

Improved derivatization protocol for simultaneous determination of alkylphenol ethoxylates and brominated flame retardants followed by gas chromatography–mass spectrometry analyses

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ABSTRACT

An improved derivatization protocol for the simultaneous determination of alkylphenol ethoxylates and brominated flame retardants with heptafluorobutyric anhydride under triethylamine amine base was investigated. The derivatization reaction was completed in 30 min at 50°C using hexane as solvent. Under these conditions, it was observed that alkylphenol ethoxylates and tetrabromobisphenol A were derivatized successfully in the presence of hexabromocyclododecane, lower congeners of polybrominated biphenyls and polybrominated diphenyl ethers. The improved protocol was applied to recovery of analytes of interest from simulated water samples after solid phase extraction. The recoveries achieved were above 60%. The limit of detection and limit of quantification ranged from 0.01–0.20 µg/l and 0.05–0.66 µg/l, respectively.

Keywords: Alkylphenol ethoxylates, brominated flame retardants, gas chromatography-mass spectrometry, heptafluorobutyric anhydride derivatization, simultaneous determination

INTRODUCTION

Alkylphenol ethoxylates (APEs) and brominated flame retardants (BFRs) are anthropogenic compounds which are often referred to as endocrine disrupting compounds (EDCs) because they have been reported to interact with oestrogen receptors (Stehmann and Schröder, 2004). Studies have shown that APE metabolites are more toxic than their parent compounds, are persistent, and accumulate in fatty tissues (Ying et al., 2002; Petrovic et al., 2003; Gatidou et al., 2007; Sibali et al., 2010). Despite their benefits for reducing fire-related injury and property damage, concern about BFRs is growing because of their occurrence and persistence, and they have been detected in the environment, biota and humans (Alaee et al., 2001; Hyötyläinen and Hartonen, 2002; Polder et al., 2008; Vetter et al., 2008; Yu et al., 2008; Odusanya et al., 2009; Segev et al., 2009; Olukunle et al., 2012).

Determination of low volatility polar compounds such as phenolic analytes has been achieved by indirect analysis using derivatization techniques because such compounds show low sensitivity and tailing in gas chromatographic analysis (Ding and Tzing, 1998; Cathum and Sabik, 2001; Hoai et al., 2003; Esperanza et al., 2004; Stehmann and Schröder, 2004; Fiedler et al., 2007; Gatidou et al., 2007; Dirtu et al., 2008). For the group of compounds covered in the present study, acetylation has been chosen because of its quantitative reactions with various hydroxyl compounds at relatively moderate conditions. In our previous report, heptafluorobutyric acid (HFBA) was used as a derivatizing agent for the determination of APE

and BFR analytes in environmental samples (Chokwe et al., 2012). The procedure described in that report used Na₂CO₃ with a derivatization time of 2 h. In another study, Dirtu et al. (2008) derivatized bisphenol A (BPA), tetrabromobisphenol A (TBBPA) and triclosan using pentafluoropropionic anhydride (PFPA) aided by triethylamine (TEA) at 70°C for 30 min. In another study, nonylphenol (NP) and BPA were derivatized within 30 min at 60°C using trifluoroacetic acid (TFAA) (Stehmann and Schröder, 2004). Derivatization of nonylphenol polyethoxylate (NPnEO) with *n*-propanol-acetylchloride at 80°C for 1 h has also been reported (Ding and Tzing, 1998). Cathum and Sabik (2001) described a derivatization procedure for alkylphenol polyethoxylate surfactants (APnEO) using pentafluorobenzoyl bromide (PFB-Br) as a derivatizing agent aided with K₂CO₃ by sonication. The derivatization took 2 h and was conducted in the dark. Gatidou et al. (2007) reported derivatization of NP, NP2EO, BPA and triclosan with bistrimethylsilylfluoroacetamide (BSTFA) at 65°C for 20 min while Fiedler et al. (2007) derivatized alkylphenolic chemicals (APs) with the same reagent at room temperature for 2 h. Furthermore, NPnEO was derivatized with BSTFA in the presence of trimethylchlorosilane (TMCS) at 70°C for 4 h (Esperanza et al., 2004). In a separate study, Hoai et al. (2003) reported derivatized NPnEOs and nonylphenoxy carboxylic acids (NPnECs) with bistrimethylsilylacetamide (BSA) at 25°C for 1 h.

In the aforementioned derivatization studies, either higher temperatures and shorter derivatization time, or lower temperatures and longer derivatization time, in the presence of different bases, were reported. It is essential that a balance between derivatization temperature and time should be determined since high temperatures and long derivatization times may not favour analytes of interest that are unstable, such as BFRs, where debromination of higher congeners to lower congeners is possible. This study, therefore, reports on

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improved protocol using HFBA enhanced by triethylamine base derivatization using hexane as the solvent and followed by gas chromatography-mass spectrometry (GC-MS) for the simultaneous determination of APE and BFR analysis. The approach developed in the present study is seen to be milder (50°C), has a shorter derivatization time (30 min) and saves sample handling since these compounds can be analysed simultaneously.

METHODS

Chemicals and standards

The derivatizing agent, HFBA, was of analytical grade, purchased from Sigma-Aldrich South Africa. The solvents methanol, dichloromethane, acetone and hexane used in the study were of GC grade and were used without further purification. The APEs and polybromobiphenyls (PBBs) were purchased from Laboratories Dr Ehrenstorfer-Schäfers, Augsburg. Only the nonylphenol ethoxylates (NPE), nonylphenol penta ethoxylates (NPPE) and octylphenol penta ethoxylates (OPPE) were of technical grade; the remaining both APEs and PBBs were of analytical grade. The polybrominated diphenyl ethers (PBDEs) mixtures were purchased from AccuStandard, USA, and were of analytical grade. TBBPA of technical grade as Firemaster BP4A, and hexabromocyclododecane (HBCD) of technical grade were purchased from AccuStandard, USA. Helium, as He 5.5 pure, was purchased from Air Products South Africa, Vereeniging.

Gas chromatography mass spectrometry (GC-MS) analysis

An Agilent 6890 gas chromatograph equipped with a 5975 mass selective detector (MSD) was used for GCMS analysis. The MS was tuned with perfluorotributylamine (PFTBA) using the auto-tune program. The GC was equipped with an Agilent autosampler. The GC separation was performed on a capillary column (Restek RTx-1614, film thickness 0.10 µm, 15 m × 0.25 mm I.D., (Chromspec cc South Africa). The GC-MS conditions used for analysis were as follows: carrier gas He; linear velocity, 40 cm/s; injector temperature, 280°C; transfer line temperature, 300°C; ion source 150°C. For the analysis, 1 µl splitless injection was carried out by autosampler. The GC temperature program conditions were as follows: initial temperature 50°C, heated to 120°C by a temperature ramp of 7.5°C/min, then 275°C by a temperature ramp of 15°C/min, then finally heated to 300°C (held for 2 min) by a temperature ramp of 25°C/min.

Derivatization

The following were added into a pyrex test tube: 80 µl of organic mixture containing APs (1 mg/l), APEs (5 mg/l), PBBs (1 mg/l), PBDEs (1 mg/l), HBCD (5 mg/l), TBBPA (5 mg/l), 0.1 ml hexane; 70 µl of 0.1 M triethylamine (TEA) and 7 µl HFBA. The test tubes were closed and completely mixed for 1 min using a vortex system. The contents were gradually heated to 50°C and the derivatization monitored by GC-MS. Derivatization was achieved within 30 min.

Thereafter, the contents were cooled, quenched with 0.3 ml of 5% aqueous solution of K₂CO₃. The organic phase was then drawn off. The aqueous phase was washed twice with 0.5 ml hexane to recover some organic fractions. After separation, the organic phase extracts were concentrated to 100 µl. Thereafter, the internal standards (Chrysene and PBB80) were added into the extract, the volume made up to 200 µl and 1 µl of a mixture of extracts and internal standards injected into the GC-MS.

Extraction of analytes from simulated water samples

Solid-phase extraction (SPE) was used as the isolation technique throughout the experiment. Before use, the SPE cartridge was conditioned with 6 ml of 30% MeOH in dichloromethane (DCM) followed by the addition of 6 ml of MeOH. About 250 ml of MilliQ water, spiked with 80 µl of organic mixture, was acidified to pH 3 with acetic acid and extracted at a flow rate of approximately 10 ml/min. After passing the sample, the cartridge was dried under vacuum for 1 h. The compounds were eluted with 3 × 2 ml of mixture of DCM-hexane. The eluates were evaporated to dryness under a gentle stream of nitrogen at 40°C. Finally the dried residues were subjected to derivatization reaction as described earlier.

RESULTS AND DISCUSSION

Derivatization

HFBA was chosen for the present study because of its rapid and quantitative reaction, the formation of stable products, excellent chromatographic properties for the targeted analytes, as well as the availability of the reagent. The derivatization reaction was monitored by GC-MS using TBBPA as shown in Fig. 1.

Both APEs and TBBPA were fully derivatized with HFBA aided by TEA in the presence of PBBs, PBDEs and HBCD. Under the scope of the derivatization, it was observed that there was no de-bromination of PBBs, PBDEs and HBCD. The GC-MS chromatogram, shown in Fig. 2, indicates that only the hydroxyl groups reacted and this was later used to determine the selected ion monitoring of the target compounds. It was also observed that derivatization depended on the analyte structure, time and solvent. In this study, the derivatization reaction for the phenolic hydroxyl group was completed faster than those for the alcoholic hydroxyl groups. This phenomenon has been reported previously (Hoai et al., 2003).

Recovery of targeted analytes from simulated water samples

The recovery results from simulated water samples, as shown in Fig. 3, ranged from 60 to 140%. These results were comparable with those reported by other researchers (Cathum and Sabik, 2001; Cai et al., 2003; Hoai et al., 2003; Gatidou et al., 2007; Dirtu et al., 2008). The obtained recovery results were considered to be satisfactory, considering that different types of analytes with different chemical structure and polarities were extracted simultaneously.

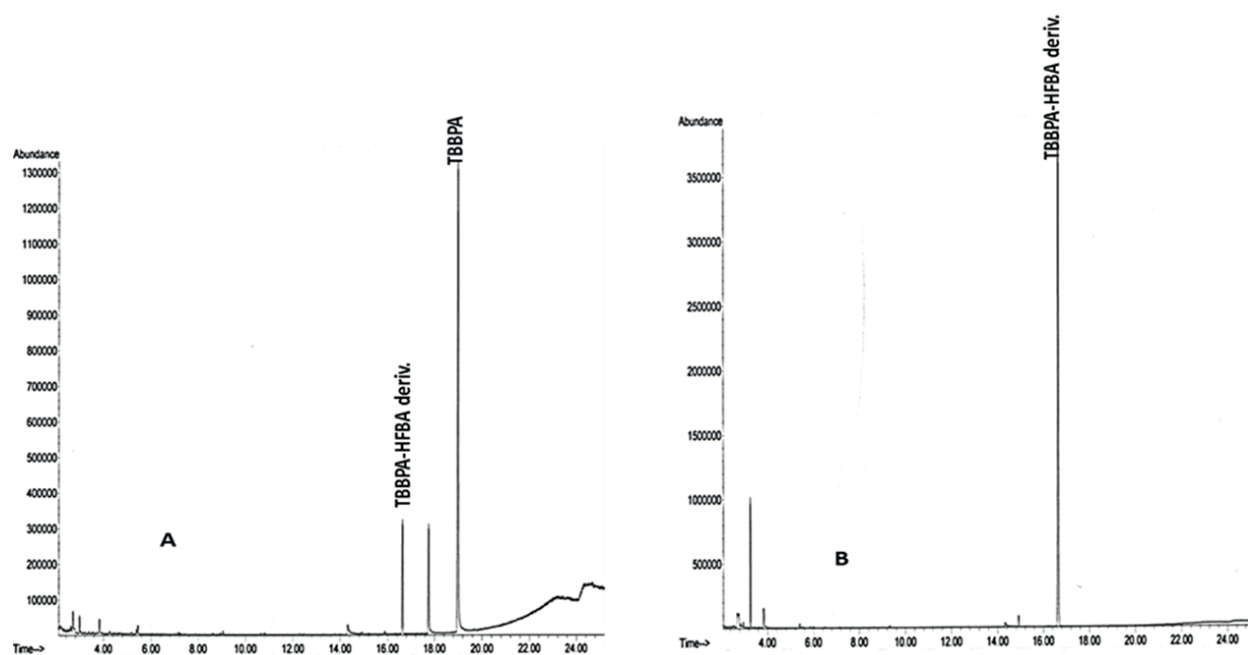


Figure 1
GC chromatogram of TBBPA derivatized after (A) 10 min and (B) 30 min at 50°C

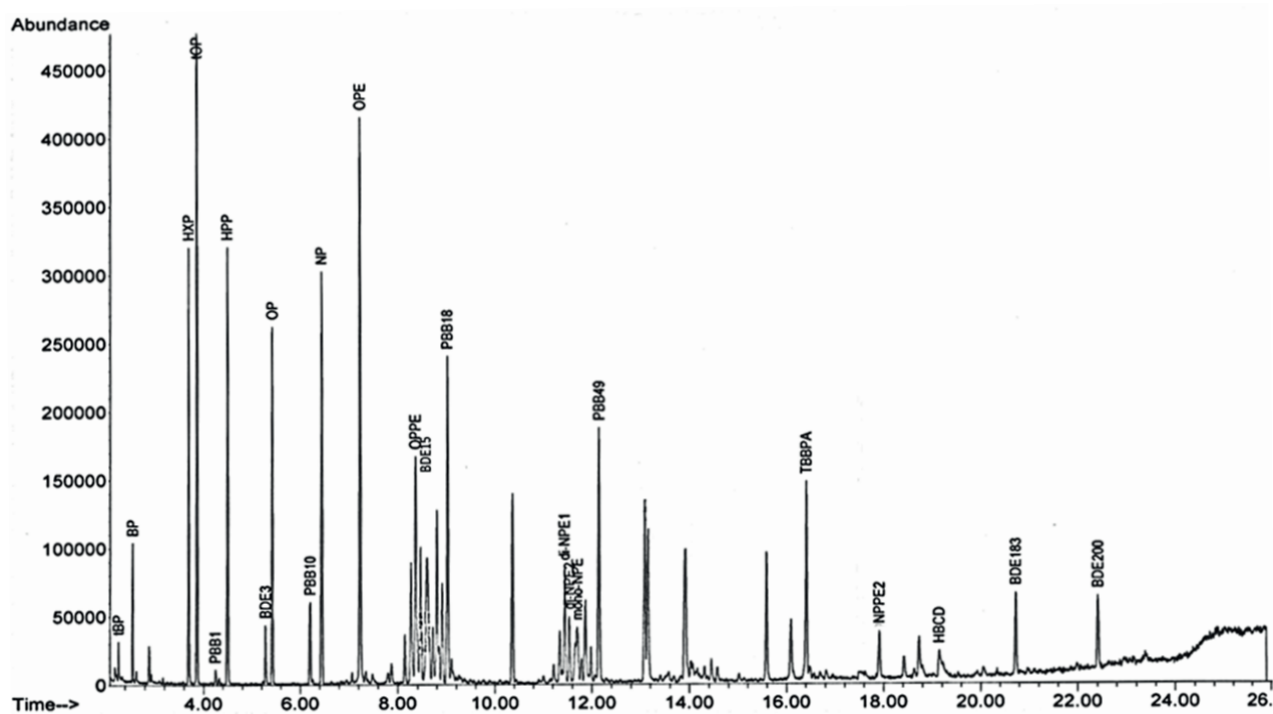


Figure 2
GC chromatogram of derivatized APEs and TBBPA in the presence of PBBs, PBDEs and HBCD standard

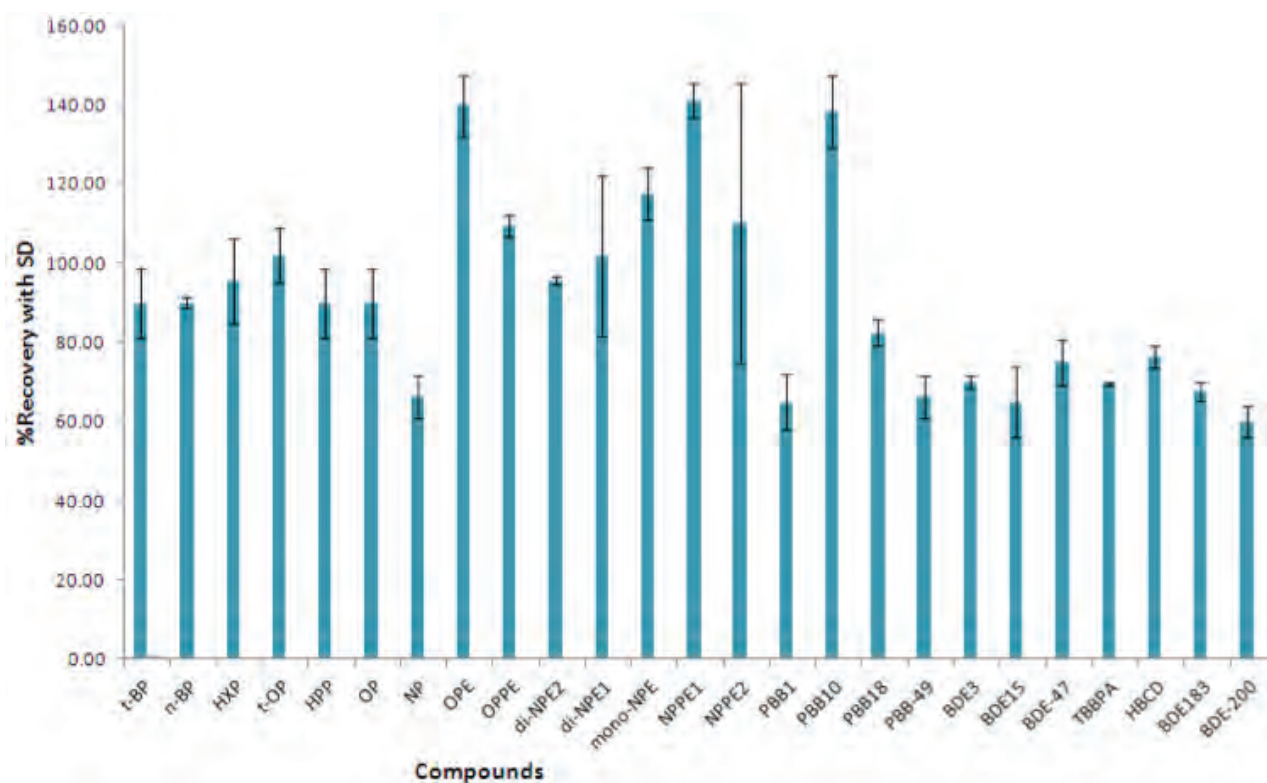


Figure 3
Mean recovery efficiencies of targeted compounds under optimum conditions. Error bars indicate standard deviation

Comparison of the derivatization protocol with other reported studies

A comparison of the derivatization procedure from this study and previously reported procedures is presented in Table 1. From the results in Table 1, BSTFA yielded a fast derivatization reaction as the reaction was complete within 20 min, but this was obtained at a higher temperature (65°C) (Gatidou et al., 2007). Another study by Fiedler et al. (2007) also used BSTFA but the reaction took place at room temperature for 2 h. These 2 studies indicated the effect of temperature on derivatization with BSTFA as the derivatization was quicker at an elevated temperature than at room temperature. Hoai et al. (2003)

obtained quicker derivatization products at room temperature with BSA than with BSTFA as the derivatized products were obtained in 1 h. In terms of the speed of derivatization, this study showed a quicker derivatization reaction, except for the studies done by Gatidou et al. (2007). With acylation reagents, this study showed similar reaction times compared to the studies where anhydride derivatizing agents were used (Stehmann and Schröder, 2004; Dirtu et al., 2008). Compared to the conditions reported in Table 1, the derivatization method developed in this study was found to save analysis time as the derivatization was complete within 30 min under very mild conditions, producing comparable limit of detection (LOD) and limit of quantification (LOQ) results.

Compounds	Derivatizing agent	Conditions	LOD (µg/ℓ)	LOQ (µg/ℓ)	Ref.
NPEO	<i>n</i> -propanol-acetyl-chloride	80°C, 1 h	NR	0.01	Ding and Tzing, 1998
APnEO	PFB-Br + K ₂ CO ₃	2h ultrasonic bath then overnight in dark place	0.001–0.002	NR	Cathum and Sabik, 2001
NPnEOs, NPnECs	BSA	25°C, 1 h	0.0025–0.018	NR	Hoai et al., 2003
NP, BPA	TFAA	60°C, 30 min	NR	NR	Stehmann and Schröder, 2004
NPnEO	BSTFA and TMCS	70°C, 4 h	NR	NR	Esperanza et al., 2004
NP, NP2EO, BPA, TCS	BSTFA	65°C, 20 min	0.03–0.41	0.11–1.34	Gatidou et al., 2007
AP	BSTFA	room temperature, 2 h	NR	NR	Fiedler et al., 2007
BPA, TCS, TBBPA	PFPA + TEA	70°C, 30 min	0.02–0.04	0.05–0.280	Dirtu et al., 2008
APs, APes, TBBPA	HFBA + Na ₂ CO ₃	55°C, 2 h	0.01–0.15	0.05–0.66	Chokwe et al., 2012
APs, APes, TBBPA	HFBA + TEA	50°C, 30 min	0.01–0.15	0.05–0.66	This study

NR = not reported; LOD = limit of detection; LOQ = limit of quantification

CONCLUSIONS

The derivatization method presented here for the simultaneous determination of APEs and TBBPA gave comparable results with other derivatization studies in the literature. The derivatization procedure in this study involved the reaction of these compounds simultaneously in the presence of lower congeners of PBBs, PBDEs and HBCD, with HFBA under triethylamine base at 50°C for 30 min. The results of this study demonstrate that the presented method has acceptable relative recoveries of 60 ± 3.86 – 141 ± 4.24 for the target compounds and adequate LOD and LOQ that ranged from 0.01–0.20 µg/ℓ and 0.05–0.66 µg/ℓ, respectively. The presented method showed milder derivatization conditions and shorter analysis time.

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