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RESEARCH PAPER

Analytical method for the evaluation of the outdoor air contamination by emerging pollutants using tree leaves as bioindicators

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Abstract In this work, an analytical method, based on sonication-assisted extraction, clean-up by dispersive solid-phase extraction and determination by liquid chromatography-tandem mass spectrometry, has been developed and validated for the simultaneous determination of 15 emerging pollutants in leaves from four ornamental tree species. Target compounds include perfluorinated organic compounds, plasticizers, surfactants, brominated flame retardant, and preservatives. The method was optimized using Box-Behnken statistical experimental design with response surface methodology and validated in terms of recovery, accuracy, precision, and method detection and quantification limits. Quantification of target compounds was carried out using matrix-matched calibration curves. The highest recoveries were achieved for the perfluorinated organic compounds (mean values up to 87%) and preservatives (up to 88%). The lowest recoveries were achieved for plasticizers (51%) and brominated flame retardant (63%). Method detection and quantification limits were in the ranges 0.01–0.09 ng/g dry matter (dm) and 0.02–0.30 ng/g dm, respectively, for most of the target compounds. The method was successfully applied to the determination of the target compounds on leaves from four tree species used as urban ornamental trees (*Citrus aurantium*, *Celtis australis*, *Platanus hispanica*, and *Jacaranda mimosifolia*).

Keywords Tree leaves · Emerging pollutants · Atmospheric pollution · Bioindicator · Dispersive solid-phase extraction · Liquid chromatography-tandem mass spectrometry

Introduction

Thousands of organic pollutants are continuously introduced into the atmosphere from anthropogenic activities such as car exhausts, spraying of pesticides, and flaring activities and from industrial sectors such as cosmetics, chemical, textile, municipal solid wastes, and others [1]. Contaminants analyzed and controlled in outdoor urban environment are usually persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAH) [2, 3], polychlorinated biphenyls (PCB) [1, 4], or organochlorine pesticides [5, 6]. However, the increasing production and usage of new products, such as plastic and textile materials, fragrances, deodorants, and other personal care products, has resulted in a continuous emission of hundreds of new organic pollutants, the so-called emerging contaminants, to the atmosphere. Some of these compounds, such as parabens [7], bisphenol A (BPA) [8], perfluorinated compounds (PFC) [9, 10], hexabromocyclododecane (HBCDD) [1], phthalates [11], or nonylphenol ethoxylates (NPE) [8], have been identified and measured in outdoor urban air at concentrations up to thousands of picograms per cubic meter [12–14]. These compounds, some of them with endocrine disrupting properties, can be present in both gaseous phase and adsorbed onto particulate matter. Therefore, due to their toxicity [1, 8, 11, 14], they could cause environmental impact and potential effects on health, not only in areas close to their emission sources but also, depending on their

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volatility and resistance to natural breakdown, could cause environmental impact on far away areas, due to their long-range transport [15].

The evaluation of the presence and distribution of these organic pollutants in outdoor air requires analytical methodologies allowing their rapid and reliable determination. Currently, most of the monitoring air quality programs are carried out by means of active or passive samplers in which pollutant accumulation onto sorbent materials occurs with or without the use of pumps, respectively [16]. Although these commercial samplers provide useful information about the distribution of organic pollutants in the inhalable, thoracic, and breathable fractions [17], their use can involve excessive acquisition and operation costs. Besides, there are not available sorbents for the reliable motorization of the broad spectrum of organic pollutants emitted to the atmosphere, especially in the case of emerging pollutants. These difficulties can be overcome with the use of tree leaves as bioindicators of atmospheric pollution [18]. They constitute an excellent and cost-effective way to assess the exposure to these compounds [19]. Moreover, the use of evergreen species would allow evaluating pollutant accumulation through long-term exposure. The types of tree leaves most used as atmospheric pollution bioindicators of organic compounds are pine needles [15, 16, 20], gingko [21], eucalyptus, populus [22], quercus [23], or bitter orange [17]. However, until now, most of these species have been used to evaluate the contamination by priority pollutants such as PCB or PAH.

Most of the analytical methods reported in the literature for the determination of organic compounds in tree leaves from urban areas are based on solid-liquid extraction [10, 15, 16, 24–26] of the organic compounds and determination by gas [10, 15, 16] or liquid chromatography [27], both coupled with mass spectrometry detector. Soxhlet extraction [24], sonication-assisted extraction (SAE) [10, 15, 25], accelerated solvent extraction [16], and microwave-assisted extraction [26]are the most used extraction methods. However, most of these methods are focused on the determination of priority organic pollutants [15–17, 25] and, to the best of our knowledge, only a few analytical methods have been reported for the determination of emerging pollutants in tree leaves as passive samplers [10, 15].

The aim of this work was to optimize and validate an analytical method for the determination of emerging pollutants in urban tree leaves. Target compounds were selected considering their toxicity [1], transport [15], persistence [1, 10, 28–30], and current legislation [1, 15]. The selected compounds were NPE (including nonylphenol monoethoxylate (NP1EO) and diethoxylate (NP2EO) and nonylphenol (NP)), two plasticizers (di(2-ethylhexyl)phthalate (DEHP) and BPA), three parabens (methylparaben (MeP), ethylparaben (EtP), and propylparaben (PrP)), six PFCs (perfluorobutanoic acid (PFBuA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS)), and a brominated flame retardant (HBCDD). The method was based on the extraction of organic pollutants by SAE, clean-up by dispersive solid-phase extraction (d-SPE) and determination by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Experimental

Chemicals and reagents

HPLC-grade water, acetone, methanol, and hexane were supplied by Romil (Barcelona, Spain). Glacial acetic acid and extrabond primary secondary amine (PSA) sorbent were supplied by Scharlab (Barcelona, Spain). Ammonium acetate, Florisil sorbent, BPA (≥ 99%), MeP (≥ 99%), EtP (≥ 99%), PrP (≥ 99%), PFOS (≥ 98%), PFOA (96%), PFHpA (99%), PFHxA (≥ 97%), PFPeA (97%), PFBuA (98%), HBCDD (95%), and propyl 4-hydroxybenzoate-$^{13}C_6$ (PrP-$^{13}C_6$) (50 mg/L in acetone) were purchased from Sigma-Aldrich (Steinheim, Germany). Bisphenol A-d_{14} (BPA-d_{14}) (50 mg/L in acetone) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Perfluoro-n-[1,2,3,4-13C4]octanoic acid (MPFOA) (50 mg/L in acetone) was supplied by Cambridge Isotope Laboratories (MA, USA). BPA-d_{14}, PrP-$^{13}C_6$ and MPFOA were used as internal standards (I.S.). Bondecyl octadecyl C18 sorbent (40 μm particle size) was provided by Agilent Technologies (CA, USA).

Individual stock standard solutions of each compound (1000 mg/L) were prepared in methanol and stored at 4 °C. Working solutions, composed by a mixture of the target compounds at 10 mg/L, were prepared by dilution of the stock standard solutions in methanol. A internal standard (I.S.) mixture working solution, at 500 μg/L for PrP-$^{13}C_6$ and MPFOA and at 1000 μg/L for BPA-d_{14}, was prepared by dilution of commercial I.S. solutions in methanol. These working solutions were used to obtain spiked samples, matrix-matched calibration standards, and solvent calibration standards. Concentrations of I.S. in calibration standards were 50 μg/L in the case of PrP-$^{13}C_6$ and PFOA-$^{13}C_8$ and 100 μg/L for BPA-d_{14}.

Sample collection

Tree species studied were an evergreen species (*Citrus aurantium*) and three deciduous species (*Jacaranda mimosifolia*, *Celtis australis*, and *Platanus hispanica*). *C. aurantium* is an evergreen species with height up to 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. *J. mimosifolia* is a subtropical tree

that grows wherever there is no risk of frost. It can reach up to 20 m height and becomes leafless in early spring. Their new leaves begin to appear from late October to early November. *C. australis* tree, also called European nettle tree or Mediterranean hackberry, is a medium tree (10–25 m) that grows in warm regions of Mediterranean coast. It loses its leaves (5–15 cm) from December to January (winter) and the new leaves begin to grow from March to April (in early spring). *P. hispanica* is a large tree (20–30 m) that grows in mild weather. Its leaves (from April to autumn) are large and have a structure with five peaks.

Samples were collected on November 2016 from two sampling sites from Seville City (south of Spain), an urban area and an extra-urban park (non-contaminated reference point). Urban sampling point was located in a high-density traffic street. The extra-urban park was located, outside the city, at 1 km approximately from the high-density traffic area. In both cases, wind direction was southeast. Sampled trees were selected taking into account the absence of anomalies, gummosis or putrefaction of the neck of the root, and infections caused by viruses and parasites. In each sampling point, 25% of trees from each species were sampled (7 *C. aurantium*, 10 *J. mimosifolia*, 6 *C. australis*, and 10 *P. hispanica*). Ten leaves, two from each cardinal point and two from the center of the tree were collected from each tree. Leaves from the same tree species and sampling point were mixed in order to obtain two laboratory samples of each species, one representative of the urban areas and other representative of non-contaminated locations. Samples were transported to the laboratory on aluminum foil, cut and crushed using a crusher, lyophilized (0.01 mbar vacuum after being frozen at − 18 °C for 24 h), pulverized, and sieved (< 1 mm). A non-contaminated sample of *C. aurantium* leaves was used for method optimization and validation.

Sample treatment

Sample (0.5 g) was weighed into a 10-mL glass centrifuge tube, mixed with 0.2 g of magnesium sulfate and 0.05 g of sodium chloride and extracted twice with two aliquots of 6 mL of acetone/acetonitrile (70:30, *v*/*v*) mixture. In each extraction, tubes were vigorously vortex-mixed during 60 s, sonicated for 15 min at 30 °C, and centrifuged at 4000 rpm for 25 min. The extracts were combined and cleaned-up by d-SPE. For this purpose, the extracts were transferred to a 10-mL centrifuge tube and 0.28 g of Florisil sorbent were added. The mixture was hand-shaken for 2 min and centrifuged at 4000 rpm for 20 min. The extract was transferred into a 10-mL centrifuge tube, evaporated to dryness by a gentle nitrogen stream (XcelVap Evaporation/Concentration System from Horizon Technology Inc. (Salem, NH)), dissolved in 0.25 mL of methanol containing 50 μg/L of PrP-$^{13}C_6$ and MFOA and 100 μg/L of BPA-d_{14}, and filtered through a 0.22-μm filter.

LC-MS/MS

Analytical determination was performed on an Agilent 1200 series HPLC (Agilent, USA) coupled to a 6410 triple quadrupole (QqQ) mass spectrometer (MS) equipped with an electrospray ionization source (Agilent, USA) using a previously developed method [31]. Separation was carried out using a HALO C18 (50 × 4.6 mm i.d.; 2.7 μm) analytical column (Teknokroma, Spain) protected by a HALO C18 (5 × 4.6 mm i.d.; 2.7 μm) guard column (Teknokroma, Spain).

Ionization was carried out using the following settings: MS capillary voltage, 3000 V; drying gas flow rate, 9 L/min; drying gas temperature, 350 °C; and nebulizer pressure, 40 psi. Instrument control and data acquisition were carried out with the MassHunter software (Agilent, USA). Detection was performed in multiple reaction-monitoring mode (MRM). MS/MS parameters were optimized by injection, without column, of 10 mg/L individual standard solutions of target compounds and I.S. using different combinations of aqueous phase (water (0.1% formic acid), aqueous solution of ammonium acetate, aqueous solution of sodium formate (0.1% formic acid)) and organic phase (methanol or acetonitrile (0.1% formic acid)). Both positive and negative ionization modes were monitored. Mobile phase (methanol (solvent A) and 10 mM ammonium acetate aqueous solution (solvent B)) were selected according the abundance of the measured transitions. Considering these abundances, two transitions were selected for each compound. The most intense transition was used for quantification. The less intense transition and the relation between both transitions were used for confirmation. Optimized MS/MS parameters for MRM analysis are shown in the Electronic Supplementary Material (ESM) (Table S1).

Analytes were separated by gradient elution at a flow rate of 0.6 mL/min. Column temperature was kept at 35 °C. The elution program was as follows: 0–14 min, linear gradient from 28 to 70% of solvent A; 14–19 min, linear gradient from 70 to 80% of solvent A; 19–25 min, linear gradient from 80 to 100% of solvent A; 25–27 min, isocratic 100% solvent A and, finally, back to initial conditions (28% of solvent A) in 2 min.

Quantification was carried out using matrix-matched calibration curves. Calibration curves were constructed, in the concentration range expected for each compound in the studied samples, by linear regression of the peak area ratio of the analyte and its corresponding I.S. against their concentrations.

Method performance

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

Extraction process recovery (R) was evaluated by comparison of the peak areas of the target compounds in samples spiked, before and after extraction, at three concentration levels in triplicate. Low, medium, and high spiking concentrations were selected according to the concentrations usually measured in studied samples. Blank samples (non-spiked samples) were measured for blank correction of spiked sample signals.

Matrix effect was evaluated by comparison of solvent calibration and matrix-matched calibration slopes. Solvent calibration curves were obtained by the injection of nine standard solutions in triplicate at concentration levels in the range of concentrations expected after sample extraction. Matrix-matched calibration curves were obtained by injection of spiked extracts in triplicate. Extracts were spiked at the same concentration levels used for solvent calibration curves. Precision of the method was expressed as the RSD of the concentrations determined in spiked samples at three concentration levels in triplicate. Instrumental detection (IDL) and quantification (IQL) limits were calculated as the concentration of analyte corresponding to a signal-to-noise ratio of 3:1 and 10:1, respectively. IDL and IQL were determined by the injection in triplicate of spiked sample extracts (free of the target compounds) at low concentration levels. MDL and MQL were determined from IDL and IQL values applying the concentration factor of sample treatment and the extraction process recovery.

Results and discussion

Optimization of sample treatment

Method optimization was focused on the key variables of SAE (type and volume of extraction solvent and number of extractions) and d-SPE clean-up (type and amount of sorbent). SAE and d-SPE clean-up were optimized separately by a Box-Behnken response surface design using the Statgraphic Plus software version 5.1 (Statpoint Technologies Inc., Warrenton, VA, USA). All studies were carried out using non-contaminated samples spiked with target compounds at 125 ng/g dry matter (dm) in triplicate, except in the case of DEHP and NP. Due to the higher concentrations of DEHP and NP usually measured in leaves samples, they were spiked at a higher concentration (500 ng/g dm).

Optimization of SAE

For SAE optimization, three levels were selected for each variable: extraction solvent (methanol, acetonitrile, and acetone), solvent volume (2, 4, and 6 mL), and number of extractions (1, 2, and 3). Extraction time and sonication bath temperature were fixed at 10 min and 30 °C, respectively. A total of 15-run Box-Behnken experimental design was carried out (Table S2 in the ESM). The design included three replicates at the center point. A second-order response surface was obtained. Data were analyzed using ANOVA, which provided determination coefficients (R^2) greater than 0.90 in all cases. Pareto charts were also obtained (Fig. S1 in the ESM). Statistically significant effects of the variables were screened using a Student's *t* test (Fig. S2 in the ESM). The vertical reference blue line represents significant effects on the extraction efficiency (confidence level higher than 95%). According to Pareto plots, only BPA and NP extractions were not significantly influenced by the studied parameters. The most significant effects for PFC extraction are type of solvent, number of extractions, and their interactions; for plasticizer extraction, they are the type and volume of solvent and their interactions, and for surfactant, brominated flame retardant, and preservative extraction, the most significant effects are type and volume of solvent, number of extractions, and their interactions. Moreover, for all cases, except for NP, positive correlations were obtained between extraction of analytes and the studied parameters.

The combination of the optimized experimental values for each compound was obtained using the desirability function. Responses for each compound in the experiments of the Box-Behnken design were first normalized between 0 and 1, and the global desirability function was defined as the geometric mean for each response. Figure 1 shows the response surface plots corresponding to the desirability function when optimizing the factors affecting the extraction step: (A) type of solvent vs. solvent volume (number of extractions: 1), (B) solvent vs. number of extractions (solvent volume: 6 mL), and (C) solvent volume vs. number of extractions (acetone/acetonitrile mixture: 70:30, *v*/*v*). Regarding to the optimization of the extraction solvent (Fig. 1a), and considering 1 for methanol, 2 for acetone and 3 for acetonitrile, the optimal result (2.29, Table S3 in the ESM) corresponds to a mixture of acetone/acetonitrile (70:30, *v*/*v*) as extraction solvent. Considering the number of extractions and solvent volume (Fig. 1b, c), the optimal values were 1.93 and 6 mL, respectively. According to these results, the optimal extraction efficiencies were achieved applying two extractions with 6 mL of acetone/acetonitrile (70:30, *v*/*v*) mixture. Therefore, these values were selected as extraction conditions.

Optimization of d-SPE clean-up

The type and amount of sorbent applied was optimized. Three sorbents with different retention characteristics were tested: C18, usually applied to remove non-polar interfering substances [32]; PSA (primary secondary amine), applied for the removal of polar acids, polar pigments, and fatty acids [33]; and Florisil used to remove lipids from biota samples [34]. For each sorbent, three levels were selected: 0, 400, and

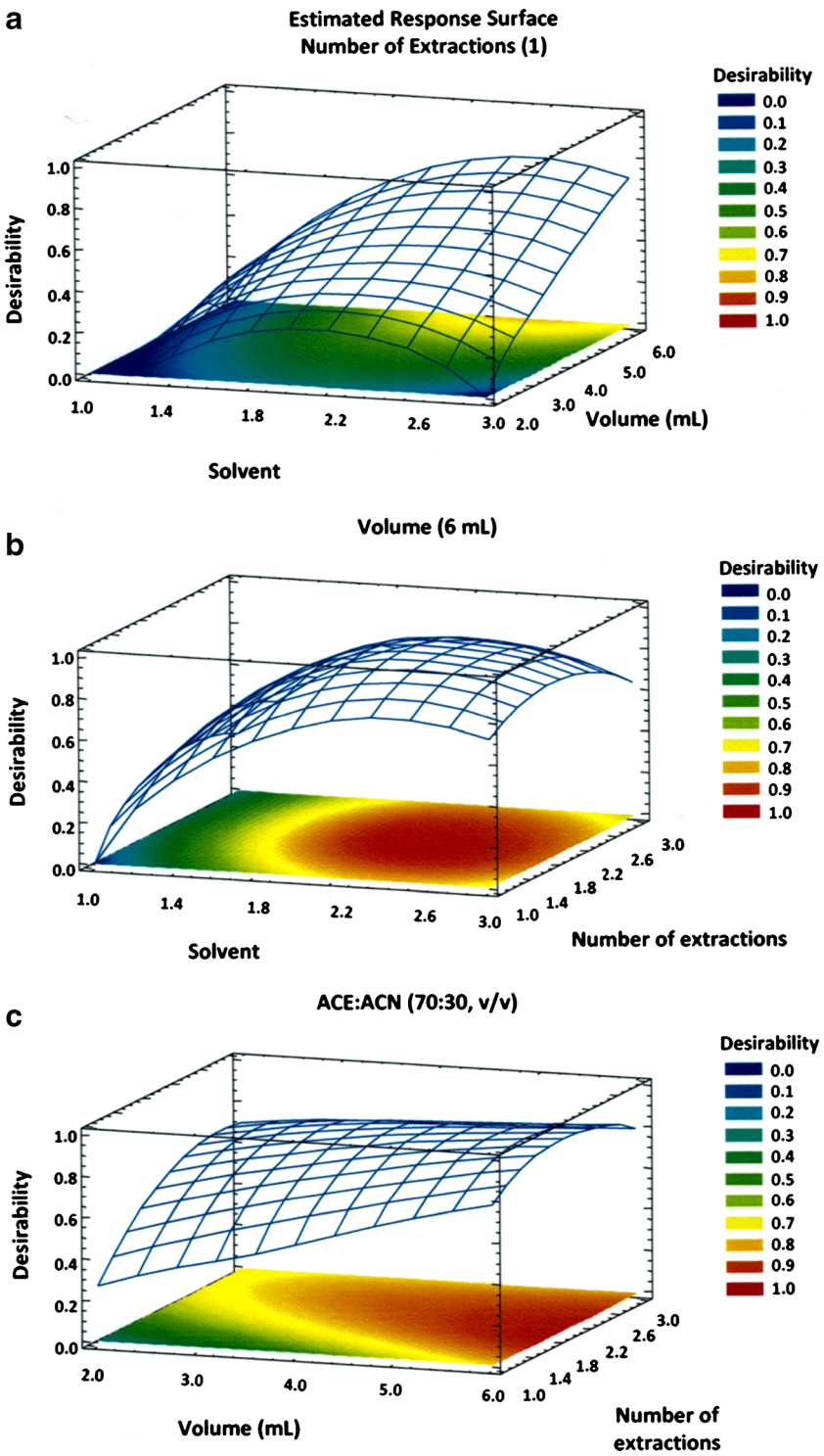

Fig. 1 Response surface plots, corresponding to the desirability function, when optimizing the following pair of factors affecting the extraction step: **a** solvent vs. solvent volume, **b** solvent vs. number of extractions, and **c** solvent volume vs. number of extractions. Samples were spiked at 250 ng/g. ACN, acetonitrile; ACE, acetone

800 mg (Table S4 in the ESM). Pareto charts were obtained (Fig. S2 in the ESM) and statistically significant effects were evaluated using Student's *t* test. PSA amount and some of their interactions were the most important variables for PFC and plasticizers. These interactions were negative for most of the studied compounds, except for the plasticizer DEHP, and especially in the case of PFC. In the case of the preservatives and surfactants, the most significant variable was C18 amount (ESM Fig. S2). C18 amount had a negative influence for most of the target compounds. This fact could indicate a negative effect in the recovery of these compounds using these sorbents, mainly due to their retention onto the sorbent.

Considering the response surface plots corresponding to the desirability function when optimizing d-SPE sorbent (Fig. 2), in

the case of the amount of PSA fixed to 0 g (Fig. 2a: C18 amount vs. Florisil amount), the optimal results were achieved using 0.28 g of Florisil (Table S5 in the ESM). When the amount of Florisil was fixed at 0.28 g (Fig. 2b: C18 amount vs. PSA amount), the optimal results were achieved for 0 g of C18 and 0 g of PSA. According to these results, 0.28 g of Florisil were selected for d-SPE clean-up. These data are consistent with those published by Sharif et al. [35]. They obtained, without an experimental design, recoveries in order of Florisil < C18 < SAX/PSA. The optimized extraction method allows the extraction of the target compounds using low volumes of extraction solvent and do not require the use of chlorinated solvents, which are commonly used for the extraction and determination of priority and emerging pollutants from tree leaves [15, 26, 27].

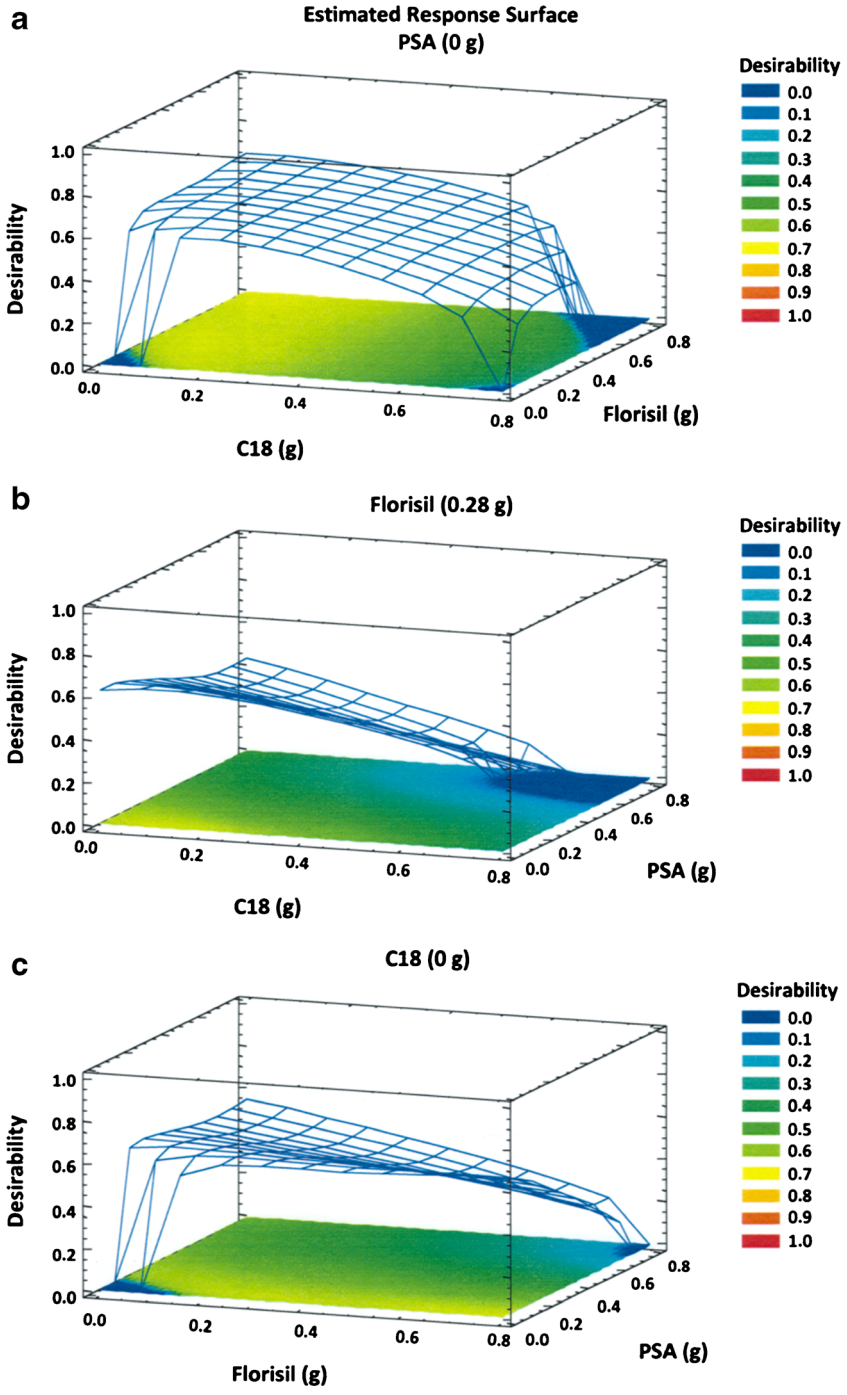

Fig. 2 Response surface plots corresponding to the desirability function, when optimizing the factors affecting the extraction step: **a** C18 amount vs. Florisil amount, **b** C18 amount vs. PSA amount, and **c** Florisil amount vs. PSA amount

Method validation

Method validation was carried out by the calculation of extraction process recovery, matrix effect, linearity, accuracy, precision, and method detection and quantification limits.

Recovery was evaluated at three concentrations levels: 25, 125, and 250 ng/g dm for all the studied compounds, except for NP and DEHP that were evaluated at 125, 500, and 750 ng/g dm. Recoveries achieved ranged from 53.6 to 106%, in the case of PFC; from 41.0 to 50.9%, in the case of plasticizers; from 39.5 to 94.6%, for NPE; from 24.9 to 62.7%, for HBCDD; and from 46.9 to 99.5% in the case of parabens. There was no clear relationship between the obtained recoveries and the spiked concentration levels. However, acceptable recoveries were obtained at all of the spiked levels. These results show the utility of the method over a wide concentration range. The highest recoveries were achieved for PFCs (mean values up to 87%), followed by surfactants (up to 87%), preservatives (up to 88%), and plasticizers and HBCDD (up to 47%) (Table 1). These recoveries were comparable with those reported by other authors for PFCs [10] and for brominated flame retardants [15]. Moreover, the obtained recoveries were similar to those reported for priority pollutants such as PAH [25, 26], PCBs, or organochloride pesticides [15] in other tree species used as bioindicators. Precision of the proposed method, expressed as relative standard deviation, was lower than 10% for all the studied compounds, except for DEHP (15%) and HBCDD (13%).

Matrix effect was evaluated by applying Student's *t* test to comparison of solvent calibration (methanol) and matrix-matched calibration curve slopes. Moreover, the variances of the calibration curves, estimated as $s_{y/x}^2$, were compared using the Fischer *F* test (Table S6 in the ESM). Statistical differences were found for calibration curve slopes of DEHP and NP. According to these results, matrix-matched calibration curves were used for all the studied compounds. Linearity, evaluated by the injection of nine matrix-matched calibration standards at concentrations between MQL to 750 ng/g dm, resulted in $r^2 > 0.996$.

Accuracy was evaluated from spiked samples at three concentration levels (Table 2). Blank samples (unspiked samples) were also analyzed and their signals were subtracted to spiked sample signals. To ensure the quality of obtained results, unspiked samples with high concentration of studied analytes (area found in unspiked samples higher than 10% of the area measured in spiked samples) were rejected. Target compounds were quantified using matrix-matched calibration curves. As shown in Table 2, accuracy was between 82 and 117%. MDL and MQL were in the ranges from 0.01 to 0.09 ng/g dm and

Table 1 Linearity, method detection (MDL) and quantitation (MQL) limits, recovery (R, %), and precision (expressed as relative standard deviation, RSD (%)) of the proposed method

Compound	Linearity		Low level		Medium level		High level		MDL(ng/g dm)	MQL(ng/g dm)
	LDR*(ng/g)	R^2	R	RSD	R	RSD	R	RSD		
Fluorinated organic compounds										
PFBuA	0.30–250	0.999	76.7	3.8	55.7	3.2	61.4	3.5	0.09	0.30
PFPeA	0.11–250	0.999	91.5	6.1	75.8	4.2	74.7	3.4	0.03	0.11
PFHxA	0.12–250	0.999	94.3	4.1	53.6	2.8	64.3	2.8	0.04	0.12
PFHpA	0.09–250	0.999	81.5	6.5	101	6.0	70.3	5.5	0.03	0.09
PFOA	0.17–250	0.999	109	1.6	70.7	0.9	80.3	1.2	0.05	0.17
PFOS	0.09–250	0.999	103	4.8	70.6	3.5	72.5	4.1	0.03	0.09
Plasticizers										
BPA	28.9–250	0.996	45.1	1.4	50.4	1.0	41.0	1.6	8.67	28.9
DEHP	8.00–750	0.999	44.3	15	46.1	13	50.9	12	2.43	8.00
Surfactants										
NP	27.4–750	0.998	39.5	10	64.5	8.2	64.8	8.6	8.21	27.4
NP1EO	0.12–250	0.998	82.9	1.3	78.1	1.1	75.2	0.7	0.04	0.12
NP2EO	0.02–250	0.999	74.9	2.0	90.1	1.2	94.6	0.7	0.01	0.02
Brominated flame retardant										
HBCDD	15.7–250	0.998	24.9	15	46.9	15	62.7	10	4.70	15.7
Preservatives										
MeP	0.22–250	0.998	52.4	5.3	67.6	4.1	53.3	4.4	0.07	0.22
EtP	0.25–250	0.997	46.9	5.7	67.0	4.9	54.3	3.6	0.08	0.25
PrP	0.19–250	0.998	79.2	5.3	99.5	4.8	84.1	5.2	0.06	0.19

Table 2 Spiked level (ng/g dm), found concentration (ng/g dm), accuracy (%), and precision, measured as relative standard deviation (RSD, %), of the proposed method

Compound	Low level				Medium level				High level			
	Spiked concentration	Found concentration	Accuracy	RSD	Spiked concentration	Found concentration	Accuracy	RSD	Spiked concentration	Found concentration	Accuracy	RSD
PFBuA	24.5	23.1	94	1.8	123	115	93	8.8	245	233	95	4.6
PFPeA	23.5	23.0	98	7.2	118	109	93	4.2	235	240	102	0.9
PFHxA	23.8	25.1	105	10	119	131	110	0.5	238	215	91	2.0
PFHpA	27.0	33.8	112	4.8	135	133	99	8.0	270	258	96	6.3
PFOA	32.5	28.4	87	11	163	151	93	6.3	325	288	89	3.7
PFOS	23.8	27.4	111	5.2	119	124	104	5.0	238	240	101	4.7
BPA	24.3	26.4	109	4.7	121	136	109	5.2	243	238	98	5.6
DEHP	93.5	109	117	5.1	469	552	117	7.6	938	771	82	3.1
NP	93.5	96.5	103	11	469	544	116	7.9	938	1074	114	3.7
NP1EO	32.5	35.9	110	8.6	163	169	104	6.7	325	330	101	8.5
NP2EO	32.5	36.5	112	14	163	189	116	9.0	325	342	105	3.9
HBCDD	25.0	21.9	88	8.3	125	104	83	5.8	250	212	85	5.9
MeP	25.5	32.6	115	6.3	128	132	103	4.3	255	241	95	3.9
EtP	25.5	28.9	113	14	128	135	106	5.9	255	289	113	1.6
PrP	24.5	27.7	112	11	123	138	112	0.7	245	262	106	4.8

Table 3 Concentrations of target compounds (ng/g dm) measured in *C. aurantium*, *C. australis*, *P. hispanica*, and *J. mimosifolia* from parks and high-density streets from Seville City

Compounds	*C. aurantium*		*C. australis*		*P. hispanica*		*J. mimosifolia*	
	Park	High-density street	Park	High-density street	Park	High-density street	Park	High-density street
Fluorinated organic compounds								
PFBuA	1.05	1.87	2.20	1.58	1.96	0.89	<MDL	<MDL
PFPeA	0.15	1.00	2.19	0.22	0.92	<MDL	<MDL	<MDL
PFHxA	<MQL	0.50	0.78	0.25	<MDL	<MDL	<MDL	<MDL
PFHpA	<MDL	0.17	0.19	0.15	<MDL	<MDL	<MDL	<MDL
PFOA	0.19	0.25	0.38	0.22	<MDL	<MDL	<MDL	<MDL
PFOS	<MQL	0.25	0.76	0.31	<MDL	<MDL	1.06	<MDL
Plasticizers								
BPA	<MQL	<MQL	<MQL	<MQL	34.2	<MDL	<MDL	<MDL
DEHP	224	132	556	362	371	<MDL	137	181
Surfactants								
NP	141	238	206	325	159	183	99.6	87.1
NP1EO	0.92	<MDL	2.94	3.15	1.02	1.85	1.83	0.95
NP2EO	<MDL	2.22	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Brominated flame retardant								
HBCDD	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Preservatives								
MeP	0.30	<MDL	15.1	21.9	19.6	39.3	<MDL	<MDL
EtP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PrP	1.15	<MDL	2.35	0.61	<MDL	<MDL	<MDL	<MDL

from 0.02 to 0.30 ng/g dm, respectively, for all target compounds, except for BPA, DEHP, NP, and HBCDD, whose MDL and MQL were of 8.67, 2.43, 8.22, and 4.70 ng/g dm and 28.9, 8.00, 28.3, and 15.9 ng/g dm, respectively (Table 1). These values allow the determination of target compounds at the concentrations expected in studied samples according to the concentrations reported in gaseous fraction [10, 15].

Application to real samples

The proposed method was applied to the determination of the target compounds in leaves of *C. aurantium*, *C. australis*, *P. hispanica*, and *J. mimosifolia* collected from parks and high-density traffic streets of Seville City (South of Spain). Results are shown in Table 3. Figure 3 shows a chromatogram obtained from extract of *C. aurantium*. The highest concentrations were found in *C. aurantium* and *C. australis*, in which all studied compounds, except NP2EO, HBCDD, and EtP, were detected. The lowest concentrations were found in *J. mimosifolia* in which only NP, NP1EO, and DEHP were detected both in high-density traffic streets and parks.

The differences between leaves from each tree species can be due to a higher atmospheric exposition of some leaves, such as leaves from *C. aurantium* (an evergreen species) and *C. australis* (leaves were collected at their last stage of life), compared to those from *J. mimosifolia* and *P. hispanica* that were collected in their medium stage of life.

The highest concentrations corresponded to DEHP and NPEs, followed by parabens and PFC. This distribution was similar to those found in outdoor urban air [1, 10, 14, 36], and the measured concentration were, in the case of PFC, slightly lower than those reported by other authors in leaves from pine needles [20]. The results obtained in this work show the applicability of the proposed method to determinate the concentrations of these emerging pollutants in leaves from different trees. This methodology could even provide a basis for future research about the use of tree leaves as bioindicator of the atmospheric contamination by emerging organic contaminants.

Conclusion

An analytical method has been proposed for the determination of 15 emerging organic pollutants, including PFC, plasticizers, preservatives, a brominated flame retardant, and surfactants, in leaves of urban ornamental trees. Sample treatment

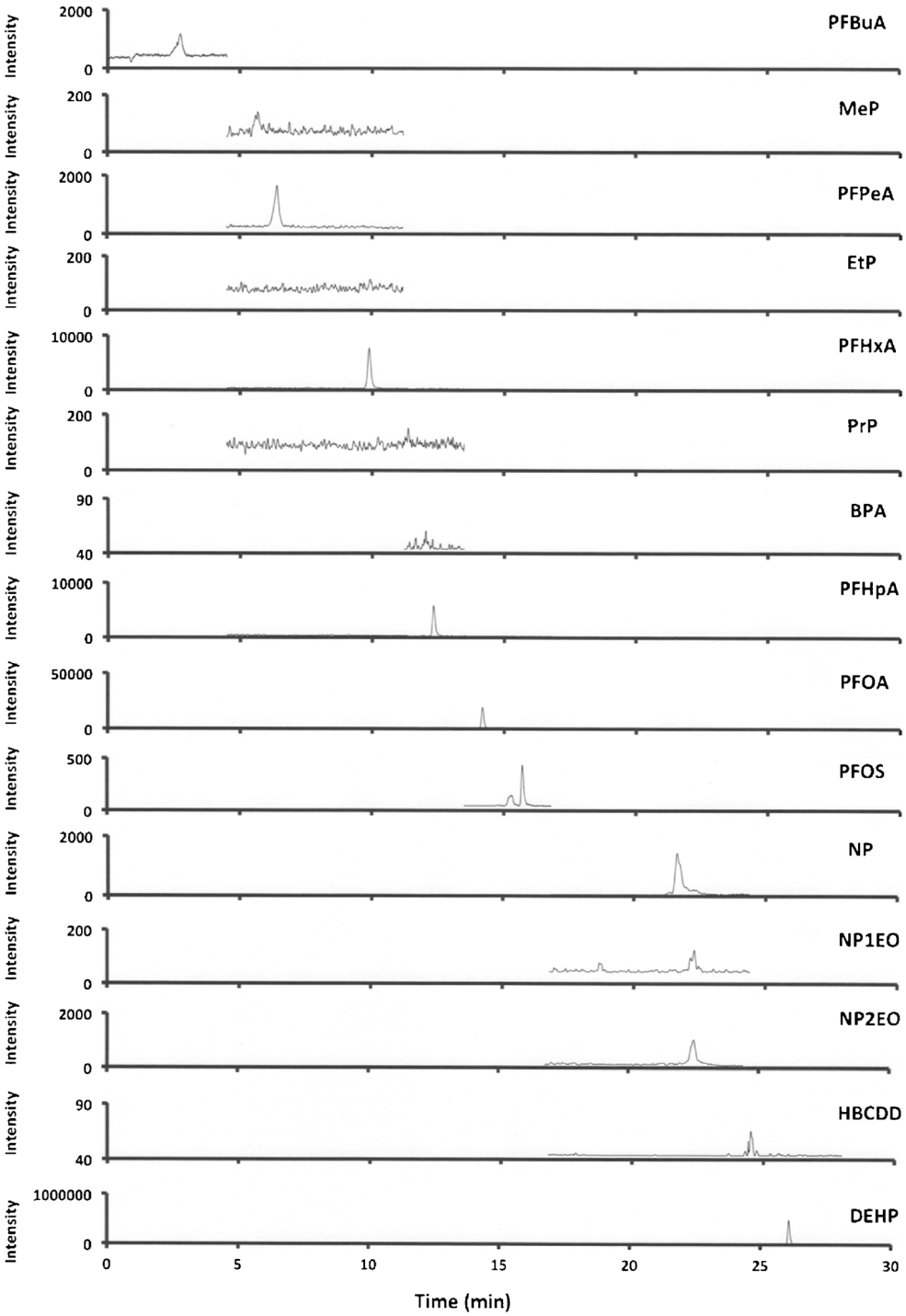

Fig. 3 Chromatogram of extract of *C. aurantium*

was based on SAE and clean-up by d-SPE. Determination was carried out by LC-MS/MS. Recoveries were higher than 60% for the most of the target compounds. Precision was lower than 10% and accuracy, evaluated in the whole linear range, varied from 82 to 117%. MDL and MQL were in the ranges 0.01–0.09 and 0.02–0.30 ng/g dm for most of the target compounds, except for BPA, NP, and HBCDD whose MDL and MQL were lower than 8.70 and 29.0 ng/g, respectively. The proposed method has been demonstrated to be a useful tool for biomonitoring of emerging organic pollutants in outdoor air using leaves of urban trees. The method constitutes a starting point to the use of tree leaves as bioindicators of urban air pollution by emerging contaminants and, despite the establishment of a link between the levels of emerging pollutants measured in tree leaves and their atmospheric concentrations is not straightforward, the proposed methods constitute a starting

point for the use of tree leaves for the evaluation of the concentration of emerging pollutants in atmosphere.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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