LC-ESI-IT-MS/MS ANALYSIS OF ORGANOPHOSPHOROUS FLAME RETARDANTS AND PLASTICISERS IN SELECTED ENGLISH MARINE SEDIMENTS AND SURFACE WATERS

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Introduction

Organophosphorus (OP) esters are utilised as flame retarding compounds for combustion inhibition and as plasticisers to provide products with desirable technical and physical properties [1]. The more commonly used organophosphorus flame retardants (OPFRs) are the halogenated (chlorinated) or non-halogenated alkyl- and aryl-phosphate esters [2]. As the production of some brominated (penta- and octabrominated diphenyl ethers) flame retardants have now been phased out, the development and manufacture of alternatives such as OPFRs has risen over recent years and it is anticipated that the global consumption for OPFRs will increase [3]. During 2005, approximately 275 000 tonnes of chlorinated phosphates *plus* non-halogenated phosphorous-based chemicals were consumed within the European flame retardant market [4]. Where flame impedance is required, the OPFR may be added to the surface of the product and is thus, not chemically or covalently bound to the surface matrix [4]. OPFRs have the potential to migrate to the open environment by processes of abrasion, diffusion, leaching, and volatilisation [5-9]. Numerous investigations have demonstrated a range of OPFR and plasticisers in sediments and surface waters [7, 10-13]. As potential sources for their introduction to the aquatic environment, OPFRs have been quantified in influents, effluents and settled sludges of wastewater treatment plants [14-18]. For several chlorinated OP esters, no elimination by treatment has been reported [15].

To date, and for the analysis of OP-based FRs and plasticisers, there are only several examples of published methods describing the use of reversed-phase (RP), liquid chromatography with electrospray ionisation (ESI) and tandem mass spectrometry (LC-MS/MS) [13,18,19]. The objective of this work was to develop an analytical method employing RPLC-ESI-Ion Trap (IT)-MS/MS for the purpose of determining 13 target OP compounds. After establishing method performance characteristics of the selected extraction techniques for marine sediment and water matrices, the aim of the work was to apply the method to real samples from several English locations. To our knowledge, this is the first study to describe field observations of concentrations and profiles of these chemicals derived from RPLC-ESI-IT-MS/MS in a selection of English aquatic and abiotic samples.

Materials and methods

Extraction and clean up of sediment and surface water samples

All solvents were HPLC-grade (Rathburns Chemicals, Scotland, United Kingdom). OPFRS and plasticisers (Table 1) were purchased from QMX Laboratories (Thaxted, UK), ACROS Organics (Geel, Belgium) or Sigma-Aldrich (Dorset, UK). *Strata-X* (200 mg) solid phase extraction (SPE) cartridges were obtained from Phenomenex (Cheshire, UK). Mixtures of calibration standards were prepared from stock solutions and in the range of 1-500 ng/mL with the addition of deuterated internal standards (IS; 2 µg/mL). Grab sediment samples were collected under the 2005-06 UK National Marine Monitoring Programme and from locations including the River Mersey and Liverpool Bay (NW England), Tees Bay and off the Tees and Tyne estuaries (NE England) and the Thames estuary; the upper 3 cm of the sediments were retained. Thawed, air-dried and ground (<2 mm) 15 g sub-samples were extracted via accelerated solvent extraction (*ASE 300*; Dionex Corp., United States) after fortification with IS. Extractions were performed using dichloromethane/acetone 1:1 (v/v) under the following

conditions: 90 °C, 1500 psi, two static cycles incorporating 5 min heating, 5 min static followed by a 60 % flush and a 60 s purge. Extract clean up by gel permeation chromatography was then undertaken using an *Agilent 1100* LC (Agilent Technologies, Germany) and two, in-series, styrene/DVB polymeric, 25 x 300 mm *PL EnviroPrep* columns (Polymer Laboratories Ltd, UK;) to remove sulphur and co-extracted lipid interferences. OPFRs and plasticisers were found to elute between 31 and 40 min and this fraction was collected and then transferred to methanol for analysis. Target analytes from filtered (0.45 µm), 1 L surface water samples (*plus* 2 µg IS) were isolated using SPE. Cartridges were eluted with 3 x 4 mL methanol and the combined eluents were concentrated by evaporation. Procedural blanks consisting of *Hydromatrix* (for ASE) or high purity water were prepared within each batch of experimental or real sample analysis for the purpose of quality control (QC).

Liquid chromatography and mass spectrometry

Determinations of OP analytes were performed using a *Surveyor LC* (ThermoFinnigan, CA, US) and separations were made with a *Gemini C18(2)* column (250 x 2.0 mm; 5 µm + pre-column; Phenomenex). Acceptable separation was achieved using a linear gradient (0.2 mL/min; 45 °C; 20 µL injection volume) consisting of solvent A (water:methanol; 80:20) and B (methanol), each with 0.2 % formic acid. From time 0 min, solvent B was increased from 55 to 70 % (over 5 min) and then to 100 % (over 10 min); this was held for 10 min, after which B was decreased to 55 % (in 0.1 min) and the column was then equilibrated for 9.9 min. Experiments using 20 mM ammonium acetate as an organic modifier were also conducted. MS/MS analyses were performed using a *LCQ Advantage* ion trap MS (ThermoFinnigan) and operated in positive ion mode. Flow injection analyses of individual compounds established optimal fragmentation energies for transition ions. A capillary temperature of 220 °C was found to be optimum and typical settings were: sheath (nitrogen) flow-55 arbitrary units (AU); spray current - 0.29 µA; minimum injection time: 200 ms. For quantification, eight levels of calibrations, relative to two IS' were used. Calibration data were monitored through the use of a QC solvent-based OPFR standard solution at 250 ng/mL and where the observed value was within ± 20 % of the expected concentrations, the analysis was deemed acceptable. Target compounds were identified by the presence of two transition ions generated during SRM.

Determination of recovery performance characteristics

The recovery efficiency of the entire (ASE + GPC) method for sediments was established by fortifying 15 g (n=5) of OPFR-free, offshore marine sediment with the analytes [*ca* 500 ng each; equivalent to 30 µg/kg dry weight (d.w.)]. For surface water samples, recovery efficiencies for OPFRs were determined by spiking 1 L (n=6), high purity water samples with the suite of analytes (*ca* 500 ng each) and then isolating and eluting the compounds from methanol-conditioned cartridges. For both sediments and waters, recoveries were determined by establishing the response ratios between the peak area of each analyte and relative to that of the peak areas of the IS for each sample. Thus, recoveries were adjusted in-line with the recoveries of the IS. The linearity and repeatability of the calibration plots were determined by generating three separate calibration curves on the same (or *intra*) day and as well as on separate (*inter*) days. Instrumental limits of detection (LOD) were measured by injecting serially diluted calibration solutions and LODs were evaluated using a signal-to-noise (S/N) ratio of 3. Method limits of quantification (LOQ) were calculated using spiked sediment (15 g) or spiked, 1 L water samples and then evaluated using a signal to noise S/N ratio of 10.

Results and discussion

Analytical method performance characteristics

The *Gemini* column gave good chromatographic separation for the compounds under investigation and the addition of 0.2 % formic acid in the mobile phases was found to enhance compound ionisation. Retention times were in the range of 4.5 to 24.8 minutes. However, a suppression effect on signal response of 17 to 85 % across the range of analytes was found when 20 mM ammonium acetate was applied. The effective separation of the compounds enabled the use of scan segmentation within the MS/MS method and optimum sensitivity was attained. The use of SRM enabled greater selectivity over SIM and this was useful for their detection and confirmation in complex matrices such as marine sediments.

For linearity studies, regression coefficients (r^2) ranged from 0.985 to 0.999 for all compounds. The RSDs for *inter-day* calibrations were < 8 %. Instrumental LODs for each compound are shown in Table 1 and absolute masses of analytes ranged from 20 to 228 pg on-column. LOQ values ranged from 1 to 10 ng/L for water and 1 to 11 µg/kg d.w. for sediment.

Performance of ASE and GPC clean up methodologies for sediments and SPE for water samples
As a technique for extracting OPFR and OP-based plasticisers from marine sediments, ASE was selected over other traditional extraction techniques (*e.g.*, Soxhlet or physical shaking) due to its ease of use, low solvent consumption and cost effectiveness. The use of 100 mL extraction vessels enabled the extraction of at least 15 g of dried sediment and this permitted a large concentration step to be applied to improve sensitivity for the determination of trace (sub µg/kg) levels of the these compounds. Target analyte percentage recoveries of OP-based FRs and plasticiseres from spiked marine sediment ranged from 71 to 112 % (RSDs of 4 – 23 %; see Table 1).

Only one SPE sorbent (*Strata X*) was evaluated for the isolation of OPFRs and plasticisers from water samples and this product has the capacity to extract both polar and non-polar compounds. The percentage recoveries displayed by the compounds from spiked waters ranged between 71 and 117 % (RSDs of 3 - 18%; see Table 1). These recoveries were regarded as acceptable for the application of this approach to real samples. TMP and the deuterated IS - TMP-d9 were not retained by the sorbent and respectively, and poor recoveries of 5 and 2 % were found. These were eliminated from further studies.

Application of the method to the determination of OPFRs in sediment and riverine samples
Of the 13 marine sediment samples extracted and analysed, eight of the 14 OP-based FRs and plasticisers were found (TCPP, TBP, TCEPhi, TTP, TEHP, TPhP, TBPO, TBEP) and all other determinants were < LOD. Concentrations ranged from 1.2 to 179 µg/kg (d.w.) and TCPP was the most frequently found analyte, with respective median and maximum concentrations of 47 and 179 µg/kg (d.w.), the latter being measured in sediments off the Tees/Tyne estuaries, northeast England. The following median concentrations were determined: 4.8[TBP], 7.7[TCEPhi], 12[TTP], 4.8[TEHP] 9.9[TPhP], 9.0[TBPO] and 10[TBEP] µg/kg d.w.

Five (TCPP, TCEP, TPP, TBEP, TCEPhi) of the 14 OP compounds were also found in the single, riverine surface water sample taken from the River Mersey (north west England). Concentrations ranged from 6.2 to 1217 ng/L and TCPP showed the highest concentrations. Mean (n=3) concentrations of 9.4 (TBEP), 4.5 (TCEPhi) 20 (TPP), 1143 (TCPP) and 26(TCEP) ng/L were calculated.

The analytical method displayed satisfactory sensitivity and selectivity for the compounds under investigation. The acceptable recoveries and repeatability of the extraction and clean up methods indicated that it is fit for marine environmental monitoring. The concentration data derived from a limited survey of some of the more 'industrial' rivers and estuaries of England suggests that a detailed study of these chemicals is required to describe the spatial distribution and profiles of these chemicals and to investigate whether their uptake in the biotic components is pertinent.

Table 1. Linear range, instrumental limits of detection of OP-based FRs and plasticisers and their mean recoveries from fortified marine sediment and water.

Analyte	Acronym	Linear range (ng/mL)	Instrumental LOD (pg on-column)	Percentage recoveries and (% RSD)	
				sediment	water
Trimethyl phosphate	TMP	11.2-562	224	nd	5 (46)
Triethyl phosphate	TEP	5.1-513	102	74 (4)	104 (8)
Tripropyl phosphate	TPrP	1.2-571	24	71 (12)	71 (11)
Tributyl phosphate	TBP	1.1-542	22	82 (19	112 (12)
Tributyl phosphine oxide	TBPO	5.1-513	102	79 (11)	103 (18)
Tris-(2-chloroethyl) phosphite	TCEPhi	0.9-495	18	100 (15)	94 (4)
Tris-(2-chloroethyl) phosphate	TCEP	10.9-549	218	101 (8)	116 (3)
Tetraethylethylene diphosphonate	TEEyDP	5.4-538	108	71 (13)	117 (8)
Triphenyl phosphate	TPhP	6.2-622	124	86 (17	105 (4)
Tris-(2-chloroiospropyl) phosphate	TCPP	11.4-571	228	105 (18)	115 (4)
Tricresyl phosphate isomer mix	TTP	1.0-511	20	78 (23)	103 (6)
Dioctylphenyl phosphonate	DOPhP	5.6-557	112	73 (22)	100 (7)
Tris-(2-butoxyethyl) phosphate	TBEP	5.3-533	106	100 (15)	106 (5)
Tris-(2-ethylhexyl) phosphate	TEHP	1.0-511	20	91 (8)	97 (12)
Internal standards (IS)					
Trimethyl-d9 phosphate	TMP-d9	nd	nd	nd	2 (35)
Tri-n-butyl-d27 phosphate	TBP-d27	nd	nd	112 (11)	109 (9)

nd – not determined

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