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Silicone rod extraction followed by liquid desorption–large volume injection-programmable temperature vaporiser–gas chromatography–mass spectrometry for trace analysis of priority organic pollutants in environmental water samples

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ABSTRACT

In this study a priority organic pollutants usually found in environmental water samples were considered to accomplish two extraction and analysis approaches. Among those compounds organochlorine compounds, pesticides, phthalates, phenols and residues of pharmaceutical and personal care products were included. The extraction and analysis steps were based on silicone rod extraction (SR) followed by liquid desorption in combination with large volume injection-programmable temperature vaporiser (LVI-PTV) and gas chromatography–mass spectrometry (GC–MS). Variables affecting the analytical response as a function of the programmable temperature vaporiser (PTV) parameters were firstly optimised following an experimental design approach. The SR extraction and desorption conditions were assessed afterwards, including matrix modification, time extraction, and stripping solvent composition. Subsequently, the possibility of performing membrane enclosed sorptive coating extraction (MESCO) as a modified extraction approach was also evaluated. The optimised method showed low method detection limits (3–35 ng L^{-1}), acceptable accuracy (78–114%) and precision values ($<13\%$) for most of the studied analytes regardless of the aqueous matrix. Finally, the developed approach was successfully applied to the determination of target analytes in aqueous environmental matrices including estuarine and wastewater samples.

1. Introduction

The huge amount of anthropogenic organic pollutants spilled continuously into water bodies is one of the main environmental concerns worldwide. The capacity of certain persistent and emerging organic pollutants to be bioaccumulated and to induce lethal toxicity on species results in biodiversity loss and threat to human health [1]. In an attempt to protect the quality of water bodies against the risk of the non-controlled waste of organic pollutants, specific legislations such as the European Water Framework Directive (WFD, 2000/60/EC) [2] are currently in force in the European Union. Though the primary focus is addressed towards the priority pollutants, organochlorine compounds, pesticides, phthalates, phenols, estrogens and residues of pharmaceutical and personal care products constitute a complex blend of chemicals often found in wastewaters. In fact, many of those compounds are included in the monitoring programs to assess the "good state" of water bodies. Additionally, the environmental requirements not only push down the detection limits to the low ng L^{-1} levels but also increase the data-quality criteria to meet compliance methods and results [3].

Current sample pre-treatment approaches for multiresidue analysis in water samples are focussed towards the minimisation of solvent use and automation of the analysis in order to improve the robustness of the entire analytical procedure [4]. Amongst alternative pre-concentration and isolation techniques, those based on solid sorbents have achieved wide acceptance over the classical solid phase extraction (SPE) or liquid–liquid extraction (LLE). The development of solid-phase microextraction (SPME) in the 1990s established the basis for the progress of many other microextraction techniques based on the same principle [5], i.e., equilibrium partitioning of the analytes between the sample and a small amount of an extracting phase [6]. Among these new techniques, stir bar sorptive extraction (SBSE), introduced by Baltussen et al. in 1999 [7], is particularly attractive for the microextraction of non-polar pollutants (log $K_{ow} > 3.0$) from aqueous matrices [8]. Though SBSE and SPME are robust and easy to

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manage, the higher amount of the acceptor phase (polydimethylsiloxane (PDMS)) in SBSE is the main difference, resulting in higher extraction efficiency, remarkable sensitivity at the sub-ppt level and high reproducibility [8].

Despite the benefits of these techniques, they are tightly linked with certain manufacturers so the need to develop new low-cost sorbent materials is highlighted. In this sense, the usefulness of technical silicone sorbents as silicone tubes (STs) or silicone rods (SRs) as low cost alternative devices to SBSE was firstly introduced by Popp et al. for the extraction of polycyclic aromatic hydrocarbons [9] and polychlorinated biphenyl compounds [10]. Thereafter SR extraction was applied mainly to the extraction of chlorophenols [11], chlorobenzenes [12] and some pharmaceuticals [13] in water samples (see Table 1). The extraction efficiency of these silicone based materials is similar to those obtained by SBSE and they meet analytical requirements in terms of purity, inertness and thermal stability [14]. The main advantage of the STs and SRs is their low cost (ca. 5 cent of euro per piece) and therefore, they can be discarded after a single use, eliminating carry over problems. Moreover, SRs and STs can easily be adjusted to specific needs of analytical requirements enabling their use in different applications, such as, as sorptive materials for passive sampling purposes [14]. In this sense, Vrana et al. [15] introduced the application of pieces of silicone-based sorptive materials as collecting phase of a passive sampler enclosed in a membrane bag. Different forms of the so-called Membrane Enclosed Silicone Collector (MESCO) were developed for the analysis of organic micropollutants in surface water and groundwater from then on [16]. The most recent version of MESCO sampler combines the advantage of a high capacity collector (i.e., appropriate sorptive material) with those of more stable polymeric membranes, such as low-density polyethylene (LDPE) [16]. However, additional investigations must be performed to study the effectiveness of different MESCO configurations in order to enhance the extraction efficiency before being used as passive samplers.

Similar to SBSE, SRs extraction has been used in most applications in combination with thermal desorption and gas chromatography (GC). However, thermo-desorption can lead to material degradation causing noisy chromatographic backgrounds [14]. An alternative is the use of a small volume of an organic solvent for desorption (liquid desorption (LD)) to minimise material decomposition and interferences with the target analytes [17]. One way to improve sensitivity of the solvent back desorption combined with GC is the use of large volume injection (LVI) in a programmable temperature vaporiser (PTV). In this sense, LVI-PTV–GC–MS was successfully applied to the analysis of organic pollutants in environmental water samples regardless of the pre-concentration technique [6,11,18,19].

The present work was focussed on the application of SRs for sorptive extraction of several persistent and emerging organic compounds (hexachlorocyclohexane compounds, organochlorine and organophosphorous pesticides, polycyclic compounds, octylphenols and phthalates) from aqueous samples in combination with solvent desorption followed by LVI-PTV–GC–MS analysis. The feasibility of MESCO approach was also studied, especially the evaluation of factors affecting the extraction efficiency. The applicability of the optimised multi-residue methodology was evaluated by analysing the target compounds in real environmental water matrices (i.e., wastewater and estuarine water samples) as well as a basis for a passive sampling method.

2. Experimental procedure

2.1. Sampling

In order to test the performance of the method in real environmental waters, surface water samples and an effluent water of an urban wastewater treatment plant (WWTP) were analysed.

Table 1
Overview of analytical methods based on the use of silicone rods and silicone tubes for the extraction of organic pollutants in environmental water samples.

Compounds	Sample	Volume (mL)	Extraction material	Extraction time (h)	Desorption conditions	Analysis	Apparent recovery (%)	LOD ($ng\ L^{-1}$)	Ref.
PAHs	River water, Groundwater	15	SE, SR (10 mm × 1 mm, 8 μL)	3	LD ($AcN:H_2O$, 100 μL), sonication 10 min).	LC–FLD	62–97	0.1–1.2	[8]
PCBs, Chlorobenzenes	Groundwater	100	SE, SR (80 mm × 2 mm, 250 μL)	4	TD	GC–MS	55–89	0.2–0.6	[9]
Pharmaceuticals (antipyrine, carbamazepine, diclofenac, ibuprofen, bezafibrate)	Water	480	SE, SR (20 mm × 2 mm, 62 μL)	34 (days)	LD (MeOH, sonication 10 min (x5))	LC–ESI–MS	n.a.	3000–16000	[12]
OPPs, triazines, PCBs	River water	10	SE, ST (10 mm × 4.5 mm (o.d) × 3.5 mm (i.d), 63 μL))	0.7	LD (cHex and EtAc, 200 μL, sonication 15 min)	LVI–GC–MS	55–89	0.5–5	[10]
Chlorobenzenes	Groundwater, river water	50	HSSE, ST (15 mm × 2 mm (o.d) × 1 mm (i.d), 35 μL))	1	TD	GC–MS	9–46 (extraction efficiency)	2–12	[11]
PBDEs	River water, Seawater, Treated wastewater	80	HSSE, SR (10 mm × 2 mm, 31 μL)	14	LD (diethyl ether, 1 mL, sonication 5 min (× 3))	GC–μECD	69–93	low $ng\ L^{-1}$	[36]
Halogenated Anisoles	Tap water, River Water	80	HSSE, SR (10 mm × 2 mm, 31 μL)	14	LD (n-pentane, 1 mL, sonication 5 min (× 3))	GC–μECD GC–MS–MS	n.a.	0.8–20 0.5–1.5	[37]

AcN: acetonitrile; *cHex*: cyclohexane; *EtAc:* Ethyl acetate; *GC–MS*: gas chromatography–mass spectrometry; *GC–MS–MS*: gas chromatography tandem mass spectrometry; *GC–μECD*: gas chromatography–micro electron capture detector; *HSSE:* headspace sorptive extraction; *LC–ESI–MS*: liquid chromatography–electrospray ionisation–mass spectrometry; *LC–FLD*: liquid chromatography–fluorescence detector; *LD*: liquid desorption; *LOD*: limit of detection; *LVI–GC–MS*: large volume injection–gas chromatography–mass spectrometry; *MeOH*: methanol; n.a: not available; OPPs; organophosphorous pesticides; *PAHs*: polycyclic aromatic hydrocarbons; *PBDEs*: polybrominated diphenyl ethers; *PCBs*: polychlorobenzenes; *SE*: sorptive extraction; *SR*: silicone rod; *ST*: silicone tube; *TD:* thermal desorption.

Table 2
Studied analytes including CAS number, purity, molecular weight, *m/z* values of quantifier and qualifier fragment ions, chromatographic retention times and supplier.

Family	Compound	Purity (%)	MW	*m/z* quantifier and (qualifier)	tR (min)	Supplier
Phenols	4-tert-octylphenol (4-tOP)[a]	99.9	206.32	135 (107)	10.88	Alfa-Aesar GmbH&Co. (Karlsruhe, Germany)
	4-octylphenol (4-OP)[a]	99	206.32	107 (135)	13.67	
Hexachlorocyclohexanes	α-hexachlorocyclohexane (α-HCH)[a]	98	290.8	181 (183)	12.51	Dr.Ehrenstorfer GmbH (Augsburg, Germany)
	β-hexachlorocyclohexane (β-HCH)[a]	100	290.8	181 (183)	13.48	
	γ-hexachlorocyclohexane (γ-HCH)[a]	98.6	290.8	181 (183)	13.65	
	δ-hexachlorocyclohexane (δ-HCH)[a]	99	290.8	181 (183)	13.67	
Polycyclic musks	Galaxolide (HHCB)[a]	53.5	258.4	243 (258)	15.56	LGC Standards GmbH (Wesel, Germany)
	Tonalide (AHTN)[a]	97.9	258.4	243 (258)	15.84	
Organophosphorous pesticides	Chlorpyrifos (Clor)[b]	99.9	350.6	197 (199)	18.71	Fluka, Pestanal®
	Chlorfenvinphos (Clorf)[b]	97.3	359.57	267 (269)	20.82	
Organochlorine pesticides	2,4-DDE[b]	99	318	246 (243)	21.64	Dr.Ehrenstorfer GmbH (Augsburg, Germany
	4,4-DDE[b]	98.5	318	246 (243)	23.32	
	2,4-DDD + 4,4-DDD[b]	99.5	320.1	235 (237)	23.71	
	2,4-DDT + 4,4-DDT[b]	98.5	354.5	235 (237)	25.63	
Phthalates	Benzyl-butyl-phthalate (BBP)[c]	96	312.36	149 (206)	27.64	Alfa-Aesar GmbH&Co. (Karlsruhe, Germany)
	Bis(2-ethylhexyl)-phthalate (DEHP)[c]	99.99	390.56	149(167)	31.7	
	Di-n-octyl-phthalate (DOP)[c]	99	390.56	149(279)	33.8	

[a] Compound corrected with [$^2H_{15}$] Musk Xylene supplied by Dr.Ehrenstorfer GmbH (Augsburg, Germany).
[b] Compound corrected with [2H8]-4,4′-DDT, supplied by Sigma-Aldrich (Seelze, Germany).
[c] Compound corrected with [2H3] DEHP, supplied by Sigma-Aldrich (Seelze, Germany).

River sample water from estuary of Bilbao (Basque Country, northern Spain) was collected in June 2012 and the effluent water sample was collected in July 2012 at the metropolitan WWTP of Bilbao, which is the largest WWTP in the Basque Country collecting industrial and urban wastewater from ca. 1 million inhabitants. Samples were collected in pre-cleaned amber glass bottles and carried to the laboratory in cooled boxes (4 °C). After collection, samples were filtered using 0.45 μm cellulose filters (Whatman™, Buckinghamshire, UK), stored at 4 °C before treatment and analysed within 48 h.

2.2. Reagents and materials

All the laboratory material was carefully cleaned with abundant pure water (<0.2 S cm^{-1}, Millipore, USA) and without using detergent to avoid possible interferences from those products. The material was sonicated under clean acetone (Q.P., Panreac Química, Spain) for an hour and then rinsed with ultrapure water (<0.057 S cm^{-1}, Milli-Q model, Millipore, USA). After all, the glass material was dried in an oven at 400 °C for approximately 4 h.

Regarding the chemical standards used in this work were: four hexachlorocyclohexane compounds (α-HCH, β-HCH, γ-HCH and δ-HCH), four organochlorine pesticides (2,4′-DDE, 4,4′-DDE, 2,4′-DDD and 4,4′-DDT), two organophosphorous pesticides (chlorpyrifos (Clor) and chlorfenvinphos (Clorf), two polycyclic musks (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN, tonalide®) and 1,3,4,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta-(γ)-2-benzopyran (HHCB, galaxolide®)), two octylphenols (4-n-Octylphenol (4-nOP) and 4-t-Octylphenol (4-tOP)) and three phthalate compounds(di-n-octyl-phthalate (DOP), benzyl-butyl-phthalate (BBP) and bis(2-ethylhexyl)-phthalate (DEHP)). The deuterated compounds used as surrogates were: $^2H_8$-4,4′-DDT ([$^2H_8$]-Pestanal®) and $^2H_3$-bis(2-ethylhexyl) phthalate ([$^2H_3$]-DEHP), and $^2H_{15}$ musk xylene ([$^2H_{15}$]-MX). The chemical properties of these compounds are summarised in Table 2. Individual stock solutions from each standard were dissolved to prepare ≈1000 μg g^{-1} stock solutions in 2-propanol (HPLC-grade, 99.8%, LabScan, Dublin, Ireland), which were stored in amber vials at −20 °C. Mixed fresh solutions with ≈50 μg mL^{-1} of each target compound were prepared monthly and lower concentration solutions were daily prepared according to the experimentation.

The solvents, n-hexane (Hex), dichloromethane (DCM), ethyl acetate (EtAc), cyclohexane (cHex) (HPLC-grade) and methanol (MeOH) (Anhydrous, HPLC-grade) were supplied by LabScan. Humic acids (technical grade) used to study the matrix effect were obtained from Fluka (CAS number. 1415-93-6, Sigma-Aldrich, Germany).

The commercial silicone elastomer (in flexible rod form of 2.0 mm i.d., 1.23 g mL^{-1}) used for sorptive extraction was purchased from Goodfellow (Huntingdon, England). SRs with a length of 1 cm were cut in the laboratory with a sterile sharp blade. After that, the SRs were weighed (≈38 mg or nominal volumen close to 30 μL) and those with mass differences higher than 3% were discarded. Before the SRs were used they were sonicated with 1 mL of a mixture of DCM:MeOH (1:1) for 5 min and afterwards, they were cleaned three times using fresh solvent mixture. Finally, the SRs were conditioned at 250 °C for 180 min under a nitrogen stream (ca. 30 mL min^{-1}) and they were kept in closed vials [20].

Low density polyethylene (LDPE) was obtained in a local supermarket, where it was sold as freezing bag for food (with a membrane thickness of 0.02 mm) in order to make our own individual membranes used as enclosed coating of the SR for the MESCO experiments. The homemade membranes (3 cm length × 1 cm width) were tailor-made using a shrink-wrapping device. After sealing the edges, the overlaying foil ends were carefully cut to minimise the exceeding polymeric material in which the analytes could be absorb.

2.3. Silicone rod sorptive extraction

The scheme of the SR extraction procedure is illustrated in Fig. 1. The pre-concentration of the analytes from water samples was performed using the SRs in 150 mL conventional headspace glass vials. Assays for the optimisation of the extraction procedure were performed using 150 mL of ultra-pure water spiked at 100 ng L^{-1} of each target compound. Deuterated analogues used

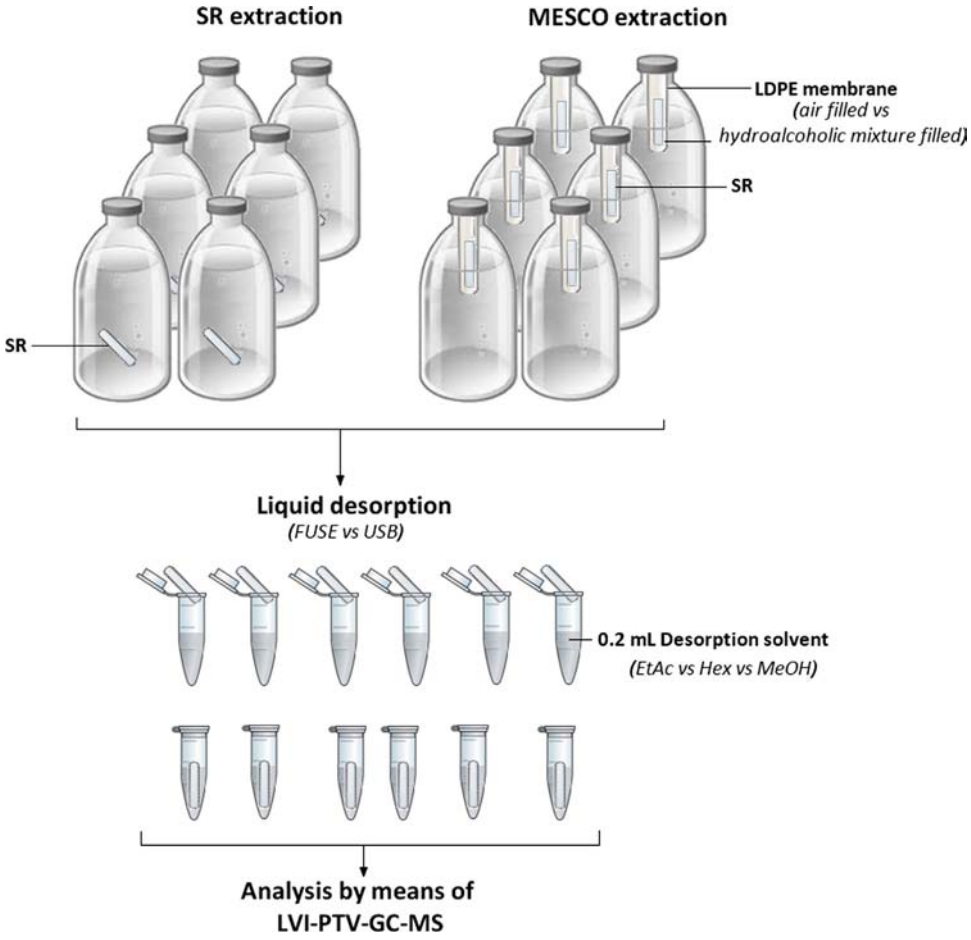

Fig. 1. SR and MESCO–SR extraction scheme.

as surrogates were added prior to the extraction and then, SRs were immersed in water sample sealing the vials with PTFE septa and aluminium crimp caps. The sorption of the target analytes was performed overnight at room temperature and at 800 rpm using a magnetic 15 position stirring plate from Gerstel (Gerstel GmbH & Co. KG, Germany).

Once the sorption step was over, the SR was removed and rinsed with ultra-pure water and dried with a clean tissue. The factors affecting liquid desorption (LD) of the sorptive material were also tested, i.e., the effect of stripping solvent nature and the effect of sonication mechanism in the desorption step. To accomplish this, the SRs were placed into a 500 μL safe-lock eppendorf® tube (Eppendorf Ibérica S.L.U., Madrid) filled with 200 μL of an appropriate solvent and assuring the complete immersion of the SR. The effectiveness of ultrasound bath (USB, Axtor by Lovango) and focussed ultrasound cup boosters (FUSB, Cupbooster BR 3.0, Bandeling, Berlin, Germany) was evaluated. Under the optimal conditions, the SRs were desorbed with 200 μL of EtAc for 16 min using ultrasounds bath (2 × 8 min). Finally, the SR was removed and the obtained extract was analysed by means of LVI-PTV–GC–MS.

2.4. Membrane enclosed sorptive coating extraction

Silicone rods were enclosed in a LDPE membrane, a hydrophobic membrane, resistant to solvents and biodegradation processes [21]. The extraction procedure was performed in 150 mL glass vials using SRs coated by home-made air-filled LDPE membrane bags or filled up with 200 μL of different hydro-alcoholic mixtures. Inside the LDPE bag a clean SR was introduced and the edges were thermally sealed. LDPE membranes with the SRs inside were attached to a metal funnel, fixed with Teflon rings (Gerstel) and placed in the bottleneck assuring that the membrane was thoroughly in contact with the water sample (see Fig. 1). Before the extraction, deuterated analogues were added as surrogates and then, the vials were closed with PTFE septa and aluminium crimp caps. Extraction vials were stirred using a magnetic 15 position stirring plate from Gerstel at 800 rpm overnight. Once the desorption step was over, the SRs were removed from the LDPE membrane bags, rinsed with ultra-pure water, dried with a clean tissue and desorbed in the same conditions as used in the extraction without membrane bags.

2.5. LVI-PTV–GC–MS analysis

The analysis was performed in a 6890N gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with a LVI system, a cooled injection system (CIS 4, Gerstel), and an Agilent 5975N mass selective detector using electron impact ionisation. 40 μL of sample extract was injected using a 100 μL syringe in a CIS, which consisted of a septum-less head and an empty baffled deactivated gas liner cooled with liquid nitrogen. LVI-PTV injection parameters were optimised by means of an experimental design, and under optimum conditions, the inlet temperature was held at 50 °C while the column head pressure was fixed to 2.9 psi and the flow rate through the split vent was set at 75 mL min^{-1} to eliminate most of the solvent. At a vent time of 3 min the analytes were focussed to the column in splitless mode for 1.5 min while the temperature of the PTV injection port was increased at 12 °C s^{-1} to 300 °C and held for 5 min.

Analytes were separated on a HP-5 ms capillary column (30 m × 0.25 mm, 0.25 μm) from Agilent Technologies. The oven temperature was programmed as follows: start at 60 °C for 1 min, increase at 30 °C min^{-1} to 120 °C, a second increase at 5 °C min^{-1} to 240 °C, and a final increase at 30 °C min^{-1} to 300 °C which has held for 5 min. Hydrogen (99.9995%, AD-1020 Hydrogen Generator, Cinel Strumenti Scientifici, Padova, Italy) was used as carrier gas at a constant flow of 1.3 mL min^{-1}. The transfer line temperature was maintained at 310 °C and the ion source and the quadrupole at 230 °C and 150 °C respectively. Detection was carried out both in the scan (50–525 m/z) and in the selected ion monitoring (SIM) modes simultaneously. Table 2 shows the *m/z* values of the fragment ions monitored in the SIM mode where the first ion was used as quantifier while the second ion was considered as qualifier.

3. Results and discussion

3.1. Optimisation of LVI-PTV–GC–MS

Although the efficiency of LVI may be affected by several variables, some of them were fixed (vent flow: 75 mL min^{-1}, purge flow: 75 mL min^{-1}, splitless time: 1.5 min and injection

Table 3
Central composite design matrix and the chromatographic responses obtained for the studied compounds. A: T_{cis} (°C), B: t_{vent} (min), C: injection speed (μL s^{-1}). The replicates of the central point are marked with an (*).

Exp	Optimised variables			Responses × 10^4 (as chromatographyc peak area)															
	A	B	C	4-tOP	β-HCH	γ-HCH	4-nOP	δ-HCH	HHCB	AHTN	Clor	Clorf	2,4-DDE	4,4-DDE	2,4-DDD+ 4,4-DDD	2,4-DDT+ 4,4-DDT	BBP	DEHP	DOP
1	25	4.5	5	0.69	0.38	0.46	0.54	0.36	0.48	0.25	0.17	n.d	0.65	0.45	0.37	0.28	0.46	0.75	1.27
2*	45	3	3.5	104.52	45.93	45.24	145.93	41.95	119.17	69.43	30.05	3.23	101.66	74.22	82.07	59.49	91.81	166.04	231.95
3	11	3	3.5	52.98	27.76	35.76	58.72	22.95	58.74	31.93	14.07	1.49	53.81	39.89	40.75	27.66	41.54	72.95	103.95
4*	45	3	3.5	121.06	34.66	28.43	131.36	34.84	98.24	66.09	29.61	4.96	85.45	62.86	74.09	50.05	116.93	200.79	325.66
5	45	3	6	96.19	34.85	36.43	106.02	32.39	96.45	56.79	22.65	2.41	75.27	54.55	58.32	43.17	74.36	128.17	168.80
6	65	1.5	5	3.43	1.59	1.37	3.51	2.02	3.09	1.64	0.89	0.31	3.69	2.40	1.92	1.69	2.67	3.76	6.16
7	65	4.5	5	0.48	0.39	0.93	0.83	0.55	1.15	0.51	0.25	0.09	0.94	0.60	0.49	0.39	0.72	1.15	1.87
8	79	3	3.5	94.28	11.43	5.09	187.39	19.29	36.82	73.24	33.79	4.27	77.27	76.06	104.25	66.31	166.86	280.28	405.83
9	45	5.5	3.5	0.81	0.58	0.81	0.57	0.56	0.91	0.43	0.28	0.11	1.02	0.65	0.51	0.46	0.74	1.10	1.88
10*	45	3	3.5	112.05	43.85	43.67	123.69	41.71	128.22	76.24	29.91	3.53	96.98	70.48	76.24	60.05	107.39	172.51	230.30
11	25	4.5	2	0.61	0.47	0.63	0.67	0.46	0.70	0.35	0.22	0.07	0.81	0.53	0.40	0.36	0.60	0.92	1.49
12*	45	3	3.5	99.45	39.58	39.31	91.54	38.80	116.44	69.59	27.39	3.41	87.84	63.80	67.00	55.25	98.71	161.56	215.15
13*	45	3	3.5	101.37	41.58	42.02	102.84	39.19	121.24	70.61	26.53	2.76	89.22	64.52	67.59	54.70	97.66	160.79	206.13
14	25	1.5	5	2.03	1.36	1.75	1.71	1.27	1.98	0.99	0.52	0.15	2.20	1.42	1.04	0.96	1.52	2.27	3.74
15	25	1.5	2	2.37	1.62	2.10	2.32	1.51	2.27	1.12	0.61	0.22	2.58	1.67	1.23	1.16	1.93	2.76	4.56
16	65	4.5	2	0.60	0.52	0.77	0.65	0.47	0.69	0.36	0.18	0.08	0.76	0.50	0.38	0.34	0.59	0.87	1.40
17	65	1.5	2	2.30	1.57	1.77	2.21	1.52	2.17	1.07	0.55	0.21	2.46	1.64	1.18	1.09	1.96	2.80	4.58
18	45	0.5	3.5	1.81	1.61	1.97	1.96	1.56	2.14	1.10	0.54	0.19	2.50	1.65	1.14	1.10	1.82	2.66	4.37
19	45	3	1	68.88	41.42	48.27	78.77	37.03	111.40	64.47	19.20	2.26	79.26	57.25	56.80	45.00	88.04	143.54	171.48

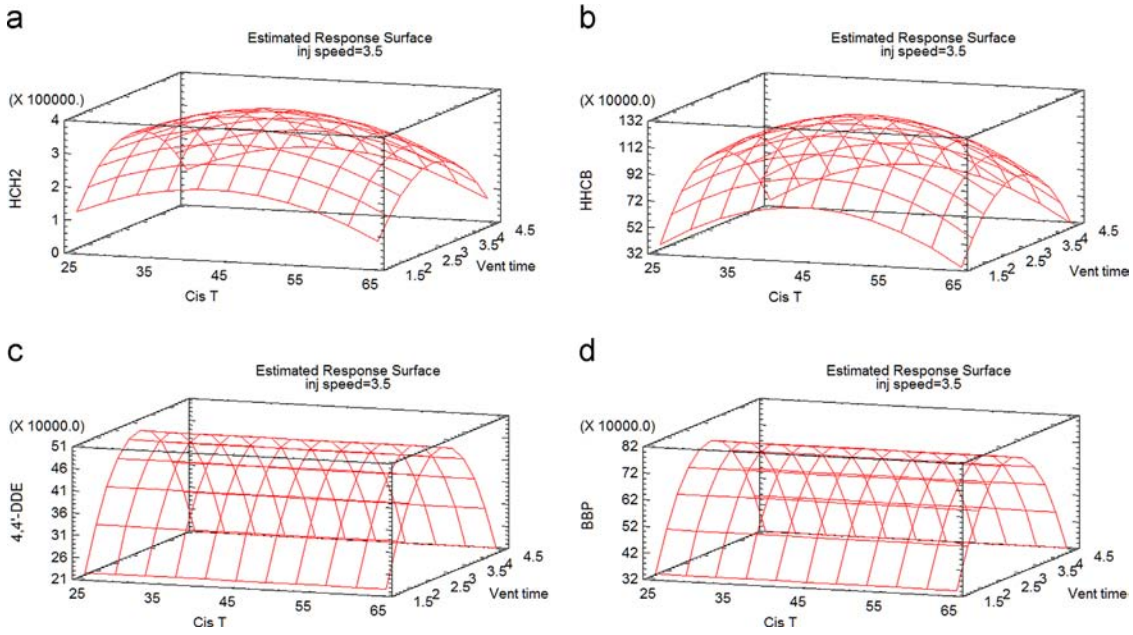

Fig. 2. Response surface obtained for (a) β-HCH, (b) HHCB, (c) 4,4′-DDE and (d) BBP using significant variables (p-value < 0.05): T_{cis} and t_{vent}. Injection speed was fixed at 3.5 μL s^{-1}.

volume: 45 μL) based on the conclusions obtained in a previous work [22], and only the injection speed (v_{inj}, μL s^{-1}), cryo-focussing temperature (T_{cis}, °C) and vent time (t_{vent}, min) were optimised. To achieve this goal, a Central Composite Design (CCD) was performed using the Statgraphics software (Centurion XV, Statpoint Tech. Inc. USA) covering the following factor space: v_{inj} (1–6 μL s^{-1}), T_{cis} (10–80 °C) and t_{vent} (0.5–5 min) (see Table 3). The goal of the optimisation process was the maximisation of chromatographic peak area using mixed stock solutions in EtAc. The precision of the measurements was estimated from the four replicates of the central point (Relative Standard Deviation (RSD) values were between 3% and 10% for all target compounds except for 4-nOP).

The analysis of the results by means of the analysis of variance and response surfaces showed that the injection speed was not statistically significant at 95% of confidence level ($p > 0.05$) for any of the studied analytes. T_{cis} and t_{vent} variables showed a significant effect on the analytes response so responses surfaces were built with these variables. Fig. 2 illustrates the response surfaces obtained for an analyte of each family. Vent time was significant for most of the studied analytes getting the highest value at an intermediate value, i.e., 3 min. The cryo-focussing temperature was significant for some pesticides, musk compounds and HCH isomers, for which the highest yields were obtained at low temperatures. Finally, it was fitted at 50 °C, as a consensus between the best chromatographic signals and N_2 (l) consumption. The obtained values are in good agreement with the optimum values obtained for compounds with similar characteristics in works described elsewhere [18,19].

3.2. Optimisation of SR–LD method

In order to get the optimum extraction conditions several parameters affecting SR–LD efficiency were tested. Thus, matrix modifications, extraction time and LD conditions were studied by performing systematic assays. After getting the optimum conditions the feasibility of performing MESCO analyses was also evaluated.

3.2.1. Optimisation of LD conditions

In a first approach, the best LD conditions which assure the complete desorption of the analytes from the SR were evaluated, i.e., stripping solvent nature and desorption procedure.

MeOH, acetonitrile or aqueous buffers are the most widely used solvents for LD of semi-volatile compounds analysed by SR in order to avoid high swelling of the sorptive material [8]. However, the use of non-polar solvents, more suitable for LVI-PTV–GC–MS analysis such as cHex or EtAc, is also possible because the swelling of the material does not affect their desorption characteristics [14,17,23]. Therefore, the efficiency of Hex, cHex and EtAc as stripping solvents was compared. As shown in Fig. 3, EtAc offered the best desorption abilities because it showed the maximum extraction efficiency, and at the same time, it showed lower swelling behaviour, acceptable chromatographic peak shapes and precision (RSD < 15%). Therefore, the SRs were desorbed with EtAc in the following steps.

Although the LD times at room temperature range often from 15 to 30 min, this time can be reduced by applying external energy sources such as shaking, sonication or increasing temperatures [8]. Ultrasound baths are mostly used for LD purposes [8,23], but the use of other systems such as focussed ultrasonic cup boosters (FUSB) can enhance the repeatability of the process focusing the ultrasound energy to each sample separately in a small ultrasonic bath (< 15 mL) [24]. On this basis, each SR was desorbed three times successively with 200 μL of fresh EtAc by means of USB during 16 min and FUSB during 15 s [24]. Desorption efficiency (%) of USB and FUSB was calculated as the amount of each fraction related to the total amount desorbed after three consecutive extractions. As shown in Fig. 4, both sonication approaches were statistically comparable (p-value > 0.05) for all studied compounds in terms of recovery (desorption efficiency: higher than 80% and 70% in the first extraction for USB and FUSB respectively) and reproducibility (RSD values lower than 20% and 30% for USB and FUSB respectively), which assured a quantitative desorption in a unique step. These results, together with the fact that USB device allows the simultaneous desorption of all the samples, make USB extraction the best option to get the highest desorption efficiencies in the shortest analysis time. Thus, the SRs

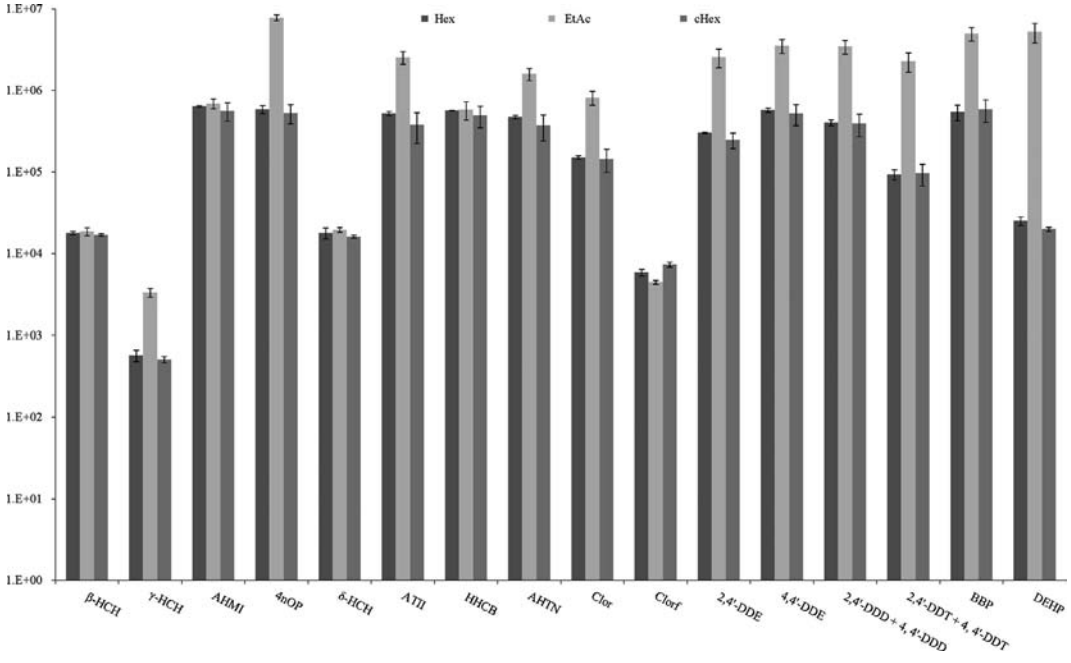

Fig. 3. Comparison of the chromatographic average responses (n=3, 95% confidence level) obtained for the different solvents used for liquid desorption of SRs: n-hexane (Hex), ethylacetate (EtAc) and cyclohexane (cHex).

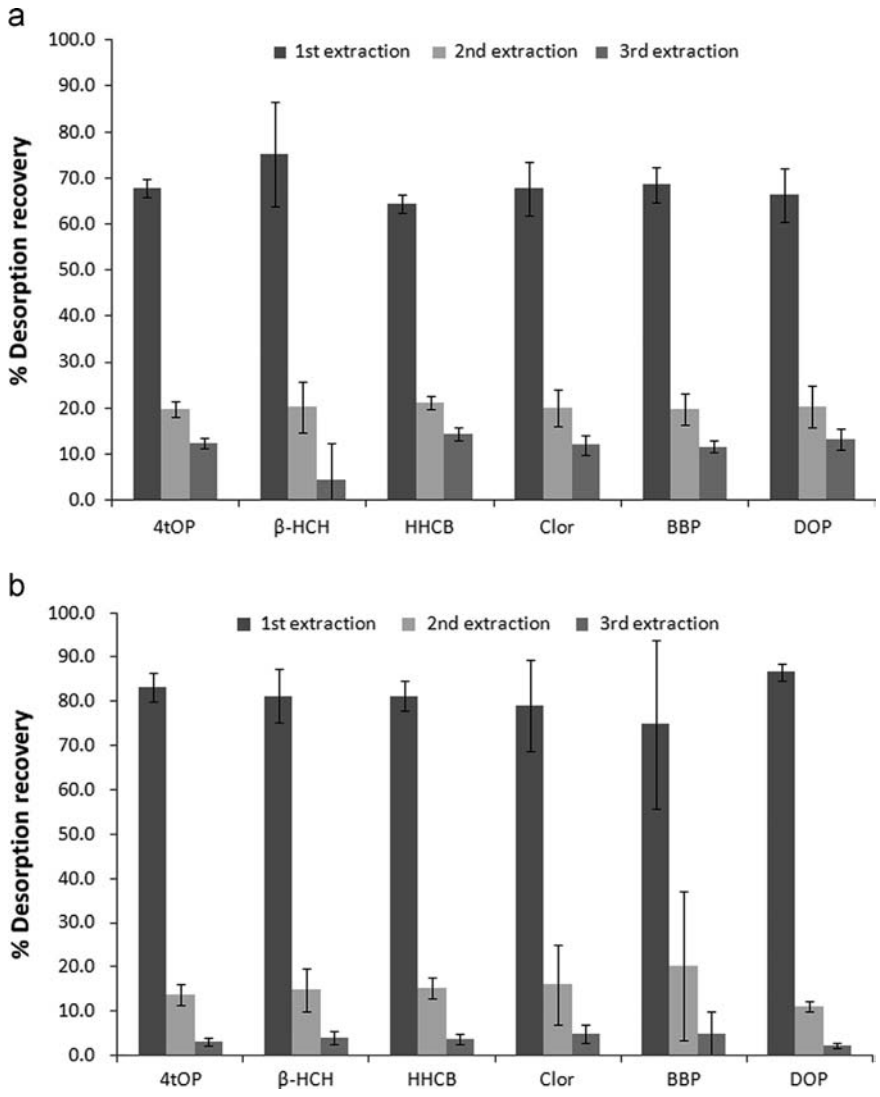

Fig. 4. SR Desorption recovery % of three successive extraction ($n=3$, 95% confidence level) obtained by using: (a) focussed ultrasound cup booster (FUSB) and (b) ultrasound bath (USB).

were desorbed with 200 μL of EtAc and assisted with an ultrasonic bath for 16 min (2 × 8 min) in subsequent experiments.

3.2.2. Optimisation of SR extraction

Among several parameters that can be optimised for micro-extraction of organic compounds using SR, the addition of NaCl and MeOH often show high influence in the extraction efficiency. The evaluation of extraction conditions using SR was carried out using the previously optimised liquid desorption conditions as well as the LVI-PTV–GC–MS conditions optimised beforehand.

The addition of inert salts such as NaCl modifies the ionic strength of the donor phase and, depending on the analyte nature, a salting-out effect can be observed. It is well established that the addition of NaCl reduces the extraction efficiency of non-polar compounds ($\log K_{ow} > 3.5$) due to the increase in the viscosity of the sample, and hence, leading to slower extraction kinetics [8,25]. In this sense, in other sorptive methods developed with environmental water samples, NaCl was not added in order to avoid the reduction of the extraction efficiency of organochlorine pesticides [26], polycyclic musks [27], octylphenols [6] and phthalates [28]. Therefore, the addition of inert salts was not considered for further experiments. However, there is a high controversy about the MeOH addition. MeOH is often added to the aqueous sample in order to reduce the adsorption of certain organic analytes onto the glassware with the trade off of increasing the solubility of the analyte in the donor phase [6,8]. Hence, the effect of MeOH addition was evaluated by adding MeOH at 0%, 5% and 10% into 150 mL ultrapure water spiked at 250 ng L^{-1}. Similarly to other works found in the literature [26,27], the increase of MeOH percentage produced a slight decrease in the response that was statistically not significant (p-value > 0.05) for all the target compounds (see Fig. 5). Therefore, the water samples were directly extracted without adding any extra reagent.

Another important parameter affecting sorptive extractions is extraction-time. Therefore, once the extraction parameters were fixed, extraction time profiles were evaluated from 5 to 1440 min using 150 mL of Milli-Q water spiked at 500 ng L^{-1} in duplicate (see Fig. 6). The equilibrium was reached for all compounds at around 420 min (7 h), so further experiments were conducted overnight to ensure equilibrium conditions. Although the extraction time can result long, but still comparable with other works using sorptive extraction techniques [8,29,30], the extraction method offers a good compromise between sensitivity and feasibility since several samples can be extracted simultaneously.

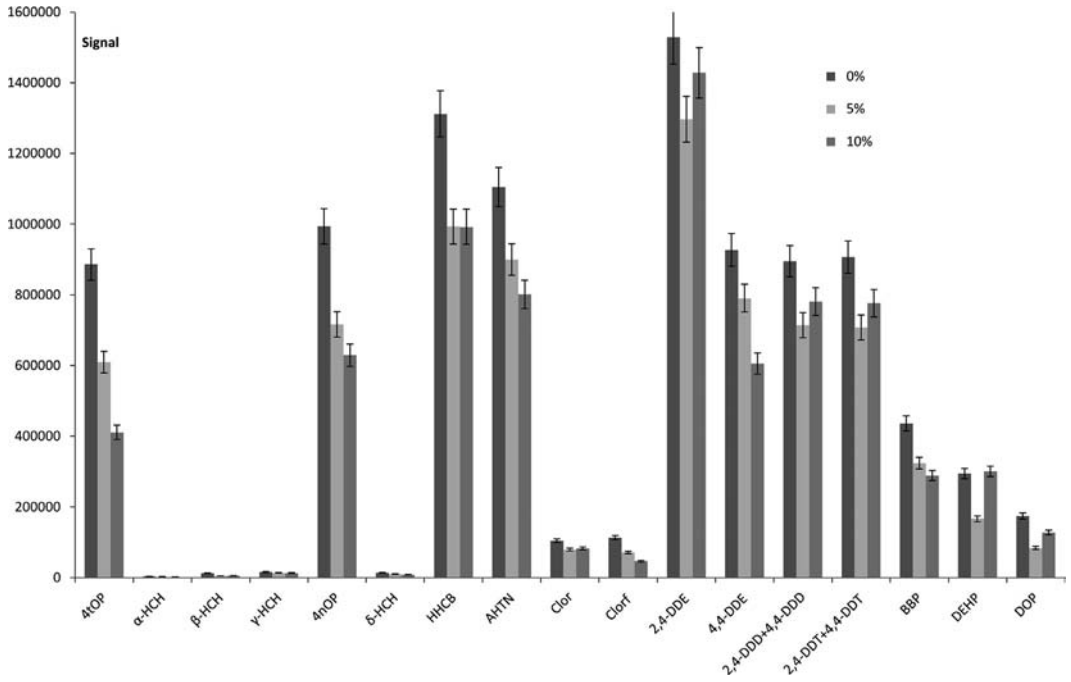

Fig. 5. Effect of MeOH addition (0%, 5% and 10%) in the extraction of the target compounds using SRs ($n=3$, 95% confidence level).

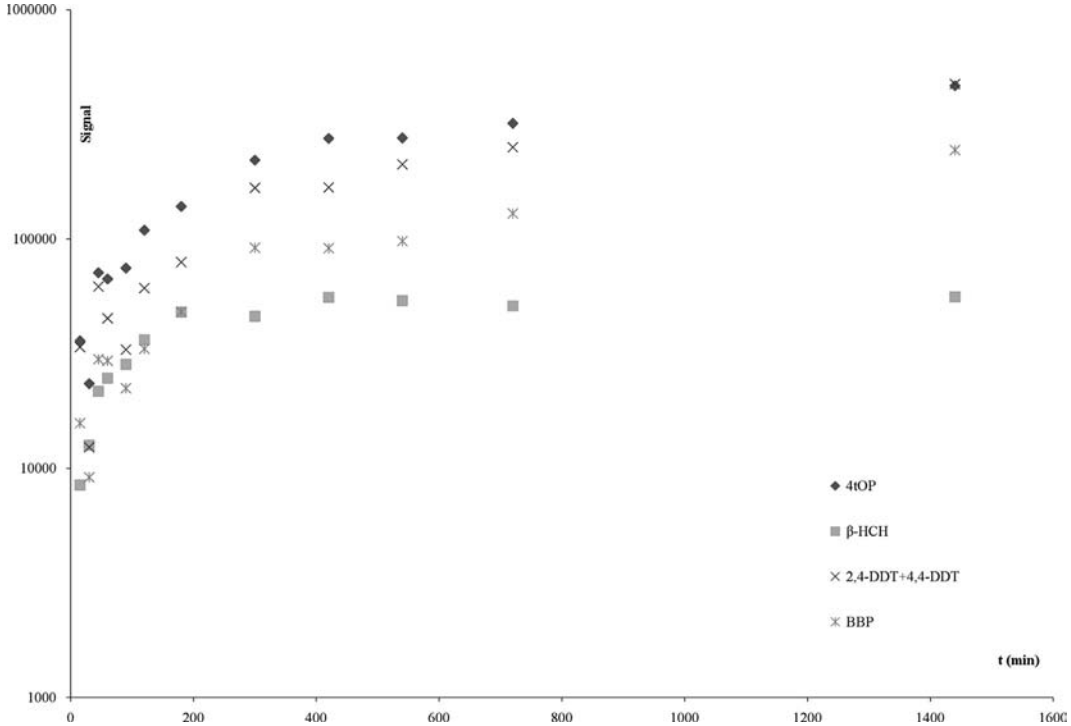

Fig. 6. Time profiles for some of the studied compounds (4-tOP, β-HCH, 2,4-DDT+4,4-DDT and BBP).

3.2.3. *Optimisation of MESCO–LD method*

The MESCOs employing SRs as passive sampler and LDPE as membrane bag material are the most promising configurations [14] even if applications using different silicone materials (twister bars and silicone tubes) and cellulose membrane bags can be also found in the literature [15,20,31]. Besides these factors, current investigations deal with the optimisation of the solvent nature in the inner part of the membrane. Thus, different sampling device configurations were tested in order to get the higher extraction yields. The sampling devices evaluated in this study consisted on SRs enclosed in a hydrophilic semi-permeable membrane (LDPE) filled with different isopropanol:water mixtures (i.e, 0:100, 25:75, 50:50, 75:25 and 100:0) and air-filled by triplicate. In the case of filling the membrane bag, a water solution modified with a small amount of isopropanol (i.e. isopropanol:water 25:75) assured the highest extraction efficiency for most of the target compounds except for BBP and DOP (data not shown). However, the assays using air-filled membrane bags yielded statistically higher recoveries ($p<0.05$) for almost all the target compounds in comparison to those obtained previously (see Fig. 7). Similar conclusion

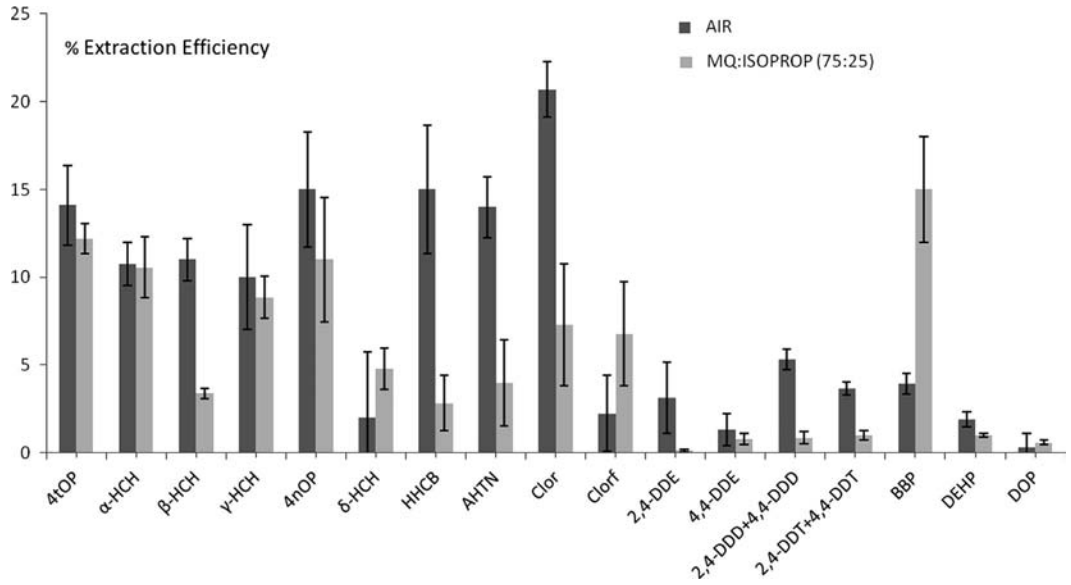

Fig. 7. Extraction efficiency of different MESCO extraction set-ups ($n=3$, 95% confidence level): membrane filled with Milli-Q:isopropanol (75:25) and solvent-less membrane.

Table 4
Main method parameters for the SR–LD–LVI-PTV–GC–MS and MESCO–LD–LVI-PTV–GC–MS procedures.

Analyte	LVI-PTV–GC–MS		SR–LVI-PTV–GC–MS					MESCO–LVI-PTV–GC–MS				
	r^2	% RSD ($n=3$;100 ng/mL)	% RSD ($n=3$; 100 ng/L)	LOD (ng/L)	MDL WWTP (ng/L, 99%)	Extraction Efficiency (%)	Apparent Recovery (%)	% RSD ($n=3$; 100 ng/L)	LOD (ng/L)	MDL WWTP (ng/L, 99%)	Extraction Efficiency (%)	Apparent Recovery (%)
4-tOP	0.9999	2	4	1	9	11	112	9	13	17	2	78
4-nOP	0.9947	6	7	1	6	19	107	24	4	6	10	101
β-HCH	0.9999	7	8	26	71	7	84	19	34	122	16	58
γ-HCH	0.9999	2	6	25	32	10	108	16	17	26	7	20
δ-HCH	0.9947	3	7	65	90	4	123	n.d	n.d	n.d.	n.d	n.d
AHTN	0.9975	5	6	15	33	19	104	12	10	33	11	80
HHCB	0.9966	4	5	11	33	20	101	7	15	33	10	89
Clor	0.9993	4	6	15	31	33	110	12	21	40	21	144
Clorf	0.9985	8	8	7	10	31	118	19	14	28	4	86
2,4 DDE	0.998	6	6	1	3	16	114	23	1	6	4	81
4,4' DDE	0.9981	5	6	1	3	10	121	26	1	8	3	78
2,4 DDD	0.9987	8	9	1	4	22	110	30	1	19	4	113
2,4 DDT	0.9987	6	8	1	4	20	109	32	5	10	3	114
BBP	0.9972	2	7	6	17	22	106	31	7	23	2	96
DEHP	0.999	2	20	15	35	13	123	21	20	39	7	82
DOP	0.9989	6	14	15	17	3	131	29	10	66	2	95

n.d.—not determined.

was also obtained elsewhere where MESCO was used for the monitorisation of organic pollutants in water samples [31]. Thus, the figures of merit for MESCO–LD assays were further determined using air filled membrane bags.

3.3. Figures of merit

Due to the lack of certified reference materials spiked water samples were used to test the analytical features of the methods (see Table 4).

Procedural calibration curves were performed in the range of 10–200 ng L^{-1} in 150 mL of Milli-Q water whereas the external calibration curves were built with a set of 6 standards containing concentrations ranged from blanks to 300 ng mL^{-1}. Deuterated analogues used as surrogates were also calibrated in order to get the recoveries of the target compounds. Linearity of external calibration curves was good for all the target compounds with coefficients of determination (r^2) close to 0.999 for synthetic musks, phthalates, pesticides and octylphenols and close to 0.995 for HCHs respectively. The linearity of procedural calibration curves was also good obtaining r^2 values between 0.992 and 0.998 for all the studied compounds.

Reproducibility of the chromatographic analysis was lower than 8% based on the measurement of three samples at intermediate external calibration solutions (100 ng mL^{-1}) for all the target compounds. The reproducibility of the whole methods were evaluated using spiked Milli-Q water at 100 ng L^{-1}. All the values were between 4% and 9% for all the analytes extracted using SRs except for DEHP and DOP ($>15\%$) and between 7% and 30% for MESCO assays. In general, the RSD values obtained for MESCO analyses were higher than those obtained using SRs.

Extraction efficiency for SR and MESCO was calculated using 150 mL of Milli-Q water spiked at 100 ng L^{-1} and comparing this concentration with the concentration obtained using an external calibration procedure. As summarised in Table 4, the extraction efficiencies for all the target analytes were significantly lower for

MESCO (between 2% and 21%) in comparison with those obtained using SR (between 10% and 33% except for HCH isomers and DOP). The apparent recoveries were obtained by comparing the spiked concentration of the studied compounds with the concentration obtained from the procedural calibration curve (i.e., built with Milli-Q spiked water samples and submitted to the whole procedure). The SR extractions provided acceptable results since the recoveries were between 84% and 123% for all the compounds except for DOP (131%). Regarding to MESCO assays, the apparent recoveries were between 78% and 114% for all target compounds except for HCH isomers.

Limits of detection (LODs) were calculated as the average signal of the Milli-Q water samples ($n=5$) plus three times their standard deviation. As a general rule, the LODs obtained for all the target analytes were lower than 15 ng L^{-1} except for HCH isomers (between 25 and 65 ng L^{-1} using SR and between 27 and 35 ng L^{-1} using MESCO) and were similar to those found in the literature [18,32,33]. The method detection limits (MDLs) were calculated after spiking effluent WWTP water samples at the corresponding LOD for each analyte according to US Environmental Protection Agency (EPA) guidelines [34] and extracted by means of SR and MESCO. As summarised in Table 4, the MDL values obtained after SR extraction were between 3 and 35 ng L^{-1} for all target compounds except for HCHs (32–90 ng L^{-1}). Regarding the MDLs for samples extracted using MESCO, the obtained values were in the same range of those obtained by SR extraction, i.e., between 6 and 65 ng L^{-1}. This fact becomes MESCO set up suitable for the extraction of the target compounds (except for HCH isomers) from environmental water samples and to support passive sampling analysis.

3.4. Evaluation of the matrix effect

The accuracy of the method can be substantially influenced by the matrix of real environmental samples. High levels of dissolved or suspended organic matter present in water samples may compete with the silicone material and thus, the extraction yield might vary from sample to sample. Among the different strategies found in the literature to minimise these effects the use of deuterated analogues is the most preferred one [18]. Consequently, the matrix effect was evaluated by triplicate comparing the responses of analytes in spiked blank water samples (at 100 ng L^{-1}) with those obtained for target compounds spiked at the same level (at 100 ng L^{-1}) in presence of different concentrations of humic acids (0, 50, 100, 250 and 500 mg L^{-1}). To assure the interaction of the target compounds with the synthetic matrix, samples were spiked and stirred for 15 min before the extraction was carried out.

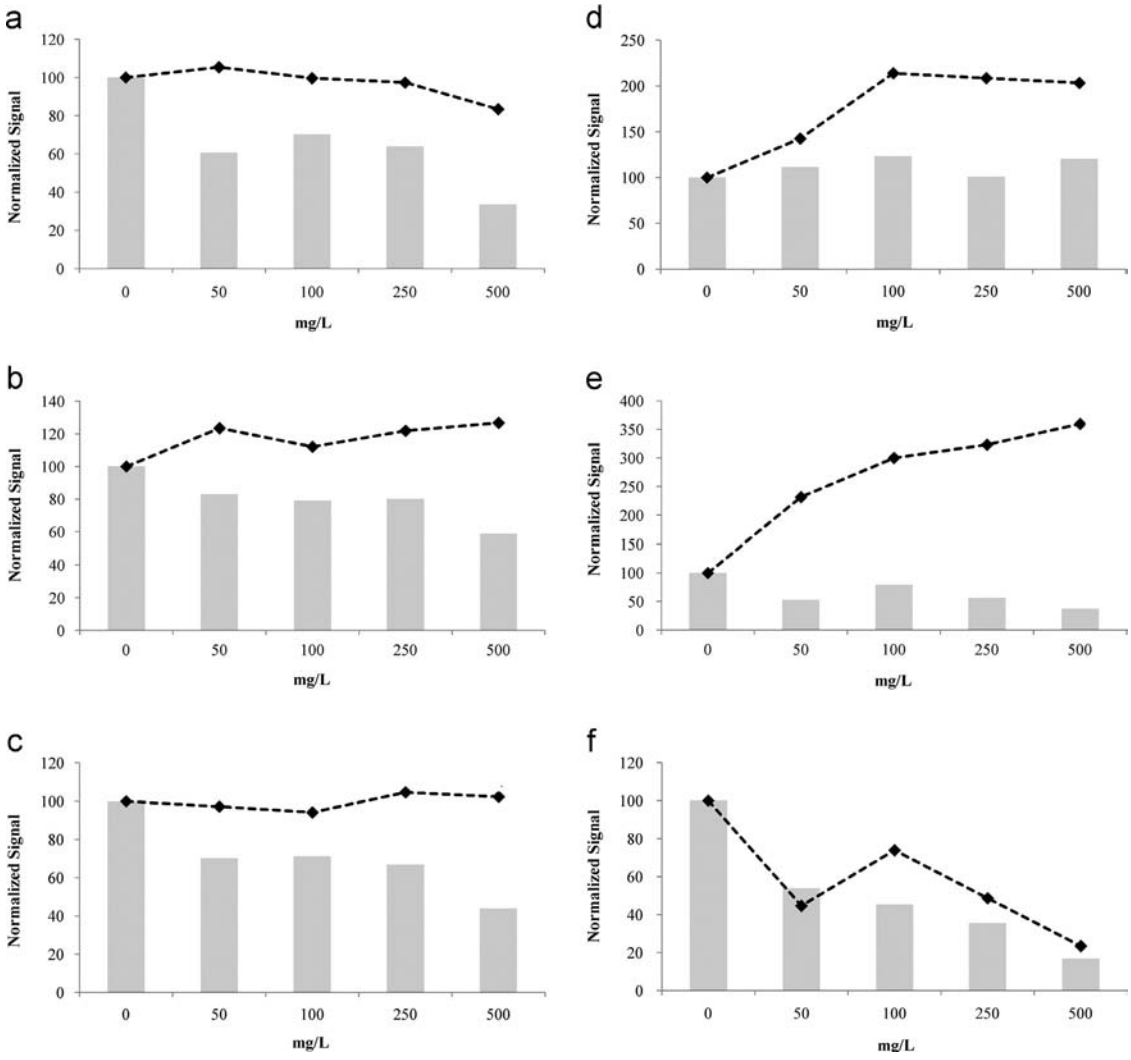

Fig. 8. Influence of humic acids on non-corrected (data shown as bars) and corrected recoveries using corresponding deuterated analogue (data shown as dotted line) for some of the target compounds: (a) 4-tOP; (b) δ-HCH; (c) HHCB; (d) DEHP, (e) Clor and (f) BBP.

Fig. 8 shows the results of chromatographic peak areas normalised to 100% for one target analyte of each family with and without correction with the corresponding deuterated analogue (see Table 2 for details of deuterated compounds). Only for a few cases (DEHP and DOP) the observed influence of humic acids was no significant (see Fig. 8d). In general terms, the behaviour of the rest of the studied analytes showed the same trend: the higher the organic matter concentration, the higher decrease of chromatographic signal. This decrease was quite well corrected for all the target compounds with the use of the corresponding deuterated analogue (see Fig. 8a–c) except for chlorpyriphos and BBP (see Fig. 8e and f). In those particular cases, the lack of correction can be attributed more to the suitability of the specific deuterated analogue used to correct the matrix effect than to the method itself.

3.5. Application to real samples

Estuarine and urban WWTP effluent water samples were analysed by triplicate by means of the optimised SR–LVI-PTV–GC–MS approach and twelve of the seventeen target compounds were detected in the water samples. As expected, effluent wastewaters showed higher concentration values than in estuarine samples. This can confirm the fact that the most organic pollutants are not removed during the water treatment process, as it is well documented in the literature [35]. Namely, musk compounds, octylphenols and organophosphorous pesticides were the main pollutants detected at higher concentrations in effluent wastewater samples, i.e. HHCB at 1270 ± 67 ng L^{-1}, AHTN at 175 ± 7 ng L^{-1}, 4-tOP at 17.7 ± 0.8 ng L^{-1}, 4-nOP at 8 ± 1 ng L^{-1} and chlorpyriphos and chlophenvinphos at 57 ± 7 ng L^{-1} and 8 ± 2 ng L^{-1} respectively (concentration results expressed as 95% of confidence level). Phthalate compounds were detected in the range of 15–447 ng L^{-1}. In the case of hexachlorocyclohexane isomers and organochlorine pesticides, the concentrations were much lower (between 8 and 55 ng L^{-1}) while the rest of target analytes were found at concentrations lower than method detection limit, which is in agreement with those values found in the literature [19]. The occurrence of the studied analytes in estuarine water samples was negligible since all the target analytes were bellow method detection limits.

4. Conclusions

The proposed method consists of a silicone rod sorptive extraction of 17 non-polar organic pollutants followed by a liquid desorption using ultrasonic bath and LVI-PTV–GC–MS analysis. The optimised extraction approach using SRs shows acceptable precision, accuracy, and limits of detection low enough (at low ng L^{-1} level) for the determination of target analytes in wastewater and estuarine samples.

Based on this method, a multiresidue analytical method covering a wider range of pollutants seems to be feasible. Additionally, the use of low cost SR polymers and the user-friendly procedures make this method valuable for routine analysis. The suitability of SR for the extraction of organic compounds was good established for the extraction of PAHs, PCBs, PBDEs and pharmaceuticals or used as passive sampling purposes in the literature, but not for the simultaneous extraction of several organic contaminants belonging to different families. Hence, this work supports the suitability of SRs for the extraction of hexachlorocyclohexane compounds, organochlorine and organophosphorous pesticides, polycyclic compounds, octylphenols and phthalates in environmental water samples as an alternative to SBSE.

Moreover, air-filled LDPE–MESCO can become a feasible alternative as passive samplers for environmental water monitoring programs even for this variety of organic compounds. However, this approach requires further research to consider bio-fouling processes on the surface of the membrane.

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