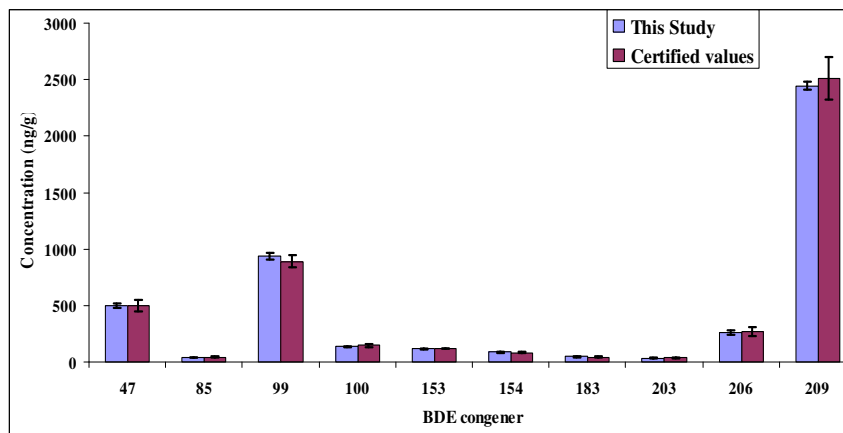


Figure 3: LC-APPI-MS/MS chromatogram of a car dust sample.

