Quantification of polybrominated diphenyl ether (PBDE) congeners in wastewater by gas chromatography with electron capture detector (GC-ECD)

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Abstract

This paper describes a gas chromatography coupled with an electron capture detector (GC-ECD) method that was developed for screening and reliable quantification of some selected PBDE congeners (BDE 28, 47, 99, 100, 153, 154, 183 and 209) in wastewater. Emphasis was placed on the ability of the method to simultaneously analyse low to high BDE congeners in a single run, whilst being comparatively cost-effective to gas chromatography coupled with mass spectrometry (GC-MS) methods commonly employed today.

Different solid phase extraction (SPE) cartridges (Oasis HLB, Isolute PAH, and Isolute C18) were tested for efficient analyte extraction in terms of recovery and resultant clean extracts free of unwanted compounds. Isolute PAH performed better, and in combination with optimized GC-ECD conditions permitted satisfactory determination of PBDE congeners at trace levels (method detection limits (MDLs) of 0.6 ng/L to 11 ng/L) in water samples. Method accuracy and precision were evaluated by recovery experiments using laboratory spiked water samples at two concentrations (3 ng/L and 10 ng/L).

The method was employed to evaluate the fate and removal of selected PBDE congeners in a conventional activated sludge wastewater treatment plant (WWTP) in Northern England. PBDEs were detected in influent and effluent samples at 0 – 113 ng/L and 0 – 18 ng/L respectively, and 75% total PBDE removal was achieved by the WWTP.

Keywords: Polybrominated diphenyl ethers; Solid phase extraction; Gas chromatography; Wastewater analysis

1. Introduction

Brominated flame retardants (BFRs) are added to many household items such as furniture, upholstery, plastic, electronic devices and textiles; they consist of several groups of compounds, including polybrominated diphenyl ethers (PBDEs), hexabromoclyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) [1-3]. PBDEs are the second most produced BFR in the world, accounting for 33% of global production, while TBBPA accounts for 60% [4]. According to the United Nations Environment Programme, the total global production of all PBDEs from 1970 to 2005 was between 1.3 million and 1.5 million tonnes with deca-mix BDE formulation accounting for about 85 % of this number [5]. PBDEs are persistent and hydrophobic compounds that tend to bio-accumulate; their occurrence in the environment has been of growing concern due to their toxicological effects including disruption of the thyroid hormone function in humans and wildlife, which has led to a ban on their production and usage in the European Union (EU) and the United States of America (USA) [4, 6]. In the EU, production, import and use of commercial PBDE formulations, including pentaBDE and OctaBDE, was banned in 2003 [7, 8], and this was extended to a worldwide ban in 2009 at the Stockholm Convention of Persistent Organic Pollutants [9]. Despite this ban, these PBDEs are still being deposited into the environment, as they are not covalently bound to the applied products and are released with usage [10, 11]. Deca-BDE formulation is currently being phased out in the EU and US, but still extensively produced in China and the resulting products are distributed globally [12].

Effluents from WWTPs have been identified as a major source of PBDE into the environment. WWTPs receive PBDEs through municipal wastewater (discharged during production, application and release from in-use domestic products) and surface runoff [10, 12]. Therefore, an effective analytical methodology to extract and quantify the presence of PDBEs in wastewater is required.

Several methods, including conventional liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid phase extraction (SPE), solid phase micro-extraction (SPME) ultrasound assisted extraction and cloud point extraction (CPE) have been used to extract PBDEs from water and semi-solids [4, 13, 14]. Most of these methods have limitations, including excessive use of solvents, and/or require further sample clean up using multilayer column chromatography or gel permeation chromatography to reach lower detection limits [14, 15]. SPE is the most common extraction method used today as it offers low solvent usage and broad applicability. Careful selection of appropriate sorbent and an optimized elution protocol can produce clean extracts that require no further clean-up [16, 17]. For unequivocal identification and quantification of brominated flame-retardants including PBDEs in environmental samples, gas chromatography coupled with mass spectrophotometry (GC-MS) operating in either electron ionization (EI) or electron capture negative ionization (ECNI) mode is often used [13, 18, 19]. However, GC-ECD (electron capture detector) has also been employed for quantification of PBDEs in environmental samples [15, 20, 21].

Determination of PBDEs in environmental samples is most commonly carried out by GC-MS mainly because of its selectivity, but GC-ECD is advantageous as it is cheaper, more user friendly and more sensitive, due to lower detection limits [22]. However, the ECD is prone to halogenated interference, since identification and resulting quantification of compounds is solely based on retention time; hence ECD based methods suffer from limited selectivity [22]. This shortcoming of the GC-ECD method can, however, be effectively minimised by carefully selecting GC columns and a clean-up method that produces high quality extracts free of interfering compounds. GC-ECD system is cheaper than GC-MS systems for micropollutant analysis in terms of purchasing, maintenance and costs per sample. For example, a basic model GC-ECD system by Agilent Technologies costs half the price of a GC-MS system in both capital (about $45,000 for GC-ECD and $91,000 for GC-MS) and maintenance cost ($1200 for

GC-ECD and $2600 for GC-MS per maintenance visit). Additionally, private laboratories carrying out analysis of total polychlorinated biphenyls (PCB) and congeners in soil and sediments reported that per sample GC-MS costs ($500 - $1000) are twice those of GC-ECD ($250 - $750) [23]. This method will be even more useful in low-middle income countries (LMICs) where shortage of studies on chemical pollution has been attributed to difficulties and high costs associated with environmental analysis [24].

To the best of our knowledge, only a few researchers have applied GC-ECD to analyse PBDEs in water and wastewater [15, 20], and none of them has used SPE for sample concentration and extraction. The authors from previous studies employed LLE, CPE or molecularly imprinted SPME and were unable to analyse BDE 209 (which is a major component of the decaBDE mix mentioned above) together with other lower molecular weight congeners in one single run: a separate GC capillary column and temperature program was generally required. In this work, a cost-effective yet functional analytical technique was developed using the combination of SPE and GC-ECD for the determination of selected low to high molecular weight PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209) (Table *1*) in wastewater; special attention was given to the determination of BDE-28 to BDE 209 in a single run. The method, which involved using a multilayer SPE column for extraction and analytical performance, was evaluated on the basis of its detection limits, linear working range and repeatability. The method was then applied to determine the levels and removal of PBDEs in a municipal wastewater treatment plant in Northern England.

2. Materials and methods

2.1 Materials and reagents

A certified standard solution mix of PBDEs (> 98% purity) containing eight primary congeners including BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183 and BDE 209 was obtained from Accustandard Inc via Kinesis (UK). The concentration of the congeners was 2.5 µg/ml in isooctane, except BDE 209 which was present at 25 µg/ml. BDE 77 (50 µg/ml in isooctane), PCB 209 (10 µg/ml in heptane) and 4PC-BDE-208 (50 µg/ml in toluene), which were used as surrogate standards, were purchased from Accustandard (via Kinesis UK), Sigma Aldrich (UK), and Wellington Laboratories (via Greyhound Chromatography UK) respectively, with purities higher than 98%.

Stock solutions were prepared by dissolving the reference and surrogate standard in acetone (at 500 ng/ml). Working solutions were then prepared by diluting the stock solutions in acetone for sample fortification and in ethyl acetate for instrumental analysis. All solutions were stored at 4 ^{o}C, and were allowed to reach room temperature for 15 minutes before use. Ultra-trace grade of acetone, ethyl acetate and isopropanol were obtained from Sigma Aldrich (UK). Oasis HLB cartridges (200mg, 6cc) were purchased from Waters (UK); Isolute C_{18} (1g, 6 ml), ABN and Isolute PAH cartridges (1.5g, 6 ml) were from Biotage (UK).

2.2 Sample collection

Grab wastewater samples (raw influent and final effluent) were collected from a nitrifying activated sludge treatment plant in North East England with a population equivalent of 22,500. Samples were collected in cleaned and disinfected (with 1% Virkron for 24 hours, then rinsed multiple times with distilled water to get rid of chlorine residues) HDPE containers - analysis of containers did not show any contamination with target compounds. Samples were stored at

4 ^{o}C upon arrival to the laboratory and were used within 24 hours. To account for the concentration of the chemicals in both aqueous and particulate matter, half of the samples were filtered through glass microfiber filter to estimate the concentration of the chemicals in the aqueous phase alone (Sartorius MGB filters, 0.7mm thick, 1.0 µm particle retention) before being passed through the SPE cartridges. The other half of the samples was not filtered before SPE.

2.3 GC Instrumentation

Analyses were performed on an Agilent 7890A gas chromatography system equipped with micro electron capture detector (µECD) and an Agilent 7683B automatic injector. A DB-5MS fused silica capillary column (15m x 0.25mm I.D. x 0.1µm film thickness) from J & W Scientific, USA was used for chromatographic separation. The inlet was fitted with an SGE single taper deactivated glass liner and samples were injected (1 µl) in split/splitless mode (1 min. splitless, then 30ml/min split). The inlet and detector temperature was set at 300 ^{o}C and 290 °C respectively. H_2 (99.999%) was used as carrier gas in constant flow mode (flow-rate 1 ml/min, velocity 91.3 cm sec^{-1}). The µECD was used with N_2 (99.999%) make-up gas at a flow-rate of 30 ml/min. The GC oven was heated from 100 °C (initial hold time 1 min) to 150 °C (hold time 0 min) at 50 °C/min. and then to 290 °C (final hold time 15 min) at 12.5 ^{o}C/min. Data was acquired and processed using a Thermo Atlas chromatographic data system (version. 8.3). Analytes were identified solely by their retention time and quantified by their integrated peak area.

The retention time of all eight PBDE congeners was confirmed using an Agilent 7890A GC split/split less injector linked to an Agilent 5975C MSD to ensure correct identification of analyte. This GC-MS was equipped with the same DB-5MS capillary column (15m x 0.25mm I.D. x 0.1µm film thickness) used above. Analysis was done in EI mode, full scan and SIM

spectrum was acquired. Ions (m/z) corresponding to the fragmentation of each BDE congeners was monitored to add a level of confidence in the identification of the analytes (Table *2*).

2.4 SPE procedure and optimization

To achieve the low detection limits required for the quantification of analytes at environmental levels, the performance of SPE cartridges including Oasis HLB (200mg, 6ml), Isolute C18 (1g, 6ml) and Isolute PAH (1.5g, 6ml) was evaluated for the extraction of PBDEs from wastewater samples. Oasis HLB and C18 cartridges are popular choices for the extraction of PBDEs from environmental samples and clean-up [16, 17, 25, 26]. The extraction procedure was modified from a Biotage application note for analysing polycyclic aromatic hydrocarbons (PAHs) in water [27]. Briefly, isopropanol (1% v/v) was added to 100 ml of filtered wastewater sample without pH adjustment for extraction of PBDEs in the aqueous phase, followed by addition of surrogate standards at 5 ng/L each for BDE 77, PCB 209 and 50 ng/L for 4PC-BDE-208, before passing through the SPE. Cartridges were conditioned with 5 ml of isopropanol followed by deionized water containing 2 % isopropanol (v/v) at a flow rate of 5 ml/min. Samples were then passed through the cartridges at a flow rate of 10 ml/min. Sample bottles were then rinsed with acetone to prevent loss of analyte to the glass walls, and then diluted with 90 ml of deionized water before passing through the cartridges.This also serves as a prewash step to remove unwanted impurities including chlorophyll from the cartridges. The cartridge was finally washed with 10 % isopropanol (v/v) and dried under vacuum for 45 minutes. Elution was performed by passing 5 ml of ethyl acetate through the cartridge twice (10 ml total volume). The extract was then evaporated under a gentle stream of nitrogen at 30 ^{o}C using Labconco Rapidvap Evaporator to 500 µl before injection into the GC-ECD. Furthermore, between 50 ml – 200 ml of spiked water samples were tested, and 100 ml sample load performed better in terms of adequate analyte extraction/retention and low background noise. Different volumes of ethyl acetate were also tested to optimize analyte elution, and 10 ml (5 ml twice) showed optimum

result and was employed in further work. For extraction of PBDEs in both the aqueous and particulate matter (PM) phase, 20 ml and 50 ml of unfiltered influent and effluent was diluted to 100 ml with deionized water respectively, and was processed as the filtered samples above.

2.5 Method validation study

Analytes were identified mainly by their retention time and the calibration curves were generated by injecting reference PBDE standard in triplicate at five concentration levels: from 0.5 to 10 ng/ml for all PBDEs except BDE 209 which ranged from 5 – 100 ng/ml, because of its lower sensitivity to the ECD detector and relatively higher environmental concentrations. Linearity was observed when the correlation coefficient was > 0.99. To accurately predict the concentration of an unknown analyte in a sample- especially at trace levels, an appropriate calibration model is important. Selecting an appropriate calibration model involves a decision as to either allow the calibration curve pass through a point of intercept on the y-axis (so that $y = mx + c$) or force the curve to go through the origin (so that $y = mx$) [28]. Regression statistics of the calibration data on Microsoft Excel 2013 can be used to make this decision. This decision is based on closeness of the y-intercept to zero, and can be tested statistically using standard error (SE) [28]. The standard error of the y-intercept (SE_y) obtained by the regression analysis is based upon the variability at the y-intercept and can be used to test if the curve passes through zero such that;

When y-intercept > SE_y, use intercept such that $y = mx + c$

When y-intercept ≤ SE_y, force curve through the origin, such that $y = mx$ ($c = 0$).

The determined calibration curve model and equation for all eight BDE congeners is given in Table 3.

PCBs have similar physical-chemical properties to BDEs [29], therefore rare PCB congeners can be used as surrogates for PBDE determination. BDE 77 and PCB 209 were primarily selected for internal standard quantification for BDE 28 to BDE 183 because of their absence from wastewater; these compounds have been used for this purpose in previous studies [15, 17, 25]. However, BDE 77 was dismissed as a surrogate when initial analysis of environmental samples showed its presence in wastewater. 4PC-BDE-208 was employed as surrogate standard for BDE 209. This compound was proposed by Wellington Laboratories (Canada) as the perfect surrogate for decaBDEs when an instrumental method without the capability to differentiate between mass-labelled and parent compound is employed, such as an GC-ECD method [30]. Additionally, the similarity in structure/chemical composition to BDEs, and absence in environmental samples supports its suitability as a surrogate standard. The effect of complex sample matrix on GC-ECD analysis was investigated by spiking the PBDE congeners at 10 ng/L into deionized water (DI), influent and effluent (n = 3). The spiking was performed after sample extraction with SPE and prior to GC sample injection. DI water was used as blank/absolute recovery and this recovery was compared to recoveries recorded in the influent and effluent samples.

Method accuracy was evaluated by performing recovery experiments in blanks (deionized water, n = 3) and matrix samples (final effluent, n = 3) at two fortification levels (3 ng/L and 10 ng/L, 30 ng/L and 100 ng/L for BDE 209). The repeatability of the method was determined by the relative standard deviation (% RSD) from the recovery experiments in the fortified blank and matrix sample [31, 32]. Instrumental limits of detection (IDL) were established as the lowest analyte concentration that gave a signal to noise ratio of three (s/n = 3) upon the injection of standard solutions, and was determined on the Atlas software. The method detection limit (MDL) was determined according to EPA method 1984 [33]. Briefly, analytes were spiked at a concentration of between one to five times of IDL in blank (DI water, n = 7) and matrix sample

(effluent, n = 7), then analysed on the GC-ECD. The resultant standard deviation was multiplied by the students' T-value that corresponds to six degrees of freedom to estimate the MDL.

$$MDL = T_{(n-1,1-\propto=0.99)} * (S)$$

Where: $T_{(n-1,1-\propto=0.99)}$ = students' T value for a 99% confidence level, and a standard deviation estimate with n – 1 degrees of freedom.

S = standard deviation of replicate analyses.

The method quantification limit (MQL) was set at three times the MDL.

3. Results and discussion

3.1 Chromatographic performance

Splitless injection mode is preferred for PBDE analysis due to the relatively low environmental levels of these compounds, and a high injection temperature (300 ^{o}C and above) is recommended to minimise discrimination of high molecular weight congeners and thermal degradation [22]. Chromatographic separation optimization is necessary when analysing a wide range of BDE congeners (from triBDEs to decaBDEs) as this ensures good separation of compounds of interest when using a one-column approach. Employing a short column (15 m) with a short internal diameter ($\leq$ 0.25 mm) allowed the detection and quantification all eight BDE congeners including BDE 209 that easily degrades in GC column when subjected to high temperatures, without compromising separation, as reported by other authors [8, 14]. Using a relatively high carrier gas flow rate further helped to reduce the degradation of BDE 209 within the GC inlet and column. This optimization made it possible to use one GC column instead of two separate GC columns: one for lighter BDEs and one for BDE 209, as used in previous studies for GC-ECD analysis [15, 20]. The optimized temperature program in addition to the capillary column applied allowed for the separation of the BDE congeners in under 16.5 min, with BDE 28 eluting first at 5.3 min and BDE 209 at 16.4 mins (Figure *1*; Figure *2*).

3.2 SPE optimization and method performance

The extraction procedure was optimized by testing different SPE cartridges for their efficiency in analyte retention. Cartridges tested included Oasis HLB (200 mg), HLB prime, C18 (1 g), and Isolute PAH (1.5 g). Oasis HLB and C18 have been popular choices in the literature for extraction of BDEs from water samples [16, 17, 25]. The result of preliminary recovery experiments of eight PBDE congeners spiked in water samples showed that Isolute PAH performed better than the other cartridges, especially in filtered effluent samples. With Isolute PAH recovery of

analytes was between 69 – 126 % with a relative standard deviation (RSD) of less than 13% (Table 4), and mean recoveries in effluent were significantly greater than with the Oasis HLB cartridge (Mann-Whitney test, $P < 0.05$) and slightly but not statistically significantly greater than with Isolute C18 (Mann-Whitney Test, $P = 0.06$). The recoveries were not significantly different in DI water. Extracts from the Isolute PAH cartridge showed the least interference and lower background noise. The cartridge comprises an octadecyl layer (1000 mg) with an amino based sorbent (500 mg) that helps remove polar interferences such as humic acids from the effluent according to Biotage (the cartridge manufacturer), thereby reducing background noise and improving analyte recovery. Therefore, the Isolute PAH was selected for further optimization and subsequent analysis.

The matrix effect test showed that peak signals of the PBDE congeners were enhanced in influent and effluent samples, except for BDE 99, which was suppressed in effluent (Figure *3*). The matrix effect was about -31% (BDE 99) to 91% (BDE 209), and 3.2% (BDE 99) to 65% (BDE 209) in influent and effluent samples respectively (Figure *3*). The matrix effect also appears to be generally higher in influent than in effluent, which might be expected due to the presence of more organic matter in the influent. This observed signal modulation implies that surrogate standards are needed to accurately quantify PBDE congeners in wastewater.

Further recovery studies were carried out on effluents with the Isolute PAH cartridge at two fortification levels; 3 ng/L and 10 ng/L for all BDEs, except BDE 209, which was studied at 30 ng/L and 100 ng/L because of its relatively higher concentrations in wastewater. Recoveries were surrogate standard-corrected using PCB 209 and 4PC-BDE-208 as standards. The US EPA method 1614 recommends analyte recovery of 60 – 140 % for BDE 28 – 183, and 50 – 200% for BDE 209, with an RSD less than 40 % for initial demonstration of method precision and accuracy [34]. At 3 ng/L fortification level (Table 5) good recoveries (78- 135%) were

recorded for all analytes except BDE 47 (176%). Recoveries of PBDEs at 10 ng/L were mostly between the acceptable ranges of 60 – 129 %, except BDE 183 with recovery of 150 % (**Error! Reference source not found.**). RSDs were generally lower than 20% thereby showing method precision.

As shown in Table 4, the MDL of the eight PBDE congeners in water ranged from 0.14 ng/L to 10 ng/L in deionized water and 0.2 ng/L to 10.8 ng/L in effluent samples. These values are below the reported levels of these chemicals in wastewater around the world [10, 25, 35], hence the method can be used globally to quantify them accurately. The method detection limits achieved were also well below the maximum allowable concentration environmental quality standards (MAC-EQSs; 140 ng/L and 14 ng/L for inland and other surface waters respectively) for PBDEs (sum of congeners BDEs 28, 47, 99, 100, 153 and 154) as proposed in the EU Water Framework Directive (WFD) [36].

3.3 Application to wastewater samples

The developed method was employed to quantify the concentrations of eight primary PBDE congeners in both the dissolved and particulate matter phase of municipal wastewater, and assess the mass removal rate achieved by the treatment plant. The PBDE congener profile in influent and effluent showed the presence of seven congeners; BDE 100 was not detected (Figure *4*). The total (aqueous and particulate matter) concentration of the individual PBDEs was between 2.1±0.3 – 111±10.7 ng/L (∑PBDE = 169 ng/L) in raw influent and 1.6±0.3 – 17.7±1.4 ng/L (∑PBDE = 43 ng/L, ∑PBDE EU WFD congeners = 19 ng/L) in the final effluent (Table 6). These concentrations are an order of magnitude lower than the EU WFD MAC-EQS and are similar to reported levels in Canada [10] Australia [37], and China [25]. Risk assessment for BDE 209 was performed as reported by Cristale *et al*., 2013 [16] since there is no specific EQS value for this single congener. The predicted no effect concentration (PNEC) for aquatic

297 organisms (including fishes, daphnids and algae) was calculated as 4.8 ng/L and maximum
298 measured concentration (MC) was used to obtain a risk quotient (RQ = MC/PNEC). Low to
299 significant adverse effect of BDE 209 was indicated when $1.0 \leq RQ < 10$, and $10 \leq RQ < 100$
300 respectively. In the present study, low potential for adverse effects on aquatic organisms was
301 observed for BDE 209 in the effluent of the WWTP, with an RQ of 3.7.

302 The concentration of BDE 209 represented 66% of the total PBDE concentration in influent
303 and 40% in effluent samples (Figure *4*). BDE 209 has been reported to dominate the total
304 concentration of PBDE found in wastewater around the world [10, 35, 38]. Furthermore, only BDE
305 209 was detected in a river receiving effluent from wastewater treatment plants in the United
306 Kingdom, to a concentration of up to 290 ng/L which were associated with significant levels
307 of risk to aquatic wildlife [16]. About 82% and 49% of the total PBDE was present in the
308 particulate phase of the influent and effluent respectively (Figure *5*). This is as expected due to
309 the high log K_{OC} values (Table *1*) of PBDEs, which gives rise to a high association with
310 suspended solids. This observation is in line with previous findings that over 90% of PBDEs
311 in influent tends to absorb to sludge in WWTP [39, 40]. About 75% removal of total PBDE
312 concentration was achieved by the WWTP; this removal is most likely due to partitioning and
313 settling out with the sludge in the primary and secondary sedimentary tanks [39]. PBDE
314 concentrations in sludge are reported to vary over one order of magnitude [19, 41], while
315 concentrations in anaerobically treated sludge are unknown. The potential risks PBDEs pose
316 via sludge applications to soil are therefore also unknown. Furthermore, the distribution of
317 PBDE over the dissolved and particulate matter (PM) phases in influent and effluent samples
318 (Figure *5*) indicates that PBDEs were mostly removed via the PM phase in the WWTP. It must
319 be noted that wastewater treatment is the preserve of high-income countries and cities, with
320 80% of the world's wastewater going untreated into receiving watercourses [42]. Concentrations

of PBDEs in domestic wastewater may be at levels that cause a risk to aquatic wildlife in highly populated urban centres, especially in low to middle income countries.

4. Conclusions

In this study, a novel SPE-GC-ECD method was developed for the analysis of eight primary PBDE congeners in wastewater. The extraction procedure was optimized by testing different SPE cartridges including Oasis HLB, Isolute C18 and Isolute PAH. The Isolute PAH cartridge proved to be superior in sample clean up and extracting low and high molecular weight PBDEs in wastewater, perhaps due to its unique combination of a C18 and amino based sorbent. The detection of the PBDEs by this method was corroborated using GC-MS. The low detection limits obtained allowed for determination of PBDEs at environmentally relevant levels, and, importantly, were well below the proposed MAC-EQSs for PBDEs set by the EU Water Framework Directive. Although the method presented here may have limitations due to a lack of selectivity of the GC-ECD compared to a GC-MS/MS system, it is a more cost-effective solution for quantifying PBDE concentrations in wastewater, especially if widespread monitoring is required. GC-ECD is two-fold cheaper than GC-MS in capital costs and up to four-fold cheaper in operational costs since all PBDE congeners can be analysed in a single run. This method thereby opens up PBDE analysis to laboratories without a GC-MS system or can allow laboratories to commit GC-MS systems to other functions. This will be especially useful in developing countries with limited resources to carry out environmental analyses and could form the basis of a traffic light system whereby water bodies potentially at risk could first be flagged using this method for subsequent confirmation using the more expensive GC-MS method. If we rely solely on the best most expensive methods, we would miss the broader picture of a global chemical pollution; which of-course starts with the ability to detect and measure these chemicals in water.

The GC-ECD method was employed for the quantification of PBDEs at trace levels in the influent and effluent of a UK conventional activated sludge WWTP. All BDEs of interest were

detected in both influent and effluent samples at trace concentrations, that is, in the 2-20 ng/L range, except BDE 209, which was present at about tenfold higher concentrations, and BDE 100, which was not detected. BDE 209 was the most abundant analyte, and 50 – 80% of the BDEs partitioned onto the particulate matter. Finally, about 75% removal of the total PBDE concentration was achieved by the WWTP, resulting in levels assessed to pose little but not negligible risk to aquatic wildlife. PBDE removal was most likely due to adsorption onto sludge during secondary treatment. Urban areas with little or no wastewater treatment might not be afforded a similar level of environmental protection against PBDEs as offered by the WWTP in our study area.

Conflict of interest

The authors have no potential conflicts of interest.

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Table 1. Names, abbreviations, physical and chemical properties of PBDE congeners investigated in this study.

PBDE	Acronym	Molecular formula	Molecular weight (g/mol) [b]	Solubility (g/L) at 25°C, pH 7 [b]	Log K_{OW} [a]
2,4,4'-TriBDE	BDE 28	$C_{12}H_7Br_3O$	406.90	7.7×10^{-4}	5.88
2,2',4,4'-TetraBDE	BDE 47	$C_{12}H_6Br_4O$	485.79	2.5×10^{-4}	6.77
2,2',4,4',5-PentaBDE	BDE 99	$C_{12}H_5Br_5O$	564.69	6.2×10^{-5}	7.66
2,2',4,4',6-PentaBDE	BDE 100	$C_{12}H_5Br_5O$	564.69	7.3×10^{-5}	7.66
2,2',4,4',5,5'-HexaBDE	BDE 153	$C_{12}H_4Br_6O$	643.58	1.6×10^{-5}	8.55
2,2',4,4',5,6'-HexaBDE	BDE 154	$C_{12}H_4Br_6O$	643.58	1.9×10^{-5}	8.55
2,2',3,4,4',5',6-HeptaBDE	BDE 183	$C_{12}H_3Br_7O$	722.48	5.6×10^{-6}	9.44
DecaBDE	BDE 209	$C_{12}Br_{10}O$	959.17	1.4×10^{-6}	12.11

Adopted from a [17], b [7]

Table 2. Comparison between the retention times and monitoring ions of PBDE congeners of interest using GC-MS and GC-ECD.

Analyte	GC-ECD t_R (min)	GC-MS t_R (min)	EI-MS/SIM (m/z)	
			Ion 1	**Ion 2**
BDE 28	5.26	5.35	246	246
BDE 47	6.69	6.83	326	324
BDE 99	7.76	7.92	405.5	403.5
BDE 100	8.13	8.30	405.5	403.5
BDE 153	8.98	9.17	483.5	481.5
BDE 154	9.47	9.66	483.5	481.5
BDE 183	10.67	10.87	563.5	561.5
BDE 209	16.08	16.76	811.5	809.5

t_R = retention time

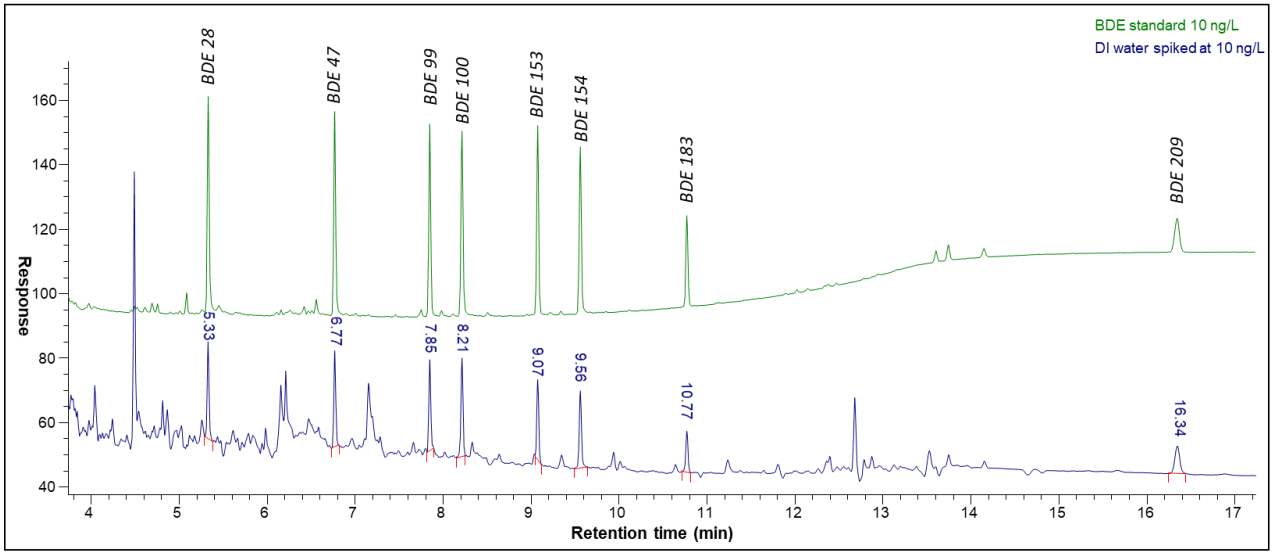


Figure 1. Example GC-ECD Chromatogram of eight primary BDE congeners spiked in deionized water (blue trace) at 10 ng/L (except BDE 209 spiked at 100 ng/L) over-layed by PBDE analytical standard (green trace). The PBDEs were spiked in deionized water before SPE.

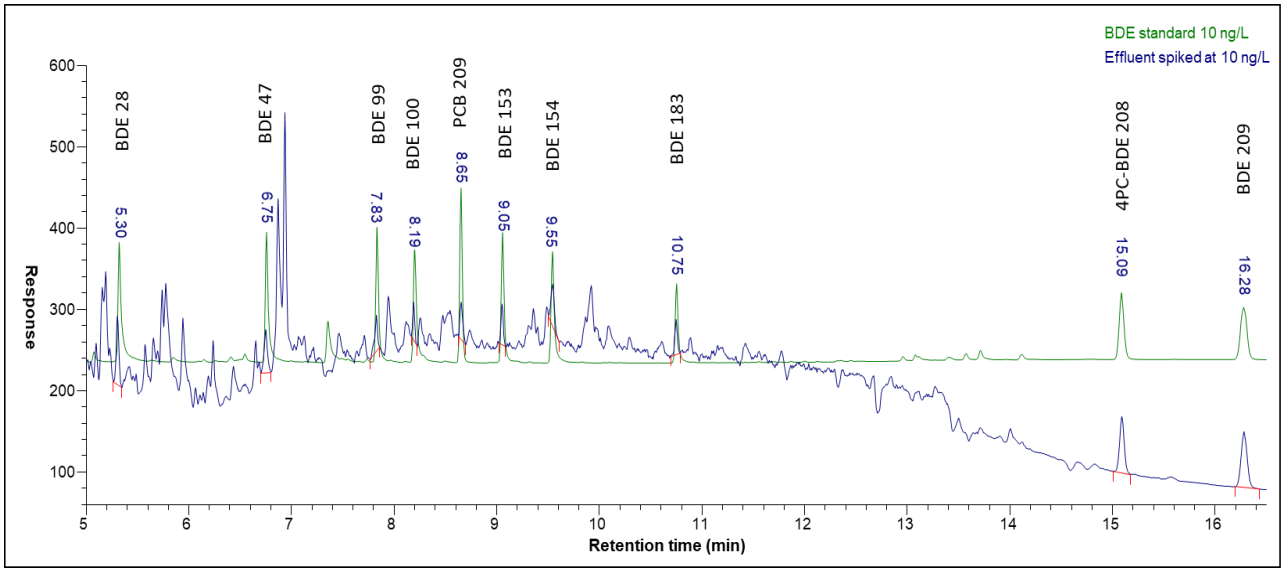


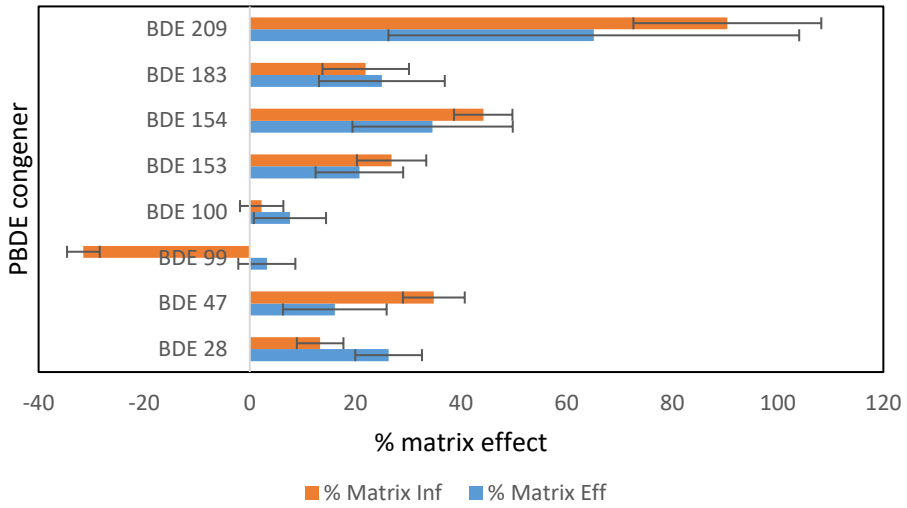
Figure 2. Example GC-ECD Chromatogram of eight primary BDE congeners and two surrogate standards in effluent (blue trace) spiked at 10 ng/L (except BDE 209 and 4PC-BDE-209 spiked at 100 ng/L) over-layed by PBDE analytical standard (green trace). The PBDEs were spiked before SPE

Table 3. Determined calibration curve model and equations for the 8 PBDE congeners

Compound	R^2	y-intercept	Standard Error (SEy)	Calibration model	Calibration equation
BDE 28	0.9992	0.106	0.049	y = mx + c	y = 0.1335x – 0.1065
BDE 47	0.9991	0.150	0.078	y = mx + c	y = 0.1391x – 0.1497
BDE 99	0.9980	0.150	0.083	y = mx + c	y = 0.1391x – 0.1501
BDE 100	0.9982	0.157	0.070	y = mx + c	y = 0.1242x – 0.1568
BDE 153	0.9993	0.010	0.045	y = mx + c	y = 0.0061x – 0.0398
BDE 154	0.9988	0.122	0.053	y = mx + c	y = 0.1152x – 0.1220
BDE 183	0.9978	0.085	0.042	y = mx + c	y = 0.0670x – 0.0847
BDE 209	0.9995	0.045	0.046	y = mx + c	y = 0.0058x – 0.0942

Table 4. Recoveries and relative standard deviation (RSD) of PBDEs spiked in deionised water and effluent at 10 ng/L. RSD in bracket

BDE	Recoveries in DI water (%)			Recoveries in effluent (%)		
	Isolute C 18	Isolute PAH	Oasis HLB	Isolute C 18	Isolute PAH	Oasis HLB
BDE 28	85 (1.7)	54 (0.7)	60 (7.1)	71 (12.5)	78 (5.8)	46 (8.1)
BDE 47	75 (8.2)	61 (13.9)	65 (7.5)	84 (10.9)	79 (0.6)	44 (13.1)
BDE 99	76 (16.7)	73 (1.6)	70 (9.1)	63 (2.1)	82 (0.8)	52 (10.6)
BDE 100	71 (17.8)	58 (3.3)	79 (4.5)	52 (1.0)	69 (0.2)	33 (0.6)
BDE 153	59 (18.1)	56 (0.9)	53 (10.1)	45 (2.5)	94 (8.9)	42 (1.8)
BDE 154	58 (19.6)	53 (1.2)	51 (6.3)	70 (27.2)	97 (12.5)	59 (8.6)
BDE 183	59 (8.3)	60 (2.7)	62 (7.9)	53 (12.7)	126 (0.1)	35 (3.5)
BDE 209	52 (23.8)	96 (0.1)	65 (10.2)	169 (13.3)	90 (12.8)	122 (6.2)
AVERAGE	66.88	63.88	63.13	75.88	89.38	54.13
SD	11.46	14.40	9.00	39.66	17.44	28.69



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485 Figure 3. Observed matrix effect in the analysis of PBDEs in influent and effluent samples. Inf
486 and Eff represents influent and effluent respectively

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489 Table 5. Surrogate corrected recovery at 3 ng/L and 10 ng/L in effluent, instrumental
490 detection limit (IDL) and method detection limit of PBDE congeners in deionized (DI) water
491 and effluent. Surrogate standards are indicated in italics.

Chemical	Corrected recovery in effluent (%)		IDL* (pg/µl)	MDL (ng/L)	
	3 ng/L	10 ng/L		DI water	Effluent
BDE 28	78 (16.1)	90 (7.0)	0.2	0.68	0.66
BDE 47	176 (11.7)	129 (15.2)	0.2	0.54	2.57
BDE 99	74 (6.4)	116 (4.7)	0.5	0.33	2.54
BDE 100	62 (5.1)	83 (7.3)	0.5	0.27	1.89
BDE 153	78 (1.8)	112 (9.1)	0.5	0.14	0.20
BDE 154	131 (7.5)	60 (6.7)	0.5	0.44	4.19
BDE 183	86 (8.2)	150 (6.7)	1.0	0.53	1.31
BDE 209	135 (3.4)	113 (2.3)	5.0	10.04	10.76
PCB 209	-	-	0.2	0.28	1.09
4PC-BDE-208	-	-	5.0	4.37	6.12

492 *IDL was determined with PBDE analytical standard

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Table 6. Concentration of BDE congeners in effluent and influent of a UK activated sludge WWTP

PBDE congener	Influent (ng/L)	Effluent (ng/L)	% Removal
BDE 28¶	11.0 (1.3)	4.4 (0.2)	60.0
BDE 47¶	7.1 (0.2)	6.0 (0.1)	15.5
BDE 99¶	10.7 (0.4)	2.8 (0.8)	73.8
BDE 100¶	< 1.89	< 1.89	-
BDE 153¶	7.1 (0.6)	1.6 (0.3)	77.5
BDE 154¶	2.1 (0.3)	4.0 (1.3)	0
BDE 183	20.0 (0.2)	6.3 (0.5)	68.5
BDE 209	111.3 (10.7)	17.7 (1.4)	84.1
∑PBDE	169.4	42.9	74.7
∑PBDE EU MAC-EQS	-	18.8	29.9*

¶ PBDE congeners included in the EU WFD * Average % removal

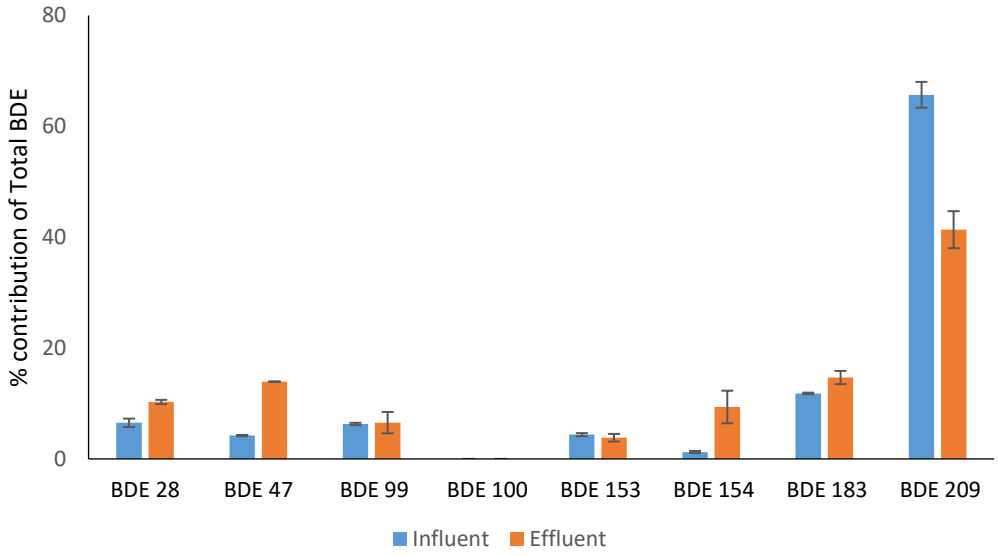

Figure 4. Distribution of BDE congeners in influent and effluent samples from a UK WWTP

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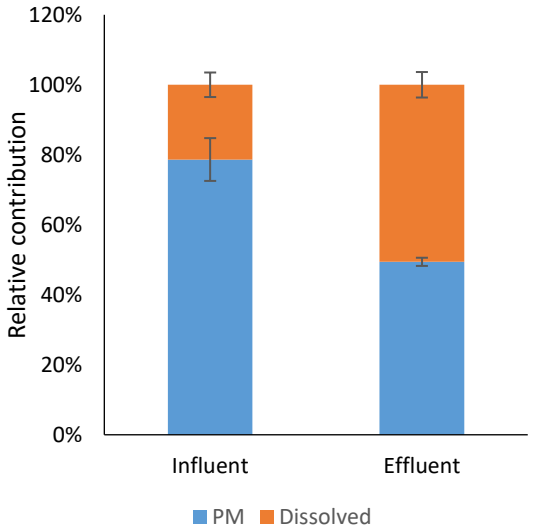

504 Figure 5. Partitioning of PBDEs over
505 particulate matter (PM) and dissolved phases of
506 influent and effluent samples from a UK
507 wastewater treatment plant.

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