Study of the retention behaviour of monofluorinated analogues of Polybrominated Diphenylethers (F-PBDEs) in gas chromatography

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Introduction

Polybrominated diphenyl ethers (PBDEs) are globally distributed in the environment. They have been used as flame retardants for over 2 decades [1]. PBDEs have been found to bioaccumulate and there is also a concern about the health effects of PBDE exposure due to their potential endocrine disrupting properties. PBDEs are structurally similar to polychlorinated biphenyls (PCBs) and tend to concentrate in the lipid tissue. This has lead to extensive monitoring of PBDEs in all environmental matrices. The sample preparation and instrumental determination may lead to systematic and non-systematic (random) errors. It is generally accepted that the accurate determination of micro contaminants in such complex mixtures requires the use of internal standards (ISs). The benefit of using ISs with physico-chemical properties similar to those of the target compounds, is that both systematic and random errors will be minimized.

Preliminary work on monofluorinated polycyclic aromatic hydrocarbons (F-PAHs) as ISs in gas chromatography (GC) is very promising [2], and therefore monofluorinated polybrominated diphenylethers (F-PBDE were synthesized [3] to determine if they could also be used as internal standards. F-PBDEs can be used as internal standards for trace-level analysis of PBDEs and are alternatives to \textsuperscript{13}C-labelled analogues. The present study focused on the retention behavior of F-PBDEs in GC, with the intention to use them as calibration standards for electron impact mass spectrometry (EI-MS) and GC-MS in the negative chemical ionisation mode (ECNI) GC-ECNI-MS. Selected F-PBDEs can also be used as internal standards with GC-Electron Capture Detection (GC-ECD).

Materials and Methods

The following F-PBDEs were synthesized [3], (numbers behind the names refer to the IUPAC numbers of the corresponding parent PBDE): 3-Bromo-4’-fluorodiphenyl ether (2), 4-Fluoro-3,3’-dibromodiphenyl ether (11), 4’-Fluoro-2,3’,4-tribromodiphenyl ether (25), 4’-Fluoro-2,3’,6-tribromodiphenyl ether (27), 3’-Fluoro-2,4,4’-tribromodiphenyl ether (28), 6-Fluoro-2,2’,4,4’-tetrabromodiphenyl ether (47), 6-Fluoro-2,3’,4,4’-tetrabromodiphenyl ether (66), 4’-Fluoro-2,3’,4,6-tetrabromodiphenyl ether (69), 3-Fluoro-2,2’,4,4’,6-pentabromodiphenyl ether (100), 3-Fluoro-2,3’,4,4’,6-penta-bromodiphenyl ether (119), 4’-Fluoro-2,3,3’,4,5,6-hexabromodiphenyl ether (160), 3-Fluoro-2,2’,4,4’,5,6-hexabromodiphenyl ether (154), 3-Fluoro-2,2’,4,4’,5,6-heptabromodiphenyl ether (183), 6,4’-Difluoro-2,2’,3,3’,4,5,5’,6’-octabromodiphenyl ether (199), 4’-Fluoro-2,2’,3,3’,4,5,5’,6’,6’-nonabromodiphenyl ether (208).

Analysis of F-PBDEs and PBDEs (Figure 2) were carried out on a HP 6890 (Hewlett Packard, Palo Alto, CA, USA) gas chromatograph with MS detection. Briefly, 1-\textmu l was injected. Separation was performed on a Restek XTI-5 capillary column (30 m \times 0.25 mm I.D., 0.25 \textmu m film thickness). Helium was used as the carrier gas at a column head pressure of 97 kPa. The split
flow was 120 ml/min. The column temperature was programmed from 103°C (4.5 min) to 280°C at 12°C/min. The final temperature was held for 20 min. Detection was based on the simultaneous registration, at the appropriate retention time, of the signals corresponding to the two m/z values selected for each analyte.

The brominated diphenyl ethers (Figure 3) were analyzed using GC-HRMS on a HP6890 Plus gas chromatograph (GC) interfaced to a VG Autospec – Ultima NT High Resolution Mass Spectrometer. Split/Splitless injection was used with a 1.5 mm I.D. injector liner. BDEs were analyzed on a DB-5HT 15m X 0.25mm id X 0.10 um film (J&W Scientific, USA) column. The GC-HRMS system was tuned to greater than 10,000 RP (10% valley definition).

Results and discussion

The small but noticeable effect of fluorine monosubstitution on the properties of aromatic hydrocarbons, like F-PBDEs, can be qualitatively understood by considering the influence of a fluorine atom on an aromatic system. First, fluorine induces a dipole moment, though not as strong as one might expect. The small fluorine atom does not retain the complete electron charge. In addition, hyperconjugation caused by overlap of the aromatic π-orbitals and the free fluorine p-orbitals, results in a partial transfer of the charge back to the aromatic system [4]. Secondly, with respect to intermolecular interactions, as a result of the reduced polarisability, the London forces are weaker for F-PBDEs than for the parent PBDEs. The latter effect is antagonistic to the effect of an increased permanent dipole moment and is of similar magnitude. Consequently, the overall effect of fluorine substitution has two antagonist effects: the creation of a permanent dipole moment and the reduction of the London forces. As a result, F-PBDEs are surprisingly similar to their corresponding parent PBDEs in terms of physico-chemical properties. A simple model of the electronic effects of fluorine substitution is shown in Fig. 1.

Depending on the fluorine substitution in ortho-, meta- or para-position, the retention behaviour compared to the corresponding parent PBDEs is different. As shown in Fig. 2, all F-PBDEs in general have shorter or identical retention times because they are slightly more non-polar. The reduction of the London force is variably compensated by the introduction of the permanent dipole moment. This depends on the pattern of fluorine substitution. The four F-PBDEs (25), (27), (69) and (100) fluorine substituted in the para-positions, co-elute with their corresponding parent PBDE. Because of the higher degree of bromination and longer retention time, F-PBDE (160) can be partly separated. The permanent dipole moment caused by fluorine in the para position is the strongest. Here, the compensation of the reduction of the London force is almost reached. The F-PBDE congeners (28), (100) and (119) are substituted in the meta-positions. The monofluorinated congener (28) is well separated, (100) and (119) show a small overlap with the corresponding parent PBDEs. This last observation can be explained by the high degree of bromination of the fluorinated aromatic ring. Because of the extension of the delocalisation of the electron density, the impact of the permanent dipole moment created by fluorine is decreased. The F-PBDEs (47) and (66) are ortho-substituted by fluorine. They show up to three minutes shorter retention times. This has two reasons. First the impact of the permanent dipole moment is the weakest in this position. Secondly, the conformation is changed partly by steric hindrance.
Fig. 1: Model of the electronic effects of fluorine substitution on an aromatic system.

Fig. 2: GC–MS of nine F-PBDE/PBDE pairs (50 ng/ml each in iso-octane). Conditions: m/z 35–400; for other details, see Materials and Methods.

Figure 3 compares the chromatograms of native PBDEs, F-PBDEs and 13C12-PBDEs. The F-PBDEs elute 2 to 10 seconds before the native PBDEs. The 13C12-PBDEs elute 1 to 2 seconds before the native PBDEs. Both types of internal standards are complementary. The advantage of the F-PBDEs is that they can be used for ECD analysis while the 13C12-PBDEs cannot.

Conclusions

The behavior of nine F-PBDEs in GC–MS analysis is shown for the first time. The main conclusion is that mono-substitution with fluorine causes only slight differences in retention behavior depending on the ortho-, meta- or para position of fluorine substitution. Complete or partial co-elution or separation of each F-PBDE/PBDE pair can be accomplished.

This opens the way to use electron capture detection ECD instead of or in addition to MS. Furthermore, the difference between the molecular ions of the F-PBDE/PBDE pairs is m/z 18, which allows a simultaneous detection without separation using MS in the electron impact (EI) mode. F-PBDEs are valuable internal standards, especially for the analysis with MS in the negative chemical ionisation mode (ECNI). In ENCI mode the dominant ions for F-PBDEs, 13C-PBDEs and PBDEs are m/z 79 and 81, and a separation by retention time between the internal standards (F-PBDEs or 13C-PBDEs) and PBDEs is, therefore, necessary. Only the F-PBDEs can be separated by retention time from the PBDEs, and 13C-PBDEs are, therefore, not useful in ECNI-.
MS. The use of F-PBDEs as IS can also be more cost effective in comparison to $^{13}$C labelled analogs.

**Figure 3 – Native PBDEs, F-PBDEs and $^{13}$C$_{12}$-PBDEs**

Ongoing investigations show, that the use of F-PBDEs as ISs causes a highly desirable improved precision in PBDE analysis. Additional studies with dynamic $^{19}$Fluorine nuclear magnet resonance spectroscopy ($^{19}$F-NMR) and solid state-NMR provide us with information concerning the dynamic of planarity and can be useful for toxicological studies.

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**References**