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Synthesis and evaluation of molecularly imprinted polymers for extracting hydrolysis products of organophosphate flame retardants

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Abstract

A molecularly imprinted polymer (MIP) that selectively retains diphenyl phosphate was prepared using a structural analogue, ditolyl phosphate, as a template. Diphenyl phosphate is a degradation product of the flame retardant additive, triphenyl phosphate. The latter has been shown to be a common airborne contaminant in indoor environments and to be emitted from various goods such as video display units. Triphenyl phosphate induces several documented biological responses, including allergenic effects. Two different polymers, one prepared from methacrylic acid and the other from 2-vinylpyridine (2-Vpy), were investigated for their ability to recognise diphenyl phosphate. The polymers were used in solid-phase extraction cartridges (MISPE) and evaluated by comparing their recovery and breakthrough parameters with those of corresponding non-imprinted polymers (NIPs). The polymer made from the basic monomer showed the most selective recognition to the acidic analyte. Diphenyl phosphate was adsorbed to the basic MIP (2-Vpy-MIP) when methanol was used as mobile phase, and approximately 80% of the analyte was recovered when eluted from this polymer using a mixture of methanol and trifluoroacetic acid. There was a clear difference in the retention strengths of 2-Vpy-MIP and the corresponding 2-Vpy-NIP. The selectivity of the investigated 2-Vpy-MIP polymer towards a structural analogue of diphenyl phosphate, di(2-ethylhexyl) phosphate was also assessed. This compound was less strongly retained using the same experimental conditions. The results indicate that the prepared 2Vpy-MIP strongly recognises diphenyl phosphate due to the imprinting effect. This interaction probably arises mostly from an ionic interaction between the basic monomers and the acidic analyte. An LC–electrospray ionisation multiple MS method, using negative ion detection and ion-pair chromatography, was developed for separation and quantification of the strongly acidic dialkylated phosphate esters. The instrumental limit of detection was below 50 pg for all investigated compounds and the MS method was shown to be linear in the investigated range of 0.05–85 ng.

Keywords: Molecular imprinting; Solid-phase extraction; Flame retardants; Organophosphorus compounds

1. Introduction

Organophosphate esters are used in a variety of commercial products as flame retarding additives. One of these agents is triphenyl phosphate (Fig. 1), commonly used in video display units (VDUs) and other electronic equipment. Investigation of office environments with computers has shown that this compound is emitted from the VDUs into the surrounding air [1]. Triphenyl phosphate exhibits contact allergenic properties [2] and has also proved to be a potent inhibitor of human blood monocyte carboxylesterase [3].

Diphenyl phosphate has been identified as a major degradation product of triphenyl phosphate in water and sediments [4]. According to data in the literature,

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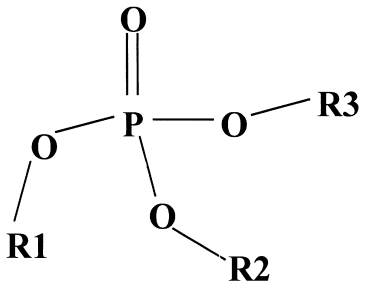

R1 = R2 = R3 = phenyl;	Triphenyl phosphate
R1 = R2 = phenyl R3 = H;	Diphenyl phosphate
R1 = R2 = butyl R3 = H;	Dibutyl phosphate
R1 = R2 = tolyl R3 = H;	Ditolyl phosphate
R1 = R2 = 2-ethylhexyl R3 =H;	Di(2-ethylhexyl) phosphate

Fig. 1. Structures of the compounds used in this study. Ditolyl phosphate was used as template, dibutyl phosphate as internal standard and diphenyl phosphate as the target analyte.

hydrolysis to the corresponding diester also appears to be a probable pathway for the metabolism of triphenyl phosphate. The metabolite di(2-chloroethyl) phosphate has been isolated as a major metabolite from the flame retardant tri(2-chloroethyl) phosphate in rat urine [5]. The same metabolite has also been detected in vitro in metabolic studies using liver and blood preparations from rats and human liver slices [6]. By analogy with the chlorinated diester, di-*p*-tolyl phosphate has been shown to be a major metabolite of tritolyl phosphate in rat urine [7].

Since the metabolites are expected to occur in trace amounts in biological samples, such as human blood or urine, highly sensitive and selective analytical methods are required for their analysis. Sample pre-treatment and clean up are the most critical steps in these kinds of analyses. Furthermore, diphenyl phosphate esters are highly polar and acidic compounds, which are difficult to extract from aqueous samples using conventional extraction methods.

The popularity of solid-phase extraction (SPE) has increased in recent years, since it is a fast and easy extraction method, which is easily automated. Another rapidly growing trend is the design and use of highly selective sorbents, such as molecularly imprinted polymers (MIPs).

Development of an MIP involves polymerisation of functional monomers in the presence of a template compound or imprint species. The monomers may interact with the template molecules either by non-covalent interactions such as ionic, reversible covalent reactions, or by metal ion-meditated interactions. The interactions are preserved during the polymerisation by co-polymerisation with cross-linkers into a highly cross-linked network. Subsequent removal of the template compound from the polymer gives rise to ''memory sites'' that are sterically and chemically complementary to the imprint species.

MIPs have been used not only for SPE (MISPE), but also as chiral stationary phases in liquid chromatography (LC) [8], capillary electrochromatography [9], various sensors [10], catalysis and immunoassays [11–13]. Several reviews have been published on the subject [14–16]. In recent years MISPE has been used for bioanalysis [17,18], food analysis [19] and environmental analysis [20–22].

Since the imprinted molecules are highly embedded in the polymer network it is difficult to completely remove the template prior to analysis of the sample. Consequently, a problem associated with MISPE when using the specific analyte of interest as a template is the template bleeding from the polymer during the desorption phase and interfering with the quantification. In this study, a structural analogue to the analyte was used as the imprint species [23] to avoid this potential problem.

The aim of this work was to investigate if MIPs could be used as SPE sorbents for selective clean up of diphenyl phosphate in different solvents. This compound and two of its analogues, ditolyl phosphate and dibutyl phosphate, have previously been determined using gas chromatography with nitrogen–phosphorus detection (GC–NPD) [4,24], and GC–flame photometric detection (FPD) [25] following methylation. However, derivatisation of complex matrices is not straightforward. Clean-up by MIP combined with determination by LC–electrospray ionisation (ESI) multiple mass spectrometry (MS^n) is an attractive approach since it enables less laborious, fast and highly sensitive analysis, suitable for

analysing biological samples. Due to its strength as a tool for identification, MS is often preferable to other detection methods, while ESI is an appropriate technique for ions, such as the investigated organophosphate anions, which allows the analytes to be quantified in their native forms. Dibutyl phosphate has previously been determined by ESI-MS^n, without chromatographic separation [26]. One objective of this work was to develop a chromatographic method for organophoshate diesters, suitable for on-line coupling to ESI-MS.

2. Materials and methods

2.1. Chemicals

A mixture of tritolyl phosphate isomers (purity 90%) and barium hydroxide monohydrate (98%) were purchased from Aldrich (Milwaukee, WI, USA). Diphenyl phosphate (97%) and di(2-ethylhexyl) phosphate (97%) were bought from Sigma–Aldrich (Steinheim, Germany). Dibutyl phosphate (97%) was obtained from Fluka (Buchs, Switzerland). Diethyl ether (analytical-reagent grade), hydrochloric acid (37%), acetonitrile (HPLC grade) and chloroform (analytical-reagent grade) were all purchased from Riedel-de Haën (Seelze, Germany). Ethanol was supplied by Kemetyl (Haninge, Sweden). Methanol (HPLC grade) was purchased from BDH (Poole, UK), and magnesium sulfate from Mallinckrodt (St. Louis, MO, USA). Methacrylic acid (MAA), 2-vinylpyridine (2-Vpy), ethyleneglycol dimethacrylate (EGDMA), acetic acid, trifluoroacetic acid (TFA) and triethylamine (TEA) were purchased from Merck-Schuchardt (Hohenbrunn, Germany). 2,2-Azobisisobutyronitrile (AIBN) was obtained from Acros Organics (Geel, Belgium). The water used was purified with a Modulab purification apparatus from Continental (San Antonio, TX, USA).

2.2. Synthesis of template

Tritolyl phosphate, 6.5 g, and $Ba(OH)_2 \cdot H_2O$, 5.1 g, were dissolved in a mixture of ethanol–water (40:60) [27]. The reaction mixture was refluxed for 30 min, cooled to ambient temperature and then chilled on ice. The obtained crystals were recrystallized twice in 35% ethanol and then washed in chilled solvent. The crystals were subsequently dissolved by addition of hydrochloric acid to a pH of approximately 2, followed by three extractions with 50 ml diethyl ether. The ether phases were pooled and then washed with a solution of saturated sodium chloride and finally dried over magnesium sulfate. After evaporation of the solvent the oily product was dried in a desiccator overnight. The product was stored at 7°C prior to use.

The purity of the obtained compound was checked by ^1H 400 MHz nuclear magnetic resonance (NMR), ^{13}C 100 MHz NMR, LC–atmospheric pressure chemical ionisation (APCI) MS, GC–flame ionisation detection (FID) and GC–NPD.

2.3. Synthesis of MIPs

Molecularly imprinted polymers were prepared following the procedure described by Andersson [17]. For the polymerisation reaction, a UV lamp (Model UVL-56, UVP Upland, USA) emitting at 365 nm was used. Ditolyl phosphate (template), 0.1 g, and AIBN (initiator), 55 mg, were weighed into a flask, dissolved in 6 ml chloroform and then briefly ultrasonicated. To synthesise an acidic polymer (MAA-MIP), EDMA (4.3 g) and MAA (0.37 g,) were added to the flask and the mixture was subjected to further ultrasonication. A basic polymer (2-Vpy-MIP) was prepared using the same procedure but with MAA replaced by 2-Vpy (0.46 g). The clear solutions were poured into glass tubes, and cooled on ice while nitrogen was bubbled through the solutions for 5 min. The tubes were then sealed and placed under UV light for 24 h at 5–7°C, while being rotated periodically to ensure homogenous polymerization. The tubes were then smashed and the hard polymers were soaked in methanol for 4 h to remove unreacted monomers. The hard polymers were ground manually and then sieved under water through a 25-μm sieve (Retsch, Haan, Germany). The retained particles were collected and allowed to sediment twice from methanol in order to separate the desired fraction from the finest particles. Non-imprinted polymers (NIPs) were synthesised in the absence of template compound, but using the same conditions as for the syntheses of MIPs.

The imprinted polymers were washed with 100 ml methanol, then 4×100 ml methanol–acetic acid (4:1), 100 ml methanol, 2×100 ml methanol–triethylamine (99:1) and, finally, 6×100 ml methanol. Each fraction was evaporated to approximately 1 ml and passed through 0.45-μm filters (Millipore, Bedford, MA, USA) prior to analysis. The presence of residual template was investigated using HPLC with UV detection. No template was detected in the last fractions eluted by methanol. The washed polymer particles were then dried in a desiccator and stored at ambient temperature prior to use. The HPLC apparatus used was a Varian 9002 solvent delivery system equipped with a Rheodyne injector, a 20-μl loop, a Nucleosil C_{18} column (150×4.6 mm, 5 μm particle size, Chromtech, Cheshire, UK) and a Carlo Erba microUVIS 20 detector (Milan, Italy) set at 267 nm. The mobile phase used was a mixture of methanol–water (80:20) at a flow-rate of 1 ml/min

2.4. Chromatographic evaluation

2-Vpy-MIPs in MeOH–water (4:1) were packed in a slurry into 100×2.1 mm stainless steel columns. For both MIP and NIP columns the eluent was acetonitrile containing 1% TEA. The amount of diphenyl phosphate injected for each analysis was 50 ng. Detection was carried out by MS using selected ion monitoring (SIM) in the m/z range 247.7–250.7. Capacity factors (k') were calculated as $(t-t_0)/t_0$ where t is the retention time of diphenyl phosphate, and t_0 the retention time corresponding to the column void volume.

2.5. Evaluation of MISPE

Both MIPs and the corresponding NIPs were packed in SPE columns using similar procedures. A suspension of 40 mg of the polymer in MeOH was packed in 3 ml extraction cartridges (I.D. 9 mm, polypropylene, Sorbent, Stockholm, Sweden), and secured by polyethylene frits (25 mm, Sorbent) placed above and below the sorbent bed. The ability of duplicate columns to extract diphenyl phosphate was evaluated, using two protocols for each MIP. Prior to extraction, each column was conditioned using 3 ml of the same solvent as for the introduction of the sample. In the first protocol, for both MAA-MIP and 2-Vpy-MIP, 2 μg of diphenyl phosphate was applied to the column in 1 ml chloroform, the column was washed with 3×1 ml methanol and eluted with 2×1 ml MeOH–TFA (99:1). In the second protocol for the MAA-MIP cartridges, 2 μg of diphenyl phosphate was again applied to the column in 1 ml chloroform, but the column was washed with 3×1 ml acetonitrile and eluted with 2×1 ml methanol. Finally, in the second protocol for the 2-Vpy-MIP cartridges 2 μg of diphenyl phosphate was applied to the column in 1 ml methanol, the column was washed with 2×1 ml methanol and 2×1 ml MeOH–TFA (99:1) and then eluted with 3×1 ml MeOH–TFA (94:6). Each fraction was collected in a glass tube and an internal standard (I.S.), 1.14 μg dibutyl phosphate dissolved in 10 ml methanol, was added. The fractions were then evaporated to dryness under a gentle stream of N_2 at 40°C. The residues were redissolved in 1 ml MeOH–water (60:40). All fractions were analysed by LC–ESI-MS.

Quantification of the recoveries was based on the responses of the analyte relative to the internal standard. The relative responses were compared to those obtained for an external standard, containing known amounts of analyte and internal standard.

2.6. Optimisation of the elution solvent

Different proportions of TFA–methanol were evaluated as eluents. Empty SPE cartridges were packed as previously described using 40 mg of the imprinted polymer, 2-Vpy. After extraction of the analyte from 1 ml chloroform, elution was performed using 3×1 ml of a MeOH–TFA mixture at four different volume ratios, i.e., 99:1, 98:2, 96:4 and 94:6. Duplicate columns were used for each eluent composition. All fractions were analysed by LC–ESI-MS. The recoveries were quantified as described in Section 2.5.

2.7. Evaluation of the selectivity of the 2-Vpy-MIP to a structural analogue, di(2-ethylhexyl) phosphate

A standard containing 1.3 μg di(2-ethylhexyl) phosphate in 1 ml methanol was applied to both 2-Vpy-MIP and 2-Vpy-NIP columns. Duplicate columns were used for each polymer. The cartridges

were then washed by 2×1 ml methanol, followed by 1 ml MeOH–TFA (99:1), and the elution was performed using 3×1 ml MeOH–TFA (94:6).

All fractions were analysed by LC–ESI-MS. The recoveries were quantified as described in Section 2.5.

2.8. LC–MS

The mass spectrometer was a Finnigan LCQ ion trap (Thermoquest, San Jose, CA, USA). The HPLC system consisted of a Rheos Model 4000 pump (Flux Instruments, Switzerland) equipped with an auto-injector, a 5-μl loop and an X-tierra C_{18} analytical column (150×2.1 mm, 3.5 μm particle size, Waters, Milford, MA, USA). The mobile phase consisted of 0.5 m*M* TEA in methanol–water (60:40) adjusted to pH 5 with acetic acid. The flow rate was 200 μl/min. Di(2-ethylhexyl) phosphate was eluted using a step gradient of 3 min A–B (40:60), 10 min A–B (5:95) and finally 5 min A–B (40:60), where A was 0.5 m*M* TEA in MeOH–water (6:94) and B was MeOH–water (96:4).

For ESI, the mass spectrometer was operated in the negative mode using the following conditions: spray voltage, 4.5 kV; capillary temperature, 300°C; capillary voltage, −30 V; tube lens offset, 10 V; sheath gas flow (N_2), 60 (arbitrary units); auxillary gas flow (N_2), 60 (arbitrary units). The collision energy was set to 32–43% of the maximum value. All the other parameters were set to default values.

For APCI the mass spectrometer was operated in the positive mode using the following settings: full scan *m*/*z* 50–2000; vaporizer temperature, 400°C; discharge current, 5.0 μA; capillary temperature, 150°C; capillary voltage, 4.0 V; tube lens offset, 30 V; sheath gas flow, (N_2) 80 (arbitrary units); auxillary gas flow, (N_2) 10 (arbitrary units). All the other parameters were set to default values.

3. Results and discussion

3.1. Hydrolysis of tritolyl phosphate

The synthesis of ditolyl phosphate resulted in crystalline *p*-tolyl phosphate and a mixture of *o*- and *m*-isomers appearing as an oil [27]. The oil was obtained in larger amounts, and was also easier to handle than the crystals. Therefore, the mixed fraction of *o*- and *m*-isomers was chosen for use as a template substance for the MIP synthesis. The yield was approximately 16%, including *o*- and *m*-isomers. The exact yield of each isomer could not be determined since the composition of the isomers in the reagent mixture was not known.

Analysis by GC–NPD, GC–FID, LC–APCI-MS and NMR showed that the purity of the product was higher than 99%. Neither tritolyl phosphates nor monoesters could be detected, indicating complete hydrolysis to a stable product. A quasi-molecular ion $[M+H]^+$ of *m*/*z* 279 and a methanol adduct of *m*/*z* 311 was obtained by LC–APCI-MS. NMR spectra showed that the oil consisted of two isomers.

3.2. Chromatographic evaluation

The affinities of the 2-Vpy-MIP and NIP columns for diphenyl phosphate were investigated using different mobile phases. Diphenyl phosphate could not be eluted using either pure water, methanol or acetonitrile as a mobile phase, suggesting that there was a strong ionic interaction between the analyte and the functional monomer used for synthesis of the polymer. The addition of TEA to the mobile phase allowed the elution of diphenyl phosphate from both columns, but with significantly different retention times (Fig. 2).

Using acetonitrile containing 1% TEA as a mobile phase, the calculated k' values were 4.3 and 1.3 for the MIP and NIP columns, respectively. The symmetry of the peak obtained when injecting 50 ng of diphenyl phosphate on the MIP column indicates that this stationary phase has a high capacity, i.e., a large number of selective sites were generated in the imprinting process. The selectivity of the MIP polymer calculated in terms of imprint factor (the ratio between the k' values of the MIP and NIP columns) was 3.3. These results demonstrate the potential of using this polymer in MISPE cartridges.

3.3. Evaluation of MISPE

Two different polymers were synthesised for MISPE evaluation, one with MAA (MAA-MIP) and the other with 2-Vpy (2-Vpy-MIP) as the functional

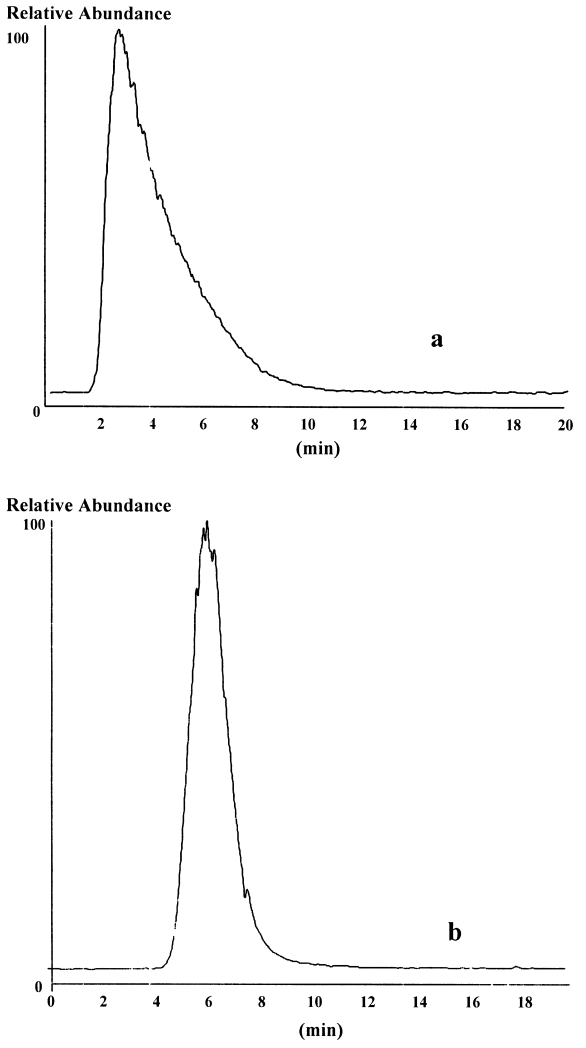

Fig. 2. LC–MS-SIM chromatograms obtained when injecting 50 ng of diphenyl phosphate into (a) an NIP column and (b) an MIP column.

monomer. However, when applying the first extraction protocol, no difference in selectivity was observed between MAA-MIP and the corresponding non-imprinted polymer (MAA-NIP). On the other hand, when using the same extraction protocol, significantly higher selectivity was obtained for the 2-Vpy-MIP compared to 2-Vpy-NIP, which gave total recoveries of nearly 100 and 30%, respectively.

In order to increase the retention on the MAA-MIP compared to its corresponding NIP, washing had to be performed using an aprotic solvent, i.e., acetonitrile instead of the protic methanol. Since the aim is to apply MIP to biological samples, selectivity in protic solutions is desirable. Due to its stronger retention of diphenyl phosphate in methanol, the 2-Vpy-MIP was chosen for further evaluation.

The eluents in the extraction protocol for 2-Vpy-MIP were modified in order to increase recovery. To elute the strongly acidic diphenyl phosphate, a strong competitor for the ionic interaction is needed. TFA was chosen for this, since it has shown to be a strong ionic modifier for other MISPE applications [28,29]. The recovery of diphenyl phosphate increased as the TFA content rose, as shown in Fig. 3a.

Using the modified extraction protocol, significantly greater selectivity was obtained for the MIP compared to the non-imprinted polymer. Breakthrough occurred in the extraction step with the latter. Furthermore, for NIP approximately 92% of the analyte was eluted prior to the elution step

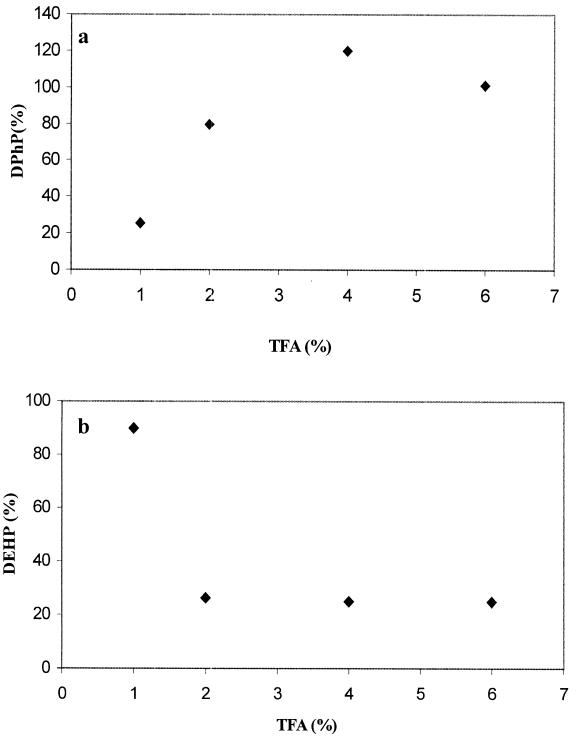

Fig. 3. Percentage recovery of (a) diphenyl phosphate (DPhP) and (b) di(2-ethylhexyl) phosphate (DEHP) from the 2-Vpy-MISPE using methanol with different concentrations of TFA as elution solvent. Elution was performed using duplicate columns.

Table 1
Evaluation of 2-Vpy-MISPE selectivity for diphenyl phosphate

Solvent		Content (%) of DPhP			
		MIP		NIP	
		Column 1	Column 2	Column 1	Column 2
Methanol	Breakthrough	0	0	33.2	0
Methanol	Wash 1	0	0	42.6	57.0
Methanol	Wash 2	0	4.6	0	0
MeOH–TFA (99:1)	Wash 3	0	13.3	12.1	39.1
MeOH–TFA (94:6)	Elution 1	7.6	20.6	0	0
MeOH–TFA (94:6)	Elution 2	32.0	23.3	0	0
MeOH–TFA (94:6)	Elution 3	46.3	23.4	0	0
Total		86.0	85.3	88.0	96.1

Extraction of 2.1 μg DPhP (diphenyl phosphate) from 1 ml methanol, washing with 2×1 ml methanol and 1 ml MeOH–TFA (99:1), elution with 3×1 ml MeOH–TFA (94:6). Columns 1 and 2 are duplicate MISPE columns. Each value is an average of two measurements.

compared to 0–18% for the MIP. Table 1 shows the recoveries for the different steps in the extraction protocol. The results for 2-Vpy-MIP illustrate the possibility of washing polar interfering compounds from samples using protic solvents, while retaining the analyte on the column.

3.4. Selectivity study using a structural analogue, di(2-ethylhexyl) phosphate

Most likely, the recognition of diphenyl phosphate by 2-Vpy-MIP is mainly due to strong ionic interactions between the highly acidic analyte and the basic monomeric site of the polymer. A detailed evaluation of the specificity of a polymer to a certain compound involves evaluation of several structural analogues, structurally related compounds and compounds that are not structurally related. In this study only one analogue, di(2-ethylhexyl) phosphate, was investigated since only a few diphosphate esters are commercially available. This compound is less acidic than diphenyl phosphate, and its substituents have a more lipophilic character, so theoretically it should be less strongly retained on the MIP. As shown in Table 2, the interaction with di(2-ethylhexyl) phosphate due to the imprinting effect was weaker, resulting in approximately 20–30% of the analyte being desorbed prior to the elution step. Furthermore, the overall recovery was significantly lower than for diphenyl phosphate. Although di(2-ethylhexyl) phos-

Table 2
Evaluation of 2-Vpy-MISPE selectivity for di(2-ethylhexyl) phosphate

Solvent		Content (%) of DEHP			
		MIP		NIP	
		Column 1	Column 2	Column 1	Column 2
Methanol	Breakthrough	0	0	40.4	16.2
Methanol	Wash 1	0	0	52.1	27.4
Methanol	Wash 2	4.5	2.8	14.0	30.4
MeOH–TFA (99:1)	Wash 3	24.6	16.4	5.8	26.7
MeOH–TFA (94:6)	Elution 1	16.7	15.8	0	0
MeOH–TFA (94:6)	Elution 2	0	6.5	0	0
MeOH–TFA (94:6)	Elution 3	0	0	0	0
Total		45.9	41.5	112.2	100.8

Extraction of 1.3 μg di(2-ethylhexyl) phosphate from 1 ml methanol, washing with 2×1 ml methanol and 1 ml MeOH–TFA (99:1), elution with 3×1 ml MeOH–TFA (94:6). Columns 1 and 2 are duplicate MISPE columns. Each value is an average of two measurements.

phate was less strongly retained than diphenyl phosphate in the adsorption and washing steps, there was still a significant difference between its retention on the MIP and the NIP. This is most likely due to the similarities between the two diesters in chemical structure and functionality.

The effect of TFA on the elution recovery was investigated as for diphenyl phosphate. In contrast to the latter compound, the recovery of di(2-ethylhexyl) phosphate decreased with increasing concentration of TFA in the eluent, as illustrated in Fig. 3b. This could be due to degradation but has to be further investigated.

When comparing the results presented in Fig. 3a and b, it is clear that more TFA is needed to compete for the imprinted sites for diphenyl phosphate than for di(2-ethylhexyl) phosphate. Only 1% TFA is needed to elute di(2-ethylhexyl) phosphate from the MIP. The stronger interaction for diphenyl phosphate may be due either to its more acidic properties or to better shape recognition. The results also show the importance of using an optimised elution protocol for each analyte. Compounds with similar structures may still differ in chemical properties, such as polarity or acidity, and thus differ in retention strengths.

3.5. LC–ESI-MS

A method for LC–ESI-MS–MS was developed. Selected reaction monitoring (SRM) was chosen for its higher selectivity and considerably higher signal-to-noise ratios compared to one-dimensional MS. This is especially important when applying the method to biological samples containing trace levels of analytes in highly complex matrices. The target compounds were quantified by summing the intensities from their product ions. An MS chromatogram is shown in Fig. 4. The method was linear (seven data points, correlation coefficients higher than 0.996) for all studied diphosphate esters in the investigated range, i.e., 0.05–85 ng. The limits of detection (LODs), calculated using a signal-to-noise ratio of 3, were found to be 25 pg for diphenyl phosphate and dibutyl phosphate (I.S.), and 50 pg for ditolyl phosphate and di(2-ethylhexyl) phosphate.

All diesters generated stable deprotonated quasi-molecular ions, with no characteristic fragments or

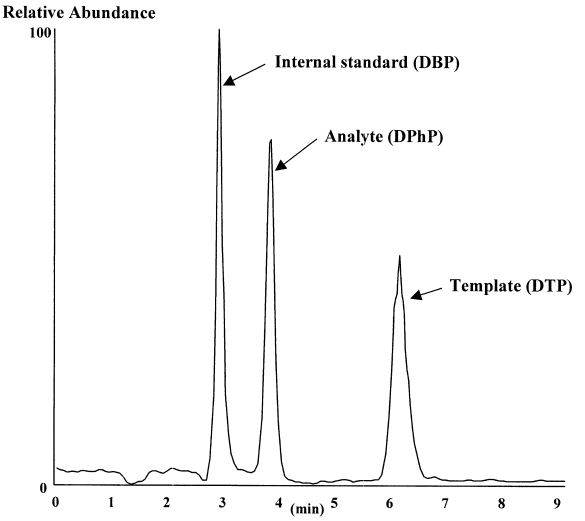

Fig. 4. Total ion mass chromatogram (derived using MS in SRM mode) of diphenyl phosphate, dibutyl phosphate and ditolyl phosphate.

solvent cluster ions under negative ESI-MS conditions. Among the product ions formed by collision-induced dissociation (CID) were the corresponding monoesters. An SRM spectrum is shown for the investigated diesters in Fig. 5.

The chromatographic conditions were optimised with the aim of separating diphenyl phosphate, ditolyl phosphate and dibutyl phosphate (I.S.). In order to enable retention of the highly acidic analytes on the lipophilic LC stationary phase, an ion-pairing chromatographic method was developed. Volatile additives were chosen to enable mass spectrometric detection. Two ion-pairing agents were evaluated and the separation and MS detection were optimised, the latter using the signal-to-noise ratios and the intensities of the signals. The agents investigated were TEA and ammonium acetate, added to the mobile phase at concentrations of 0.1–10 m*M*. TEA has previously been used as an ion-pairing agent for LC–ESI-MS determination of aromatic sulfonates [30].

The highest signal-to-noise ratios were obtained for TEA at a concentration of 0.5 m*M*. However, for both TEA and ammonium acetate, the intensities of the signal were shown to decrease with increasing concentrations of the ion-pairing agent due to the

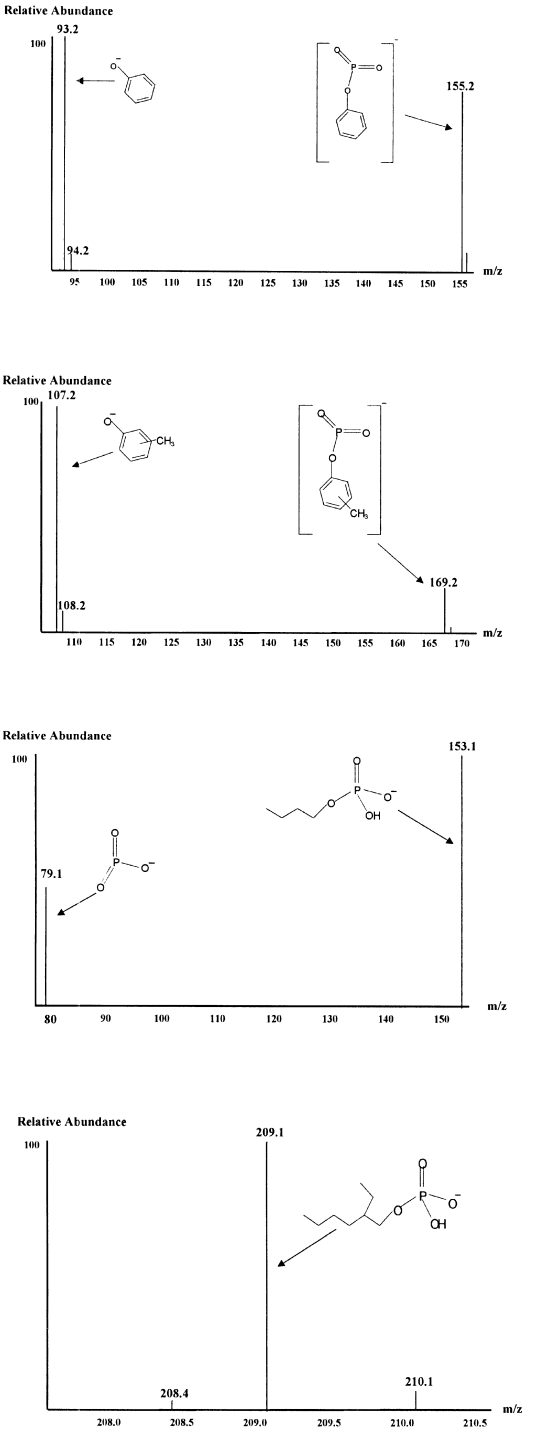

Fig. 5. Selected reaction monitoring mass spectrum of the investigated diphosphate esters. Proposed structures of the ions are shown in the spectrum.

large amounts of anions competing for the MS detection.

4. Conclusions

Development of convenient methods for quantifying acidic metabolites of organophosphate esters, such as diphosphate esters in human blood or urine, is highly important for assessing the risks associated with exposure to these compounds. This work has shown the potential of the MISPE technique for selective enrichment of ionic diphosphate esters in protic matrices. The combined MISPE and ion-pairing-LC–ESI-MS–MS technique also has potential as a fast and easy clean-up method for the very polar and acidic diphosphate esters in complex matrices such as biological samples. High sensitivity and selectivity is obtained by the combination of negative detection and SRM. However, the applicability of the presented method to biological samples is currently being investigated.

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References

[1] H. Carlsson, U. Nilsson, C. Östman, Environ. Sci. Technol. 34 (2000) 3885.
[2] J.G. Camarasa, E. Serra-Baldrich, Contact Dermatitis 15 (1992) 264.
[3] A.M. Saboori, D.M. Lang, D.S. Newcombe, Chem. Biol. Interact. 80 (1991) 327.
[4] D.C.G. Muir, N.P. Grift, J. Assoc. Off. Anal. Chem. 66 (1983) 684.
[5] L.T. Burka, J.M. Sanders, D.W. Herr, H.B. Matthews, Drug Metab. Dispos. 19 (1990) 443.
[6] D.E. Chapman, S.R. Michener, G. Powis, Fundam. Appl. Toxicol. 17 (1991) 215.

[7] H. Kurebayashi, A. Tanaka, T. Yamaha, Toxicol. Appl. Pharmacol. 77 (1985) 395.
[8] K. Hosoya, Y. Shirasu, K. Kimata, N. Tanaka, Anal. Chem. 70 (1998) 943.
[9] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 817 (1998) 5.
[10] S. Kröger, A.P.F. Turner, K. Mosbach, K. Haupt, Anal. Chem. 71 (1999) 3698.
[11] C.J. Slade, J. Mol. Catal. B 9 (2000) 97.
[12] O. Ramström, K. Mosbach, Curr. Opin. Chem. Biol. 3 (1999) 759.
[13] I. Surugiu, L.Ye.E. Yilmaz, A. Dzgoev, B. Danielsson, K. Mosbach, K. Haupt, Analyst 125 (2000) 13.
[14] L.I. Andersson, J. Chromatogr. B 1 (1999) 1.
[15] B. Sellergren, Trends Anal. Chem. 18 (1999) 164.
[16] C.J. Allender, K.R. Brain, C.M. Heard, Prog. Med. Chem. 36 (1999) 235.
[17] L.I. Andersson, Analyst 125 (2000) 1515.
[18] C. Berggren, S. Bayoudh, D. Sherrington, K. Ensing, J. Chromatogr. A 889 (2000) 105.
[19] M.T. Muldoon, L.H. Stanker, Anal. Chem. 69 (1997) 803.
[20] J. Matsui, K. Fujiwara, S. Ugata, T. Takeuchi, J. Chromatogr. A 889 (2000) 25.
[21] C. Baggiani, F. Trotta, G. Giraudi, C. Giovanni, A. Vanni, Anal. Commun. 36 (1999) 263.
[22] I. Ferrer, F. Lanza, A. Tolokan, V. Horvath, B. Sellergren, G. Horvai, D. Barcelo, Anal. Chem. 72 (2000) 3934.
[23] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57.
[24] D.C.G. Muir, N.P. Grift, Chemosphere 10 (1981) 847.
[25] M.A. Ali, A.M. Al-Ani, Analyst 116 (1991) 1067.
[26] C. Lamouroux, H. Virelizier, C. Moulin, J.C. Tabet, C.K. Janowski, Anal. Chem. 72 (2000) 1186.
[27] V. Bruecke, Biochem. Z. 253 (1930) 470.
[28] W.M. Mullett, E.P.C. Lai, B. Sellergren, Anal. Commun. 36 (1999) 217.
[29] P. Martin, I.D. Wilson, D.E. Morgan, G.R. Jones, K. Jones, Anal. Commun. 34 (1997) 45.
[30] M.C. Alonso, M. Castillo, D. Barcelo, Anal. Chem. 71 (1999) 2586.