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A NEW LC-MS METHOD FOR THE DETECTION AND QUANTIFICATION OF HEXABROMOCYCLODODECANE DIASTEREOISOMERS AND TETRABROMOBISPHENOL-A FLAME RETARDANTS IN ENVIRONMENTAL SAMPLES

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Introduction

There is a growing interest amongst environmental laboratories in the determination of brominated flame retardants (BFRs), and a growing number of institutes perform polybrominated diphenylether (PBDE) determinations routinely[1]. More recently, the high volume BFRs hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A), have received greater attention, particularly as the use of penta-mix PBDE's has been restricted in Europe. 'HBCD' is a technical mixture containing three diastereoisomers, α-, β- and γ-HBCD[2]. In the commercial product Saytex HP-900^{TM} (Albemarle Europe SPRL, Belgium), the γ 'isomer' is typically the most abundant compound (70.4 %) followed by α-HBCD (16.2 %), and then the β 'isomer' (12.8 %)[3]. Where HBCD is an additive BFR and is directly applied to the product requiring flame retardancy, TBBP-A is reactive and is covalently bonded to the product matrix. Although both HBCD and TBBP-A can be analysed by gas chromatography with electron capture detection or negative ion chemical ionisation mass spectrometry (GC-NICI-MS), there are inherent problems associated with these techniques. Thermal rearrangement, as well as decomposition of the three diastereoisomers of HBCD occurs especially at the high temperatures (>160 °C) that are often applied during GC injection and separation[2]. In addition to this, contemporary GC columns are unable to resolve the three individual compounds. GC is also used for the determination of TBBP-A although due to its polar nature, a derivatisation step is required to improve the chromatographic behaviour of the compound[4], and this can be time consuming especially during routine analyses. Co-elution of HBCDs and TBBP-A with PBDEs can also be a problem during GC separation especially if only, as is often the case, the bromine isotopes at 79/81 amu are monitored following NICI.

High performance liquid chromatography (HPLC) interfaced with MS is a suitable alternative to GC-NICI-MS determination as it circumvents problems of thermally-induced reactions and 'isomeric' rearrangements, and chemicals of a wide range of polarity as well as molecular weights can be separated[5], and then selectively detected. The extraction and clean-up (adsorption column

chromatography) of HBCD and TBBP-A from environmental matrices are similar to those methods used for PBDEs, but due to its higher polarity, modifications are necessary to obtain a cleaned-up fraction containing TBBP-A.

To further our understanding of the environmental fate and behaviour of HBCD, as well as to accurately quantify residues, a diastereoisomer-specific determination is essential. A method that also allows for the simultaneous determination of TBBP-A without derivatiation is also preferred. Three institutions have collaborated on the development of a method of analysis to present a framework of approaches for the measurement of these commonly used BFRs.

Materials and Methods

Liquid-solid extraction by Soxhlet, Ultra Turrax^{TM} or Erlenmeyer shake flask methods were applied to sediment and biota samples using binary solvent mixtures of acetone:*n*-hexane (1:1 or 3:1 v/v) or acetone:pentane (1:1). Samples of biota were extracted from their wet state, and sediments were extracted from either wet or air-dried states. Several clean-up techniques were investigated and these ranged from conventional adsorption chromatography using deactivated alumina and silica, fractionation of crude extracts using acidic (conc. sulphuric acid) and basic (phosphate buffer) solution washings, and gel permeation chromatography using divinylbenzene gels. Labelled $^{13}C_{12}$ TBBP-A was added to sample extracts as a surrogate standard to account for losses of TBBP-A during the extract clean up stage.

Analysis of individual HBCDs and TBBP-A was performed using liquid chromatography with atmospheric pressure ionisation and single quadrupole or ion trap mass spectrometry. Chromatographic separation was performed using a C_{18} reverse phase mini-bore column and analyte ionisation was achieved using electrospray (ESI) or atmospheric chemical ionisation (APcI) interfaces. Full scan analyses as well as selected ion monitoring of the deprotonated $[M-H]^-$ ion of HBCD (m/z 640.7), TBBP-A (m/z 540.9) and $^{13}C_{12}$ TBBP-A (m/z 554.8) were conducted. A selection of aqueous mobile phase modifiers (ammonium acetate, ammonium hydroxide, and formic acid) was investigated to assess the effect on HBCD anion formation. The effect of ion transfer capillary temperatures was also evaluated over a temperature range from 130 to 400 °C. Quantification was based on peak areas and multi-point calibration curves.

Results and Discussion

Diastereoisomers of HBCD and TBBP-A are amenable to classic extraction procedures deployed for the routine analysis of persistent and halogenated organic compounds such as PCBs and PBDEs. The types of extraction methods applied in this study were chosen because of their simplicity, robustness and low cost. Due to the efficacy over single extraction solvents, combinations of semi-polar (acetone) and non-polar (*n*-hexane or pentane) were used to ensure quantitative recovery of the compounds under investigation.

Although α- and γ-HBCD could be recovered using 1.5 % deactivated silica gel column clean-up techniques, the elution of β-HBCD required large volumes of a polar solvent such as diethyl ether in pentane (15:85 v/v). This restricts its application in terms of cost and time efficiency as well as increasing the potential for the co-elution of unwanted, polar interferences. Due to its higher polarity, TBBP-A is absorbed more strongly to the silica thus permitting its collection as a separate fraction to HBCDs as well as to PBDEs and PCBs. When analysing for TBBP-A and PBDEs using GC-NICI-MS, it is desirable to obtain two fractions to isolate both as TBBP-A and BDE-153 can co-elute during chromatographic separation. A fractionation step incorporating a basic

solution wash of the crude sample extract was also investigated. However, the numerous volume transfers proved to be a rate-limiting step in the chain of procedures and led to variable losses of TBBP-A. Care is also required to minimise the amounts of co-extracted lipids and their breakdown products following treatment with conc. sulphuric acid. It is recommended that frequent deactivation of the GC injector liner or the removal of the first 0.5 to 1 m of the GC column is practised as theses zones become readily activated with TBBP-A and/or co-extracted polar interferences.

The application of LC-MS to the detection and quantification of HBCDs and TBBP-A proved to be a viable alternative to GC-NICI-MS for their determination in environmental samples. Soft or mild negative ionisation was necessary for the generation of the deprotonated ions. Positive ionisation had very little effect in terms of the production of protonated ions. For HBCDs, a strong [M-H]- ion at m/z 640.7 was obtained using ESI, and a weaker, water adduct ion at m/z 676 ion $[M\text{-}H+H_2O]^-$ was also apparent. The presence of the latter ion in real world samples was variable and may reflect an ion suppression effect due to the sample matrix composition. Gradient mobile phase programmes using flow rates of 200 μL min^{-1} and combinations of acetonitrile, methanol and water resulted in baseline separation of all three HBCD diastereoisomers (Figure 1a).

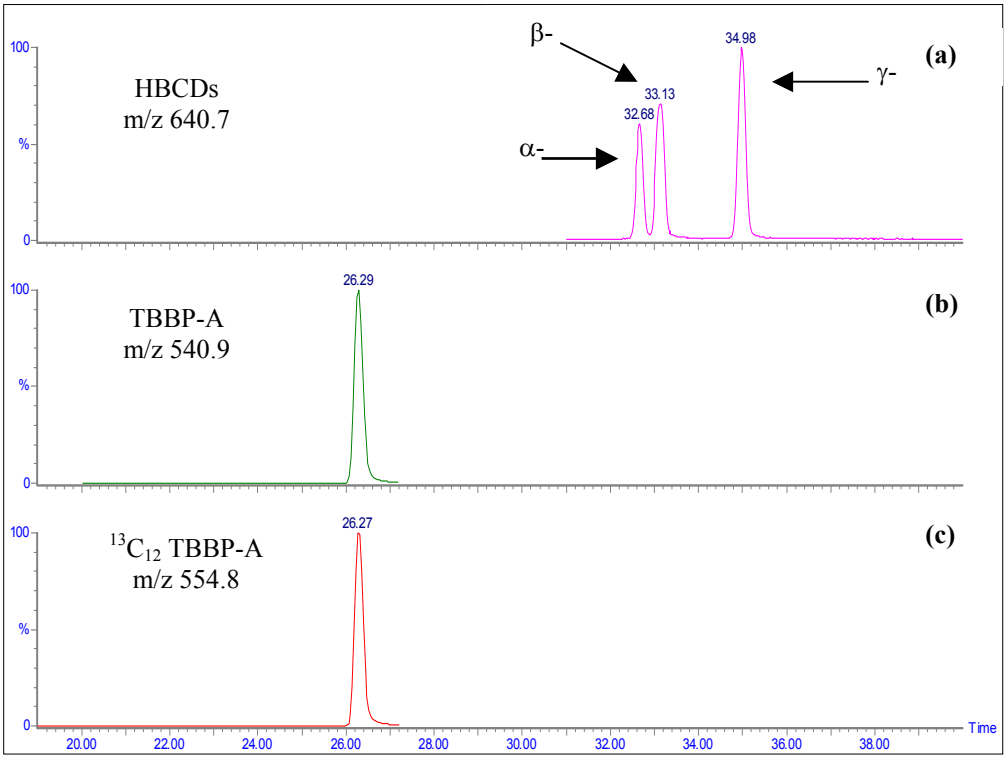

Figure 1. Time scheduled SIM chromatograms of (a) α-, β-, γ- diastereoisomers of the HBCD technical mixture, (b) TBBP-A, and (c) $^{13}C_{12}$ TBBP-A.

A binary (acetonitrile *plus* water) mobile phase gradient was developed for the separation of TBBP-A, $^{13}C_{12}$ TBBP-A (Figure 1b and c), and HBCDs using time scheduled, SIM analysis. The use of a Luna (150 x 2.0 mm, 5 μm; Phenomenex, Cheshire, UK) proved to be robust in terms of retention time stability and a RSD of ~1 % (*n*=20 injections) was calculated for all analytes prepared in a matrix-matched solution.

In equivalent-mass response terms, the deprotonated ions of TBBP-A and the labelled surrogate compound were stronger than those responses obtained for the HBCD diastereoisomers. A comparison between ESI and APcI indicated that the former technique was at least ten times more sensitive for all analytes. The optimum ion transfer capillary temperature for maximum HBCD response was found to be 160 °C. For LC separation, and where aqueous phase modifiers were investigated, 10 mM ammonium acetate gave the highest increase in ion abundance.

A linear calibration curve could be fitted for TBBP-A over three orders of concentration whereas a second order curve was more appropriate for the HBCDs. The linearity of the HBCD curves was limited to only two orders of concentration. On six separate occasions, a laboratory reference material (Brown trout; *Salmo trutta*) extract was analysed for HBCDs, and a mean RSD of 4.5 % was determined for the summation of the three compounds. A typical lower limit of quantitation using a 1 g sediment sample intake mass, a 200 μL final extract volume and a 15 μL injection volume is 1.2 μg Kg^{-1}dry weight.

Conclusions

This is the first time LC-MS has been applied to the detection and quantification of the individual HBCD diastereoisomers. The inclusion of TBBP-A in this method was also possible using time scheduled SIM of the deprotonated ion. The analytical method has been validated and applied to extracts of sediments, fresh and marine water biota as well as a range of difficult matrices including sewage sludges and marine mammals[6]. With the decreasing capital costs of bench-top quadrupole and ion trap platforms, LC-MS is a viable and robust technique for the measurement of these BFRs in environmental samples.

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