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Solid-phase extraction combined with large volume injection-programmable temperature vaporization–gas chromatography–mass spectrometry for the multiresidue determination of priority and emerging organic pollutants in wastewater

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ABSTRACT

In the present work the simultaneous extraction for the multiresidue determination in wastewater samples of organic compounds such as polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), pesticides, polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PEs), alkylphenols (APs), bisphenol A (BPA) or hormones included in different lists of priority and emerging pollutants because of their action as endocrine disrupting compounds (EDCs) was developed. Different solid phase extraction (SPE) variables such as the nature of the solid phase (Oasis-HLC, C18 and Lichrolut), the sample volume, the addition of MeOH and NaCl, the pH of the water phase and the volume of the eluent solvent were optimized in order to analyze simultaneously the priority and emerging families of pollutants mentioned above. Good recoveries were obtained for Milli-Q water (80–120%), however, since the use of deuterated analogues and dilution of the sample did not correct the matrix effect, additional SPE clean-up step using Florisil® cartridges was necessary to obtain good results for wastewater samples (80–125%). In order to improve the limits of detection (LODs), large volume injection (LVI) using programmable temperature vaporizer (PTV) coupled to gas chromatography–mass spectrometry (GC–MS) was also optimized. Since analytes losses in the case of the most volatile congeners occurred during the derivatization step and no separation of the derivatized and the non-derivatized analytes was possible during SPE elution, two different injections were optimized for each analyte group. LODs were in good agreement with those found in the literature and relative standard deviations (RSDs) were in the 10–25% range for Milli-Q and 12–30% for wastewater samples. The method was finally applied to the determination of target analytes in three different wastewater treatment plants (WWTPs, Bakio, Gernika and Galindo (Spain)) and in one water purification plant (WPP) in Zornotza (Spain).

1. Introduction

Water pollution is one of the most outstanding environmental concerns for the European Union (EU) due to the excessive use of anthropogenic compounds. Trying to protect human health and the environment against the risk of the non-controlled use or waste of organic compounds, a new legislation such as REACH (Registration, Evaluation and Authorization of Chemical Substances EC1907/2006) is taking place in the EU since June 2007 [1]. One of the bases of the REACH is to have robust analytical methods capable of identifying target compounds in order to permit the progressive elimination of the most harmful compounds. While REACH legislation takes care about all the chemical substances without regarding their end-up, more specific legislations for water quality are needed. In that sense, the European Water Frame Directive (WFD, 2000/60/EC) [2] is one of the laws currently in force in the EU.

Sometimes the treatments used at the wastewater treatment plants (WWTPs) are not exhaustive enough to completely remove organic compounds. Therefore, the effluents of urban waters become a source of many different organic pollutants, some of them are toxic for the environment [3]. Different contaminants, such as alkylphenols (APs) [4], phthalates (PEs) [5], phenols [6], polycyclic aromatic hydrocarbons (PAHs) [7] and flame retardants [8] among others, have been found in wastewaters. According to the Spanish Royal Decree of 2007 [9], the reuse of wastewaters is allowed if they pass the minimum quality criteria according to their usage, however, organic compounds are not included. The treated waters can be used in agricultural irrigations [10], for municipal and industrial purposes (food industry is not included), for environmental aims,

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such as recharging the aquifers, or they can be directly discharged into rivers or the sea [11].

Two of the most established pre-concentration methods for the simultaneous analysis of organic compounds are liquid–liquid extraction [8,12] and solid-phase extraction (SPE) [13–19]. For many years, silica bonded phases such as C_{18} have been used in the case of SPE but, nowadays, in order to gain more specificity, different polymeric phases, such as OASIS-HLB or Lichrolut, are well accepted.

As SPE pre-concentration is mostly non-specific, other matrix components co-elute with the target analytes [20]. Taking all this into account, a clean-up step is sometimes necessary for complex matrices such as wastewater. According to the literature (see Table 1), different cleaning procedures have been used for wastewater samples such as SPE extraction (usually with silica nature cartridges, but others stationary phases are also used, for instance NH_2, PSA, CN or diol [21,22]) or gel permeation chromatography (GPC) [11]. Sometimes, the clean-up procedure is not enough to eliminate completely the matrix effect and different calibration methods have to be considered, i.e. matrix-matched calibration [11,23], standard additions [24] or the use of isotope-labeled analytes [25]. In some cases, a combination of more than one calibration method can be used; for instance, the use of matrix-matched calibration with the use of isotope-labeled is a well-accepted combination.

GC is described as a powerful technique for the separation, identification and quantification of organic compounds at the nanogram level [22,23], but as all the instrumental techniques, it also has some disadvantages. Analytes that are too polar, non volatile or thermolabile, cannot be straightly analyzed by GC [21]. In those cases, a derivatization step can be included to increase the chromatographic response [31,33]. Besides, in order to improve the sensibility of GC, large volume injection (LVI) in a programmable temperature vaporizer (PTV) can be used instead of the classical split/splitless inlet [22,27,36].

The aim of the present work was to develop a multiresidue analytical method for the determination of different target organic compounds including PEs, pesticides, PAHs, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), APs, bisphenol A (BPA) and certain hormones in wastewater based on SPE pre-concentration and LVI-PTV–GC coupled to mass spectrometry (MS) analysis. The developed method was applied for the determination of the target analytes in influent and effluent samples from three WWTPs and one water purification plant (WPP) (all located in Biscay, Spain) every month from March 2011 to July 2011.

2. Experimental

2.1. Cleaning

With the purpose of avoiding the contamination of the laboratory material and the samples, the material was rinsed with abundant water and then maintained in acetone for 24 h. Afterwards, the material was rinsed with Milli-Q water (<0.05 μS/cm, Milli-Q model 185, Millipore, Bedford, MA, USA). Finally, all the glass material, except the volumetric one, was dried in the oven at 120 °C for at least 4 h.

In the case of test glass tubes, the same cleaning procedure was employed but they were dried in a muffle at 400 °C for at least 4 h.

2.2. Reagents and materials

Chlorinated biphenyls (CBs) 30, 65, 142 and 200 (in isooctane), polybrominated diphenyl (PBD) 103 (in cyclohexane), brominated diphenyl ethers (BDEs) (in cyclohexane) and pesticide Mix 11 and 164 (in cyclohexane) were purchased at 10 ng/μL concentration from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

TLC polycyclic aromatic hydrocarbons mix ((1:1) in dichloromethane:benzene), SS EPA phthalate ester mix (in *n*-hexane) and semi-volatile internal standard mix (in dichloromethane) at 2000 ng μL^{-1} and CEN PCB congener mix (in heptane) at 10 ng μL^{-1} were obtained from Supelco (Bellefonte, PA, USA).

The solid reagents clorphyriphos (99.9%), trifluralin (99.2%), simazine (99.0%), clorfenvinphos (97.3%), alachlor (99.2%), atrazine (98.8%), n-octylphenol (99%), BPA (99+ %), diethylstilbestrol (99.9%), cis-andostrerone (98.2%), equilin (97.6%), testosterone (99.9%), 19-norethisterone (99.5%), progesterone (99.6%), coprostan-3-ol (98%), cholesterol (99%), stigmasterol (97.4%), equilenin (99%), [$^2H_4$]-diethyl phthalate (99.7%), [$^2H_5$]-atrazine (99.6%), [$^2H_4$]-bis(2-ethylhexyl)phthalate (98.9%), [$^2H_4$]-nonylphenol (97%), [$^2H_4$]-equilin (97.6%) and [$^2H_6$]-cholesterol (98%) were purchased from Sigma–Aldrich (Steinheim, Germany), while nonylphenols technical mixture (94%), estrone (99.5%), mestranol (99.4%), 17α-ethynylestradiol (99.4%) and 17β-estradiol (99.7%) were obtained from Riedel-de HaënSeelze (Steinheim, Germany) and 4-*tert*-octylphenol (99.4%) and [$^2H_6$]-BPA from Supelco.

All chemical standards were stored at 4 °C in the dark and the stock solutions at −20 °C. 100 ng μL^{-1} dilutions were prepared in anhydrous methanol weekly, while the dilutions at lower concentrations were prepared daily according to the experimentation.

Isooctane, ethyl acetate (EtOAc), *n*-hexane, methanol (MeOH), dichloromethane (DCM) and toluene (all HPLC grade) were purchased from LabScan (Dublin, Ireland).

Anhydrous pyridine (99.8%) was obtained from Sigma–Aldrich (Steinheim, Germany) and the derivatization reagent, N,O-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA + 1% TMCS, Sylon BFT, 99:1) from Supelco.

Waters OASIS-HLB (hydrophilic–lipophilic-balanced) SPE cartridges (200 mg, 6 mL, both plastic and glass cartridges) and C_{18} (200 mg) cartridges were purchased from Waters (Milford, USA), Lichrolut cartridges (200 mg) from Merck (Darmstadt, Germany) and LC-Florisil (1 and 2 g) cartridges from Supelco. The SPE step was performed with a Visiprep® SPE manifold from Supelco.

Sodium chloride was purchased from Panreac (Reixac, Barcelona, Spain). 125 μm (125 mm diameter) and 11 μm (70 mm diameter) paper filters, 1.2 μm (90 mm diameter) glass micro-fiber filters and 0.45 μm (47 mm diameter) cellulose nitrate membrane filters were purchased from Whatman (Maidstone, UK).

2.3. Sampling

Influent and effluent spot samples were collected at WWTPs of Galindo, Gernika and Bakio and at the WPP of Zornotza every month from March 2011 to July 2011. In the case of Galindo and Bakio, water samples (24 h integrative) were collected. In all the cases water was collected in pre-cleaned amber glass bottles and transported to the laboratory in cooled boxes. The analyses were performed within 48 h after sampling.

2.4. Sample pre-concentration

A 500-mL aliquot of wastewater was passed at 3 mL min^{-1} through a 200-mg OASIS-HLB cartridge, which had been previously conditioned with 5 mL of EtOAc, 5 mL of MeOH and 5 mL of Milli-Q water. After the sample was loaded, 5 mL of (95:5) Milli-Q: MeOH mixture were added with cleaning purposes and, then, the cartridges were dried for 1 h under vacuum. Finally, the analytes were eluted using 8 mL of EtOAc and 8 mL of *n*-hexane and collected in a single vial. After elution, the extract was concentrated at

Table 1
Review of SPE based methods used in the determination of the analytes under study in water samples. Recovery, relative standard deviation (RSD) and limit of detection (LOD) included when available.

Analyte	Pre-concentration	Clean-up	Sample matrix	Technique	Recovery (%)	RSD (%)	LOD (ng L^{-1})	Ref.
PAHs	C_{18}	No	Wastewater (100 mL)	GC–MS–MS	–	–	–	[19]
	C_{18}	No	Milli-Q (100 mL)	GC–MS–MS	68–117	2–23	1–100	[26]
	Styrene-divinylbenzene	No	Irrigation stream water (50 mL)	PTV–GC–MS	102	1	36	[27]
	OASIS (60-mg)	No	Wastewater (50 mL)	GC–MS	67–109	1–26	0.4–263	[28]
	OASIS-HLB (500-mg)	No	Wastewater (1000 mL)	GC–MS	67–83	7–11	2–8	[29]
PCBs	OASIS (60-mg)	No	Wastewater (50 mL)	GC–MS	88–124	1–12	15–68	[28]
PBDEs	OASIS (60-mg)	No	Wastewater (50 mL)	GC–MS	77–117	4–18	5–375	[28]
PEs	Styrene-divinylbenzene	No	Irrigation stream water (50 mL)	PTV–GC–MS	72–97	4–32	5–10	[27]
	C_{18}	Silica	Wastewater/surface-ground water (300 mL)	GC–MS	91–108	10–11	0.5–161	[21]
	OASIS (60-mg)	No	Wastewater (50 mL)	GC–MS	73–116	7–28	3–21	[28]
Organochloride pesticides	C_{18}	No	Wastewater (100 mL)	GC–MS	–	–	–	[19]
	C_{18}	No	Milli-Q (100 mL)	GC–MS	56–111	3–23	5–130	[26]
	Styrene-divinylbenzene	No	Irrigation stream water (50 mL)	PTV–GC–MS	64–111	2–8	1–10	[27]
	C_{18}	No	Effluent Wastewater,surface and ground water (300 mL)	GC–MS	60–116	1–7	0.5–161	[21]
	C_{18}	GPC	Effluent wastewater (250 mL)	LVI-PTV–GC–MS	50–120	<25	10–500	[11]
Alachlor	Lichrolut	Matrix matched calibration	Water (500 mL)	GC–MS	105	6	3.4	[23]
Triazine	C_{18}	No	Wastewater (100 mL)	GC–MS	–	–	–	[19]
	C_{18}	No	Milli-Q (100 mL)	GC–MS	101–108	4–8	10–30	[26]
Triazine	Styrene-divinylbenzene	No	Irrigation stream water (50 mL)	PTV–GC–MS	29	2	20	[27]
	Lichrolut	Matrix matched calibration	Water (500 mL)	GC–MS	94–105	2–15	2–29	[23]
APs	Bond elut-ENV	No	Aquifer (1000 mL)	GC–MS	13–104	1–6	2–115	[30]
	OASIS-60 mg	No	Drinking, surface Wastewater (1000 mL)	GC–MS	91–100	3–11	0.3–4.5	[34]
Herbicide	C_{18}	No	Wastewater (100 mL)	GC–MS	–	–	–	[19]
	C_{18}	No	Wastewater (100 mL)	GC–MS	–	–	–	[19]
	C_{18}	No	Milli-Q (100 mL)	GC–MS	74–124	7–12	5–20	[26]
	OASIS-HLB (200-mg)	No	Surface water (2500 mL)	GC–MS/MS	65–109	8–11	0.8–2.6	[31]
	OASIS (60-mg)	No	Wastewater (50 mL)	GC–MS	102–112	1	18–166	[28]
	OASIS-HLB (500-mg)	Diol	Effluent wastewater, estuarine and coastal water (1000 mL)	LVI-PTV–GC–MS	89–99	2–21	0.1–1	[22]
	OASIS-HLB (200 mg)	No	River and seawater (1000 mL)	GC–MS	46–113	<20	0.8–2.6	[16]
	C_{18} + polymeric material	No	Surface water (500 mL)	GC–MS	69–74	10	6	[32]
	C_{18}	No	Wastewater (100 mL)	GC–MS	31–36	4	30	[33]
BPA	C_{18}	Silica	Effluent Wastewater, surface and ground water (300 mL)	GC–MS	0.2	7.7	5.4	[21]
	OASIS-HLB (200-mg)	No	Surface water (2500 mL)	GC–MS/MS	96–112	1	0.5	[31]
	OASIS-60 mg (50 mL)	No	Wastewater (50 mL)	GC–MS	107	26	54	[28]
	OASIS-HLB (500-mg)	Diol	Effluent wastewater, estuarine and coastal water (1000 mL)	LVI-PTV–GC–MS	97–108	11–27	3–19.5	[22]
	C_{18} + polymeric material	No	Surface water (500 mL)	GC–MS	58	10	5	[32]
	OASIS-HLB (200 mg)	No	River and seawater (1000 mL)	GC–MS	81–95	<20	5.3	[16]
	C_{18}	No	Wastewater (100 mL)	GC–MS	87–93	5	140	[33]
Hormones	OASIS-HLB (200-mg)	No	Surface water (2500 mL)	GC–MS/MS	72–119	4–17	0.3–3.4	[31]
	OASIS-HLB (200-mg)	Florisil	Effluent and influent wastewater (500 mL)	LC–MS/MS	91–128	4–36	0.4–3	[35]
	C_{18} + polymeric material	No	Surface water (500 mL)	GC–MS	106	16	50–300	[32]
	OASIS-HLB (200-mg)	No	River and seawater (1000 mL)	GC–MS	40–116	<20	0.3–3.4	[16]
	OASIS-HLB (200–mg)	Florisil	Wastewater (100 mL)	LVI-PTV–GC–MS	74–114	2–18	0.04–6	[36]

–: No data available.

approximately 1 mL. During evaporation isooctane was added in order to minimize analyte losses and to guarantee that the concentrated extract was in a non-polar solvent before the clean-up step.

2.5. Clean-up of the samples

The concentrated extract was loaded onto a 1-g Florisil cartridge, which had been previously conditioned using 5 mL of *n*-hexane.

Target analytes were eluted in 15 mL of a mixture of (65:35) *n*-hexane:toluene, followed with 15 mL of a (40:40:20) EtOAc:DCM:MeOH mixture. The eluate was divided into two aliquots of 12 mL each. One of the aliquots was evaporated to dryness and reconstituted in 100 μL of *n*-hexane before LVI-PTV–GC–MS analysis of PCBs, PBBs, pesticides, PEs and PAHs. The second 12-mL aliquot was evaporated to dryness and submitted to derivatization for the analysis of APs, BPA and hormones.

2.6. Derivatization

A 12-mL aliquot of the cleaned extract was derivatized as follows: the extract was taken to dryness and 50 μL of BSTFA + 1% TMCS and 125 μL of pyridine were added. The samples were heated for 1 h at 70 °C in an oven. The derivatized aliquot was further

evaporated and the sample was reconstituted in 100 μL of *n*-hexane before LVI-GC–MS analysis of APs, BPA and hormones.

2.7. LVI-PTV–GC–MS

In the case of the non-derivatized analytes, 50-μL sample was injected at 3.5 μL s^{-1} in a cooled (60 °C) PTV at 7.7 psi vent pressure using a 100 μL syringe placed in a MPS2 autosampler (Gerstel GmbK & Co. KG, Mülheim an der Ruhr, Germany). *n*-Hexane was purged out with a vent flow of 75 mL min^{-1} for 3 min (vent time). Then, splitless mode was programmed for 1.5 min, while the temperature increased at 12 °C s^{-1} to 300 °C, where it was held for 5 min.

Analytes were introduced into a HP5 MS (30 m × 0.25 mm × 0.25 μm) capillary column placed in 6890 Gas Chromatograph (Agilent Technologies, Avondale, USA) equipped with an Agilent 5975 Electron Impact Ionization Mass Spectrometer. The following oven temperature program was used for the separation of the analytes: 60 °C (hold 1 min), temperature increase at 7.0 C min^{-1} up to 300 °C, where it was finally held for 15 min. Helium (99.995%, Carburos Metálicos, Barcelona, Spain) was used as carrier gas at a constant flow rate of 1.2 mL min^{-1}.

In the case of the derivatized analytes, 50-μL sample was injected at 3.5 μL s^{-1} in a cooled PTV (45 °C) at 7.7 psi vent pressure using a 100 μL syringe placed in a MPS2 autosampler. *n*-Hexane was purged out with a vent flow of 75 mL min^{-1} for 3 min (vent time), then, splitless mode was programmed for 1.5 min, while the temperature increased at 12 °C s^{-1} to 300 °C, where it was held for 1 min.

Analytes were introduced into a HP5 MS (30 m × 0.25 mm × 0.25 μm) capillary column placed in 6890 Gas Chromatograph equipped with an Agilent 5975 Electron Impact Ionization Mass Spectrometer. The following oven temperature program was used for the separation of the analytes: 50 °C (hold 5 min), temperature increase at 10.0 °C min^{-1} up to 220 °C, a second increase of 3.2 °C min^{-1} up to 300 °C where it was finally held for 5 min. Helium (99.995%) was used as carrier gas at constant flow of 1.2 mL min^{-1}.

In both cases, the transfer line temperature was maintained at 310 °C, and the ion source and quadrupole at 230 °C and 150 °C, respectively. Measurements were performed both in the scan (50–525 *m*/*z*) and in the SIM (Selected Ion Monitoring) modes.

Tables 2–4 summarize the *m*/*z* values monitored for the derivatized, the non-derivatized analytes and the deuterated analogues, respectively. The first ion was used as quantifier and the second as qualifier.

3. Results and discussion

3.1. Optimization of LVI-PTV–GC–MS

The present work aimed to analyze simultaneously up to 75 analytes divided in different families: PCBs, PBBs, PEs, PAHs, APs, BPA and hormones. Some of the target analytes (APs, BPA and hormones) should be derivatized in order to obtain an optimum chromatographic signal during GC separation.

Before optimizing LVI of all the 75 analytes, a series of experiments were performed in order to check whether all the target analytes could be submitted to the derivatization step previous to the chromatographic step. Neither transformation nor degradation of the analytes was observed. However, major losses of the most volatile analytes (Nap, Ace, Acy, DMP and Ace) were observed. In some cases losses exceeded 50%. Thus, during LVI-PTV–GC–MS analysis two different aliquots were injected, one containing the derivatized analytes and the other the non-derivatized compounds.

Three variables were studied during LVI-PTV–GC–MS: injection speed (1–6 μL s^{-1}), cryofocusing temperature (35–85 °C) and vent time (0.5–5.5 min). Vent flow (50 mL min^{-1}), vent pressure (7.7 psi), injection volume (50 μL), purge to split vent (100 mL min^{-1}) and splitless time (1.5 min) were fixed according to a previous optimization in our research group [36].

Variables were studied using a central composite design and the responses obtained (data not shown) were adjusted to a nonlinear equation using Statgraphics Centurion XV program:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 \quad (1)$$

In the case of the non-derivatized analytes, vent time was significant for some pesticides and PAHs (see Fig. 1(a) for Nap) and it showed the highest values at an intermediate value of this parameter (3 min). Injection speed was significant for most of the non-derivatized analytes (see Fig. 1(b) for Atr) and, once again, the highest signals were obtained at a medium value of 3.0 μL s^{-1}. Finally, cryofocusing temperature was only significant for Pyr, Atr and γ-HCH, showing the best yields at 60 °C.

In the case of the derivatized target compounds, vent time was significant for APs, hormones and BPA, showing a maximum at 3-min (see Fig. 1(c) for 4tOP), similarly to the previous results for the non-derivatized analytes. In the case of the injection speed, it was not significant for any of the derivatized analytes and it was decided to fit it at the central value (3.5 μL s^{-1}). In the case of the cryofocusing temperature, when significant, the highest signals were obtained at low values of this parameter (see Fig. 1(c)) and, finally, it was fitted at 45 °C as a consensus between optimum analytical signal and nitrogen consumption when analyzing derivatized target compounds.

3.2. Optimization of SPE pre-concentration

During SPE optimization the following variables were studied: the nature of the solid phase, the sample volume, the addition of MeOH and NaCl, the pH of the water phase and the nature and the volume of the eluent solvent.

Three different reverse phase cartridges were chosen for the study according to the literature: 200 mg OASIS HLB, 200-mg C_{18} and 200-mg Lichrolut (see Table 1). A 100-mL aliquot of Milli-Q water was spiked with the target analytes at 2.5 mg L^{-1} and all the experiments were performed at pH 7 with no addition of neither MeOH nor NaCl. All the experiments were carried out in triplicate. Average of the corrected signals (surrogates were added after the elution step in order to compensate all the losses occurring after the elution step) of several analytes can be observed in Figs. 2 and 3.

Although Lichrolut and C_{18} provided higher extraction efficiencies in the case of certain analytes, in general, no significant differences were observed for a 95% confidence level and, finally, OASIS-HLB was chosen due to its availability in our laboratory.

After OASIS-HLB was chosen as the best option, the addition of MeOH and NaCl was studied. MeOH is added to avoid the adsorption of certain organic analytes onto the wall of the glassware [11,37], although in some cases the solubility of the analyte in the donor phase is increased [38,39] and, therefore, extraction efficiency decreases. In the case of the addition of an inert salt such as NaCl, the ionic strength of the donor phase is modified and, depending on the analyte nature, a salting-out or salting-in effect can be observed [40,41].

For that reason, NaCl and MeOH addition were studied using a central composite design, both variables in the 0–20% range. A 100-mL aliquot of Milli-Q water was spiked at 2.5 mg L^{-1} for all the

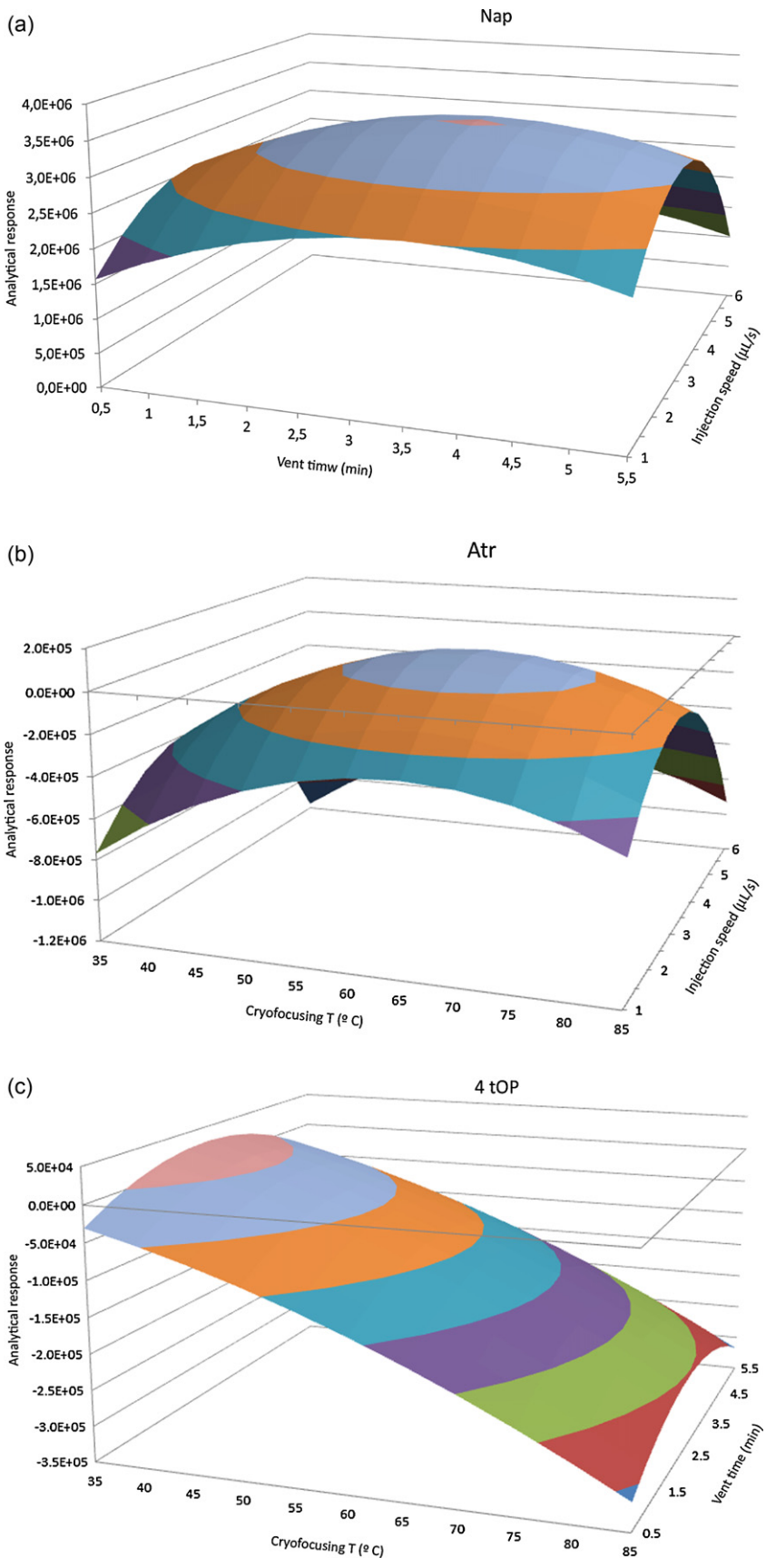

Fig. 1. Response surface for (a) Nap at a cryofocusing temperature of 60 °C, (b) Atr when vent time was fixed at 3.5 min and (c) 4tOP when injection speed was fixed at 3.5 $\mu L\,s^{-1}$.

Table 2
Abbreviations, purity, log K_{ow}, pK_a and m/z values for the derivatized analytes.

Analyte	Abbrev.	Purity	log K_{ow}	pK_a	m/z (quantifier)	m/z (qualifier)
4-*tert*-Octylphenol	4tOP	99.4	5.28	–	207	208
Nonylphenols (technical mixture)	NPs	94	5.76	10.1	207	208
n-Octylphenol	nOP	99	4.12	–	179	278
Bisphenol-A	BPA	99	3.32	9.7	357	358
Diethylstilbestrol	DES	99.9	5.07	–	412	413
Cis-androsterone	ADT	98.2	3.69	15.1	272	271
Estrone	E1	99.5	3.13	10.3	342	257
Equilin	EQ	97.6	3.53	10.1	340	216
17β-Estradiol	E2	–	4.01	10.3	416	285
Testosterone	TT	99.9	3.32	15.1	360	270
Mestranol	MeEE2	99.4	5.18	13.1	367	282
19-Norethisterone	NT	99.5	2.97	13.1	355	356
17α-Ethynylestradiol	EE2	99.4	3.67	10.2	425	440
Estriol	E3	99.7	2.45	10.2	504	345
Equilenin	EQN	99	3.76	9.7	338	295

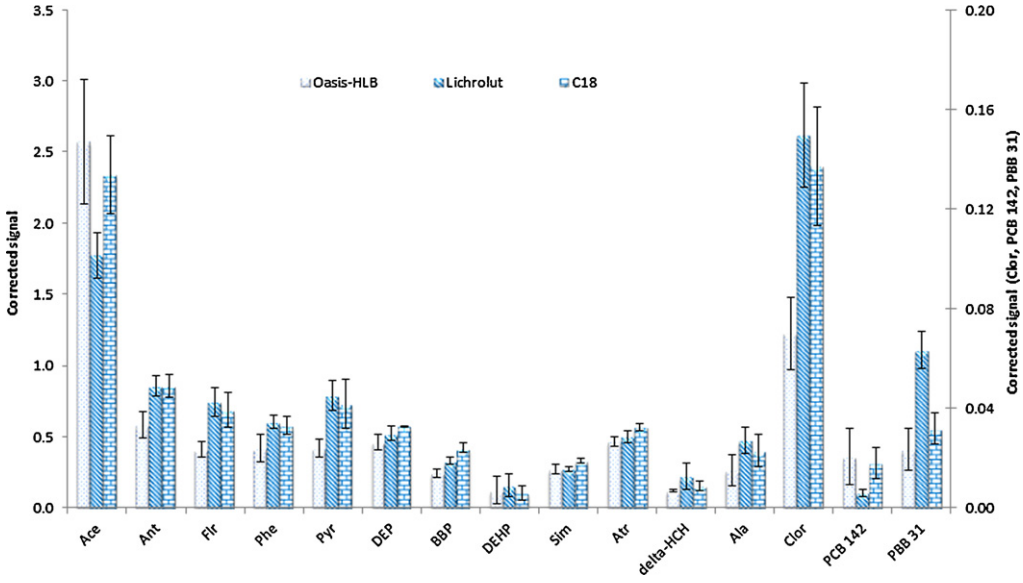

Fig. 2. Corrected average signals ($n = 3$) and their standard deviation observed for the different stationary phases studied during SPE for the non-derivatized analytes. Signals for Clor, PCB142 and PBB31 can be read in the right *Y*-axis, while the signal for the rest of compounds are read in the left *Y*-axis.

compounds and they were passed through an OASIS-HLB cartridge at neutral pH and eluted with 8 mL of EtOAc. Different amounts of NaCl and MeOH were added to the Milli-Q water according to the experimental design. Deuterated analogues were added to the EtOAc extract after the extraction step. The signals obtained were adjusted to a non-linear equation using the Stratgraphics software.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_1X_2 \quad (2)$$

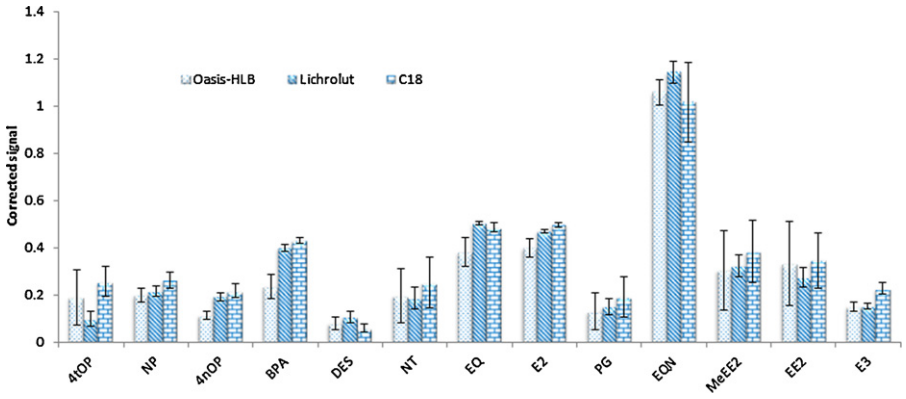

Fig. 3. Corrected average signals ($n = 3$) and their standard deviation observed for the different stationary phases studied during SPE for some of the derivatized analytes.

Table 3
Abbreviations, purity, log K_{ow}, pK_a and m/z values for the non-derivatized analytes.

Analyte	Abbrev.	Purity	log K_{ow}	pK_a	m/z (quantifier)	m/z (qualifier)
2,4,6-Trichlorobiphenyl	PCB 30	99	5.47	–	256	258
2,3,5,6-Tetrachlorobiphenyl	PCB 65	99	6.34	–	292	220
2,2′,3,4,5,6-Hexachlorobiphenyl	PCB 142	99	[a]	–	360	290
2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	PCB 200	99	8.91	–	430	358
2,2′,5-Trichlorobiphenyl	PCB 18	100	5.55	–	186	256
2,4,4′-Tricholobiphenyl	PCB 28	100	5.62	–	256	258
2,4′,5-Tricholobiphenyl	PCB 31	100	5.69	–	256	258
2,2′,3,5-Tetrachlorobiphenyl	PCB 43	98	6.34	–	292	220
2,2′,5,5′-Tetrachlorobiphenyl	PCB 52	99	6.09	–	292	220
2,2′,4,5,5′-Pentachlorobiphenyl	PCB 101	98	6.80	–	326	254
2,3′,4,4′,5-Pentachlorobiphenyl	PCB 118	100	7.12	–	326	254
2,2′,3,4,4′,5′-Hexachlorobiphenyl	PCB 138	99	7.44	–	360	290
2,2′,3,4′,5′,6-Hexachlorobiphenyl	PCB 149	99	7.28	–	360	290
2,2′,4,4′5,5′-Hexachlorobiphenyl	PCB 153	100	6.80	–	360	290
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	PCB 180	99	8.27	–	396	394
2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl	PCB 194	97	8.68	–	430	428
2,4-Dibromobiphenyl	PBB 7	100	5.40	–	152	312
2,4′,5-Tribromobiphenyl	PBB 31	100	6.35	–	390	232
2,2′,4,5′,6-Pentabromobiphenyl	PBB 103	100	6.57	–	469	548
2,2′,4,4′,5,5′-Hexabromobiphenyl	PBB 153	98.2	6.39	–	468	628
Alpha hexachlorobenzene	α-HCH	99	3.72	–	181	183
Beta hexachlorobenzene	β-HCH	99	3.78	–	181	183
Ganma hexachlorobenzene (lindane)	γ-HCH	99	3.72	–	181	183
Delta hexachlorobenzene	δ-HCH	99	4.14	–	181	183
1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]	2,4′-DDE	99	6.00	–	246	318
1,1′-(Dichloroethenylidene)bis(4-chlorobenzene)	4,4′-DDE	99	6.51	–	246	318
1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]	2,4′-DDD	99	5.87	–	235	237
2,2-Bis(p-chlorophenyl)ethane;Benzene	4,4′-DDD	99	6.02	–	235	237
1-Chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]	2,4′-DDT	99	6.79	–	235	237
1,1′-(2,2,2-Trichloroethylidene)bis[4-chloro]	4,4′-DDT	99	6.79	–	235	237
Chlorfenvinphos Z + E isomer	Clorf	97.3	3.81	–	267	323
Chlorpyriphos	Clor	99.9	4.96	–	197	199
Trifluralin	Trf	99.2	5.34	–	306	264
Alachlor	Ala	99.2	3.52	–	200	215
Simazine	Sim	99.0	2.18	1.62	201	186
Atrazine	Atr	98.8	2.61	1.70	200	215
Dimethyl phthalate	DMP	99	1.60	–	163	128
Diethyl phthalate	DEP	99	2.42	–	149	177
Di-n-butyl phthalate	DBP	99	4.50	–	149	205
n-Butyl benzyl phthalate	BBP	96	4.73	–	149	206
Bis(2-ethylhexyl) phthalate	DEHP	99	7.60	–	149	167
Di-n-octyl phthalate	DOP	99	8.10	–	149	207
Naphthalene	Nap	99	3.30	–	128	136
Acenaphthylene	Acy	99	3.94	–	152	153
Acenaphthene	Ace	99	3.92	–	153	154
Fluorene	Flu	99	4.18	–	166	165
Phenanthrene	Phe	95	4.56	–	178	179
Anthracene	Ant	99	4.45	–	178	179
Fluoranthene	Flr	99	5.16	–	202	200
Pyrene	Pyr	97	4.88	–	202	246
Benzo[a]anthracene	B[a]A	99	5.79	–	228	229
Chrysene	Cry	99	5.81	–	228	229
Benzo[b]fluoranthene	B[b]F	99	5.78	–	252	253
Benzo[k]fluoranthene	B[K]F	99	6.64	–	252	253
Benzo[a]pyrene	B[a]P	99	6.13	–	252	253
Dibenzo[a,h]anthracene	D[a,h]A	99	6.75	–	276	278
Benzo[ghi]perylene	B[ghi]P	99	6.63	–	277	278
Indeno[1,2,3-cd]pyrene	Ind	99	6.70	–	276	278

[a] Not available.

Fig. 4(a)–(d) show the response surfaces for Clor, E1, DEP and Ind, respectively.

Some works in the literature try to correlate MeOH and NaCl addition with log K_{ow} (see values in Tables 2 and 3 for derivatized and non-derivatized analytes, respectively). According to the literature [37], the addition of NaCl does not improve the extraction efficiency of analytes that have log K_{ow} higher than 3.5 and, sometimes, the recovery is reduced. On the contrary, polar analytes show higher recoveries with the modification of the ionic strength. In the case of MeOH addition, the analytes with high log K_{ow} show higher recoveries than the ones with low values. However, in this case no general rule could be concluded and as the number of analytes showing a positive or a negative effect after the addition of either MeOH or NaCl was similar (see Fig. 4(a)–(d)) no addition of neither NaCl nor MeOH was chosen as consensus conditions. Actually NaCl addition has a negative effect on 12 analytes, positive in nine and none effect on 39 of the 60 analytes studied during this optimization process. Similarly, in the case of MeOH addition it had a negative effect on 5 analytes, positive on 6 and none effect on 49 of the 60 analytes studied.

Once the nature of the SPE sorbent and MeOH and NaCl were fitted, the adjustment of the pH was studied. pH is usually studied when the target analytes have acidic/basic properties (see Table 2 for derivatized and Table 3 for non-derivatized analytes). In the

Table 4
Abbreviation, purity and *m*/*z* values of fragment ions of the deuterated compounds, as well as the target analytes that each one corrected.

Deuterated analogues	Purity (%)	*m*/*z*	Corrected compounds
[$^2H_8$]-Nap	100	136	Nap
[$^2H_{10}$]-Ace	100	164	Acy, Ace, Flu
[$^2H_{10}$]-Phe	100	188	Phe, Ant, Flr, PCB 30, 18, 31, 28, 43, 52, 65, PBB 7
[$^2H_{12}$]-Cry	100	240	Pyr, B[a]A, Cry, PCB 101, 153, 118, 142, 138, 149, 194, PBB 31, 103
[$^2H_{10}$]-Per[a]	100	264	B[b]F, B[k]F, B[a]P, D[a,h]A, B[ghi]P, Ind, PCB 200, PBB 153
[$^2H_4$]-DEHP	98.9	153	DMP, DEP, DBP
[$^2H_4$]-DEP	99.7	153	BBP, DEHP, DOP
[$^2H_5$]-Atr	99.6	200	Pesticides
[$^2H_4$]-NP	97	183	APs
[$^2H_4$]-BPA	99.9	368	BPA, DES
[$^2H_6$]-EQ	–	340	ADT, E1, EQ, EQN
[$^2H_4$]-E2	98	416	E2,TT, MeEE2, NT, EE2, E3

[a] Per: perylene.

present work, three different pH values were studied: neutral, pH = 3 (after acidification with 3 mol L^{-1} HCl) and pH = 12 (after adjustment with 3 mol L^{-1} NaOH). All the experiments were performed in triplicate.

In general, acidic or neutral conditions provided the best extraction yields (data not shown). However, since humic substances present in wastewater are best extracted at low pH values, neutral pH was chosen in order to minimize matrix effect [32]. Therefore, no pH adjustment of the real samples analyzed was performed since the average pH was 7.5 (6.7–8.4 range).

Sample loading volume was also a parameter that needed to be optimized in order to load as much sample as possible to obtain better limits of detection (LOD) without the breakthrough of the cartridges. Different loading volumes have been used in the literature, which range from 50 mL to 1000 mL [19,23,28,29,42]. In this sense, three different loading volumes (100 mL, 250 mL and 500 mL) were studied under optimized conditions and in triplicate. All the analytes were spiked at 100 ng L^{-1}.

According to the results (data not shown) for non-derivatized and derivatized analytes, respectively, 500-mL aliquots yielded the highest responses and, thus, no breakthrough effect could be assumed. Since all the target analytes could not be submitted to the derivatization step due to losses of the most volatile compounds, separation during the elution step of those two groups was studied.

Three different elution solvents or mixtures were studied according to the literature: EtOAc, MeOH:acetone (50:50, v/v) mixture and EtOAc:acetone (50:50, v/v) mixture [11,32]. 2-mL aliquots of the elution solvents was collected in separate vials up to 10 mL. All the fractions were analyzed separately. From the results obtained (data not shown), it could be concluded that almost all the analytes were eluted after the addition of 8 mL of any of the elution solvents studied, except for BPA and the

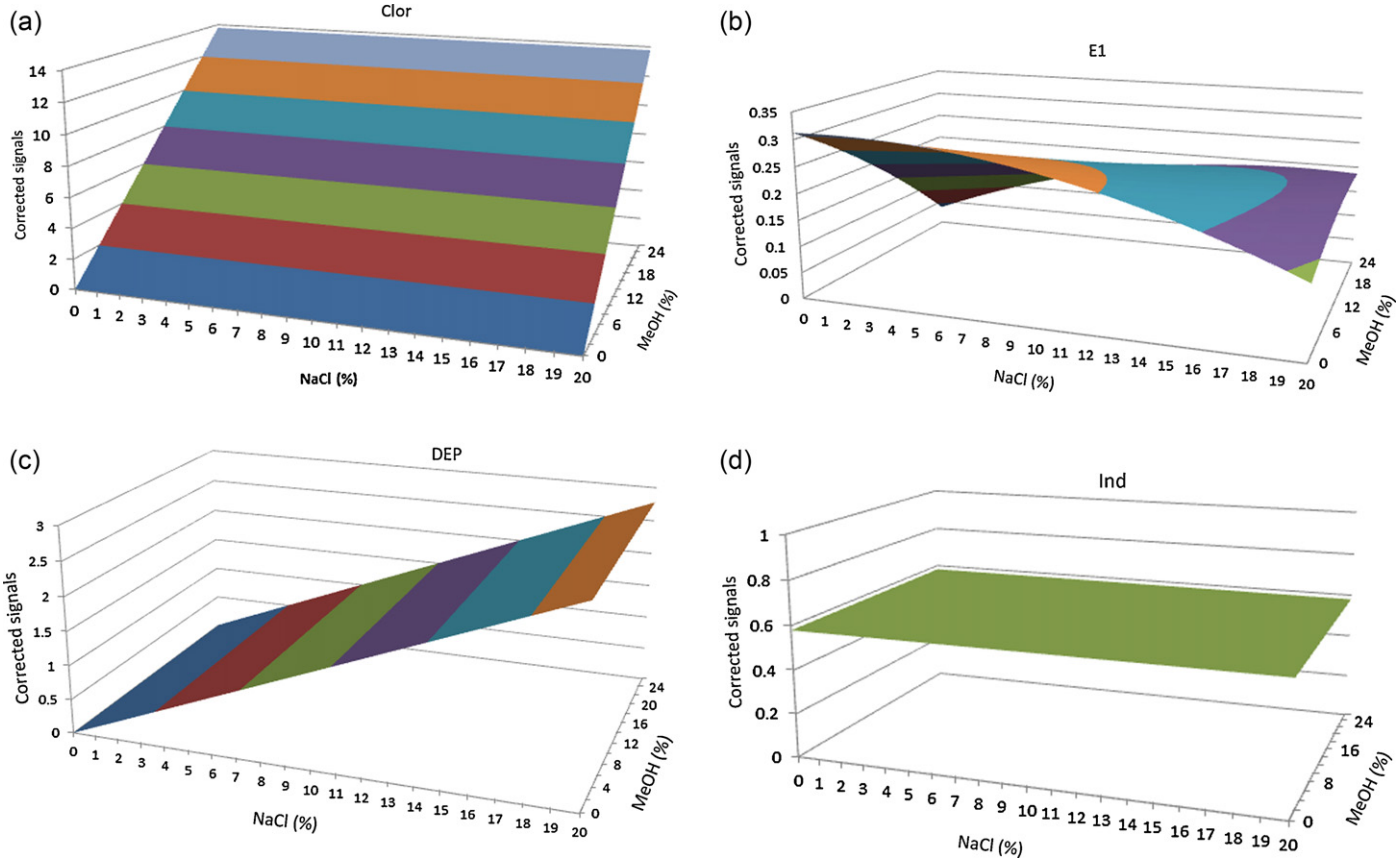

Fig. 4. Response surface for Clor (a), E1 (b), DEP (c) and Ind (d) during NaCl and MeOH optimization.

Table 5
Average ($n=3$) recovery (%) obtained for the derivatized target analytes spiked in Milli-Q water and LOD for Milli-Q blank samples. AA-EQS and EQS-MAC values have been included.

Analyte	Recovery (%)	LOD (ng L^{-1})	AA-EQS/EQS-MAC (ng L^{-1})[a]
4tOP	97	55	100/not applicable
NPs	121	1	300/2000
nOP	99	1	–
BPA	69	12	–
DES	84	1	–
ADT	88	1	–
E1	94	1	–
EQ	99	1	–
E2	101	1	–
TT	85	5	–
MeEE2	97	5	–
EE2	94	1	–
E3	120	1	–
EQN	102	8	–

[a]Data from Directive 2008/105/EC of the European Parliament and of the Council.
–: not proposed.

highest molecular weight PAHs and PCBs. Thus, separation of the derivatized and non-derivatized analytes was not possible and pure EtOAc was chosen since it is easier to evaporate compared to MeOH:acetone (50:50, v/v) and no need of mixture preparation was necessary. Besides, in order to completely elute all the target analytes, the addition of 8 mL of *n*-hexane was necessary. Recoveries improved up to 35% for PAHs and ~100% for PCBs after addition of *n*-hexane.

3.3. Validation of the method using Milli-Q water

Once the SPE procedure and the LVI-PTV parameters were optimized, the method validation was carried out. As no certified reference materials are available for the target compounds, spiked Milli-Q water was used to obtain the recoveries and relative standard deviations (RSD) of the method. Milli-Q water was spiked at 1000 ng L^{-1} for PEs, 400 ng L^{-1} for APs, 200 ng L^{-1} for BPA, 80 ng L^{-1} for hormones and 100 ng L^{-1} for PCBs, PBBs, pesticides and PAHs.

Experimental calibration curves were performed in the range of 250–40,000 ng L^{-1} for PEs, 20–1200 ng L^{-1} for NPs, 10–800 ng L^{-1} for BPA, 6–200 ng L^{-1} for hormones and 15–8000 ng L^{-1} for PCBs, PBBs, PAHs and pesticides. The signals obtained were corrected with their corresponding deuterated analogues and r^2 values higher than 0.990 were obtained for most of the analytes.

The average ($n=3$) recoveries obtained (see Table 5 for the derivatized compounds and Table 6 for the non-derivatized analytes) were in the 80–120% range for all the target analytes, except for PCB 101, PCB 118, PCB 153, PCB 200 and B[b]F that were around 65%, and PCB 18 and Ace that were 166 and 135%, respectively. In the case of PCBs, the best recovery values were obtained without correction with the corresponding surrogate. Deuterated PAHs used for correction did not seem to be the best surrogates in the case of most of the PCBs. The values obtained were similar to those found in the literature, as can be observed from the values included in Table 1.

RSDs in the 10–25% range were obtained for most of the analytes, similar to the values obtained in the literature (see Table 1).

LODs were calculated as the signal of the average signal of three blanks plus 3 times the standard deviation. When no area was observed at the retention time of the target analytes, 3 times the baseline noise was used as signal for LOD calculation. The values are included in Table 5 for the derivatized and in Table 6 for the non-derivatized analytes, respectively.

Glass OASIS-HLB cartridges were also studied in order to improve LODs for target analytes such as PEs and BPA but no significant improvement was observed and plastic cartridges were further used.

In general, the LODs obtained for all the target analytes were in the low ng L^{-1} levels except for PEs. All those values were similar to the ones observed in the literature for pesticides (0.5–25 ng L^{-1}) [21,43,44], PAHs (0.2–10 ng L^{-1}) [37,43], PCBs (4–43 ng L^{-1}) [37], BPA (12 ng L^{-1}) [21,37], APs (1–55 ng L^{-1}) [7,21,37], hormones (6–9 ng L^{-1}) [37,45] and PEs (7–736 ng L^{-1}) [7,37]. Other works show better LODs for analytes such as BPA [33,45] or estrogens [46], but it should be underlined that in this work up to 75 analytes were simultaneously extracted although analyzed after two injections.

3.4. Matrix effect

In order to study possible matrix effects during the analysis of wastewater samples, influent and effluent samples collected at the WWTP of Galindo (Bizkaia) were spiked at 100 ng L^{-1} (PCBs, PBBs, Ala, Trf, Atr, Sim, DES, EQN and E3), 250 ng L^{-1} (Clor), 500 ng L^{-1} (4tOP), 700 ng L^{-1} (PAHs), 800 ng L^{-1} (α-HCH, β-HCH, γ-HCH and δ-HCH), 1000 ng L^{-1} (2,4′-DDD, 4,4′-DDD, 2,4′-DDE, 4,4′-DDE, 2,4′-DDT, 4,4′-DDT, Clorf, BPA, ADT, EQ and nOP), 2000 ng L^{-1} (E1), 9000 ng L^{-1} (NP, E2 and MeEE2) and 40,000 ng L^{-1} (PEs) concentration and submitted to the optimized pre-concentration and detection steps. Spiking concentrations were chosen as 10 times the concentrations observed for the non-spiked samples, and when no signal was observed in the sample 100 ng L^{-1} concentration was chosen for the spiking. The signals obtained were compared to those obtained with Milli-Q water spiked at the same concentration, after substraction of the signal observed in the non-spiked aliquots. The values obtained for diluted (1:25) and non-diluted samples can be observed in Figs. 5 and 6 for the influent and effluent, respectively.

Different situations could be observed. Firstly, for certain analytes (see Ant and DOP in Figs. 5 and 6) good results were obtained both for diluted and non-diluted samples after correction with the corresponding deuterated analogue. For several analytes, dilution of the sample improved the results (see B[a]A, Cry, Ind and DES in Fig. 5). Finally, in the case of most of the derivatized analytes, recoveries were lower than 100%, even after dilution of the sample.

According to the results, we concluded that there was a matrix effect either during the extraction or the derivatization step. According to the literature, different approaches can be used in order to overcome the matrix effect. In some cases, dilution of the samples was used [47] but it did not work in our case for all the analytes. In other works, matrix-matched calibration was used [21] and finally, in others, a clean-up of the sample was performed either using GPC [11] or SPE clean-up using different cartridges such as silica, diol or Florisil [22,29,32,36]. In our case, and due to a previous experience of our research group [36], 1-g Florisil cartridges were studied both for influent and effluent water samples (see Fig. 7). In the work by Vallejo et al. [36] estrogenic compounds were only determined and in the present work in order to elute more non-polar compounds, a previous elution with (65:35) n-hexane:toluene was included [48]. As can be observed, good results were obtained for most of the analytes except for PBBs, 4,4′-DDT and Phe. In order to improve those results, 2-g cartridges were studied, but no significant improvement was observed.

RSD values for wastewater samples were higher than those observed for Milli-Q water, in the range of 12–30%.

3.5. Application to real samples

Monitorization of the target analytes was carried out in three different WWTPs (Bakio, Galindo and Gernika) as well as in a WPP (Zornotza), all of them located in Biscay (North of Spain). The choice

Table 6
Average ($n = 3$) recovery (%)obtained for the non-derivatized target analytes spiked in Milli-Q water and LOD for Milli-Q blank samples. AA-EQS and EQS-MAC values have been included.

Analyte	Recovery (%)	LOD (ng L^{-1})	AA-EQS/EQS-MAC (ng L^{-1})[a]	Analyte	Recovery (%)	LOD (ng L^{-1})	AA-EQS/EQS-MAC (ng L^{-1})[a]
PCB 43	97	5	–	Clor	112	56	30/100
PCB 52	84	4	–	Trf	76	1	30/not applicable
PCB 65	102	4	–	Ala	83	11	300/700
PCB 101	57	2	–	Sim	86	1	1000/4000
PCB 118	53	3	–	Atr	89	10	600/2000
PCB 138	82	3	–	DMP	78	156	–
PCB 142	115	3	–	DEP	94	122	–
PCB 149	122	1	–	DBP	94	198	–
PCB 153	53	2	–	BBP	84	322	–
PCB 180	108	4	–	DEHP	100	130	1300/not applicable
PCB 194	109	3	–	DOP	101	136	–
PCB 200	54	4	–	Nap	104	3	2400/not applicable
PBB 7	95	5	–	Acy	94	1	–
PBB 31	115	3	–	Ace	135	7	–
PBB 103	83	6	–	Flu	109	1	100/1000
PBB 153	85	8	–	Phe	90	3	–
α-HCH	68	3	20/40	Ant	98	5	100/400
β-HCH	89	1		Flr	68	1	100/1000
γ-HCH	103	4		Pyr	108	6	–
δ-HCH	98	1		B[a]A	83	8	–
2,4′-DDE	101	4	–	Cry	128	6	–
4,4′-DDE	94	6	–	B[b]F	59	28	Σ30/not applicable
2,4′-DDD	77	25	–	B[K]F	103	6	
4,4′-DDD	101	12	–	B[a]P	72	7	50/100
2,4′-DDT	93	9	25/not applicable	D[a,h]A	121	6	–
4,4′-DDT	98	11	10/not applicable	B[ghi]P	104	4	Σ2/not applicable
Clorf	96	7	100/300	Ind	93	5	

[a] Data from Directive 2008/105/EC of the European Parliament and of the Council.
–: not proposed.

of the WWTPs was a function of their geographical situation and the amount and type of wastewater treated (i.e. rural, industrial or urban sources). Galindo is the largest WWTP in Biscay. This plant handles a daily average wastewater flow of 350,000 m^3 with a maximum throughput of 12 m^3 s^{-1} that is discharged into the Nerbioi–Ibaizabal estuary. This plant collects industrial and urban wastewater from the metropolitan and surrounding areas of Bilbao. The WWTP located in Gernika collects similar types of water as Galindo but population is lower, and WWTP in Bakio is quite different. In Bakio the flow of wastewater changes from summer (2400 m^3 day^{-1}) to winter (480 m^3 day^{-1}) due to the evident population increase during the summer period, which changes from ~2000 to 12,000 habitants [49].

PBBs and PBDEs were at levels lower than the LODs of the developed method at all the sampling stations and campaigns and were not included in Table 7. In the case of PCBs, CB-43 was found at the highest concentration in all the sampling points, except for Gernika, where the higher chlorinated isomers (CB-142, CB-138 and CB-180) were found at the highest concentration. The abundance of CB-43 isomer could be attributed to the degradation of higher chlorinated isomers [50]. Concentration of CB-43 was higher in the effluent than in the influent in all the cases except for Gernika. The concentration sum of the seven studied PCBs was in the 49–1616 ng L^{-1} range, much higher than those reported in Norway (3.3–4.1 ng L^{-1}) [7] but similar to those found in Greece (100 ng L^{-1} average) [51] and in Paris (380–1300 ng L^{-1}) [52].

In the case of PEs, in most of the cases, DBP, DEP and DMP were found at the highest concentration, while DEHP was found at concentrations lower than the LOD in most of the cases and when detected, the values were lower than the Environmental

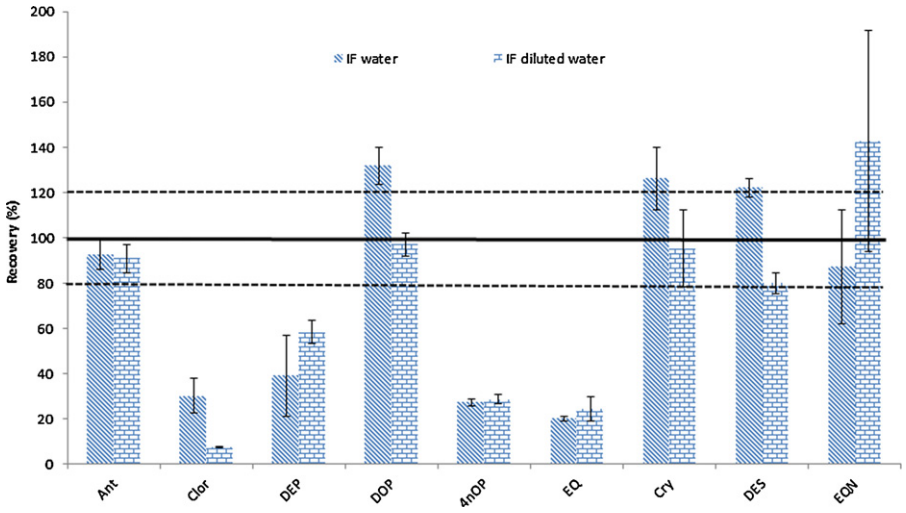

Fig. 5. Recovery of the target analytes in influent water sample without clean-up.

Table 7
Maximum and minimum concentrations (ng L^{-1}) found in both influent (IF) and effluent (EF) wastewaters of the 3 WWTPs (Bakio, Galindo and Gernika) and the WPP (Zornotza) from March to July 2011.

Family	Analyte	Bakio				Galindo				Gernika				Zornotza			
		EF		IF		EF		IF		EF		IF		EF		IF	
		MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
PEs	DMP	<LOD	7183	<LOD	7471	6250	11,691	310	9130	2068	11,223	361	93,332	7974	13,637	3940	10,553
	DEP	226	400	2109	5757	201	1023	139	5910	361	1056	124	4448	147	508	<LOD	956
	DBP	217	7917	226	1987	<LOD	10,897	<LOD	764	213	1343	593	2241	198	1897	233	1541
	BBP	<LOD	<LOD	246	312	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1141	1141	<LOD	<LOD
	DEHP	391	413	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	307	307	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	DOP	205	30,755	235	5903	215	266	266	266	233	292	220	252	3259	9063	<LOD	3538
Pest 1	2,4′-DDE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5	219	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4,4′-DDE	7	9	<LOD	<LOD	<LOD	<LOD	1259	2097	7	223	8	101	<LOD	<LOD	<LOD	<LOD
	2,4′-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4,4′-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	603	1057	74	295	18	72	<LOD	<LOD	<LOD	<LOD
	2,4′-DDD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	197	562	98	171	75	75	<LOD	<LOD	<LOD	<LOD
	4,4′-DDD	35	98	<LOD	<LOD	<LOD	<LOD	642	1701	37	226	45	80	<LOD	<LOD	<LOD	<LOD
Pest 2	alpha-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	beta-HCH	156	204	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	139	195	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	ganma-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	684	1900
	delta-HCH	321	392	<LOD	279	<LOD	<LOD	<LOD	<LOD	32	386	27	27	3	5	<LOD	<LOD
Pest 3	Trf	<LOD	<LOD	<LOD	<LOD	7	1561	5	3841	2	3	3	4	2	2	2	2
	Simazine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1	9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Atr	23	33	<LOD	263	<LOD	<LOD	12	16	67	79	87	104	38	55	31	47
	Ala	18	18	ND	<LOD	<LOD	<LOD	22	27	13	18	21	48	13	17	12	13
	Clor	60	<LOD	254	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Clorf	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PAHs	Nap	<LOD	69	<LOD	55	<LOD	15	<LOD	11	<LOD	<LOD	<LOD	<LOD	<LOD	4	<LOD	5
	Acy	<LOD	277	<LOD	177	<LOD	6	<LOD	8	<LOD	1	<LOD	742	<LOD	19	<LOD	16
	Ace	<LOD	135	<LOD	334	<LOD	18	<LOD	<LOD	<LOD	22	<LOD	14	<LOD	<LOD	<LOD	<LOD
	Flu	<LOD	27	<LOD	41	<LOD	20	<LOD	68	<LOD	20	<LOD	68	<LOD	<LOD	<LOD	1
	Phe	<LOD	<LOD	<LOD	<LOD	<LOD	11	<LOD	44	<LOD	11	<LOD	44	<LOD	<LOD	<LOD	<LOD
	Ant	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8	<LOD	<LOD	<LOD	8	<LOD	<LOD	<LOD	<LOD
	Flr	<LOD	4	<LOD	5	<LOD	4	<LOD	16	<LOD	5	<LOD	16	<LOD	5	<LOD	4
	Pyr	<LOD	<LOD	<LOD	<LOD	<LOD	106	<LOD	310	<LOD	106	<LOD	310	<LOD	2	<LOD	<LOD
	B(a)A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2	<LOD	7	<LOD	1	<LOD	<LOD
	Cry	<LOD	<LOD	<LOD	<LOD	<LOD	612	<LOD	21	<LOD	1	<LOD	6	<LOD	1	<LOD	<LOD
	B(b)F	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3	<LOD	5	<LOD	3	<LOD	<LOD
	B(k)F	<LOD	43	<LOD	57	<LOD	130	<LOD	120	<LOD	130	<LOD	120	<LOD	98	<LOD	107
	B(a)P	<LOD	<LOD	<LOD	<LOD	<LOD	69	<LOD	8	<LOD	15	<LOD	8	<LOD	13	<LOD	13
	D(a,h)A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4	<LOD	<LOD	<LOD	<LOD
	B(ghi)P	<LOD	5	<LOD	7	<LOD	300	<LOD	77	<LOD	11	<LOD	77	<LOD	20	<LOD	<LOD
	Ind pyr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PCBs	pcb 43	55	329	7	81	85	636	9	294	18	143	53	461	214	325	43	63
	pcb 52	<LOD	<LOD	11	11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	pcb 65	<LOD	<LOD	15	15	<LOD	<LOD	<LOD	<LOD	10	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	pcb 101	2	3	5	107	4	9	6	7	4	147	2	22	3	6	<LOD	<LOD
	pcb 142	0.6	1	10	83	<LOD	<LOD	<LOD	<LOD	5	321	4	55	6	6	<LOD	<LOD
	pcb 138	<LOD	0,5	8	57	<LOD	<LOD	<LOD	<LOD	6	393	5	62	<LOD	<LOD	<LOD	<LOD
	pcb 180	23	35	55	76	4	91	34	44	5	602	6	90	59	92	39	52
BPA	BPA	12	974	34	270	20	248	374	10,424	92	2615	119	2835	79	36,228	21	1940
Hormones	DES1	1.0	1.5	<LOD	<LOD	ND	<LOD	3.6	7.9	1.2	3.2	86	157	<LOD	<LOD	<LOD	<LOD
	DES2	<LOD	<LOD	<LOD	6	ND	<LOD	<LOD	<LOD	7	7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	ADT	11	169	30	94	<LOD	<LOD	30	30	21	5604	<LOD	<LOD	6	6	18	18
	E1	<LOD	78	2	42	2	1031	1	183	48	574	2	178	<LOD	23	<LOD	57
	E2	4	274	3	22	<LOD	<LOD	76	449	22	54	5	16	<LOD	<LOD	<LOD	<LOD
	TT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	15	58	7	411	<LOD	<LOD	<LOD	12
	EQN	20	188	82	331	<LOD	<LOD	10	133	<LOD	702	<LOD	<LOD	<LOD	<LOD	<LOD	668
	MeEE2	<LOD	<LOD	6	234	<LOD	3894	<LOD	<LOD	35	53	24	72	16	174	25	363
	NT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	74	74	4	4	<LOD	<LOD	9	9
	EE2	7	40	3	29	<LOD	232	3	40	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	E3	3	210	2	83	2	<LOD	43	81	5	21	6	23	3	6	4	7
APs	tOP	157	2959	164	3720	477	62,148	222	1786	2087	47,326	763	20,890	80	964	294	1117
	NP	3	516	3	203	16	1230	2	132	2	28,917	4	18,896	2	208	1	88
	OP	10	137	79	514	<LOD	<LOD	390	2994	172	4912	264	2629	318	484	<LOD	<LOD
	4nNP	259	359	69	81	<LOD	<LOD	28	90	9	18	4	176	66	91	16	98

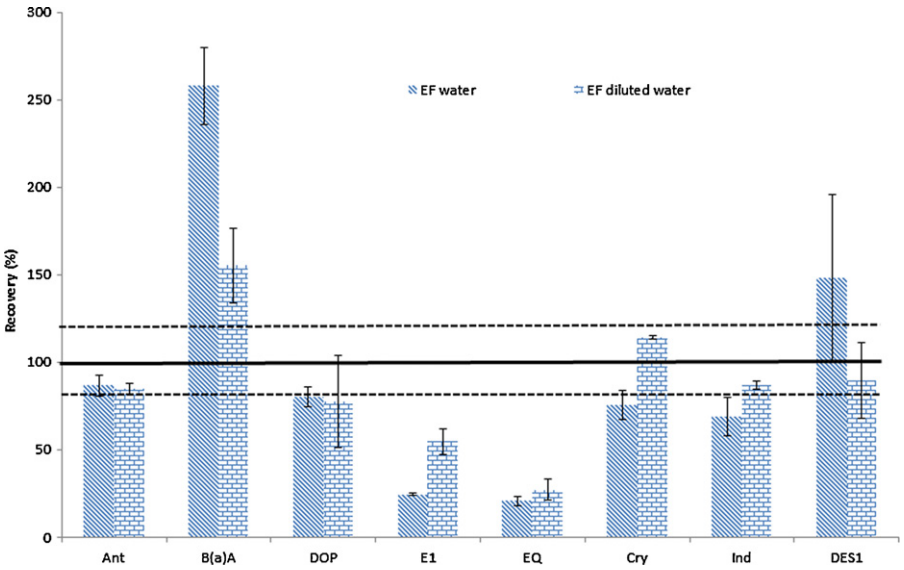

Fig. 6. Recovery of the target analytes in effluent water sample without clean-up.

Quality Standard expressed as annual average value (AA-EQS) of 1300 ng L^{-1} established by the WFD [2]. The concentration of the sum of 6 PEs in the influents and effluents of the three WWTPs and the WPP were similar to those found in five WWTPs in Norway (1.3–23 μg L^{-1}) [7] and lower than those found in Denmark (14–46 μg L^{-1}) [5], in Finland (98–122 μg L^{-1}) [53] or in Catalonia (12–83 μg L^{-1}) (Spain) [54]. Similarly to Vogelsang et al. [7] and Prieto et al. [37], DEP was found at the highest concentrations in most of the samples.

In the case of PAHs, the sum of the 16 isomers studied was in the LOD-1427 ng L^{-1} range, similarly to the values reported in Norway (200–1300 ng L^{-1}) [7] and lower than those found in Catalonia (Spain) (1341–2172 ng L^{-1}) [54]. Concentrations of Ant, Nap and B[a]P never exceeded AA-EQS values and only the sum of B[b]F and B[k]F and the sum of B[ghi]P and Ind exceeded in a few samples these AA-EQS values. The most carcinogenic B[a]P was found at low concentrations, in the LOD-7 ng L^{-1} range, similarly to the values observed in Norway [7].

In the case of APs, AA-EQS values were exceeded in the three WWTPs studied for both NPs and nOP and in some cases even the Environmental Quality Standard expressed as a maximum allowable concentration (EQS-MAC) was exceeded for NPs. APs concentrations were usually higher at the effluent than at the influent. NPs concentrations were in the LOD-1230 ng L^{-1} range, lower than those found by Fauser et al. in Denmark (2800–10,200 ng L^{-1}) [5], Sánchez-Avila et al. (658–11,361 ng L^{-1}) [54] and Vogelsang et al. in Norway (200–7000 ng L^{-1}) [7], but similar to those found in Portugal (680–2120 ng L^{-1}) [55]. Concentrations for nOP (78–2487 ng L^{-1}) were similar to those found in Catalonia (603–3690 ng L^{-1}) [53] but much higher than those found in Portugal (3.7–52 ng L^{-1}) [55].

BPA concentrations in the four sampling points were in the 12–7048 ng L^{-1}, much higher than those found in Las Palmas de Gran Canarias (6.4–13.8 ng L^{-1}) [45], in Portugal (120–461 ng L^{-1}) [55] and in a municipal WWTP in Greece (160–220 ng L^{-1}) [33] and more similar to those found in Catalonia (603–3690 ng L^{-1}) [54].

Concentration ranges for the hormones are included in Table 7. Except for some outlier values, in general, the concentrations found for E2, E1, E3, MeEE2 and EE2 are in the low ng L^{-1} range and are similar to those found in the literature [3,37,45,46,56–59].

DDT isomers were mostly detected in the WWTP located in Gernika. Concentrations for 4,4′DDT were in the 74–295 ng L^{-1}

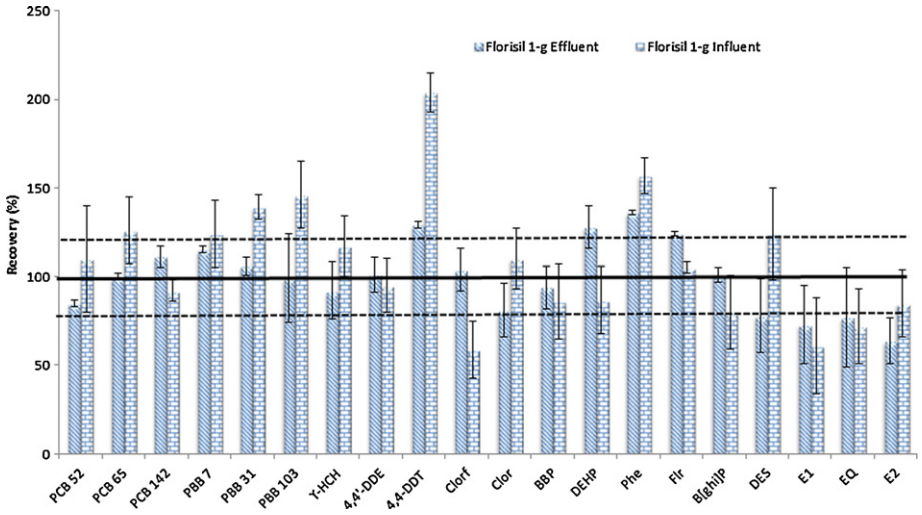

Fig. 7. Recovery of the target analytes in influent and effluent water samples after Florisil clean-up.

(effluent) and in the 8–101 ng L^{-1} (influent), always exceeding the AA-EQS value (10 ng L^{-1}) and concentrations observed in Catalonia (Spain) [54].

In the case of hexachlorocyclohexane isomers, the concentrations detected were lower than the LOD in most of the sampling sites and campaigns but, when detected, concentrations exceeded both the EQS-AA and EQS-MAC established by the WFD. Concentrations found for γ-HCH were always lower that the LOD and lower than those found in Catalonia (Spain) [54].

For the rest of pesticides monitored (Clorf, Clor, Atr, Sim, Ala and Trf) concentrations were lower than EQS-AA and EAS-MAC in all the cases. Concentrations for Sim (LOD-9 ng/L) were much lower than those found in Ireland (<17–510 ng L^{-1}) but similar to those found in China [60]. Concentrations for Atr (LOD-263 ng L^{-1}) were similar to those reported in Ireland (<17–190 ng L^{-1}) [44] and in China (184–238 ng L^{-1}) [60]. Finally, concentrations for Ala (LOD-48 ng L^{-1}) were in the same range as those detected in China (17–74 ng L^{-1}) [60].

4. Conclusions

A new method was developed for the analysis of several priority and emerging organic pollutants based on SPE and LVI-PTV–GC–MS. The analysis method includes 75 analytes (PCBs, PBBs, PAHs, pesticides, PEs, BPA, APs and hormones). The simultaneous analysis in a single injection was not possible since losses of the most volatile analytes occurred during the derivatization step and the separation on the SPE step was not possible. For this reason, after elution from the clean-up step, the eluate was divided into two aliquots, one for the non-derivatized analytes (PAHs, PCBs, PBBs, PEs, PBDEs and pesticides) determination and the second aliquot for the derivatized analytes (hormones, NPs and BPA) determination.

Matrix effect in wastewater samples could not always be corrected by the use of deuterated analogues or dilution of the sample. The best results were obtained after the clean-up of the sample using Florisil cartridges.

LODs and LOQs obtained for all the analytes were at low ng L^{-1} level except for PEs that were at mid ng L^{-1} concentrations. All the values were similar to those observed in the literature.

The developed method was applied to wastewater samples from 3 WWTPs and 1 WPP of northern Spain and concentrations were compared to environmental quality standards (when available) or concentrations found in other locations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2012.05.022.

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