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Development of an analytical method for the simultaneous determination of 50 semi-volatile organic contaminants in wastewaters

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Optimization and validation of an analytical method for the analysis of semi-volatile organic compounds (SVOCs) in wastewater effluent samples by GC–MS

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ABSTRACT

This work describes the development of a robust analytical methodology for the simultaneous determination of 50 semi-volatile organic compounds (SVOCs) in wastewater effluent samples by solid-phase extraction (SPE) followed by gas chromatography coupled to mass spectrometry (GC–MS) analysis. In this work, we studied extensively whether the validated SPE method used for the analysis of polar compounds in wastewaters could be extended to the analysis of non-polar compounds in the same analytical run. To that aim, the effect of different organic solvents in the SPE process (i.e., sample conditioning prior to SPE, elution solvent and evaporation steps) was evaluated. In this sense, the addition of methanol to wastewater samples before the extraction, the use of hexane:toluene (4:1, v/v) mixture for the quantitative elution of target compounds, and the addition of isooctane during the evaporation were required to minimize analyte losses during SPE and enhance extraction yields. Overall, the developed methodology showed a good performance for the determination of 50 SVOCs, and was further applied to the analysis of real wastewater effluent samples.

- A validated SPE method for polar compounds was extended to the analysis of non-polar compounds.
- Elution with hex:tol (4:1, v/v) and the addition of isooctane during the evaporation yield good recoveries.
- The developed methodology was suitable for the determination of 50 SVOCs in aqueous samples.

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Name and reference of original method:	*Suspect screening workflow comparison for the analysis of organic xenobiotics in environmental water samples* [1]*; Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion* [2]
Resource availability:	*NA*

Method details

Background

The increased use of daily consumer products, as well as industrial production, has led to an increase in the emission of a variety of contaminants into the environment [1]. Thus, a large number of contaminants known as contaminants of emerging concern (CECs) are released into the aquatic environment causing different ecotoxicological effects including habitat loss, reduction of biodiversity or accumulation in the food chain of various predators [3]. Based on the results of many research works [4,5], those contaminants are not completely eliminated in wastewater treatment plants (WWTPs), and consequently, the disposal and treatment of such contaminants is a key concern in the field of water treatment and reuse amongst the group of contaminants so-called CECs a wide range of compounds with different physicochemical properties are included such as semi-volatile organic compounds (SVOCs) (e.g. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalate esters, organochlorine pesticides and other environmental endocrine disruptors) [6]. The vast majority of SVOCs are non-polar compounds, which exhibit toxicological characteristics and low mobility leading to the accumulation in biological tissues and different environmental compartments. Several decades ago, the danger posed by the progressive entry of hydrophobic organic substances into the biosphere was recognized and activities that promote the substantial reduction of their use were proposed from then on. However, non-polar pollutants continue to pose a serious threat to the environment [7], being some classes of SVOCs largely classified as priority substances.

Within this context, the aim of the present work was the optimization and validation of an analytical method for the simultaneous analysis of 50 SVOCs (i.e. PAHs, PCBs, phthalate esters, photoinitiators, UV filters, musk fragrances and pesticides) in aqueous samples via gas chromatography coupled mass spectrometry (GC–MS) based on research group's previous experience [1,2]. The factors affecting compounds' preconcentration using solid-phase based extraction (i.e., elution and evaporation steps) were evaluated in order to improve the extraction yield. The optimized protocol was employed for the analysis of SVOCs in WWTP effluent samples submitted to different treatments in the WWTP.

Chemicals and reagents

The list of 50 target compounds, purchased from Sigma Aldrich (St. Louis, MO, USA), analysed in this work is provided in Table S1 and included in the supplementary material. The table includes information about the family they belong (PCBs, musk fragrances, insecticides, PAH and phthalate esters, amongst others), names, abbreviation, molecular formula and molecular weight, log K_{ow}, supplier, solvent and surrogate used to correct the recovery for each analyte.

Seven working solutions of a concentration range of 50 – 310 mg/g were prepared by weighing (Sartorius-Stedim, Barcelona, Spain) in isopropanol (99.8%, Merck KGaA, Darmstadt, Germany) from the standard solutions included in Table S1. From those working solutions, a mixture solution containing all the analytes (concentration of 2 mg/kg) was prepared monthly for validation of the analytical procedure. All standard and working solutions were kept in the freezer at −20 ˚C until further use.

The preconcentration and extraction of the samples was performed using home-made triphasic solid phase extraction (SPE) cartridges. Empty polypropylene cartridges (12 mL, Supelco, Bellefonte, PA, USA) were filled with polypropylene (PP) frits (Supelco) and 250 mg of the following sorbents: cationic exchange (Sepra ZT-WCX, 30 µm, 85 Å, Phenomenex, California, USA), anionic exchange (Sepra ZT-WAX, 30 µm, 85 Å, Phenomenex, California, USA) and reverse phase (Chromabond© HRX, 85 µm, 55–65 Å, Macherey-Nagel, Düren, Germany) in a 1:1:3 ratio, respectively.

The solvents used during the SPE step were methanol (MeOH, HPLC grade, Macron Fine Chemicals, Avantor, Poland), ethyl acetate (EtOAc, HPLC grade, Macron Fine Chemicals, Avantor, Poland), ammonia (25% NH_3, Sigma Aldrich), formic acid (FA, > 98% purity, Panreac AppliChem, Barcelona, Spain), hexane (hex, HPLC grade, 95%, Macron Fine Chemicals, Avantor, Poland) and toluene (tol, HPLC grade, 99.9%, Lab-Scan Analytical Sciences, Gliwice, Poland).

All standard solutions and extracts were vigorously shaken with a Vortex agitator (Reax top, Heidolph, Schwabach, Germany) before using.

Analytical protocol

The extraction protocol was adapted from Gonzalez-Gaya et al. [1] for SVOCs determination. Aged secondary effluent samples collected in the Alcalá de Henares (East and West, Madrid) [4] and Markina (Biscay) WWTPs were used to validate the analytical method. Concretely, method optimization was carried out using a pool of the three aged WWTP effluents. 100-mL aliquots of pool samples (n = 6) were spiked before the extraction step with the 50 target compounds at two concentration levels (i.e., to get 75

and 200 ng/g in the final extract) to study the recoveries of the analytical method under the different conditions tested. In addition, non-spiked pool wastewater ($n = 3$) were treated in parallel and a mixture containing all the target analytes at a concentration level of 200 ng/g (given in mass concentration units as the standards were prepared weighing all the solutions) was added to the final extract in order to study the matrix effect at the detection step. Milli-Q water samples ($n = 3$) were treated as procedural blank samples. In all the assays, 5 mL of MeOH were added to the sample prior to its extraction in order to avoid the adhering of the target compounds to the flask walls.

For the extraction step, home-made SPE cartridges (250 mg, ZT-WCX:ZT-WAX:HR-X 1:1:3) were prepared by weighing on the analytical balance. First, the PP frit was introduced into each cartridge with the help of a teflon plunger (Supelco, Bellefonte, PA, USA), and then we positioned it vertically introducing it in a glass jar to sequentially add the different sorbents. Then, we introduced another PP frit on the top of the phases and applied pressure with the plunger to compact the sorbents. The use of one frit between each sorbent (4 PP frits vs 2 PP frits) was discarded according to the previously experience of the research group since longer times of sample loading and cartridge drying steps were required. Anyway, it is important to mention that the three layers corresponding to the three sorbents were still visible. SPE cartridges were conditioned using 5 mL of MeOH:EtOAc (1:1) and 5 mL of Milli-Q water before their use in a 20-position SPE Vacuum Manifold (Agilent Technologies, Avondale, PA, USA). Afterwards, samples were loaded into their corresponding cartridge through polytetrafluoroethylene (PTFE) reservoirs (Supelco) using a flow rate of approximately 2 mL/min, which was controlled by a vacuum pump. Once the samples were loaded, the cartridges were dried and the compounds eluted.

Optimization of different steps affecting the SPE process (i.e. elution and evaporation) was performed for the simultaneous analysis of the 50 target compounds. Regarding the extraction step, the addition of MeOH to the aqueous samples prior to their loading onto the SPE cartridges and the use of different organic solvents and/or mixtures for the elution of compounds from the SPE cartridges were studied. Regarding the evaporation step, the addition of isooctane (HPLC grade, Macron Fine Chemicals, Avantor, Poland) just before and during the complete evaporation of the extracts was also studied in order to avoid losses.

Extracts evaporation to dryness was performed using a Turbo Vap LV evaporator (Zymark, Hopkinton, USA) under a gentle stream of nitrogen (N_2, > 99.999% purity, Messer Iberica de Gases SA, Tarragona, Spain), reconstituted in 200 μL of hexane and filtered with syringe filters (polypropylene, PP, 0.22 μm, 13 mm, Jasco Analítica, Madrid, Spain) onto amber silanized chromatographic vials (Agilent Technologies). The extracts were stored at −20 ˚C until GC–MS analysis.

Instrumental analysis

The chromatographic separation of the compounds was performed following the methodology described by Ziarrusta et al. [2] with slight modifications. The analyses were carried out using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C quadrupole mass spectrometer and an Agilent 7693 autosampler (Agilent Technologies). 2 μL of the extract were injected in the splitless mode (1.5 min), and the injection port temperature was set at 300 ˚C. The separation was carried out in an Agilent HP-5 MS capillary column (30 m x 0.25 mm, 0.25 μm) using helium (99.9995%, Messer Ibérica de Gases SA, Tarragona, Spain) as a carrier gas at a constant flow of 1.3 mL/min. The oven temperature was programmed as follows: start at 60 ˚C for 1 min, increase at 3 ˚C /min to 170, increase again at 5 ˚C /min to 300 ˚C (held 20 min) and a final increase at 30 ˚C /min to 310 ˚C, which was held for 4 min.

The MS worked in the electron impact (EI) mode with electron energy of 70 eV. The temperature of the interface was kept at 310 ˚C, while the temperature of the ionization source and the quadrupole detector were maintained at 230 ˚C and 150 ˚C, respectively. The quadrupole MS was operated under both selected ion monitoring (SIM) and scan acquisition modes (see Table 1). In the former, the first ion was used as quantifier while the second ion was considered as qualifier. The mass spectra were acquired in the range of 30 to 600 *m/z*.

All chromatogram files obtained were converted to a format compatible with the software used for data acquisition and treatment using Mass Hunter GC/MS translator B.07.06 (GC MSD translator). The MassHunter Qualitative (Version 10.0) and MassHunter Quantitative (Version 10.0) softwares (Agilent Technologies) were used for data acquisition and automatic integration and quantification of the chromatographic peaks.

Analytical method validation

Figures of merit for the calibration curves and the analytical method were determined. The validation of the developed analytical procedure was performed in terms of linearity and determination coefficients of the external calibration curves, instrumental and procedural limits of detection (LODs) and quantification (LOQs), absolute (R_{abs},%) and apparent recoveries (R_{appar},%), precision in terms of repeatability (relative standard deviation, RSD%) and matrix effect (ME%) at the detection.

Calibration curves and detection (LOD) and quantification (LOQ) limits

External calibration curves were prepared in hexane and were analysed by GC–MS at 10 concentration levels in the calibration range of 1 and 1000 ng/g for all the target analytes (given in mass concentration units as the standards were prepared weighing all the solutions). Those solutions were injected in triplicate to calculate instrumental LOD (LOD_{inst}) and LOQs (LOQ_{inst}). LOD_{inst} were estimated as the lowest concentration detected in all injection replicates (RSD < 30%), while LOQ_{inst} were set as the lowest

Table 1
Target compounds quantifier and qualifier transitions for the mass spectrometer in the SIM mode, retention time (tR), calibration linear range (ng/L) and quality assurance parameters in terms of instrumental and procedural limits of detection and quantification (LODinst, LOQinst, LOQinst and LOQproc).

Compound	Abbreviation	Quantifier /Qualifier	tR (min)	Calibration linear range (ng/L)	LOD_{inst} (ng/g)	LOQ_{inst} (ng/g)	LOD_{proc} (ng/L)	LOQ_{proc} (ng/L)
2,6-dichlorobiphenyl	PCB 10	152/222	15.23	LOQ-750	5.00	11.0	21.0	53.0
2,4,4-trichlorobiphenyl	PCB 28	186/256	24.13	LOQ-750	11.00	22.0	38.0	88.0
2,2′,5,5′-tetrachlorobiphenyl	PCB 52	220/292	26.27	LOQ-750	11.00	22.0	13.0	38.0
2,2′,3,4,4′,5′-hexachlorobiphenyl	PCB 138	362/290	36.26	LOQ-750	22.00	53.0	37.0	55.0
2,2′,4,4′,5,5′-hexachlorobiphenyl	PCB 153	360/290	35.15	LOQ-750	53.00	53.0	46.0	52.0
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	PCB 180	394/396	38.47	LOQ-750	53.00	53.0	39.0	45.0
Musk Ambrette	MA	253	21.92	LOQ-750	21.00	21.0	82.0	90.0
Musk Ketone	MK	279/294	26.71	LOQ-750	19.00	19.0	9.0	25.0
Celestolide	ADBI	229/57	18.08	LOQ-500	6.00	13.0	7.0	20.0
Phantolide	AHDI	229/43	19.50	LOQ-500	11.00	21.0	30.0	83.0
Traseolide	ATII	215/43	22.60	LOQ-500	20.00	47.0	46.0	123.0
Cashmeran	DPMI	191/135	11.86	LOQ-500	24.00	24.0	64.0	68.0
Tonalide	AHTN	243/46	23.01	LOQ-500	21.00	50.0	4.0	10.0
2-ethylhexyl 4-(dimethylamino)benzoate	EHA	165/148	34.37	LOQ-250	12.00	23.0	64.0	68.0
Ethyl 4-dimethylaminobenzoate	EDAB	148/193	18.19	LOQ-500	21.00	51.0	52.0	58.0
Tetraconazole		336/101	27.71	LOQ-750	54.00	76.0	48.0	54.0
Tebufenpyrad		171/318	38.77	LOQ-500	24.00	58.0	145.0	213.0
Chlorfenvinphos	Chlorf	269/325	29.44	LOQ-750	66.00	89.0	95.0	108.0
alpha-hexachlorocyclohexane	α-HCH	181/183	17.09	LOQ-500	45.00	63.0	76.0	134.0
beta-hexachlorocyclohexane	β-HCH	181/183	18.75	LOQ-750	45.00	63.0	39.0	111.0
Lindane	γ-HCH	181/183	19.26	LOQ-750	45.00	63.0	56.0	157.0
delta-hexachlorocyclohexane	δ-HCH	181/183	21.17	LOQ-500	45.00	63.0	39.0	112.0
2,4-dichlorodiphenyldichloroethylene	2,4 DDD	235/237	32.81	LOQ-500	28.00	68.0	3.0	8.0
4,4′-dichlorodiphenyldichloroethane	4,4 DDD	235/237	34.59	LOQ-500	28.00	68.0	5.0	13.0
4,4′-dichlorodiphenyldichloroethylene	4,4 DDE	246/248	32.52	LOQ-500	28.00	68.0	3.0	7.0
4,4′-dichlorodiphenyltrichloroethane	4,4 DDT	235/237	36.15	LOQ-500	28.00	68.0	69.0	73.0
Bis(2-ethylhexyl) phthalate	DEHP	149/167	39.11	LOQ-500	8.00	16.0	118.0	152.0
Dicyclohexyl phthalate	DCHP	149/43	31.55	LOQ-500	18.00	43.0	44.0	46.0
Bis(2-methoxyethyl) phthalate	DEMP	58/59	26.41	LOQ-500	51.00	73.0	128.0	230.0
Dioctyl phthalate	DOP	149/279	42.32	LOQ-750	26.00	26.0	99.0	99.0
Dibutyl phthalate	DBP	149/150	35.80	LOQ-750	10.00	20.0	171.0	321.0
Diamyl phthalate		149/167	38.86	LOQ-500	19.00	46.0	170.0	247.0
Napthalene	Nap	128	6.52	LOQ-500	3.00	3.0	2.0	5.0
Acenaphthene	Ace	154/153	11.97	LOQ-750	7.00	14.0	1.0	2.0
Acenaphthylene	Acy	152/153	11.20	LOQ-750	14.00	33.0	4.0	12.0
Fluorene	Flu	166/165	14.61	LOQ-500	14.00	33.0	17.0	22.0
Anthracene	Ant	178/179	21.66	LOQ-500	14.00	33.0	1.0	3.0
Phenanthrene	Phe	188/186	21.25	LOQ-500	14.00	33.0	17.0	2.0
Fluoranthene	Flr	202/203	30.12	LOQ-500	33.00	47.0	1.0	3.0
Pyrene	Pyr	202/203	31.52	LOQ-500	33.00	47.0	33.0	35.0
Benz[*a*]anthracene	BaA	228/229	38.63	LOQ-500	14.00	33.0	72.0	74.0
Chrysene	Chr	228/229	38.74	LOQ-500	14.00	33.0	15.0	44.0
Benzo[*b*]fluorantene	BbF	252/253	44.09	LOQ-500	14.00	33.0	143.0	149.0
Benzo[*k*]fluorantene	BkF	252/253	44.12	LOQ-500	14.00	33.0	15.0	44.0
Benzo[*a*]pyrene	BaP	252/253	45.48	LOQ-500	33.00	47.0	153.0	340.0
Benzo[*ghi*]perylene	BghiP	276/277	52.46	LOQ-500	13.00	25.0	45.0	80.0
Indeno[1,2,3-*cd*]pyrene	IcdP	276/277	51.87	LOQ-500	8.00	16.0	110.0	326.0
Bis(2-ethylhexyl) adipate	DEHA	129/37	36.59	LOQ-500	8.00	16.0	1.0	2.0
2,2-dimethoxy-2–2-phenylacetophenone	BDK	151/105	23.79	LOQ-1000	3.00	3.0	7.0	12.0
4-methylbenzophenone	4-MBP	119/196	20.02	LOQ-750	3.00	7.0	49.0	84.0

concentration level that rendered a relative standard deviation (RSD) < 30% and trueness within 70 – 130% between the theoretical concentrations and the experimental concentrations estimated from the external calibration curve.

On the other hand, procedural blanks were analysed for the calculation of the procedural LOD (LOD_{proc}) and LOQ (LOQ_{proc}). LOD_{proc} were estimated as the average response for each analyte plus three times the standard deviation, whereas LOQ_{proc} were calculated considering the average response for each analyte plus ten times the standard deviation.

Recoveries and detection-matrix effect

Analytical method R_{abs},% for each compound were determined as the percentage ratio of the concentrations of the compounds in effluent samples determined using the external calibration curve and the theoretical ones. R_{appar},% were determined using two different approaches: (i) using isotopically labelled compounds used as surrogates to correct the absolute recovery of the target

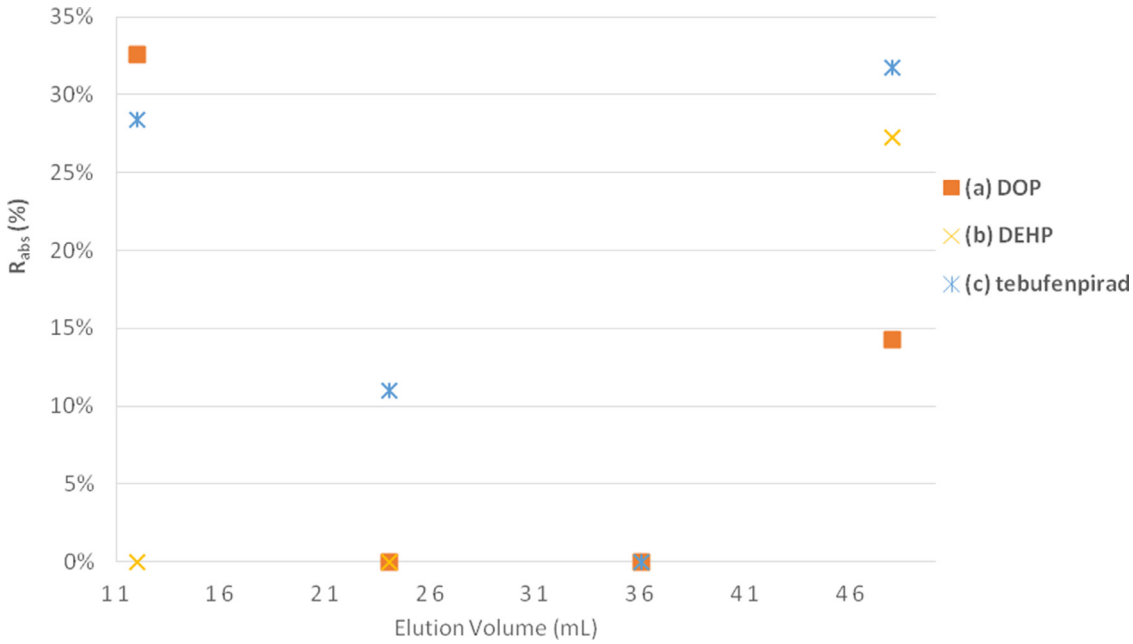

Fig. 1. Absolute recoveries (R_{abs},%) of the compounds DOP (a), DEHP (b) and tebufenpyrad (c) obtained during the optimization of the elution profile using 2% NH_3, MeOH:EtOAc (1:1, v/v) (12 mL) and 1.7% FA, MeOH:EtOAc (1:1, v/v) (12 mL), hexane (12 mL) and hex:tol (4:1, v/v) (12 mL) (elution solvent total volume of 48 mL).

compounds and (ii) matrix-matched calibration approach using a seven-point calibration curve in the range of 50 – 450 ng/g (in the final extract) prepared in a pool sample of the three mentioned WWTP effluents.

Furthermore, ME% affecting each analyte was experimentally determined following Eq. (1):

$$Matrix\ effect\ (ME\%) = \left(\frac{A}{B} - 1\right) \times\ 100 \quad (1)$$

where, B corresponds to the chromatographic peak area of the analyte in a reference standard solution and A is defined as the area of the analyte in a pool extract spiked just before the chromatographic analysis at the same compounds concentration level as the standard solution.

Results

Optimization of analytical extraction method

Extraction step

The optimization process was performed using Milli-Q water (100 mL, $n = 3$) spiked with the mixture containing all analytes at 200 ng/g concentration level in the final extracts, and Milli-Q water (100 mL, $n = 3$) blanks. All the samples were subjected to the same SPE procedure as the effluent samples (see Analytical protocol section). In this case, two different variables were evaluated to achieve quantitative extraction recoveries using a minimum elution solvent volume: (i) the addition of MeOH (5%) to samples before extraction, and (ii) the use of MeOH:EtOAc (1:1, v/v) (2% NH_3), MeOH:EtOAc (1:1, v/v) (1.7% FA), hex:tol (4:1, v/v) and hexane as elution solvents. In order to determine the elution solvent or solvents necessary for all the compounds elution, several aliquots (10 fractions) were collected in separated test tubes. Thus, 4 consecutive fractions (aliquots of 6 mL) of MeOH:EtOAc (1:1,v/v) (2% NH_3) and MeOH:EtOAc (1:1,v/v) (1.7% FA) solvents, and 6 consecutive fractions (aliquots of 4 mL) of the other two solvents (i.e., hex:tol (4:1, v/v) and hexane, three of each solvent) were injected.

The addition of MeOH before extraction led to an enhancement of the recoveries of the target compounds (~10 – 45% vs ~10 – 71% without and with MeOH addition, respectively), and thus, the addition of 5% of MeOH was considered for further experiments.

Elution solvents of MeOH:EtOAc (1:1, v/v) (1.7% FA) and pure hexane were definitely discarded as obtained negligible analyte recoveries (R_{abs} < 15%) far away from the recommended extraction recoveries (i.e.,70 – 130%) [8]. Elution with MeOH:EtOAc (1:1, v/v) (2% NH_3) and hex:tol (4:1) resulted in higher recoveries (i.e., 12 – 49% and 27 – 92%, respectively) for most of the analytes, even if some of them were below the acceptable range of 70 – 130%.

Target compounds followed overall three different trends regarding to absolute recoveries obtained using the different elution solvents. Fig. 1 illustrates those trends for one target compound per each case. DOP was recovered mainly using 2% NH_3, MeOH:EtOAc (1:1, v/v) and partially recovered using hex:tol mixture and the same pattern was observed for Chlorf, diamyl phthalate, DCHP, BDK,

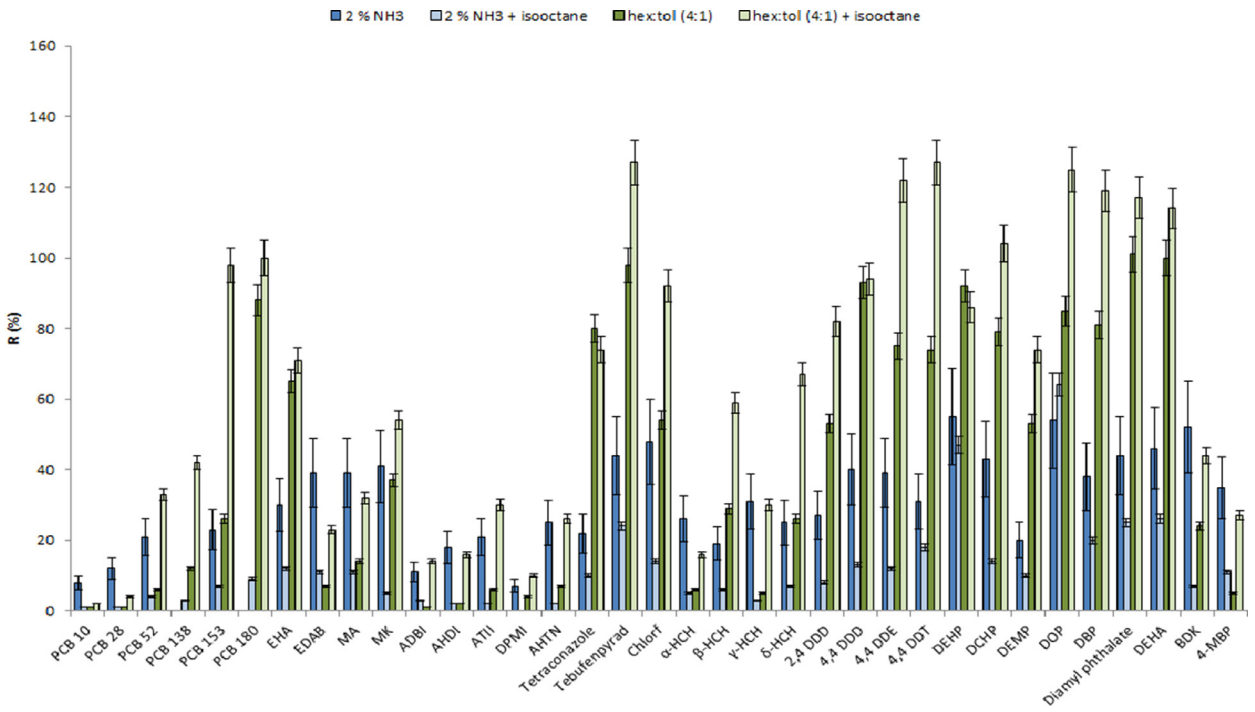

Fig. 2. Recoveries (R%) of the analytes obtained during the optimization of the evaporation step with different solvents. Error bars refer to relative standard deviations (RSD,%).

4-MeBP and 4,4-DDD. Hex:tol (4:1, v/v) was required to recover DEHP, and similar conclusions were get for PCBs, ADBI, AHDI, ATII, AHTN, PAHs, tetraconazole, HCHs, DEMP, DEHA and DPMI. Finally, although tebufenpyrad was recovered using all the tested elution solvents, the highest recoveries for that compound were obtained using hex:tol (4:1, v/v) (12 mL). Similar results were obtained for MA, MK, EHA, EDAB, DBP, 2,4-DDD, 4,4-DDE and 4,4-DDT.

In view of the above results, hex:tol (4:1, v/v) was selected as the optimal analytes elution solvent and evaluated once again in order to fix the minimum solvent necessary for the complete elution from the SPE cartridges of all the compounds. In this sense, new experiments were carried out and the analytes were eluted in fractions of 4 mL- hex:tol (4:1) (total elution volume 16 mL) was performed ($n = 3$) and individually injected. Recoveries between 27 and 92% were obtained after the elution with 12 mL of hex:tol mixture and any improvement in the analytes absolute recovery higher than 10–20% was observed in the last fraction of 4 mL. Thus, 12 mL of hex:tol (4:1) mixture was selected as optimal elution solvent volume.

Assessment of the evaporation step

Three replicates of 12 mL-aliquots of MeOH:EtOAc (1:1, v/v) (2% NH_3) and hex:tol (4:1, v/v) spiked with a mixture containing all the compounds at a concentration of 200 ng/g in the final extract were prepared and they were subjected only to the evaporation step. In order to reduce the possible loss of volatile analytes, the volume of a Pasteur pipette of isooctane (around 0.5 mL) was added three times to the test tubes (i) before the evaporation, (ii) during the evaporation and (iii) when around 0.5 mL of the extracts remained in the test tubes, and the extracts were evaporated to dryness. Dried extracts were reconstituted in 200 μL of hexane. The recoveries obtained for the analytes (except for PAHs) with and without the isooctane addition are shown in Fig. 2.

From the results summarized in Fig. 2, the addition of isooctane to the solvent mixture MeOH:EtOAc (1:1, v/v) (2% NH_3) led to a decrease in the recoveries compared to the ones obtained without isooctane, being still far from quantitative results. Conversely, in the case of hex:tol (4:1, v/v), the addition of isooctane seemed to be beneficial since the recoveries were in the range of 40% and 130% for most of the compounds. Furthermore, the longer evaporation time needed when using MeOH:EtOAc (1:1, v/v) (2% NH_3) than for hex:tol (4:1, v/v) extracts evaporation (2 h vs 40 min) led to choosing 12 mL of the hex:tol (4:1, v/v) for elution.

Quality assurance of the analytical method

The figures of merit of the method are summarized in Tables 1 and S2. The linearity of the calibration curves was confirmed over different concentration ranges for each analyte (see Table 1) with linear regression determination coefficient values (r^2) higher than 0.99 for all of the target analytes. LOD_{proc} and LOQ_{proc} values were within the range of 1 – 3405 ng/L as reported [9], while most of them showed values below 50 ng/L (see Table 1).

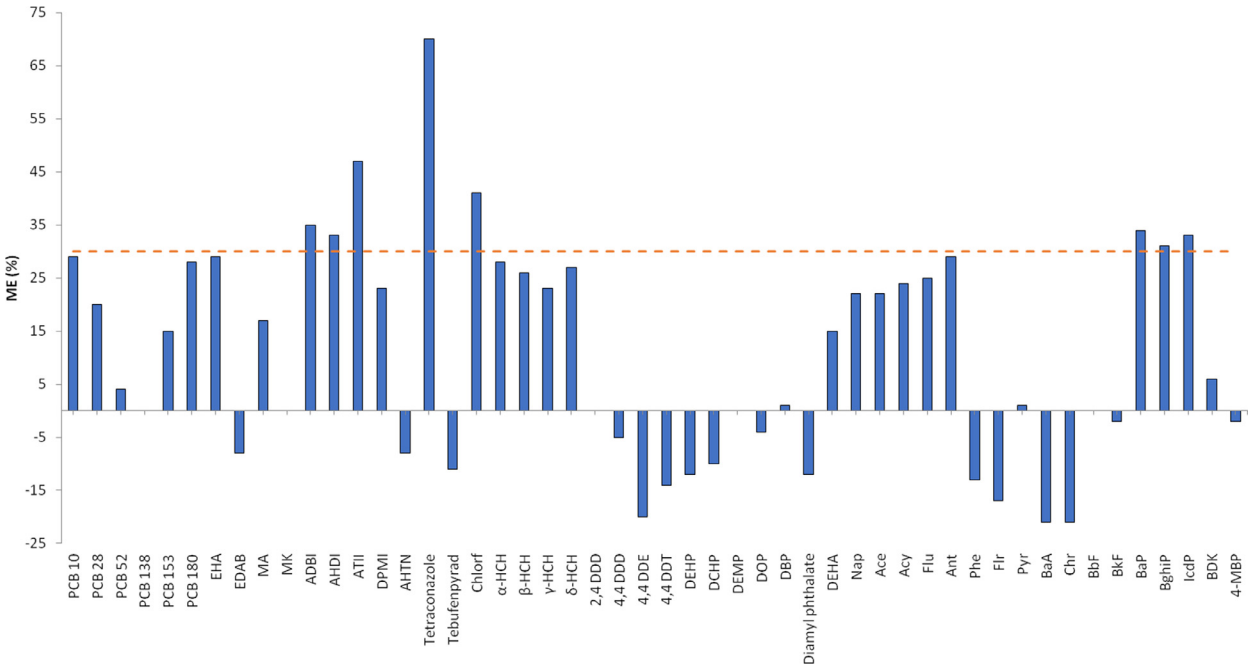

Fig. 3. Matrix effect (ME,%) of the studied analytes. Red dashed line indicates the ME threshold of 30%.

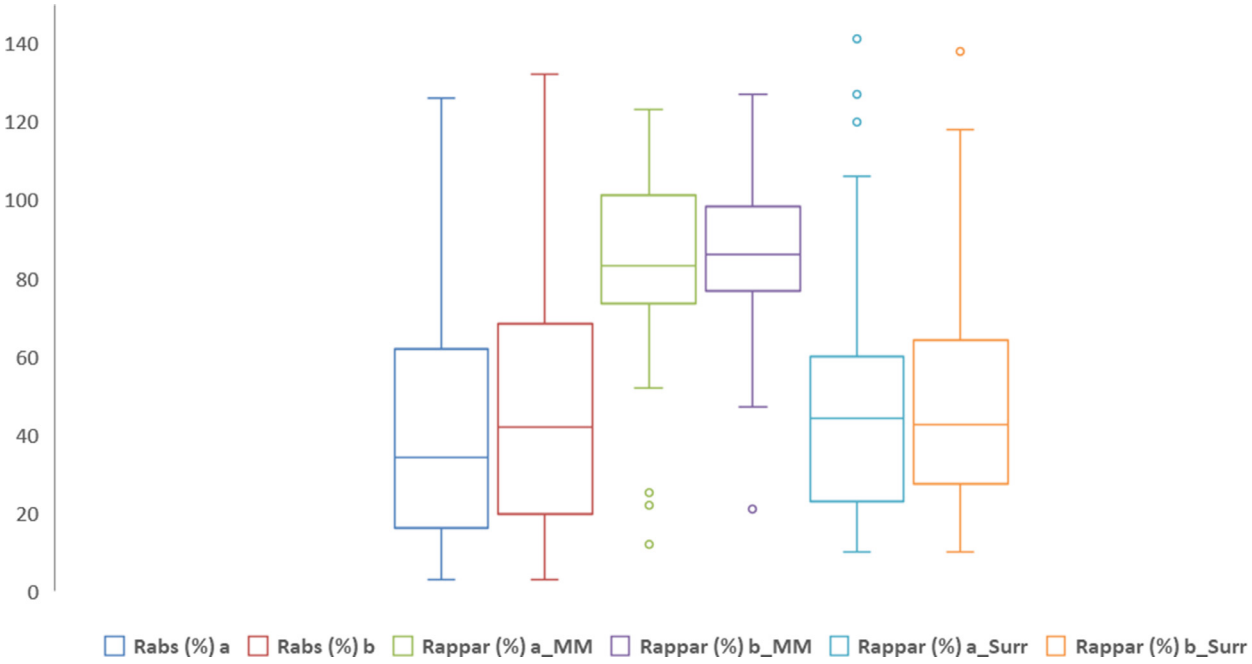

Fig. 4. Absolute (Rabs,%) and apparent recoveries (Rappar,%) using both matrix-matched calibration (MM) and surrogates corrections (Surr) obtained in WWTP effluent samples spiked at two concentration levels (a: 75 ng/g and b: 200 ng/g in the final extarct).

The ME at detection was evaluated for all of the target analytes as aforementioned. As can be seen in Fig. 3, the vast majority of the target compounds showed ME values below 30% (absolute values), assuming in that way that the matrix effect at detection is negligible. However, slight signal enhancement (ME > 30%) of some of them including ADBI, AHDI, ATII, tetraconazole, Chlorph, BaP, IcdP and BghiP was observed. RSD values below 25% were obtained for all of the analytes.

The absolute and apparent recoveries corrected by the corresponding surrogates and the matrix-matched calibration for each of the compounds under study are shown in Table S2. The chromatographic signals of those compounds present in blanks were subtracted

from the chromatographic signals of the same compounds in the spiked samples. Acceptable (see Fig. 4) absolute recoveries (64 – 132%) were only retrieved for 22% of the total compounds, whereas the remaining compounds showed R_{abs} < 70%.

The trueness of the results was assured by using two different strategies: the use of surrogates and the use of matrix-matched calibration (see Fig. 4). Comparison between the two strategies for absolute recoveries correction showed that the matrix-matched calibration was more appropriate, obtaining values in the acceptable range mentioned for most of target compounds, except for tetraconazole, Nap, Acy, BbF and BkF (R_{appar} 20 – 66%). On the contrary, the use of selected surrogates led to the correction of only 5 out of the 50 target compounds (i.e. ATII, DCHP, Ant, Pyr and DBP), suggesting the lack of suitable deuterated analogues for each compound (see Table S2). Adequate precision in terms of repeatability (RSD ≤ 30%) was obtained for all the analytes. Overall, the optimized methodology showed to be suitable for the simultaneous analysis of different SVOCs in aqueous samples including WWTP effluents.

Ethics statements

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Leire Mijangos: Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Dennis Bilbao:** Conceptualization, Methodology, Investigation, Validation. **Naroa Lopez-Herguedas:** Writing – original draft. **Natalia Ortueta:** Investigation, Formal analysis. **Maitane Olivares:** Writing – review & editing, Conceptualization, Methodology, Supervision. **Olatz Zuloaga:** Conceptualization, Methodology, Resources. **Nestor Etxebarria:** Writing – review & editing, Conceptualization, Methodology, Supervision, Validation. **Ailette Prieto:** Writing – review & editing, Conceptualization, Methodology, Supervision, Validation.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102252.

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