



# Selective pressurized extraction as single-step extraction and clean-up for the determination of organophosphate ester flame retardant in *Citrus aurantium* leaves by gas chromatography-tandem mass spectrometry

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## Abstract

In this work, an analytical method has been developed and validated for the determination of organophosphate esters (OPEs) in urban ornamental tree leaves. OPEs are flame retardants and plasticizers which are classified as health and environmental hazards substances. Their presence in urban air has been previously described. The method proposed in this work would allow the use of urban tree leaves as simple, cheap, and widely distributed in urban areas alternative to the existing active and passive sampler for sample collection. The method was based on sample treatment by selective pressurized liquid extraction (SPLE) and determination by gas chromatography with triple quadrupole mass spectrometry detector. After the optimization of the extraction solvent, the key parameters applied to SPLE (clean sorbent and sorbent amount applied for the sample clean-up, temperature, extraction cycles, and time) were optimized using a Box-Behnken response surface design. The method achieves high recoveries (higher than 60% for most of the target compounds), accuracies between 70 and 109%, and method detection and quantification limits ranged 0.05–4.96 ng/g dw (dry weight) and 0.15–14.4 ng/g dw, respectively. The method allowed the proper biomonitoring of OPE in tree leaves. Concentrations measured in analyzed samples were from 47.5 to 5477 ng/g dw (TEP). The most frequently detected compounds were triethyl phosphate tri-*n*-butyl phosphate, triphenyl phosphate, and tris(1-chloro-2-propyl)phosphate, while tris(2-ethylhexyl)phosphate was not detected in the analyzed samples. The proposed analytical method constitutes a starting point for the use of ornamental urban trees as passive sampler for the evaluation of OPE as air pollutants.

**Keywords** Organophosphate ester · Airborne contaminants · Urban ornamental trees · Passive samplers · Gas chromatography-tandem mass spectrometry

## Introduction

Organophosphate esters (OPEs) are chemicals usually used as flame retardant agents and plasticizers. These compounds are added to a wide variety of commercial products as electronic equipment, lubricants, plastics, glues, varnishes, or

furnishing fabrics [1, 2]. OPEs replaced polybrominated diphenyl ethers (PBDEs) when the Stockholm Convention classified them as persistent organic pollutants in 2009 [3]. OPEs usually are not chemically bound to the host product and, as a result, they can release to the surrounding environment through volatilization, leaching, and/or abrasion processes. Although OPEs seem to be sufficiently degradable to exhibit low persistence in the environment and low long-range atmospheric transport potential, recent investigations have pointed out their global occurrence, especially in the atmosphere compartment, even in remote regions of the world [4, 5]. For example, recently, these compounds have been detected in atmospheric aerosols for the first time, even at a high frequency of detection [6]. Moreover, their ecotoxicological risks could be high due to their potential carcinogenic, neurotoxic, or endocrine disruptors character [7, 8].

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Currently, the evaluation of atmospheric pollution by OPEs is carried out by the analysis of the vapor phase and the atmospheric particulate matter (PM) [9] collected with active or passive samplers. These sampling devices have certain drawbacks. For example, the power supply requirements of the active samplers hinder its use in remote areas. In the case of passive samplers, they should be exposed to the contaminants for a wide period of time, sometimes even months, which makes them unusable in the case of short-term contamination events. The use of tree leaves as biosamplers could be a helpful instrument since, in addition to a simple sampling; they constitute an inexpensive and widely available option for atmospheric monitoring. Until now, this practice has been extended to families of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) [10–12], polychlorinated biphenyls (PCBs) [13], organochlorine pesticides (OCPs) [14], or PBDEs [15]. More recently, it has been extended to emerging pollutants as parabens, perfluoroalkyl compounds, bisphenol A, or musk fragrances, among others [9].

The major limitations of the use of tree leaves as biosamplers of the atmospheric pollutants are the lack of analytical methods for the reliable determination of pollutants in these complex matrices and, the challenge of to establish correlations between the concentrations of the organic pollutants measured in leaves and their respective concentrations in air. The latter has been addressed recently in the case of some organic pollutants as PBDEs [16] and PAHs [17, 18] by modeling the organic pollutants uptake by vegetation from the air. However, for this purpose, it is necessary the determination of the concentrations of organic pollutants in the tree leaves. For this reason, reliable and validated analytical methods that allow the determination of the organic pollutants in this complex matrix are necessary.

In the particular case of flame retardants, the methods reported in the literature for their determination in tree leaves have been focused on the determination of PBDEs [16, 17] while others as OPEs have received little attention even though their wide consumption, persistence, and bioaccumulation.

Considering analytical methods, until now, there are no analytical methods published in the literature that allow the determination of the studied compounds in bitter orange tree leaves. Only a few analytical methods have been reported in the literature for the determination of OPEs in plant origin products, including foodstuff as cereals or derived products, fruits, and vegetables [19]. However, these samples show a different matrix to the tree leaves. This conditioned the extraction technique applied and the method performance. These analytical methods are based on sample treatments by ultrasonic-assisted extraction (USE) [20] or Soxhlet [21], followed by LC-MS/MS or GC-MS/MS. These extraction techniques, although efficient, show poor automation possibility and, in many instances, non-environmental friendly

solvents (halogenated solvents) are used. Other extraction techniques as matrix solid-phase dispersion (MSPD) [22] or pressurized liquid extraction (PLE) have been reported [23, 24], although these methods have been applied to environmental samples as sediments [23] and atmospheric particulate matter [24]. PLE not only has proved to be efficient for the extraction of OPEs from these solid environmental samples but also it allows the automatic treatment of up to 24 samples and the in-cell clean-up, reducing the time of analysis. The aim of this work was to develop a multiresidue method for the determination of OPEs in urban tree leaves. Seven OPEs were selected according to their wide consumption, their major volatility, bioaccumulation, and persistence in the environment [25]. Extraction and clean-up were carried out using selective pressurized liquid extraction (SPLE) technique and determined by gas chromatography-tandem mass spectrometry (GC-MS/MS). The main parameters affecting extraction yield and selectivity, such as the extraction solvent, extraction time, number of cycles, and clean-up sorbent, were evaluated to obtain lipid-free extracts and quantitative recoveries for OPEs. This procedure constitutes the first analytical method for the determination of OPEs in *Citrus aurantium* leaves.

## Experimental

### Chemical and reagents

High purity standards of the studied compounds, triethyl phosphate (TEP) (> 99.8%), tripropyl phosphate (TPP) (> 98.5%), tri-n-butyl phosphate (TBP) (> 99%), tris(1-chloro-2-propyl)phosphate (TCPP) (> 99%), tris(1,3-dichloro-2-propyl)phosphate (TDCPP) (> 95%), triphenyl phosphate (TPhP) (> 96%), and tris(2-ethylhexyl)phosphate (TEHP) (> 96.5%) were purchased from Sigma-Aldrich (Milan, Italy). The physicochemical properties of target compounds are summarized in Table S1 (see Electronic Supplementary Material, ESM). Isotopically labeled tributyl phosphate-d27 (TBP-d27) (> 98%), used as internal standard (IS), was provided by Dr. Ehrenstorfer, GmbH (Augsburg, Germany). HPLC-grade methanol, acetone, and hexane were supplied by Romil Ltd. (Barcelona, Spain). Primary-secondary amine (PSA) and C18 sorbents were provided by Scharlab (Barcelona, Spain). Florisil was provided by Sigma-Aldrich (Milan, Italy). PSA, Florisil, and C18 were used for clean-up optimization.

Individual stock solutions of each compound (100 mg/L) were prepared in acetone. Working solution of the mixture of the seven target compounds (1.0 mg/L) and a working solution of the IS were prepared by dilution of the individual standard stock solution using acetone as solvent. All of the solutions were sealed and stored at  $-18\text{ }^{\circ}\text{C}$  in darkness.

## Sample collection

*C. aurantium* is an evergreen specie which height reaches 10 m. It grows in Asia and North Africa, and it is particularly widespread in the southern region of Europe, especially in the south of Italy and Spain. In this work, *C. aurantium* leaves were collected, with the corresponding authorization, from three locations from Seville city (South of Spain) (Fig. S1 in ESM). The first sampling site was located in an urban park (UP) sited in the eastern part of the city (37° 22' 46.9" N, 5° 57' 10.1" W), the second sampling site was located in a high-populated area situated in the northern part (HP) (37° 25' 38.4" N, 5° 58' 54.0" W) and the third sampling site was placed in a periurban park (PP) (37° 25' 15.0" N, 5° 59' 29.1" W).

All of the trees evaluated were in similar grown status and the trees with anomalies were discarded (gummosis or putrefaction of the neck of the root, infections caused of viruses or presence of parasites). From each tree, one sample was composed of five subsamples collected from each of the cardinal points and from the center of the tree. These subsamples were composed of 3–5 young leaves (up to the 3rd node from the top of the shoots) and 3–5 old leaves (from the basal parts of the shoots) [26]. Sample from each sampling point was composed of at least 25% of the trees sited in the studied location. In the case of UP and HP locations, one composed sample from each sampling point was collected in November 2016, April 2017, and July 2017 during three consecutive days in each month. Two samples were collected from PP, in November 2016 and in July 2017. All samples were wrapped in aluminum foil cleaned with acetone and transported to the laboratory. Then, they were cut and crushed using a crusher, lyophilized at 0.01 mbar vacuum after being frozen at  $-18\text{ }^{\circ}\text{C}$  for 24 h in a Cryodos-50 liophilizer (Telstar, Terrasa, Spain), pulverized and sieved ( $<1\text{ mm}$ ). Blank sample of *C. aurantium* leaves was collected from a periurban park sited in the South area of Seville (37° 22' 25.1" N, 6° 01' 05.4" W), far away from the urban area. This sample was used for the optimization and validation of the analytical method. During these processes, bank sample was processed and measured in triplicate in order to ensure the absence of the target compounds or, if necessary, correct the blank signal.

## Optimization of the sample treatment

The extraction of the studied compounds was performed by selective pressurized liquid extraction (SPLE) using a Dionex ASE 350 system (Sunnyvale, CA, USA). Stainless steel cells with 10 mL capacity were used. The optimization of sample treatment was carried out determining the influence of the parameters affecting extraction efficiency (extraction solvent, pressure, temperature, number of extraction cycles, and extraction time) and clean-up (type and amount of sorbents)

[27, 28]. The extraction solvent was optimized separately. After that, the optimization of both the clean-up process and pressurized liquid extraction operational parameters were carried out using a multivariable design in each case. The optimization was carried out using spiked samples and spiked extracts. Blank samples were processed for blank correction.

Due to the complexity of the sample, clean-up was necessary in order to avoid the matrix effect. For this reason, 0.8 g of C18 was used as clean-up sorbent for extraction solvent optimization (three sorbents (C18, PSA, and Florisil) were tested previously by dispersive solid-phase extraction (data not shown) and C18 was selected). The optimization was carried out using samples spiked in triplicate at 100 ng/g. Cells were filled with 0.5 g of diatomaceous earth, 0.8 g of C18, 0.5 g of lyophilized sample mixed with 2 g of diatomaceous earth and diatomaceous earth until cell capacity was filled. The operational conditions were the following: extraction temperature of 100 °C, static periods of 15 min in two cycles, extraction pressure of 1500 psi, flush volume of 60% of the cell volume, and a nitrogen purge of 90s.

The optimization of clean-up sorbents was carried out by a multivariable design based on Box-Behnken response surface design using Statgraphic Plus software version 5.1 (Statpoint Technologies Inc., Warrenton, VA, USA). The use of multivariable experimental design for the optimization of sample treatment allowed the evaluation of the influence of the tested variables in the extraction of the analytes and of the potential interactions of these variables on overall recoveries. Moreover, the applied statistical design allowed reducing the number of experiments, reagent consumption, and laboratory work necessities for the optimization of the analytical method [29]. The variable (types of sorbent) was evaluated at three levels (mass of sorbent). Three sorbents were tested: (i) the first, usually applied to remove non-polar interfering substances (C18); the second, applied to the removal of polar acids, polar pigments and fatty acids (PSA (primary-secondary amine)); and the third, applied to the elimination of lipids from biota samples (Florisil) [30, 31]. The number of experiments carried out (given by the equation  $N = 2k(k-1) + C_0$ , where  $k$  is the number of variables and  $C_0$  is the number of central points) was 15 according to the three variables (Florisil, C18, and PSA) (Table S2 in ESM), the three levels for each variable (mass of sorbent: 0, 0.4, and 0.8 g) and the number of central points, set at 3. This optimization was carried out using blank sample treated with different sorbents and sorbent amounts (Table S2 in ESM). After SPLE treatment, obtained sample extracts were spiked with target compounds. ME was quantified by the comparison of the peak areas measured (in triplicate) in spiked extracts and those measured in standard solution prepared in acetone at the same concentration. Blank sample was measured for blank correction. ME was quantified using the following equation:

$$\text{ME (\%)} = ((A_{\text{sp-extract}} - A_{\text{bl-extract}}) / A_{\text{standard}}) \times 100$$

where  $A_{\text{sp-extract}}$  is the peak area measured in standard solution prepared in sample extract,  $A_{\text{bl-extract}}$  is the peak area measured in blank sample, and  $A_{\text{standard}}$  is the peak area measured in a standard solution prepared using acetone as solvent.

Once extraction solvent and clean-up sorbents were fixed, the key SPLE operational parameters (temperature, number of extraction cycles, and extraction time) were optimized. The optimization of SPLE operational parameters was carried out using samples spiked with target compounds at 100 ng/g. Extraction pressure, flush volume, and nitrogen purge were fixed on 1500 psi, 60% of the cell volume and 90s, respectively. The operational parameters were evaluated at three levels (number of cycles: 1, 2, and 3; extraction time: 5, 10, and 15 min; temperature, 80, 100, and 130 °C) (Table S3 in ESM).

### Optimized sample treatment

The extraction cell was fixed (bottom to top) with 0.5 g of diatomaceous earth, clean-up sorbents (0.6 g of C18 mixed with 0.8 g of PSA), 0.5 g of lyophilized sample mixed with 2 g of diatomaceous earth and diatomaceous earth until cell capacity was filled. One cellulose filter was placed at the bottom of the cell. The extraction was carried out using acetone as extraction solvent and one static extraction cycle of 15 min at 130 °C with a cell pressure of 1500 psi. The flush volume was 60% and the purge time 90 s. The obtained acetone extract (approximately 20 mL) was evaporated to dryness at 50 °C by a nitrogen stream in an XcelVap® automated evaporation/concentration system (Horizon Technology, Salem, New Hampshire, USA), reconstituted with 1 mL of acetone containing 200 ng/mL of the I.S. and filtered through a 0.22- $\mu\text{m}$  nylon syringe filter. After use, extraction cells were disassembled and cleaned by sonication in acetone for 10 min.

### Gas chromatography-tandem mass spectrometry

The analysis of sample extracts was carried out using an Agilent 7890B gas chromatograph coupled with an Agilent 7000D triple quadrupole mass spectrometry detector with an electron-impact ion source (Agilent Technologies, Santa Clara, CA, USA) and a multipurpose sampler (MPS2, Gerstel, Germany). Separation of the target compounds was carried out using an HP-5 ms capillary column (30 m  $\times$  0.25 mm i.d. and 0.25- $\mu\text{m}$  film thickness) from Agilent (Agilent Technologies, Santa Clara, CA, USA). High purity helium gas was used as carrier gas in a constant flow mode of 1 mL/min. The injection-port temperature was set at 300 °C and the injection volume was 1  $\mu\text{L}$  in the splitless mode.

The mass spectrometer operated with a filament current of 35  $\mu\text{A}$  and electron energy 70 eV in the positive electron ionization mode. Ion source and transfer line (auxiliary) temperatures were fixed at 230 and 280 °C, respectively.

The optimization of GC-MS/MS operational parameters was carried out in two steps: (i) MS/MS optimization and (ii) chromatographic separation optimization. In both cases, the optimization was carried out by the injection of a standard solution prepared in acetone. For MS/MS optimization, a standard solution containing 1 mg/L of the target compounds and IS was injected using full scan mode. Oven temperature program was fixed as follows: 50 °C for 2 min; a temperature ramp of 20 °C/min up to 250 °C (holding this temperature for 2 min) and ramp of 10 °C/min up to 300 °C (holding this temperature from 1 min). Chromatographic peaks, obtained in the full scan mode, were identified using NIST database and the most abundant ion of each compound was selected as precursor ion. Product ions of each compound were selected by the injection of the standard solution in product ion scan mode using the same oven temperature program. The most abundant transitions were selected. Finally, collision energy applied for each transition was optimized by the injection of standard solution, in multiple reaction-monitoring (MRM) mode, applying different collision energies (from 0 to 60 V). The optimized MS/MS parameters are shown in Table 1. The two most abundant transitions were selected. The most abundant (MRM1) was selected for quantification. The other transition (MRM2) and the ion ratio were used for confirmation according to Commission Decision 2002/657/EC [32]. Mass spectrometer autotune was performed on a weekly basis.

Regarding to the optimization of chromatographic separation, it was carried out by the injection of a standard solution of target compounds at 200 ng/mL in MRM mode with the optimized MS/MS conditions. Initial oven temperature was increased from 50 to 80 °C with respect to those used for MS/MS operational condition optimization and the gradient was modified using one ramp instead two ramps as it was used in the MS/MS optimization. Figure 1 shows a MRM chromatogram of a standard solution at 50 ng/mL of target compounds obtained using the optimal GC-MS/MS conditions.

### Method performance

The proposed method was validated by the determination of the matrix effect (ME), extraction process recovery, accuracy and precision (expressed as repeatability in terms of relative standard deviation (RSD)), linearity and method detection (MDL), and quantification (MQL) limits.

ME was evaluated by the comparison of the slopes of calibration curves constructed using standard solutions prepared in acetone (solvent calibration curve) and standard solutions prepared in extract sample (matrix-matched calibration curves). The sample extract used for the preparation of the

**Table 1** MS/MS parameters applied to the determination of organophosphate ester flame retardants

Compound	Precursor ion ( <i>m/z</i> )	Product ions (MRM1/MRM2) ( <i>m/z</i> )	Ion ratio	CE (V)	RT (min)
TEP	155	81/99	17.1	20/8	4.54
TPP	141	99/81	62.4	8/10	6.76
TBP	99	63/81	5.9	15/10	8.88
TPhP	326	215/170	71.4	20/20	13.87
TEHP	99	63/81	25.4	60/25	14.10
TCPP	125	99/81	4.9	8/8	10.06
TDCP	191	155/57	9.2	20/8	13.47
TnBP-d27	103	63/81	0.2	40/30	8.75

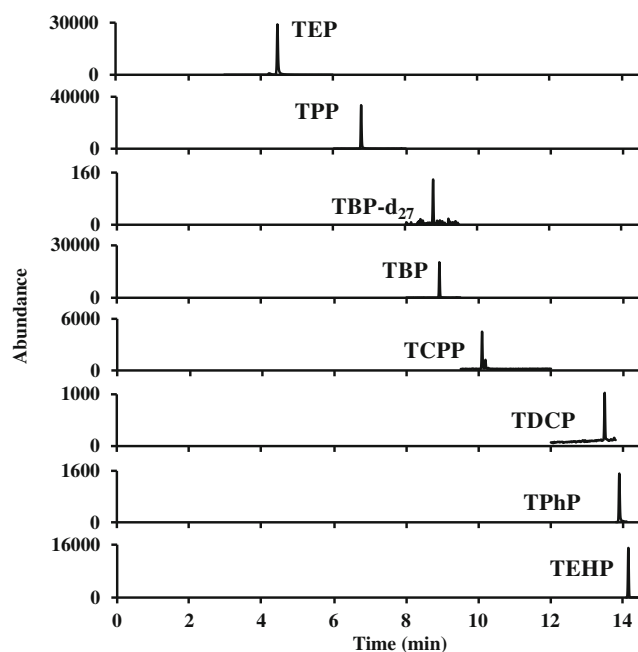
CE collision energy, RT retention time. MRM1 was used for quantification; MRM2 was used for confirmation

matrix-matched calibration standards was obtained from the blank sample, previously analyzed to ensure the absence of the analytes in the extract. Both calibration curves were constructed using the peak area ratios (between analyte and internal standard peak areas) measured on at least six calibration levels (15, 25, 100, 200, 500, 1000 ng/mL).

For each compound, ME was quantified by the comparison of the slopes obtained in both solvent and matrix-matched calibration curves. For each compound, ME was determined as it was reported by Ciofi et al. (2018) [33]:

$$ME (\%) = (b_{MMC}/b_{SC} \times 100) - 100$$

where  $b_{MMC}$  is the slope of the matrix-matched calibration curve and  $b_{SC}$  is the slope of the solvent calibration curves. The negative ME values indicate an ionic suppression of the target compound while positive ME values indicate the signal enhancement.



**Fig. 1** MRM chromatogram of a standard solution of target compounds at 50 ng/mL

The recoveries of the sample treatment procedure were determined by the comparison of the peak areas measured in samples spiked in triplicate with target compounds and those measured in sample extracts spiked at the same concentration (measured in triplicate). Recoveries were determined at three concentrations levels (low, medium, and high) as follows:

$$R (\%) = ((R_{sp-sample} - R_{bl-extract}) / R_{sp-extract}) \times 100$$

where  $R_{sp-sample}$  is the peak area ratio (between analyte and internal standard peak areas) measured in the spiked sample,  $R_{bl-extract}$  is the peak area ratio measured in blank sample extract, and  $R_{sp-extract}$  is the peak area ratio measured in a standard solution prepared in extract sample.

Accuracy was determined by the comparison of the concentrations measured in spiked blank samples (in triplicate at three concentration levels) and the true spiked concentrations. Intraday precision was determined by the analysis of samples spiked in triplicate, at different concentration levels, as the relative standard deviations of the concentrations measured in spiked samples at low, medium, and high concentration levels. The experiments were repeated after 3 and 9 days for the determination of the interday precision.

Calibration curves were generated by linear regression of peak area ratios (between analyte and internal standard peak areas) measured in seven matrix-matched calibration standards against the concentration of each compound (from IQL of each target compound to 500 ng/mL). Linearity was evaluated as the Pearson correlation coefficient of the matrix-matched calibration curves.

Instrumental detection (IDL) and quantification (IQL) limits were determined as the concentration of target compounds with a signal-to-noise ratio of 3 and 10 respectively (Table S4). IDL and IQL were determined by the injection (in triplicate) of blank sample extracts spiked at low concentration levels (0.01, 0.05, 0.10, 0.5, 1, 2, 5, and 10 ng/mL). Method detection (MDL) and quantification (MQL) limits of each target were determined from IDL and IQL considering the concentration factor achieved in sample treatment and the recovery achieved for the corresponding compound at low concentration level.

## Results and discussion

### Sample treatment optimization

According to the physicochemical characteristic of the target compounds ( $K_{ow}$  from 0.8 to 9.49 (Table S1 in ESM)) and the extraction solvents usually applied for their determination in other solid environmental samples [22–24, 34, 35], three solvents were tested for the extraction solvent optimization (two polar solvents: one protic (methanol) and other aprotic (acetone)) and one non-polar solvent (hexane)) were tested. The results are shown in Fig. 2.

Similar recoveries were achieved in the case of acetone (from 65 to 106%) and methanol (from 50 to 96%) while hexane showed poor recoveries for the most of the studied compound (recoveries lower than 25% in the case of TEP, TPP, and TDCP and from 59 to 66% in the other compounds). In the case of TEP, the recoveries achieved using methanol were slightly higher than those obtained with acetone. However, recoveries obtained in the case of TPhP and TEHP were much higher in the case of acetone than those obtained for methanol. According to these results, the use of mixtures of solvents was not necessary, in spite of their use for the extraction of these compounds in solid environmental matrices [1, 6–8, 20–22, 34–37]. Moreover, considering the volatility of acetone, higher than methanol volatility, acetone was selected as extraction solvent in order to reduce the time of solvent evaporation step.

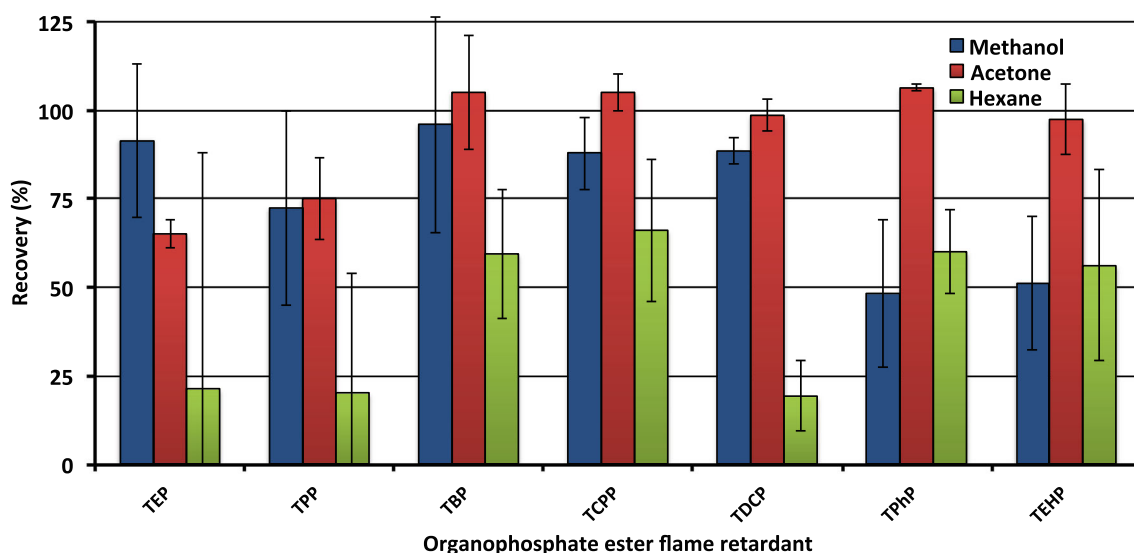
Regarding the optimization of clean-up sorbents, obtained response surface plots are shown in Fig. 3. In the case of the amount of Florisil fixed to 0 g (Fig. 3A: C18 amount vs. PSA amount), the optimal results were achieved using 0.6 g of C18 (Table S2 in ESM). When the amount of C18 was fixed at

0.6 g (Fig. 3B: PSA amount vs. Florisil amount), the optimal results were achieved for 0 g of Florisil and 0.8 g of PSA. The same results were obtained fixing PSA amount at 0.8 g (Fig. 3C: C18 amount vs Florisil amount). According to these results, 0.6 g of C18 and 0.8 g of PSA were selected as clean-up sorbents (Table S2 in ESM).

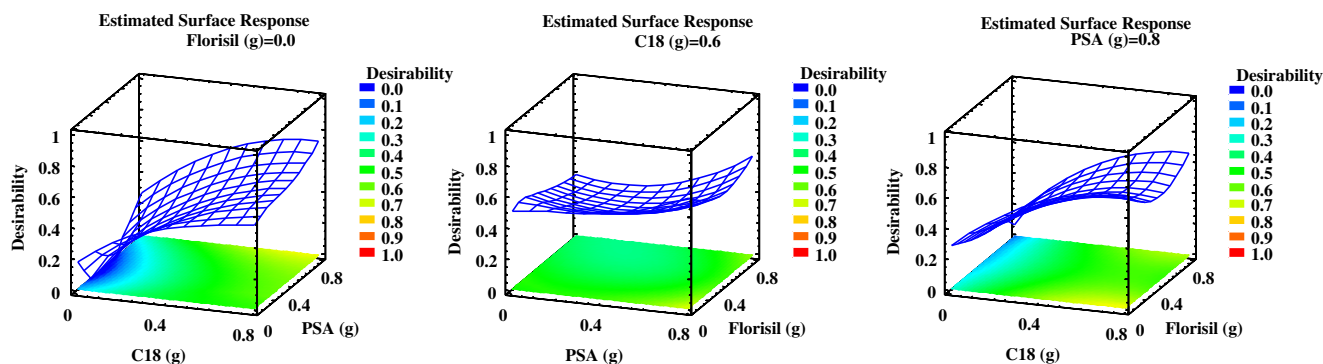
Considering the optimization of SPLE operational parameters, in the case of the number of cycles fixed at 1 (Fig. 4A: time vs temperature), the optimal conditions were achieved at 130 °C for 15 min. These results were confirmed in response surface plots constructed fixing temperature at 130 °C (Fig. 4B: time vs number of cycles) and 15 min for extraction time (Fig. 4C: temperature vs number of cycles). On the contrary, considering the number of cycles, similar results were obtained in the case of 1 and 3 cycles. According to these results, 1 extraction cycle of 15 min at 130 °C was fixed as SPLE extraction conditions.

### Method validation

Regarding the evaluation of ME, solvent and matrix-matched calibration curves are shown in Fig. S2 (see ESM). As can be seen, a high matrix effect was observed for all target compounds. This result was corroborated using Student's *t* test to compare solvent calibration and matrix-matched calibration curve slopes (Table S5 in ESM). ME (%) obtained for each target compound is showed in Table S5 in the ESM. A low matrix effect was observed (from – 22 to 22%) for most of the studied compounds, except in the case of TEHP showed a high matrix effect (73.4%). For this reason, matrix-matched calibration curves were necessary for the quantification of the target compounds.



**Fig. 2** Overall recoveries obtained from spiked tree leaves (in triplicate) using different extraction solvents (spiked concentration 100 ng/g dw). Error bars represent the minimum and maximum recoveries



**Fig. 3** Response surface plots of the global desirability function for clean-up optimization. Values obtained from tree leaves extracts spiked at 50 ng/L in triplicate

Recoveries were from 59 to 133, from 71.5 to 128%, and from 72.4 to 137% (45% TEP) in the case of the low, medium, and high spiked level. The lower recoveries were obtained for the most volatile compounds (TEP and TPP) while the recoveries for the other compounds were higher than 88% for all spiked levels. These recoveries were comparable with those reported in pine needles [38] and other environmental samples (sludge and sediments) using other extraction techniques such as solid-liquid extraction by shaken [38], Soxhlet [34, 35], or microwave-assisted extraction [39] and similar to those reported using PLE with acetone:hexane mixture as extraction solvent [37].

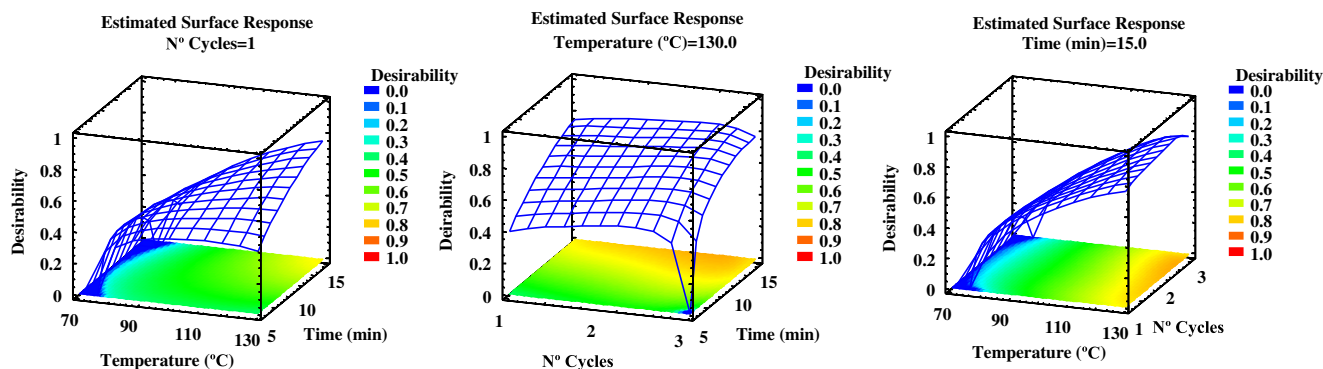
Intra- and interday precision expressed as the relative standard deviation (Table 2) were lower than 16 (TDCP) and 20% (TPhP), respectively.

Accuracy of the proposed method was calculated at three concentration levels (close to MQL (0.5, 2, and 15 ng/g), medium (50 ng/g) and high (100 ng/g)). High accuracy was obtained in the three spiked levels (mean value: 86% in the low and high spiked level and 98% in the case of medium level) (Table 3). MDL and MQL are showed in Table 2. As can be seen, MDLs were from 0.05 to 4.96 ng/g, while MQLs were from 0.15 to 14.4 ng/g.

Several methods have been developed for the analysis of OPE in environmental (soil, sediment, dust) and food samples (cereals, fruits, fish, and vegetables) (ESM Table S6). Among

applied extraction techniques are Soxhlet, USE, PLE, and MSPD using hexane, acetone, dichloromethane, methanol, and mixture of them as extraction solvent. Most of these methods included clean-up processes based on column chromatography and SPE after extraction. The analytical parameters obtained in the proposed method are in concordance with those previously reported using these techniques in other vegetable matrices as rape (reported recoveries and MQL ranged 65.1–109% and 0.05–0.33 ng/g, respectively [21]) and wheat (75.6–90.0% and 0.06–0.35 ng/g [22]) and similar to those reported in dust, soil [36, 38], and sediment [37, 39] using the same extraction technique [23, 24] (ESM Table S6). Moreover, the MDL and MQL achieved by the proposed method are in concordance with those reported in tree leaves in the case of other contaminants found at similar concentrations in atmosphere as perfluorinated organic compounds or PBDE [9, 40, 41] and lower than those reported for OPE in pine needle [38].

The proposed method allows the suitable determination of OPE using a single-step extraction and clean-up and environmental friendly solvents. This method constitutes the first analytical method allowing the biomonitoring of OPEs in *Citrus aurantium* tree leaves and the second reported in the literature for the evaluation of OPE as atmospheric pollutants using tree leaves as passive samplers.



**Fig. 4** Response surface plots of the global desirability function for SPLE conditions. Results obtained from tree leaves at 100 ng/g dw in triplicate

**Table 2** Matrix effect (ME), extraction process recovery (R), linear dynamic range (LDR), method detection limit (MDL), and method quantitation limit (MQL)

Compound	Low			Medium			High			LDR (ng/g)	MDL (ng/g)	MQL (ng/g)
	R (%)	Intraday precision (%)	Interday precision (%)	R (%)	Intraday precision (%)	Interday precision (%)	R (%)	Intraday precision (%)	Interday precision (%)			
TEP	59.1	2.8	18	71.8	8.0	10	45.1	6.2	8.2	0.3–2200	0.10	0.30
TPP	83.5	3.1	13	71.5	7.8	8.7	72.4	5.9	5.6	1.32–1380	0.36	1.32
TBP	101	12	14	109	3.1	5.3	121	3.4	5.3	0.19–826	0.06	0.19
TPhP	112	3.6	11	91.4	6.3	20	137	1.0	1.7	0.15–730	0.05	0.15
TEHP	88.2	3.7	15	118	7.8	8.1	128	5.5	4.1	0.23–780	0.07	0.23
TCPP	122	10	14	128	7.0	15	116	5.9	8.9	14.4–860	4.96	14.4
TDCPP	133	16	17	107	10	9.3	133	3.8	5.2	12.0–750	4.56	12.0

### Application of proposed method to real samples

OPEs have been analyzed in both indoor and outdoor environments. Concentrations up to 5300  $\mu\text{g/g}$  and 2300  $\text{ng/m}^3$  have been measured in indoor dust and air, respectively [42] (ESM Table S7). Lower concentrations have been measured in outdoor environment. For example, Wang et al. (2018) [36] measured concentrations up to 2140  $\text{ng/g}$  in outdoor dust while concentrations up to 20,700  $\text{pg/m}^3$  have been measured in outdoor air [38]. In this work, eight samples (three for HP, three from UP, and two from PP) were analyzed. Only TPP, TEHP, and TDCPP were not quantified in any of the analyzed samples (Table 4). The highest concentrations were measured in the case of TEP (up to 5477  $\text{ng/g dw}$ ), followed by TCPP (up to 1058  $\text{ng/g dw}$ ), TBP (up to 686  $\text{ng/g dw}$ ), and TPhP (up to 265  $\text{ng/g dw}$ ), in that order. Similar concentrations were measured in HP and UP, and higher than those measured in PP, which could be explained considering the distance to sources of these compounds of the sampled periurban park. These concentrations were higher than those reported recently by Li et al. (2019) [38] in pine needles collected near to airports. However, in this study, samples were pre-cleaned using HPLC water in order to remove the particulate matter on the

surface of the leaves. The distribution of OPEs found in this work was different than those measured by Li et al. (2019) [38]. Alkyl-OPEs were the predominant compounds, especially TEP, followed by Cl-OPEs (mainly TCPP) and aryl-OPEs (TPhP), in contrast with the distribution found in pine needles (Aryl-OPE > Alkyl-OPE > Cl-OPE). These results could show a different source of these pollutants in the studied area with respect to the results reported in pine needles [38]. Furthermore, the results obtained in this work are in concordance with other studies where TEP, TBP, or TCPP has been found as the predominant OPE in air samples [43–45].

The proposed method constitutes an interesting tool for the monitoring of OPEs in *Citrus aurantium* tree leaves and it shows the capacity of leaves as bioindicators of the presence of OPE in the atmosphere and the proposed method has shown to be useful for the biomonitors of these compounds. Furthermore, in spite of the concentrations measured in this work were in concordance with those measured in air and dust, the correlations between the concentrations measured in tree leaves and the air were not evaluated as it has been done for other contaminants [17, 18]. Further investigations measuring concentrations in leaves and air/particle matter are necessary in order to establish this correlation.

**Table 3** Accuracy of the proposed method for low, medium, and high spiked level ( $n = 3$  for each level)

Compound	Low spiked level			Medium spiked level			High spiked level		
	Spiked (ng)	Found (ng/)	Accuracy (%)	Spiked (ng)	Found (ng)	Accuracy (%)	Spiked (ng/g)	Found (ng/)	Accuracy (%)
TEP	0.5	0.37	74	50	50.8	102	100	89.9	90
TPP	2.0	1.40	70	50	48.6	97	100	88.0	88
TBP	0.5	0.41	82	50	50.6	101	100	87.6	88
TPhP	0.5	0.49	98	50	54.6	109	100	101	101
TEHP	0.5	0.52	104	50	51.8	104	100	91.5	91
TCPP	20	18.5	93	50	44.9	90	100	75.6	76
TDCPP	20	16.2	81	50	42.9	86	100	79.7	80



**Table 4** Concentrations (ng/g) of organophosphate ester flame retardants in bitter orange tree leaves from Seville city

Compound	Sampling site							
	UP1	UP2	UP3	HP1	HP2	HP4	PP1	PP2
TEP	1059	<MDL	5192	5184	5477	5349	106	93.3
TPP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MQL
TBP	686	<MDL	148	<MQL	<MQL	<MQL	<MQL	<MQL
TPhP	64.0	<MQL	47.5	265	246	137	<MDL	<MDL
TEHP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
TCPP	1058	327	321	525	410	412	<MDL	<MQL
TDCPP	<MDL	<MDL	<MQL	<MQL	<MQL	<MQL	<MDL	<MDL

## Conclusions

In this work, an analytical method was proposed for the determination of seven OPEs in leaves from urban ornamental trees. The method was based on the single-step extraction and clean-up of the contaminants by SPLE and determination by GC-MS/MS. The key variables in the SPLE have been optimized using a multivariable design based Box-Behnken response surface. The most favorable solvent for the extraction of OPE was acetone while a mixture of C18 and PSA was selected as sorbents clean-up. High recoveries (higher than 60% for most of the target compounds) and low MDL (lower than 4.96 ng/g) and MQL (lower than 14.4 ng/g) were achieved. Accuracy, evaluated in the whole linear range, varied from 70 to 109%. The proposed method was applied to the biomonitoring of OPE in urban tree leaves from Seville city. Concentrations of OPE in urban tree leaves from Seville city. Concentrations from 64.0 to 5477 ng/g dw were measured. TEP, TCPP, TBP, and TPhP were the compounds found at the highest concentrations. A distribution similar to those described in air and atmospheric particulate matter was measured (alkyl-OPEs>Cl-OPEs>Aryl-OPEs). The method has been proven to be useful for the monitoring of OPEs in tree leaves. However, further studies analyzing both leaves and air/atmospheric particulate matter samples are needed in order to establish the correlation between the concentration measured in leaves and those in the atmosphere.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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