

Optimisation of a selective method for the determination of organophosphorous triesters in outdoor particulate samples by pressurised liquid extraction and large-volume injection gas chromatography–positive chemical ionisation–tandem mass spectrometry

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Received: 21 March 2007 / Revised: 25 April 2007 / Accepted: 27 April 2007 / Published online: 30 May 2007
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Abstract A selective analytical method for the determination of nine organophosphate triesters and triphenylphosphine oxide (TPPO) in outdoor particulate matter is presented. It involves a fully automated pressurised liquid extraction (PLE) step, integrating an alumina clean-up process, and subsequent determination by large-volume injection gas chromatography–positive chemical ionisation–tandem mass spectrometry (LVI-GC–PCI–MS/MS). The extraction variables (solvent, amount of adsorbent, temperature, time and number of cycles) were optimised using a multicriteria strategy which implements a desirability function that maximises both extraction and clean-up efficiencies while searching for the best-compromise PLE conditions. The final method affords quantification limits of between 0.01 and 0.3 $\mu\text{g g}^{-1}$ and recoveries of >80%, with the exceptions of the most polar analytes, TCEP and TPPO

(~65%) for both urban dust and PM10 samples. Moreover, the method permitted the levels of these compounds in dust deposited outdoors (between LOD and 0.5 $\mu\text{g g}^{-1}$ for TEHP) and PM10 samples (between LOD and 2.4 $\mu\text{g m}^{-3}$ for TiBP) to be measured and reported for the first time.

Keywords Organophosphorous triesters · Flame retardants · Plasticizers · Urban dust · Particulate matter · PM10 · Pressurised liquid extraction · Accelerated solvent extraction · GC–MS · Experimental design · Multicriteria optimisation · Desirability function

Introduction

Phosphoric acid triesters are mainly employed as plastic additives, as flame retardants and as plasticizers [1], but they are also used in many other applications, such as in hydraulic fluids, paints and several industrial processes [2]. Usage of these chemicals is rapidly increasing and worldwide consumption of them increased from 108,000 tonnes in 1995 to 186,000 tonnes in 2001 [2]. Moreover, phosphoric acid triester usage is expected to grow even further due to the recent ban on the use of most polybrominated diphenyl ethers (PBDEs) as flame retardants; the PBDE market was even larger than the market for organophosphorous compounds [1].

When used as plastic additives, phosphoric acid triesters are not chemically bonded but simply mixed with the bulk material they are intended to protect, and so they are relatively easy to release into the surrounding environment.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-007-1338-4) contains supplementary material, which is available to authorized users.

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Thus, in the last few years, interest in the environmental fate of organophosphorous triesters has substantially increased and they have been detected in a range of environmental compartments, like several surface and wastewater samples [3–9], fish [2], indoor dust and air [10–17] and even in snow and in remote outdoor air samples in the North of Finland [18].

Organophosphorous triesters can be analysed using either gas chromatography or liquid chromatography–tandem mass spectrometry (LC–MS/MS) [3, 8, 19, 20]. Detection in gas chromatographic methods is often achieved through nitrogen–phosphorous detection (GC–NPD) [6, 13, 21, 22] because of its good selectivity and sensitivity towards P-containing analysed and also due to the fact that most aliphatic phosphoric esters yield mainly low-mass fragments when analysed by electron impact ionisation mass spectrometry (GC–EI–MS). m/z 99 is typically obtained, which corresponds to protonated phosphoric acid, while molecular ions are low in intensity in the spectrum, complicating their confirmation and quantification, since low masses are often interfered with by matrix components. In order to overcome these limitations, Björklund et al. [16] proposed the use of gas chromatography–positive chemical ionisation–tandem mass spectrometry (GC–PCI–MS/MS) for the analysis of air samples. GC–PCI–MS/MS was shown to provide similar sensitivity to GC–NPD, but with a confirmation capability and enhanced selectivity.

The sample preparation step is comparatively well developed for water [3, 6, 8, 23] and air [11, 12, 16, 17, 22]. These samples are preconcentrated by solid-phase extraction or solid-phase microextraction, for example, without the need for clean-up in many cases. However, few analytical methods have been developed for solid samples like dust, and so Soxhlet [18] and sonication [11] are relied upon, which lack validation data. More recently, microwave-assisted extraction has been proposed as a suitable alternative for the extraction of organophosphorous esters from indoor particulate matter [24]. However, this method requires laborious and extensive clean-up of extracts in order to determine the analytes by GC–NPD. Moreover, data on outdoor occurrence (particularly in particulate samples) of these organophosphorous compounds is rather limited. However, some works have postulated that they may occur in outdoor air and particulate samples as a consequence of traffic, as they are also present in various automobile and plane lubricant fluids [18, 25].

Thus, the aim of this work was to optimise a selective analytical method for the determination of organophosphate triesters and triphenylphosphine oxide (TPPO) in urban dust and airborne particulate matter. Pressurised liquid extraction (PLE) was selected as the sample preparation technique because of its high degree of automation and the

possibility of adding a clean-up step in the extraction cell [26, 27]. Finally, detection was carried out by large-volume injection (LVI)–GC–PCI–MS/MS because of its selectivity. The method was validated and applied to urban dust and PM10 (airborne particulate matter <10 μm). This method will permit the distributions of such chemicals in the outdoor environment to be studied, bearing in mind that PM10 is a major EU standard on air quality due to the ability of airborne particulate matter to penetrate into the human respiratory system [28].

Materials and methods

Chemicals and stock solutions

Analyte and internal standard names, abbreviations and other data are shown in Table 1. TiBP, TnBP, TCEP, TEHP, TPhP, TPPO and TCiP were obtained from Sigma-Aldrich (Steinheim, Switzerland). TPpP (internal standard), TDCP and EHDPP were supplied by TCI Europe (Zwijndrecht, Belgium). TCPP was kindly provided by Dr. T. Reemtsma (Technical University of Berlin). Diatomaceous earth, silica, basic alumina (WB-2), neutral alumina (WN-3) and florisil (60–100 mesh) were from Sigma-Aldrich. Adsorbents were activated overnight at either 130 °C (silica and florisil) or 350 °C (basic and neutral alumina), in preliminary experiments. Ethyl acetate and hexane, both of trace analysis grade, were purchased from Panreac (Castellar del Vallès, Spain) and Merck (Darmstadt, Germany), respectively. Individual stock solutions were prepared in acetone at the 2 mg mL⁻¹ level. Mixed standard solutions were prepared at the 20 $\mu\text{g mL}^{-1}$ level in ethyl acetate and subsequently diluted as necessary.

Samples

Settled urban dust samples were collected from the border (sidewalk and verge) of one of the main inner avenues of the city of A Coruña (Galicia, NW Spain, 43°22'04" N, 08°25'08" W). This avenue has an average traffic rate of ca. 20,000 vehicles per day. After collection, each sample was sieved and the fraction below 200 μm collected and stored in an amber vial at room temperature. A fraction of this sample (ca. 30 g) was placed into a beaker, completely covered with ethyl acetate and spiked with the analytes at the 0.5 $\mu\text{g g}^{-1}$ level in a clean area of the laboratory. Ethyl acetate was left to evaporate at room temperature until the dust was dry (after ~10 days), stirring the mixture frequently in order to obtain a homogeneous spiked material. It was then further aged for one month before its use in method optimisation and for a total period of three months before method validation.

Table 1 Names, abbreviations, physicochemical data and GC–PCI–MS/MS parameters of the studied compounds

Name ^a	Abbreviation ^a	CAS No.	log K_{ow}^b	Vapour pressure (Torr) ^b	Parent ion (m/z)	Product ions (m/z) ^c	Excitation voltage (V)
Tri-isobutyl phosphate	TiBP	126-71-6	3.60	1.28×10^{-2}	267	211 , 155, 99	0.70
Tri- <i>n</i> -butyl phosphate	TnBP	126-73-8	4.00	1.13×10^{-3}	267	211 , 155, 99	0.70
Tri(2-chloroethyl) phosphate	TCEP	115-96-8	1.44	6.13×10^{-2}	285	223 , 187, 161, 125, 99	1.00
Tri(chloropropyl) phosphate	TCPP	13674-84-5	2.59	2.02×10^{-5}	251	175 , 99	0.70
Triphenyl phosphate (IS)	TPeP (IS)	2528-38-3	5.29	1.67×10^{-5}	309	239 , 169, 99	0.85
Tris(1-chloromethyl-2-chloroethyl) phosphate	TDCP	13674-87-8	3.65	7.36×10^{-8}	321	211 , 209 , 99	0.85
Triphenyl phosphate	TPhP	115-86-6	4.59	6.28×10^{-6}	327	251, 233, 215 , 153, 152	1.25
2-Ethylhexyl diphenyl phosphate	EHDPP	856800-52-7	6.64	6.49×10^{-7}	251	233, 215 153 , 152 , 95	1.25
Tri(2-ethylhexyl) phosphate	TEHP	78-42-2	9.49	8.45×10^{-8}	–	99 , 127	–
Triphenylphosphine oxide	TPPO	791-28-6	2.83	2.6×10^{-9}	279	201 , 173	1.25
Tricresyl phosphate	TCrP	1330-78-5	5.11	6×10^{-7}	369	261, 256, 243 , 166, 165	1.40

^a IS: internal standard. TCPP and TCrP consist of technical mixtures of various isomers.

^b Data from [34] (experimental values), except EHDPP from [35] (calculated values).

^c Ions used for quantification are *italicised*. The remaining ions were used for confirmation. TEHP was measured in SIM mode.

Twenty-four-hour PM10 samples were collected at the same location, 2 m away from the avenue on a Schleicher & Schuell (Dassel, Germany) QF20 quartz fibre filter with a high-volume Graseby–Andersen PM10 sampler (Atlanta, GA, USA). The filter was activated at 400 °C for 24 h to avoid blank problems and stored in a humidity-controlled room, in accord with the EN 12341 Norm [29], before sampling. A total volume of 1764 m³ was sampled with an average PM10 concentration of 52.7 µg m⁻³. Aliquots of this filter were cut with a round-shaped cutting punch approximately the same diameter as a PLE cell. Nine of these aliquots were typically employed per extraction (~0.2 g filter), corresponding to ~1/20 filter and 80 m³ of sampled air. The representativeness of these subsamples was proven previously (data not shown) and was further supported by the RSD values obtained during validation (see “Results and discussion”).

Pressurised liquid extraction

Samples were extracted by an ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, USA) in 11-mL stainless steel cells. Several parameters that affect extraction efficiency and selectivity were tested, as explained in the “Results and discussion” section. In the optimised method, a cellulose filter was placed in the

bottom of the cell, then 5 g of (unactivated, used as received) basic alumina, a predetermined amount of sample (0.2 g for PM10 filters or 0.5 g for urban dust), and finally a second cellulose filter on the top. TPeP was spiked onto the top of this filter as a surrogate internal standard (20 µL of a 20 µg mL⁻¹ standard). Extraction was implemented in a single cycle of 10 min static time at 40 °C and 1500 psi (10,342 kPa) using ethyl acetate as extraction solvent. Flush volume (60%) and purge time (60 s) were left at their default values. The obtained extract was concentrated to a final volume of 1 mL by rotary evaporation (Rotavapor R-3000, Büchi, Flawil, Switzerland). All cellulose filters were pre-extracted with the same PLE program before use, otherwise blank problems associated with the presence of TiBP and TnBP on the filters were encountered.

In order to determine the degree of clean-up, UV absorbance measurements of 1:100 diluted extracts at 400 nm were performed during PLE optimisation with a Lambda 6 UV/Vis spectrometer (Perkin Elmer, Wellesley, MA, USA) in quartz cells of 10 mm path length.

Instrumentation

GC–NPD Determination was performed on a Perkin Elmer 8700 gas chromatograph equipped with a ZB-5 column (30 m×0.25 mm×0.25 µm) obtained from Phenomenex

(Eschborn, Germany). Manual injections (1 μL) were performed in the splitless mode (1 min splitless time). Helium (99.999%) was used as carrier gas at a constant head pressure of 130 kPa. Synthetic air and hydrogen (99.999%) were used as detector gases at pressures of 50 and 40 kPa, respectively. The column oven temperature program was as follows: the initial oven temperature was 50 $^{\circ}\text{C}$; this was maintained for 1 min and then raised at 15 $^{\circ}\text{C min}^{-1}$ to a final temperature of 290 $^{\circ}\text{C}$, which was maintained for 10 min. Injector and detector temperatures were set to 250 $^{\circ}\text{C}$ and 320 $^{\circ}\text{C}$, respectively.

GC–MS A TRACE GC gas chromatograph coupled to a PolarisQ ion trap mass spectrometer and equipped with a programmed temperature vaporiser (PTV) was used (Thermo Finnigan, Waltham, MA, USA). PTV injections (10 μL) were carried out with a CTC Combi Pal Autosampler (CTC Analytics, Zwingen, Switzerland) in the PTV, which was equipped with a glass-wool-filled liner. The PTV program was as follows: the initial temperature was 55 $^{\circ}\text{C}$; this was maintained for 0.33 min and then increased to 290 $^{\circ}\text{C}$ at 1 $^{\circ}\text{C s}^{-1}$. Separation was performed on a DB-XLB column (60 $\text{m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) with helium (99.999%) as carrier gas at a constant flow of 1.2 mL min^{-1} . The column oven temperature program started at 50 $^{\circ}\text{C}$, which was maintained for 1 min and then increased at 20 $^{\circ}\text{C/min}$ to a final temperature of 290 $^{\circ}\text{C}$, which was maintained for 15 min. Transfer line and source temperature were fixed at 300 $^{\circ}\text{C}$ and 200 $^{\circ}\text{C}$, respectively. Methane (99.999%) was employed for the positive chemical ionisation of analytes at a constant flow of 2 mL min^{-1} . All analytes were determined by PCI–MS/MS at a Q value of 0.225 by isolating the parent ion with a window of 2 m/z and applying an adequate excitation voltage (Table 1), with the exception of TEHP, which was also determined by PCI, but in selected ion monitoring (SIM) mode.

Software

Experimental design analysis was performed with the software Statgraphics Plus 5.1 for Windows (Manugistics Inc., Rockville, MD, USA).

Results and discussion

PLE optimisation

i) Preliminary experiments

Blank PLE tests were performed with an empty cell containing just the cellulose filters, extracted with ethyl acetate, two 5-min cycles at 100 $^{\circ}\text{C}$. These blank

extractions showed the occurrence of TiBP and TnBP. In view of this problem and others previously reported for these analytes [6, 24] and for PLE [30], its source was traced by extracting the different parts of the ASE cell [30] and all glassware material by sonication. Finally, ASE cellulose filters were found to be the source of this contamination. Thus, they were pre-extracted before use and this contamination was avoided. Moreover, the extraction of the quartz fibre filters used for PM10 sampling showed the same problem, which was easily avoided by performing the activation step at 400 $^{\circ}\text{C}$.

Preliminary PLE experiments were performed with 0.5 g of spiked settled urban dust, extracted as described above for blanks. After concentration to 1 mL, a dark brown extract was obtained. Although a relatively clean baseline with few interfering compounds was observed when they were injected on the GC–NPD, the continual injection of these extracts into the GC system resulted in rapid column degradation. Thus, a clean-up step was incorporated by including a 0.2 g layer of adsorbent in the extraction cell below the sample [26, 27]. The efficacies of the different adsorbents tested (silica, basic alumina, neutral alumina and florisil) were qualitatively assessed by studying the GC–NPD and GC–MS results (total ion chromatogram) and the extract colour (UV absorbance, data not shown). Among the adsorbents tested, florisil provided the worst clean-up, yielding the darkest extract and the most interfering peaks in the chromatogram. All of the other adsorbents provided similar chromatographic profiles, but both basic and neutral alumina provided a slightly clearer extract than silica. Because of the better availability of basic alumina in the laboratory, it was selected for further optimisation.

ii) Solvent composition, extraction temperature and amount of adsorbent

After adsorbent selection, the influence of extraction solvent composition, temperature and amount of adsorbent on both the efficiency of clean-up and the extraction yield were studied. This was done using a central composite design ($2^3 + \text{star}$) plus four central points, see Table 2, which allowed these three variables to be efficiently studied at five levels each, resulting in a total of 18 experiments. The extraction solvent was varied so that it covered different polarity ranges, from pure hexane to pure ethyl acetate. The amount of sorbent was varied from 0 (no adsorbent) to 5 g, which is the maximum amount that can be reasonably be added to the extraction cell, and the temperature was varied from room temperature to 150 $^{\circ}\text{C}$ (Table 2). The whole experimental plan and the normalised responses obtained are presented in the “Electronic Supplementary Material” (Table S1).

The extraction yield for each compound was studied in terms of the GC–NPD area. The degree of clean-up was

Table 2 Central composite design (2^3 +star), relative significances of factors with their signs and selected optimal values. “+++/—””: most important factor; “++/—””: second most important factor; “+/-””: least

Response	Ethyl acetate %	Temperature	Alumina
Lowest level ($-\alpha$)	0 (100% hexane)	25 °C	0.0 g
Highest level ($+\alpha$)	100 (0% hexane)	150 °C	5.0 g
TiBP	+++ *	++	—
TnBP	+++ *	++	—
TCEP	+++ *	+	— *
TCPP	+++ *	++ *	—
TDCP	+++ *	+	— *
TPhP	+++ *	+	— *
EHDPP	+++ *	+	— *
TEHP	+++ *	++	—
TPPO	+++ *	+	— *
TCrP	+++ *	+	— *
UV absorbance	+++ *	+ *	— *
Optimal values	100 (0% hexane)	40 °C	5 g

* Statistically significant factor at the 95% confidence level

studied by measuring the absorbance of the obtained extract, diluted 1:100, at 400 nm. This provides with a semi-quantitative measurement of this parameter, which is very difficult to measure in quantitative terms otherwise. The area values obtained for each compound and the UV absorbance were normalised to the highest measurement before statistical analysis. The ANOVA analysis of this experimental design showed that solvent composition is the most important factor, since it was significant and positive for all variables (Table 2). This means that, as expected, a higher ethyl acetate content, gives a higher extraction efficiency due to the polar nature of most analytes, but also a lower clean-up efficiency, as the UV absorbance is also higher. The other two parameters were less significant but also behaved in the expected way, i.e. higher temperatures and lower sorbent amounts favoured the extraction yield but worsened clean-up efficiency. The response contour plots for TCEP and UV absorbance can be found in the “Electronic Supplementary Material” (Fig. S1).

Thus, one of the best ways to approach this type of optimisation and find the best-compromise extraction conditions is to follow a multicriteria optimisation approach, which maximises extraction efficiency and minimises UV absorbance (maximising clean-up). This was accomplished by establishing a desirability function [31]. The overall desirability function (D) is the geometrical mean of the individual desirability functions (d_i) for each parameter (i) to be optimised. For each parameter i a value of “0” is assigned to a completely undesired value, whereas a value of “1” is assigned to a completely satisfactory response. Further details can be found elsewhere [31–33]. In this case, extraction efficiency was maximised (i.e. $d_i=1$ for the highest area) while UV absorbance was minimised

important factor; “+++/++/+””: factor has a positive effect on the response; “—/—/—””: factor has a negative effect on the response

(i.e. $d_i=0$ for the highest absorbance value). Furthermore, the weight of any parameter in D can be adjusted. Indeed the weight of each d_i value in D is quite empirical and must be selected according to the relevance attributed to the responses to be optimised. Table S2 presents how the weighting of UV absorbance in D affects the results obtained. Obviously, the higher this value, the more stringent the D function for clean-up, and therefore the lower the extraction efficiency and the higher the clean-up efficiency (d_i increases for UV absorbance). Thus, this parameter was weighted five times higher than the individual extraction efficiencies, otherwise the relative weight of the extraction efficiency of D would be too high since there are ten extraction efficiencies to be maximised. Hence, the total weight of recoveries in the global desirability (D) is twice the weight of the clean-up efficiency.

The maximum D obtained was 0.62 for 100% ethyl acetate, 40 °C and 5 g of alumina (Fig. 1). Under these conditions (Table S2), the predicted values of d_i were higher than 0.8 for all analytes, except for the most polar ones, TCEP ($d_i=0.47$), TPPO ($d_i=0.25$), TDCP ($d_i=0.60$), and for the UV absorbance ($d_i=0.58$). This comparatively low d_i for the less hydrophobic analytes may be explained by the stronger retention on the alumina layer required for clean-up purposes.

iii) Number of static cycles and time

Once these three conditions (solvent, temperature and alumina amount) were fixed, the other two PLE variables that were expected to affect extraction efficiency the most—the time and number of cycles—were optimised via a Doehlert experimental design (Tables 3 and S3). This

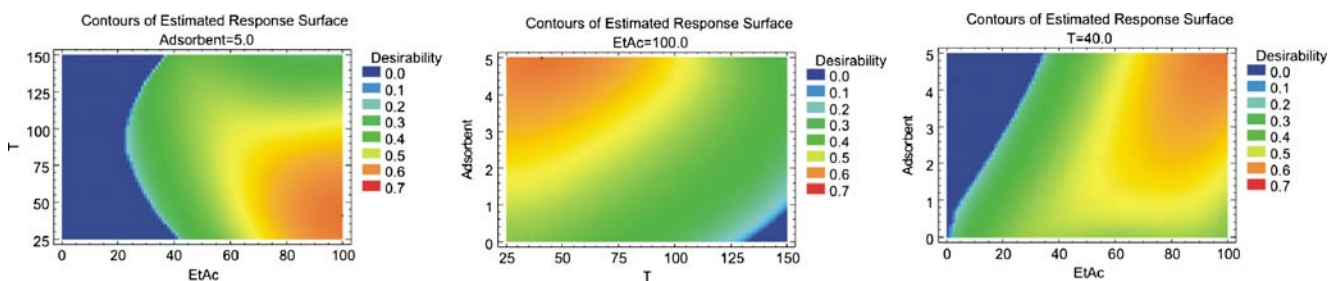


Fig. 1 Desirability (D) contour plots obtained for the central composite design

design enables optimisation by considering one factor at five levels, in this case the number of cycles (1, 2, 3, 4 and 5) and a second factor at three levels, the extraction time (2, 6 and 10 min), minimising the number of experiments. As the central point was replicated three times, the total number of experiments was nine. These experiments were once again performed with the spiked urban dust, and the variables considered were the analyte responses (areas) and the UV absorbance as a measure of clean-up, normalised to the highest experimental value. Some of the contour plots obtained can be found in Fig. S2. ANOVA analysis of the results obtained shows that neither extraction time nor number of cycles are significant at the 95 % confidence level, except for TCEP, for which extraction time has a significant positive effect (Table 3). Although not significant in most cases, the extraction time is always positive, while the number of cycles is normally negative and far from the significance boundary in the standardised effects plot (data not shown). This may account for the fact that an increase in the number of cycles does not lead to an effective increase in the total solvent volume. The global optimisation of the two variables was again accomplished

Table 3 Doehlert design, relative significance of factors with their signs and selected optimal values. “++/—”: most important factor; “+/-”: least important factor; “++/+”: factor has a positive effect on the response; “—/-”: factor has a negative effect on the response

Response	Cycles	Time
Lowest level	1	2
Highest level	5	10
TiBP	—	++
TnBP	—	++
TCEP	—	++ *
TCPP	—	++
TDCP	—	++
TPhP	—	++
EHDPP	—	++
TEHP	—	++
TPPO	—	—
TCrP	—	++
UV absorbance	—	++
Optimal values	1	10

* Statistically significant factor at the 95% confidence level

using a desirability function. In this case, all responses were weighted the same and the UV absorbance was not considered in D due to its lack of statistical significance and the fact that the main objective at this step was to maximise extraction recovery. Figure 2 shows a contour plot of the global desirability function. The maximum (Table S4) is obtained for one cycle of 10 min of static extraction, corresponding to a D value of 0.96, with all $d_i > 0.95$, except for TPPO ($d_i = 0.62$).

iv) Other parameters

Other PLE parameters like pressure, flush volume or purge time were left at their default values as they are known to have a minor effect on PLE performance. On the other hand, many authors consider that both adsorbent activation and sample dispersion are crucial to obtaining optimal recoveries [26, 27]. However, both steps are time-consuming and so their effects on extraction efficiency and clean-up were tested. This was done by extracting the spiked aged sample under the previously optimised conditions with either (a) unactivated alumina and no sample dispersant or (b) activated alumina and no sample dispersant or (c) with unactivated alumina and sample dispersant. In this last case, the sample (0.5 g) was dispersed with 1 g of diatomaceous earth. Figure 3 presents the results obtained normalised to the simplest procedure (a), i.e. neither alumina activation nor sample dispersion. From this figure, it is obvious that neither activation nor dispersion affect sample clean-up in terms of UV absorbance, while

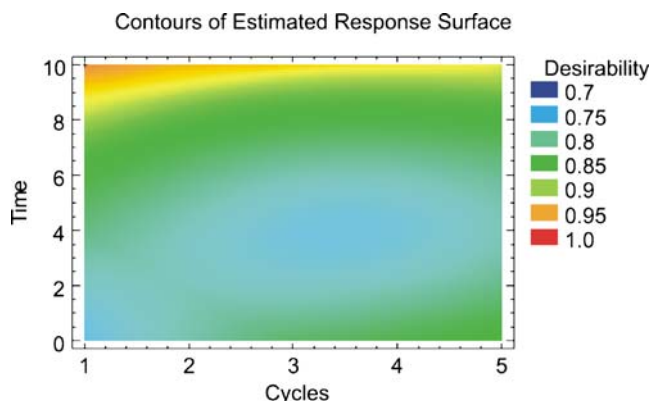


Fig. 2 Desirability (D) contour plot obtained for Doehlert design

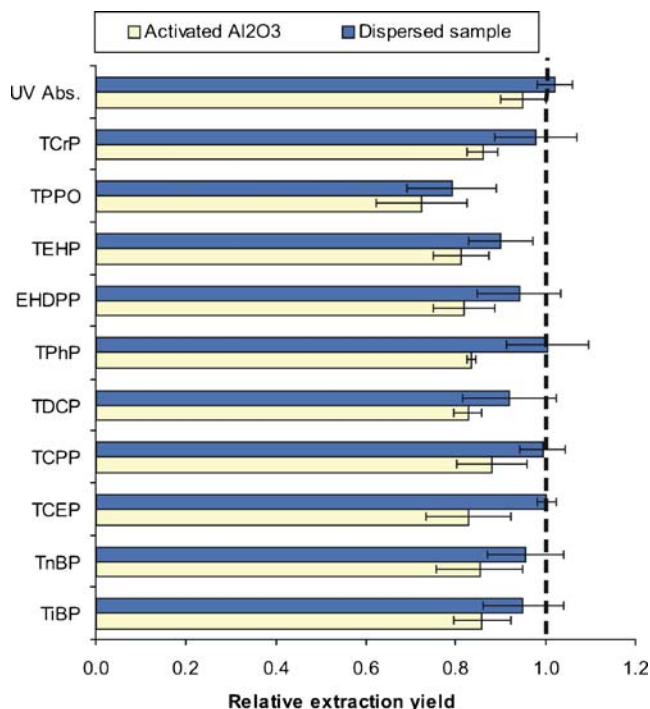
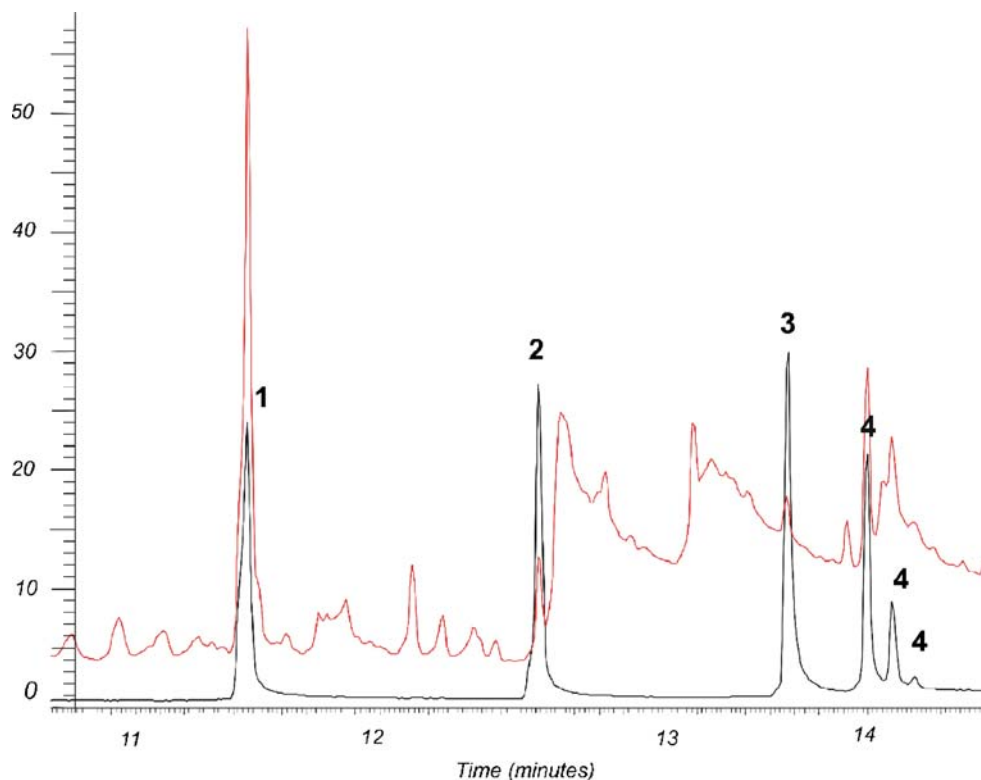


Fig. 3 Relative PLE yield normalised (to 1) to the extraction that did not use sample dispersant but did use unactivated basic alumina ($n=3$)

only a few additional early-eluting peaks appeared with diatomaceous earth, which did not interfere with analytes anyway. The activated alumina provided lower extraction yields than the unactivated one due to its stronger retention capacity. Dispersion of the sample did not improve

Fig. 4 Detail of the overlaid GC–NPD chromatogram of a PM10 sample extract and a 100 ng mL^{-1} standard. Peak assignment: 1, TiBP; 2, TnBP; 3, TCEP; 4, TCPP (three isomers)



extraction efficiency but it did however decrease the extractability of TPPO. Although diatomaceous earth is considered to be inert, TPPO is one of the most polar analytes in terms of $\log K_{ow}$ (Table 1) and it is a Lewis base and thus much more likely to be adsorbed to this matrix.

Based on these results, alumina was used as received (without activation) and was sample-dispersed in the final optimal method.

GC–NPD and GC–PCI–MS/MS

Without the integrated alumina layer, dark brown extracts were obtained for the settled dust, which resulted in rapid GC column degradation. As mentioned, this was overcome using the alumina-PLE method, which yielded yellowish extracts that were easily determinable by GC–NPD without GC performance degradation. However, in the case of suspended PM10 samples, although the extract was almost colourless and no GC column deterioration was observed, many interfering peaks appeared in the GC–NPD chromatogram (Fig. 4). This is a consequence of the compromise clean-up obtained with pure acetate that was necessary to achieve acceptable recoveries for these polar compounds. It also indicates the different natures of settled and suspended dusts, since settled dust has heavier matrix components and is less complex than suspended dust in terms of volatile organic constituents. Although the NPD is

regarded as being selective in GC analysis, its selectivity may not be enough when facing complex environmental samples, which is the case for dust samples, since they can also contain many nitrogen-containing compounds. A good example of this was pointed out by García et al. [24] in a recent publication, where the determination of these organophosphorous triesters in microwave-extracted indoor dust samples by GC–NPD was only possible after an extensive clean-up procedure that combined an initial reversed-phase and normal-phase clean-up with several solvent evaporation and reconstitution steps, resulting in a laborious method.

Hence, for PM10 samples, it was necessary to look for either an enhanced clean-up or, better still, a more selective method than GC–NPD in order to keep the sample preparation short and automated. This could be achieved, as proposed by Björklund et al. [16], by GC–PCI–MS/MS. PCI is necessary in order to get enough protonated molecular ion intensity from aliphatic triesters; otherwise EI–MS is dominated by m/z 99 (protonated phosphoric acid), which is useless for MS/MS. Although instrumental detection limits are higher than in NPD, the technique is much more selective and far surpasses the sensitivity and selectivity of GC–EI–MS detection [16]. Thus, the implementation of a GC–PCI–MS/MS method with 10 μ L LVI was investigated in order to achieve the desired degree of selectivity without sacrificing too much sensitivity.

PCI conditions were optimised by varying the flow of the reagent gas (methane) between 0.8 and 2.0 mL min^{-1} .

As shown in Fig. 5, 2.0 mL min^{-1} produced the best yield of protonated molecular ion, which would not have been produced by EI of aliphatic phosphoric acid triesters. The PCI–MS base peak corresponds to the protonated molecular ion, but for TiBP, TEHP, EHDPP, TCPP and TDCP. The aliphatic branched structure of these analytes favours the McLafferty rearrangement mechanism [3], so that a significant degree of fragmentation occurs, even under PCI conditions. As an example, Fig. 5 compares the EI and PCI spectra for the two isomers TnBP (linear aliphatic triester) and TiBP (branched). MS/MS spectra were obtained from the base peak except for TiBP, where the MH^+ ion was selected as precursor since m/z 99 is not suitable for MS/MS. Moreover, TEHP was measured in single-MS SIM mode, since even under PCI conditions only the m/z 99 ion was obtained at a level sufficient to perform MS/MS. The final operational PCI–MS/MS conditions are shown in Table 1.

A comparison of both chromatographic methods in terms of linearity, precision and limits of quantification (LOQs) is presented in Table 4. GC–NPD and LVI–GC–PCI–M/MS provide similar R^2 and RSD values, while GC–NPD provides slightly better LOQs, except for TiBP and TDCP, where the difference in sensitivity is more significant. This is due to the relatively low yield of parent ions obtained for TiBP, as discussed above, and the high number of product ions obtained for TDCP upon collision-induced dissociation (CID) which gives a lower sensitivity (which is also

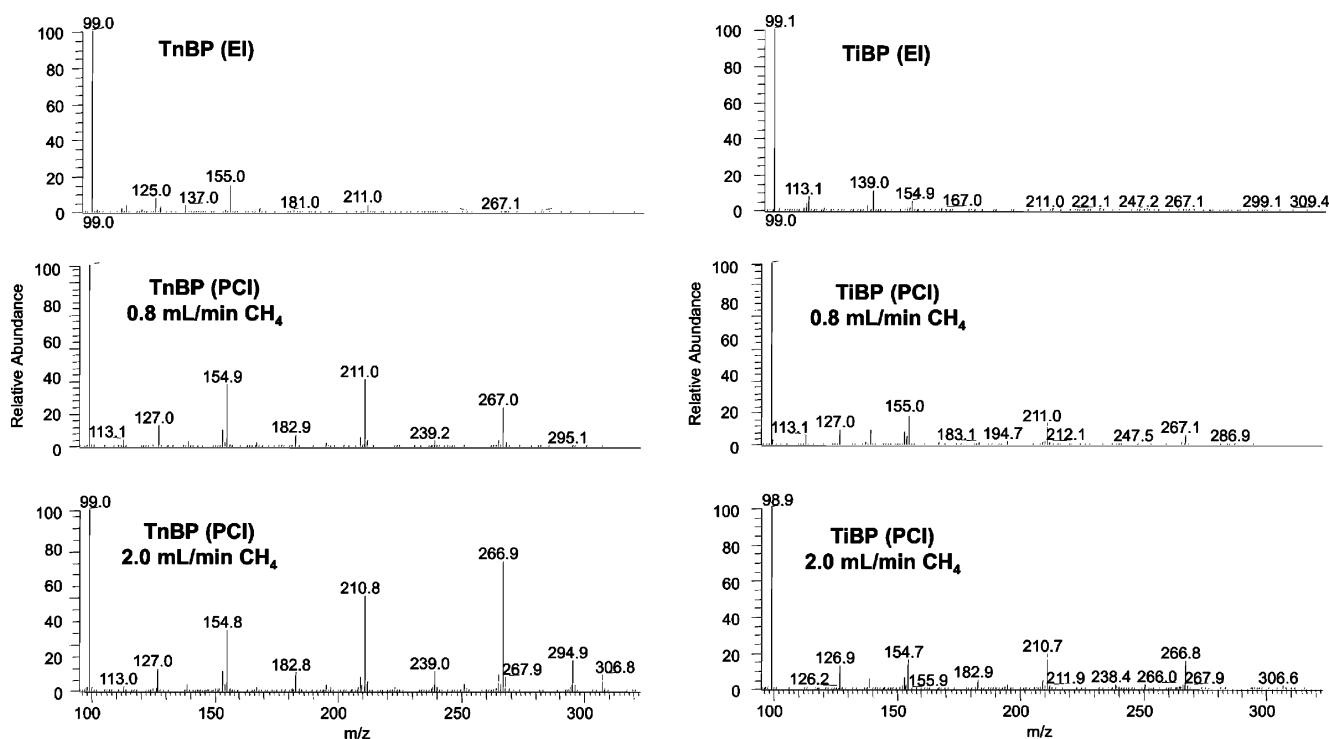


Fig. 5 Electron impact (EI) and positive chemical ionisation (PCI) spectra of tri-*n*-butyl phosphate (TnBP) and tri-*iso*-butyl phosphate (TiBP). Note that m/z 267 corresponds to the protonated molecular ion for both analytes

Table 4 Performances of the GC–NPD and LVI–GC–PCI–MS/MS chromatographic methods

	GC–NPD			LVI–GC–PCI–MS/MS		
	Linearity (R^2) ^a	Repeatability (% RSD) ^b	LOQ (ng mL ⁻¹) ^c	Linearity (R^2) ^d	Repeatability (% RSD) ^e	LOQ (ng mL ⁻¹) ^e
TiBP	0.9901	5.4	2	0.9954	4.3	50
TnBP	0.9994	4.3	2	0.9906	2.2	10
TCEP	0.9923	3.8	4	0.9990	3.0	10
TCPP	0.9869	6.5	2	0.9958	2.8	10
TDCP	0.9803	7.3	10	0.9847	5.0	100
TPhP	0.9991	2.3	10	0.9955	3.3	8
EHDPP	0.9914	3.4	5	0.9994	2.7	30
TEHP	0.9981	2.5	2	0.9931	2.5	50
TPPO	0.9855	6.0	10	0.9835	4.9	4
TCrP	0.9903	5.1	8	0.9922	4.4	20

^a 10–1000 ng mL⁻¹^b 50 ng mL⁻¹ level ($n=7$)^c S/N>10^d 20–2000 ng mL⁻¹, except EHDPP and TEHP: 50–2000 ng mL⁻¹, and TiBP and TDCP: 200–5000 ng mL⁻¹^e 200 ng mL⁻¹ level ($n=8$)

good for confirmation). Anyhow, LVI–GC–PCI–MS/MS yields the selectivity required (Fig. 6) and further confirmation of the positives is obtained by directly comparing the MS/MS spectra of samples and standards. Similar results for the sensitivities of GC–PCI–MS/MS and GC–NPD were obtained by Björklund et al. [16].

Method performance and application

The developed PLE method was finally applied to the determination of target analytes in two kind of particulate samples: a deposited urban dust and a PM10 sample, both

taken at the same location. Also, as mentioned in the “Experimental” section, these samples were used for method validation by spiking them at different levels. The spiked deposited dust sample was aged for three months before it was used for validation purposes. PM10 samples were spiked on the PLE extraction cell and left overnight before extraction as it was not feasible to age them due to the low amounts available.

Table 5 presents the concentrations found in the unspiked samples together with the recovery values from the spiked ones and the LOQ values estimated from the urban dust sample (S/N>10). LOQ values range between 0.01 and

Fig. 6 LVI–GC–PCI–MS/MS chromatograms of (a) a 100 ng mL⁻¹ standard, (b) deposited urban dust and (c) the PM10 sample. MS/MS transitions as in Table 1. Sample concentrations are given in Table 5. Peak assignment: 1, TiBP; 2, TnBP; 3, TCEP; 4, TCPP (three isomers); 5, TDCP; 6, TEHP; 7, EHDPP; 8, TPhP; 9, TPPO and 10, TCrP (four isomers)

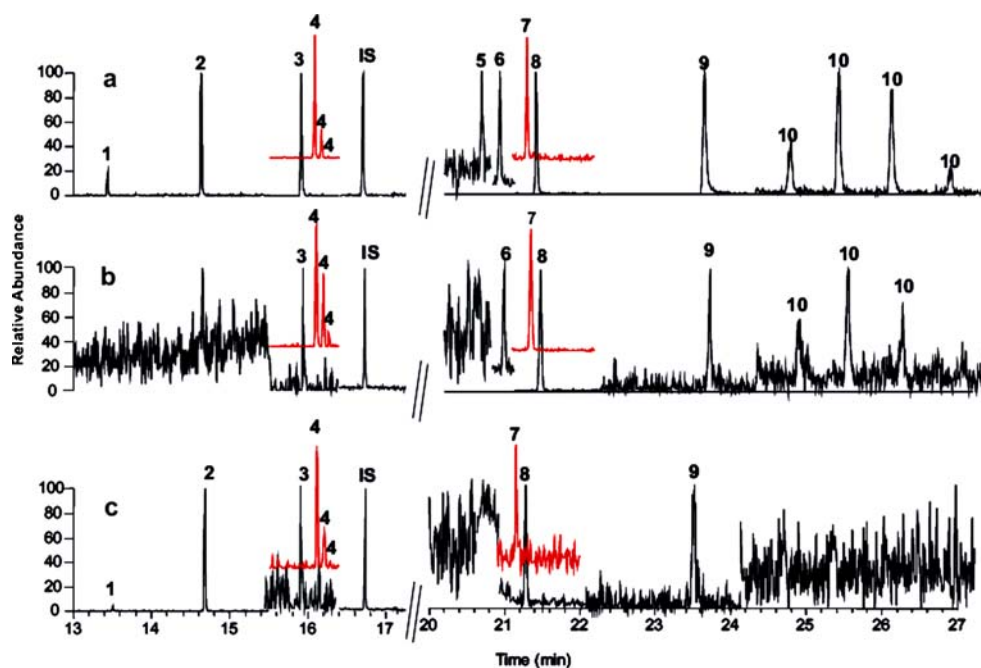


Table 5 Performance of the PLE–LVI–GC–PCI–MS/MS method and concentrations found in the analysed samples ($n=4$)

	LOQ ($\mu\text{g g}^{-1}$) ^a	LOQ (ng m^{-3}) ^b	Deposited urban dust		PM10	
			% Recovery (RSD) ^c	Conc.± S.D. ($\mu\text{g g}^{-1}$)	% Recovery (RSD) ^d	Conc.± S.D. (ng m^{-3})
TiBP	0.2	1.3	89 (10)	<LOD	82 (2.7)	2.4±0.1
TnBP	0.02	0.1	82 (6.2)	<LOD	98 (2.3)	1.5±0.1
TCEP	0.03	0.2	67 (14)	0.051±0.009	65 (7.9)	0.52±0.06
TCPP	0.03	0.2	105 (3.3)	0.32±0.05	91 (11)	1.0±0.1
TDCP	0.3	1.9	95 (12)	<LOD	84 (6.3)	<LOD
TPhP	0.02	0.1	110 (4.0)	0.290±0.03	104 (8.9)	1.06±0.02
EHDPP	0.07	0.4	107 (5.2)	0.50±0.06	101 (9.5)	0.24±0.01
TEHP	0.15	0.9	110 (12)	0.22±0.04	111 (5.7)	<LOD
TPPO	0.01	0.1	67 (5.5)	<LOQ	64 (4.4)	<LOQ
TCrP	0.05	0.3	104 (13)	<LOQ	96 (5.0)	<LOD

^a S/N>10, calculated from the settled urban dust for a 0.5 g sample

^b S/N>10, calculated from the PM10 sample for a sampling volume of 80 m³ (i.e. 0.2 g of filter)

^c Aged sample (three months) spiked at the 0.5 $\mu\text{g g}^{-1}$ level

^d Sample spiked at the 2 $\mu\text{g g}^{-1}$ level (equivalent to 5 ng m^{-3}).

0.07 $\mu\text{g g}^{-1}$, except for TiBP and TDCP (0.2 and 0.3 $\mu\text{g g}^{-1}$, respectively), which were different for the reasons discussed previously, and for TEHP (0.15 $\mu\text{g g}^{-1}$), as this compound was measured in SIM mode. These LOQ values are of the same order as those obtained by García et al. through microwave-assisted extraction and GC–NPD, which however requires a more extensive sample clean-up [24].

The analysis of the real (unspiked) samples mainly shows the presence of the heaviest compound in the deposited dust sample, while the (lighter) TiBP and TnBP are found as the highest in concentration in the PM10 sample, as shown by their chromatograms (Fig. 6) and Table 5. This agrees with the expected behaviour (the most volatile compounds are distributed towards the suspended particulate matter and gas phase), but these results need to be confirmed by a more comprehensive study. Organophosphorous triesters were found at the 0.05–0.5 $\mu\text{g g}^{-1}$ level in the deposited dust and at the 0.2–2.4 ng m^{-3} level in the PM10 sample. These concentrations are lower than those found in indoor environments, in both particulate [11, 24] and air samples [10, 12, 13, 15], and slightly higher than those reported in air samples from northern Finland [18]. Nevertheless, it is important to note that although these concentrations are lower than those from indoor samples, they may still represent a risk, as PM10 maximal permitted levels have been created for monitoring purposes by the European Union [28] due to their ability to penetrate into the human respiratory system. Also, deposited dust containing these contaminants can get into sewage due through rainwater run-off, which highlights the need for a more detailed study.

Conclusions

The optimisation of a PLE method of determining organophosphorous flame retardants and plasticizers in outdoor particulate samples has been presented. The overall conditions that represented a best compromise between extraction yield and clean-up were obtained using an experimental design strategy that implements a desirability function for optimisation. The optimal PLE method is fully automated and integrates an alumina clean-up layer that reduces the amount of the most polar (coloured) interferences in the extract. Final selectivity is gained by LVI–GC–PCI–MS/MS. This combination affords satisfactory limits of quantification (0.01–0.3 ng g^{-1}), recoveries (64–111%) and precision (RSD<15%), while it keeps the level of automation high, avoiding the extensive clean-up steps required by other methods. Subsequent application of the method to dust and PM10 samples revealed the levels of organophosphorous triesters in these kinds of samples for the first time, but a long-term study is also required.

Acknowledgements J.B.Q. and R.R. acknowledge Xunta de Galicia and Ministerio de Educación y Ciencia for their contract sponsorship through the Isidro Parga Pondal and Juan de la Cierva research programs, respectively. This work was financially supported by Ministerio de Educación y Ciencia (project no. REN2003-08603-C04-01) and Xunta de Galicia (“Programa de consolidación e estruturación de unidades de investigación competitivas, en réxime de concorrencia competitiva 2006”).

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