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 the recover of the analytes of interest from a simulated water sample after solid phase extraction. The recoveries achieved were above 60%. The limit of detection and limit of quantification ranged from 0.01–0.20 and 0.05–0.66 μgL−1, respectively. The improved derivatization procedure was also successfully applied to determine trace amounts of these compounds in environmental water samples. The concentrations of the targeted analytes from the environmental samples were determined from limit of quantification. The levels of the targeted compounds in the environmental samples ranged from nd–7.63 ± 2.83 μgL−1.

Key words | alkylphenol ethoxylates, brominated flame retardants, gas chromatography-mass spectrometry, heptafluorobutyric anhydride derivatization, simultaneous determination

INTRODUCTION

Alkylphenol ethoxylates (APEs) and brominated flame retardants (BFRs) are of anthropogenic compounds which are often referred to as endocrine disrupting compounds (EDCs) because they have been reported to interact with the oestrogen receptors (Stehmann & 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, growing concern about BFRs has risen because of their occurrence and persistence, and they have been detected in the environment, biota and in humans (Alaee et al. 2001; Hyötyläinen & 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. 2011).

Some of the most frequently used methods for the analysis of these groups of compounds include: direct analysis using liquid chromatography-mass spectrometry (LC-MS) (Petrovic et al. 2003), gas chromatography with electron capture detector (GC-ECD) (Odusanya et al. 2009), gas chromatography-mass spectrometry (GC-MS) (Azevedo et al. 2001; Olukunle et al. 2011), gas chromatography-high resolution mass spectrometry (GC-HRMS) (Alaee et al. 2001; Lacorte et al. 2010), or indirect analysis using derivatization techniques (Ding & Tzing 1998; Cathum & Sabik 2001; Hoai et al. 2003; Esperanza et al. 2004; Stehmann & Schröder 2004; Fiedler et al. 2007; Gatidou et al. 2007; Dirtu et al. 2008). Derivatization techniques have been used to determine low volatility polar compounds such as phenolic analytes since such compounds show low sensitivity and tailing in gas chromatographic analysis (Gatidou et al. 2007). For the group of compounds covered in the present study, acetylation has been chosen because of its quantitative
reactions with various hydroxyl compounds in relatively moderate conditions. In our previous paper, heptafluorobutyric anhydride (HFBA) was used as the derivatizing agent for the determination of APEs and BFRs analytes in environmental samples (Chokwe et al. 2012). However, the procedure described in that paper used Na₂CO₃ with a derivatization time of 2 h. In another study, Diri et al. (2008) derivatized BPA, Tetrabromobisphenol A (TBBPA) and triclosan using PFPA aided by triethylamine (TEA) at 70 ºC for 30 min. In another study, NP and BPA were derivatized within 30 min at 60 ºC using TFAA (Steinhann & Schröder 2004). Derivatization of Nonylphenol Ethoxylate (NPEO) with n-propanol-acetylchloride at 80 ºC for 1 h has also been reported (Ding & Tzing 1998). Cathum & Sabik (2001) described a derivatization procedure for APnEO using pentafluorobenzoyl bromide (PFB-Br) as 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 bistrimethylsilylfluoroaceta-mide (BSTFA) at 65 ºC for 20 min, while Fiedler et al. (2007) derivatized 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 ºC for 4 h (Esperanza et al. 2004). In a separate study, Hoai et al. (2003) reported derivatized NPNEOs and NPNECs with bistrimethylsilylaceta-mide (BSA) at 25 ºC for 1 h.

In the aforementioned derivatization studies, either higher temperatures and shorter derivatization time, or lower temperatures and longer derivatization times 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, that is, BFRs, where debronomination of higher congeiners to lower congeners is possible. This study, therefore, reports on improved protocol using HFBA enhanced by trimethylamine base derivatization using hexane as the solvent and followed by gas chromatography-mass spectrometry analysis, for the simultaneous determination of APEs and BFRs in environmental samples. The approach developed in the present study is seen to be milder (50 ºC), with a shorter derivatization time (30 min), and saves sample handling since these compounds can be analyzed simultaneously.

### CHEMICALS AND STANDARDS

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 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 and both the remaining APEs and 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 Product South Africa, Vereeniging.

### GC-MS analysis

An Agilent 6890 GC equipped with 5975 mass selective detector was used for GC-MS analysis. The MS was tuned with perfluorotributylamine using the auto-tune program. The GC was equipped with an Agilent autosampler. The GC separation was performed on a capillary column (Restek RT×-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⁻¹; injector temperature, 280 ºC; transfer line temperature, 500 ºC; ion source 150 ºC. For analysis, 1 µL splitless injections were carried out by autosampler. The GC temperature program conditions were as follows: initial temperature 50 ºC; heated to 120 ºC by a temperature ramp of 7.5 ºC min⁻¹, then 275 ºC by a temperature ramp of 15 ºC min⁻¹, then finally heated to 300 ºC (held for 2 min) by a temperature ramp of 25 ºC min⁻¹.

### Derivatization

Into a Pyrex test tube, 80 µL of organic mixture containing APs (1 mg L⁻¹), APEs (5 mg L⁻¹), PBBS (1 mg L⁻¹), PBDEs (1 mg L⁻¹), HBCD (5 mg L⁻¹) and TBBPA (5 mg L⁻¹),
0.1 ml hexane; 70 μL of 0.1 M TEA and 7 μL HFBA was added. 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 of hexane to recover 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 sample**

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 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⁻¹. 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 (4:1). 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.

**WATER SAMPLE COLLECTION**

Environmental water samples were collected from three sites, A, B and C, along the Grootdraai sub-catchment located in the Mpumalanga Province, South Africa, as shown in Figure 1. The sub-catchment is supplied by tributaries from Blekbokspruit and the Brummerspruit. Key
activities in the sub-catchment include flows from waste-water treatment works (WWTW), stock farming, irrigation, agricultural activities, as well as the overall increase in human population around the sub-catchment area. Water samples were collected using 250 mL Winchester brown bottles by immersing a sample bottle below the surface and allowing it to fill. The samples were placed in cooler bags, transported to the laboratory and stored in a cold room set at a temperature of 4°C until analysis. The samples were allowed to equilibrate at room temperature before use.

**Extraction of analytes from environmental water sample**

The collected samples were subjected to the extraction and derivatization procedures described above.

**Quality assurance**

The spiking method was used in the quality assurance process of analytical method due to the unavailability of certified reference material for the target compounds. MilliQ pure water was spiked with 80 μL of standard mixture of 1.0 mg L⁻¹ APs, PBBs and PBDEs; 5.0 mg L⁻¹ HBCD, TBBPA and APEs, and was taken through the same extraction and derivatization procedure mentioned above prior to GC analysis. Several quality assurance measures were also routinely used in this study, including running blanks in between samples as well as analysing samples in triplicate.

**RESULTS AND DISCUSSION**

**Derivatization**

Heptafluorobutyric anhydride 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 Figure 2.

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 Figure 3, indicates that only the hydroxyl groups reacted and this was later used to determine the selected ion monitoring of the target compounds; Table 1 shows the specific fragment ion to be used for quantification of the analytes from environmental samples. 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

![Figure 2](https://iwaponline.com/wst/article-pdf/69/12/2389/470862/2389.pdf)
the alcoholic hydroxyl groups. This phenomenon has been reported (Hoai et al. 2003).

Recovery of targeted analytes from simulated water sample

The recovery results from simulated water samples as shown in Figure 4 ranged from 60–140%. These results were comparable with those reported by other researchers (Cathum & Sabik 2001; Cai et al. 2003; Hoai et al. 2005; 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.

Comparison of the derivatization protocol with other reported studies

A comparison of the derivatization procedure from this study and previous reported procedures is presented in Table 2, from which it can be seen that 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 was done at room temperature for 2 h. These two studies indicate the effect of temperature for the derivatization with BSTFA as the derivatization was quicker at an elevated temperature than at room temperature. Hoai et al. (2005) 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 than all the others, except for the studies done by Gati- dou et al. With acylation reagents, this study showed similar reaction times compared to the studies where anhydride derivatizing agent were used (Stehmann & Schröder; Dirtu et al.). Compared to the conditions reported in Table 3, the derivatization method from this study was found to save analysis time as the derivatization was complete within 30 min under very mild condition, producing comparable LOD and LOQ results.

**LEVELS OF APES AND BFRS IN THE ENVIRONMENTAL SAMPLES**

The concentrations of the targeted analytes (analyzed in triplicate) from the environmental samples, as shown in Table 3,
were determined from LOQ. The results from environmental samples mainly indicated the presence of HBCD, as it was detected from all the sampling points, while one di-ethoxylate was detected from both the final effluent from sites B and C. Also from site C, t-OP (0.07 μg L⁻¹), OPPE (0.71 μg L⁻¹) and mono-NPE (0.60 μg L⁻¹) were detected. Gatidou et al. (2010) obtained similar results for mono-NPE (0.56 μg L⁻¹) from effluent from the WWTW of Mytilene City in Greece.

CONCLUSIONS

The derivatization method presented for the simultaneous determination of APEs and TBBPA gave comparable results to other derivatization studied 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 to 141 ± 4.24 for the target compounds, and adequate LOD and LOQ that ranged from 0.01–0.20 μg L⁻¹ and 0.05–0.66 μg L⁻¹, respectively. When the conditions developed were optimized and applied to environmental samples, most of the analytes were not detected with the exception of HBCD, which was detected from all the sites, and mono-NPE, di-NPE2, OPPE and t-OP detected only at site C. The presented method showed milder derivatization condition and shorter analysis time.

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