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Microextraction with polyethersulfone for bisphenol-A, alkylphenols and hormones determination in water samples by means of gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry analysis

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ABSTRACT

In the present work, the suitability of polyethersulfone (PES) tube was assessed for the simultaneous sorptive microextraction of commonly found endocrine disrupting compounds in natural waters such as bisphenol-A (BPA), nonylphenol technical mixture (NP mix), 4-*tert*-octylphenol (4tOP), 4-*n*-octylphenol (4-*n*OP), 17β-estradiol (E2) and 17α-ethynilestradiol (EE2). After the concentration of target compounds in the PES polymer, the analytes were recovered soaking the polymer with a suitable solvent (ethyl acetate or methanol), derivatized using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA + 1% TMCS) and determined by gas chromatography–mass spectrometry (GC–MS). The analysis was also performed without derivatization step by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS). Extraction parameters (addition of MeOH, ionic strength, extraction speed and time and desorption time) were evaluated and the optimum conditions were fixed as follows: 150 mL water samples containing a 10% (w/v) of sodium chloride and using 5 tubular PES sorbent fibers (1.5 cm length × 0.7 mm o.d.). Equilibrium conditions were achieved after 9 h, with absolute extraction efficiencies ranging from 27 to 56%. On the whole, good apparent recoveries were achieved (68–103% and 81–122% for GC–MS and LC–MS/MS, respectively) using deuterated analogues as surrogates. Achieved quantification limits (LOQs) varied between 2–154 ng/L and 2–63 ng/L for all the compounds using GC–MS and LC–MS/MS, respectively. The effect of organic matter was evaluated previous to apply the final method to the analysis of estuarine and wastewater real samples. The comparison of both methods showed that overall, PES-LC–MS/MS provided shorter sample preparation time and better LODs, but PES-*silylation*-GC–MS allowed the simultaneous determination of all the studied compounds with adequate repeatability and accuracy.

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1. Introduction

For about more than 20 years, an increasing input of new organic pollutants has been detected in the aquatic environment. Pharmaceutical residues, estrogens, pesticides and their metabolites generate together with the priority pollutants, a complex cocktail of chemicals with mostly unknown risks and consequences for the human and environmental health [1].

One of the important steps towards a realistic and cost–benefit implementation of the European Union Water Framework Directive (EU WFD 2013/39/EU) should be not only the identification but also the robust determination of the most hazardous environmental contaminants. Currently, according to the WFD the list of priority substances contains only 45 contaminants and/or contaminant groups, which is only a small fraction of a much larger number of possible hazardous pollutants that eventually reach aquatic systems [2]. From this list of 45 priority substances, a group of 21 priority hazardous compounds have been identified as being toxic, persistent and bioaccumulative substances or giving and equivalent level of concern. Alkylphenols (APs), essentially nonyl and octylphenols, and other endocrine disrupting compounds (EDCs) are included in the list but some other contaminants, such as natural and synthetic estrogens, are still out of the priority list. Bisphenol-A (BPA) is among the substances to watch. The presence of EDCs in aquatic systems is often attributed to the discharges of wastewater treatment plants (WWTPs) [3–5] but this type of scenario is deemed highly complex due to the mixture of contaminants and the high variability of their concentrations.

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One of the key issues is precisely to establish safe levels of chemical exposure, i.e. with no appreciable health risk to humans, but little is known about the fate and effects of emerging contaminants (EC) mainly due to the large diversity of chemical structures and the complexity of the chemical analysis. In fact, the literature shows that long-term exposure of trace pollutants and EC can cause adverse health effects in most organisms at very low concentrations (low ng/L) [6–8].

Among the different operational units of an analytical procedure, sample preparation, including analyte enrichment and matrix component removal, is one of the most important steps to achieve fit-for-purpose analytical results [9]. Despite recent advances in this field, conventional sample preparation procedures such as liquid–liquid extraction (LLE) or even solid-phase extraction (SPE) consume large amounts of organic solvents and require high labour-effort and time consuming because their automation grade is still low. Hence, the analytical efforts are directed to the development of fast and environmentally friendly methods for the analysis of organic pollutants in different environmental compartments, which at the same time provide accurate results and low detection limits [9,10]. One of the most used strategies for the determination of trace organic compounds in liquid matrices is centered on the miniaturization and automation of sample preparation techniques [11–15]. In addition to the well-established microextraction techniques, mainly solid phase microextraction (SPME) [11,16–18] and stir bar sorptive extraction (SBSE) [19–27], polyethersulfone (PES) polymer has emerged recently [14,28,29] as a very suitable choice for the extraction of polar species, improving significantly the extraction efficiencies provided by other polymeric materials such as polydimethylsiloxane (PDMS), among others.

The combined use of these outstanding microextraction techniques with sensitive and reliable chromatographic techniques is always necessary in order to develop analytical methods able to meet legislation requirements. Currently, the suitability of gas chromatography–mass spectrometry (GC–MS) for the identification of a wide variety of organic contaminants in several environmental matrices is widely proved in many research works [30–32]. However, the analysis of polar compounds such as hormones requires a previous derivatization step. On the contrary, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) does not require the derivatization step and offers the possibility to identify and quantify simultaneously a wide variety of organic contaminants of different polarities [33,34].

In the framework of the development validated analytical procedures to support the monitorization of natural waters and to assess the impact of chronic exposure to EDCs, the main aim of this work was to study the effectiveness of PES tubes for the simultaneous extraction of trace amounts of BPA, APs and some hormones from water samples. Therefore, parameters affecting the performance of the desorption and the extraction processes were evaluated and the extracts were quantified by both GC–MS, through a previous derivatization of the analytes, and LC–MS/MS. This way the suitability of both analytical techniques could be compared. Finally, the matrix effect required a further analysis in order to overcome the presence of organic matter in the aquatic samples and to apply the final methods to the analysis of estuarine waters and WWTPs effluents.

2. Experimental section

2.1. Reagents and materials

The target analytes, including names, abbreviations, CAS number and chemical structures are listed in Table 1.

4-*n*-Octylphenol (4nOP), [$^2H_4$]-4*n*-nonylphenol ([$^2H_4$]-NP), bisphenol-A (BPA), 17β-estradiol (E2), [$^2H_3$]-17β-estradiol ([$^2H_3$]-E2) were obtained from Sigma-Aldrich (Steinheim, Germany). Nonylphenol technical mixture (NP mix, Pestanal®) was purchased from Fluka (Steinheim, Germany), [$^2H_{16}$]-biphenol-A ([$^2H_{16}$]-BPA) and 4-*tert*-octylphenol (4-tOP) from Supelco (Walton-on-Thames, UK) and 17α-ethynylestradiol (EE2) from Riedel-de Haën (Seelze, Germany). Stock solutions for each compound and deuterated analogues were dissolved individually in anhydrous methanol in order to prepare approximately 5000 mg/L stock solutions. Chemical standards were stored at 4 °C in the dark, and stock solutions were stored at −20 °C in amber vials. 100 mg/L dilutions were prepared in anhydrous methanol monthly. Dilutions at lower concentrations were prepared daily, according to the experimentation.

Anhydrous methanol (MeOH, HPLC grade, 99.9%, Labscan, Dublin, Ireland) and sodium chloride (NaCl, Merck, Darmstadt, Germany) were used for matrix modification experiments. Humic acids (technical grade) used for matrix effect assays were obtained from Fluka (Sigma-Aldrich, Germany).

N,*O*-Bis (trimethylsilyl)trifloroacetamide with 1% of trimethylchlorosilane (BSTFA+1% TMCS, Sylon BFT, 99:1) was used as the derivatization reagent and was purchased from Supelco.

Ultra-pure water was obtained using a Milli-Q water purification system, (<0.057 S/cm, Milli-Q model 185, Millipore, Bedford, MA, USA). Ethyl acetate (EtOAC) (HPLC grade, 99.9%) was obtained from Lab Scan (Dublin).

MeOH (Optima®, LC–MS quality) used as mobile phase eluent in LC–MS/MS was obtained from Fisher scientific (Geel, Belgium). Ammonia (25% as NH_3, Panreac, Reixac, Barcelona, Spain) was used for mobile phase modifications.

Extracts were filtered before analysis with Acrodisc syringe (13 mm diameter, 0.2 μm pore size) filters (GHP membrane or PTFE) obtained from Pall Life Sciences (USA).

PES tube material used was acquired from Membrane (Wuppertal, Germany) in a tubular format (0.7-mm external diameter, 1.43 g/mL density). Pieces of this polymer (1.5 cm length, ~2 mg) were cut using a sharp blade and soaked overnight in EtOAC previous to their use as sorbent material. Afterwards, the polymer was dried with air and stored until used. Given their reduced cost (c.a. 0.05 €/unit), sorbents were discarded after each use.

2.2. Sampling

Estuarine water samples were collected from the estuary of Bilbao (+43°15′26.23″, −2°55′37.82″, Bay of Biscay, Spain) whereas wastewater samples were collected at the effluent of the WWTP of Galindo (+43°18′19.32″, −2°59′50.88″, Bay of Biscay, Spain), both in October 2013. Samples were collected in pre-washed amber bottles and carried to the laboratory in cooled boxes (4 °C). Samples were filtered through 0.45 μm cellulose filters (Whatman, Kent, UK) and kept in the fridge at 4 °C before treatment, which was performed within 24 h.

2.3. Extraction, desorption and derivatization procedures

Sample preparation conditions were optimized with 150 mL aliquots of ultrapure water (Milli-Q), previously fortified at 1 ng/mL with the target analytes, considering different experimental conditions (ionic strength, addition of organic modifier, stirring rate, extraction time and desorption time). According to the final method, water samples (150 mL) were directly poured into 150 mL extraction vessels with 10% NaCl (w/v). Afterwards, 5 pre-cleaned pieces of PES in tube format (see Section 2.1) and a PTFE covered stirrer was introduced in the vessel. Thereafter, vessels were closed and extraction was performed at room temperature with a stirring rate of 1200 rpm and overnight using a 15 position magnetic stirring plate from Gerstel (Mülheim an der Ruhr, Germany). Once the sorption step was over, the polymers were removed and rinsed with Milli-Q water in order to eliminate salt residues, and finally, dried with a clean tissue. Subsequently, the sorbents were chemically desorbed using

Table 1
Studied analytes including CAS number, chemical structures, log kow, reacting functionality and *m*/*z* values of fragment ions (GC–MS, quantifier, qualifier).

Analyte	CAS number	Chemical structure	Log k_{ow}	Reacting functionality	*m*/*z*
4-*tert*-Octylphenol [a] (4tOP)	140-66-9		5.28	–OH	207, 208
Nonylphenols mixture [a] (NP mix)	104-40-5		5.76	–OH	193, 221
4-*n*-Octylphenol [a] (4nOP)	1806-24-4		4.12	–OH	179, 278
[$^{2}H_4$]-Nonylphenol ([$^{2}H_4$]–NP)	358730-95-7			–OH	183, 296
[$^{2}H_{16}$]-Bisphenol A ([$^{2}H_{16}$]–BPA)	96210-87-6			2 × (–OD)	368, 386
Bisphenol-A [b] (BPA)	80-05-7		3.32	2 × (–OH)	357, 358
[$^{2}H_3$]-17β-Estradiol ([$^{2}H_3$]–E2)				2 × (–OH)	419,285

Table 1 (*continued*)

Analyte	CAS number	Chemical structure	Log k_{ow}	Reacting functionality	m/z
17β-Estradiol [c] (E2)	50-28-2	CH3, OH, HO	4.01	2 × (–OH)	416, 285
17α-Ethynyl estradiol [c] (EE2)	57-63-6	CH3, OH, HO	3.67	2 × (–OH)	425, 440

[a] Corrected with [$^2H_4$]-Nonylphenol.
[b] Corrected with [$^2H_{16}$]-Bisphenol A.
[c] Corrected with [$^2H_3$]-17β-Estradiol.

appropriate organic solvent. To this aim the polymers were introduced into an amber eppendorf tube containing 300 μL of the corresponding desorption solvent and soaked for 16 min in an ultrasound bath (USB Axtor by Lovango). After liquid desorption, the polymers were removed, the extract was quantitatively recovered and analyzed by means of GC–MS and LC–MS/MS.

In the case of GC–MS analyses, after the desorption step using 300 μL of EtOAC, the organic phase was transferred to a 2 mL vial, evaporated to dryness under a gentle stream of nitrogen in a Turbovap LV Evaporator (Zymark, Hopkinton, USA) and submitted to a derivatization step previous to the analysis.

For compound derivatization, pyridine (125 μL) and BSTFA + 1% TMCS (50 μL) were added to the evaporated extract and the mixture was shaken in a vortex and kept in an oven at 65 °C for 45 min. 2 μL of the extracts containing derivatized compounds were subsequently analyzed by means of GC–MS.

In the case of the assays performed by means of LC–MS/MS, the polymers were desorbed with 300 μL of MeOH filtered with GHP membrane filters and directly analyzed.

2.4. Gas chromatography–mass spectrometry analysis

2 μL of the derivatized extract was injected into a 6890N gas chromatographer (Agilent Technologies, Avondale, PA, USA) coupled to a 5973N electron impact ionization mass spectrometer and a 7683 Agilent autosampler. The analysis was performed in the splitless mode for 1.5 min at 300 °C using HP5-MS capillary column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, Avondale, PA, USA). The oven program temperature used was as follows: 60 °C (1.5 min), a temperature increase of 10 °C/min to 170 °C to continue rising at 15 °C/min to 300 °C, maintaining it for 5 min.

Hydrogen (Hydrogen generator AD-1020, Cinel Strumenti Scientifici, Padova, Italy) was used as carrier gas at constant flow of 1.3 mL/min. The MS transfer line temperature was maintained at 310 °C, the ion source and quadrupole at 230 °C and 150 °C, respectively. Measurements were performed in both SCAN (m/z: 50–525) and SIM modes and the reacting functionality and m/z values of fragment ions are also listed in Table 1.

2.5. Liquid chromatography tandem mass spectrometry with triple quadrupole detection

Underivatizated extracts were directly analyzed in an Agilent 1260 series HPLC equipped with a degasser, a binary pump, an autosampler and a column oven, and coupled to an Agilent 6430 triple quadrupole mass spectrometer equipped with ESI source (Agilent Technologies). Before analysis, all samples were filtered through 0.2 μm syringe PTFE microfilters.

The quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode. High purity nitrogen gas (99.999%, Air Liquide, Madrid, Spain) was used as nebulizer, drying and collision gas. MS/MS ionization parameters were set as follows: N_2 flow rate of 11 L/min, a capillary voltage of 4000 V, nebulizer pressure of 52 psi (358.5 kPa) and source temperature of 325 °C.

Fragmentor electric voltage and collision energy were optimized for ESI source, in the 70–175 V and 5–45 eV ranges (negative voltage) respectively, by injection of individual compounds.

MeOH: Milli-*Q* water with 0.05% NH_4OH mobile phase was used since recent results in the literature [35] have shown that more basic mobile phases (pH = 10.5) provided better sensitivity for APs determination.

Separation of analytes was carried out using an Agilent Zorbax Extend-C_{18} (2.1 mm, 50 mm, 1.8 μm) column (pH range 2.0–11.5). In all cases an UHPLC Zorbax Eclipse XDB-C_{18} pre-column (2.1 mm, 5 mm, 1.8 μm) was used. The column temperature was set to 35 °C. The injection volume was set at 10 μL and the flow rate at 0.2 mL/min.

Under optimized conditions a binary mixture consisting of Milli-*Q* water containing 0.05% NH_4OH (eluent A) and MeOH

containing 0.05% NH_4OH (eluent B) was used for gradient separation of target analytes. Linear gradient was as follows: 30% B maintained for 4 min, increased to 60% B in 3 min and to 80% B in 10 min and maintained constant for 18 min. Initial gradient conditions (30% B) were then achieved in 5 min where it was finally held for another 5 min.

Agilent 6430 Quantitative analysis software (Mass Hunter, version 05.02) was used for data treatment.

3. Results and discussion

3.1. Optimization of LC–MS/MS

MS/MS operating conditions for ESI in the negative ionization mode were optimized. For this purpose, fragmentor electric voltage and collision energy were studied in the 70–175 V and 5–45 eV ranges, respectively. Fragmentor electric voltage was chosen in order to maximize the signal of the quasi-molecular ion, while trying to minimize the formation of adducts. Cell accelerator voltage was also evaluated in the 1–7 V range (data not shown). Optimization was performed in the full scan MS mode. The fragmentor voltage and the collision energy were simultaneously optimized by means of the automatic “Optimizer” software (Mass Hunter software) option and the most sensitive two transitions were selected (see Table 2) being comparable to those found in the literature [36,37].

3.2. Optimization of sample preparation conditions

All the extracts were analyzed by means of GC–MS for the optimization of PES extraction conditions, according to the procedure described in Section 2.4.

First of all, the chemical desorption conditions were optimized in order to assure a quantitative release of the extracted analytes from PES tubes. In this case, the solvent composition and the volume were chosen and the extraction was accomplished in a standardized way, i.e. aliquots of 150 mL of deionised water samples (without NaCl or MeOH) spiked at 1 ng/mL were extracted for 4 h.

The used organic solvents may show high affinity to target compounds and may be compatible with the sorptive material. In the specific case of PES, since it is already known that PES may decompose with chlorinated solvents [28], the effectiveness of non-chlorinated polar solvents such as EtOAC and MeOH was evaluated. With this aim, chemical desorption of the sorbents was performed four times with 300 μL of EtOAC or MeOH each time for 8 min in an ultrasonic bath. Three hundred μL were chosen as the minimum volume which assures that all PES tubes were completely covered by any of the solvents. The percentage of analytes recovered in the first and second EtOAC fractions were between 85–100% of the total (sum of 1st and 2nd fractions) for all the target compounds (see Fig. 1 in the Supplementary material section). Similar results were obtained when MeOH was used (data not shown). Therefore, the desorption conditions were fixed as follows: a single desorption step with 300 μL of EtOAC (in the case of GC–MS analysis) or 300 μL of MeOH (in the case of LC–MS/MS analysis) for 16 min in an ultrasonic bath.

Thereafter, factors affecting analyte extraction were evaluated in order to achieve the optimum extraction conditions. The effect of the addition of an inert salt (NaCl) and an organic modifier (MeOH) in the extraction efficiency was simultaneously studied by means of an experimental design approach. To this aim, a Central Composite Design (CCD) was performed using the Statgraphics® Centurion XV program and covering a factor space of 0–25% for NaCl (w/v) and 0–20% for MeOH (v/v) (with three central points, i.e., 11 experiments). These assays were carried out using fortified Milli-Q water samples at 2 ng/mL concentration level and extracted overnight (ca. 12 h). The precision of the measurements was estimated from the three replicates of the central point, getting relative standard deviation (RSD %) values between 6–12% for the all studied analytes.

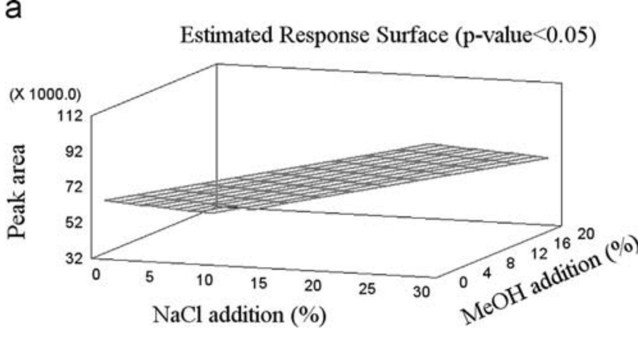
The responses obtained for the CCD were analyzed by means of multiple linear regression and response surface analysis including the significant variables (p-values <0.05). Fig. 1(a) and (b) show the response surfaces obtained for E2 and 4tOP, respectively. Overall, the addition of MeOH was statistically significant (p-value <0.05) and a negative effect was observed for all the studied compounds. This is in agreement with the literature for other sorptive extraction approaches [28,38,39]. The presence of MeOH may enhance the extraction efficiency of low polar compounds since the adsorption in the walls of glassware is reduced. On the other hand, lower extraction yields are usually expected for more polar compounds since the solubility increases in presence of an organic modifier. Therefore, in the present work no MeOH was added in further experiments.

In general, during sorptive extraction, it has been observed that for hydrophobic analytes (octanol/water partition coefficient, log $K_{ow} > 3.5$) the addition of NaCl does not improve, but even reduces, the extraction efficiency. However, polar analytes profit from higher ion strength of the sample solution and the response increases with the addition of inert salts [22]. Both trends can be

Table 2
Optimized conditions for LC–MS/MS analysis in terms of precursor ion, product ion, fragmentor, collision energy, cell accelerator voltage and polarity.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (eV)	Cell acelerator voltaje (V)	Polarity
[$^2H_4$]–NP	223.2	110.1	114	17	1	Negative
4tOP[a]	205.2	133.1	105	25	3	Negative
4tOP[b]	205.2	134.2	120	15	3	Negative
NP mix[a]	219.0	133.0	120	25	4	Negative
NP mix[b]	219.0	147.4	120	25	4	Negative
4nOP[a]	205.2	106.0	135	17	1	Negative
4nOP[b]	205.2	119	120	20	1	Negative
[$^2H_{16}$]–BPA	241.3	142.0	120	25	3	Negative
BPA[a]	227.1	212.1	100	13	5	Negative
BPA[b]	227.1	133	100	25	5	Negative
[$^2H_3$]–E2	274.2	145.0	150	41	1	Negative
E2[a]	271.2	145.1	135	41	3	Negative
E2[b]	271.2	183.2	135	41	3	Negative
EE2[a]	295.17	199.1	110	41	3	Negative
EE2[b]	294.9	269.0	81	25	3	Negative

[a] Quantifier.
[b] Qualifier.



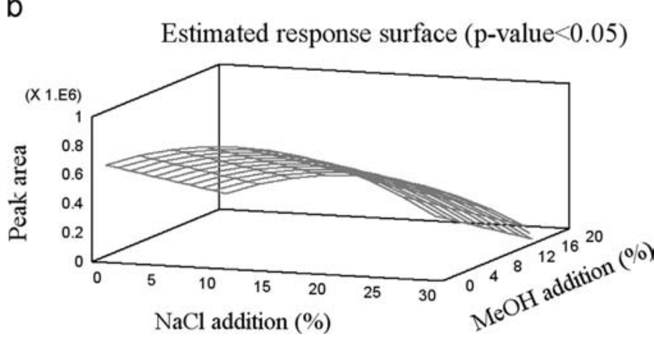


Fig. 1. Response surfaces obtained for (a) E2 and (b) 4tOP compounds after the study of NaCl and MeOH addition using a CCD design.

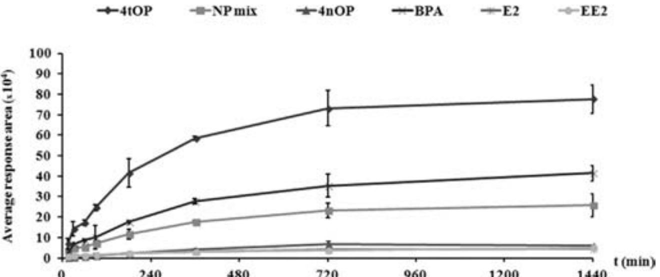


Fig. 2. Extraction time profiles ($n=3$) for the target compounds studied under the optimized conditions (10% NaCl and 1200 rpm).

explained on the basis of the salting out effect, which is particularly significant for polar species, and the negative impact of increasing the ionic strength on the kinetics of microextraction processes. In the present work it was no statistically significant (p-value >0.05) for some analytes except for E2, 4tOP and NP mix, for which a positive effect was observed. As consensus, 10% of NaCl was selected as optimum value.

The effect of stirring speed on the extraction efficiency was also studied at four levels: 600 rpm, 800 rpm, 1000 rpm and 1200 rpm. For this assessment, extractions were performed in triplicate with 150 mL of fortified Milli-Q water samples (1 ng/mL) under previously fixed extraction conditions (i.e., 10% of NaCl and no addition of MeOH during overnight). In all the cases, high stirring speed affected positively the extraction process (data not shown). Although no significant differences were observed for most of the analytes between 1000 and 1200 rpm, highest chromatographic responses were obtained at 1200 rpm in the case of 4tOP and NP mix, thus, this extraction speed was selected as optimum.

The influence of the extraction time was also investigated for the extraction of 150 mL Milli-Q water samples spiked at 1 ng/mL per compound under optimal conditions during extraction periods ranging between 15 and 1440 min. As shown in Fig. 2, the equilibrium was reached for all compounds after 720 min, extracting the samples overnight. Even if the extraction time is quite long, it is still comparable to those used with other common extraction techniques [27].

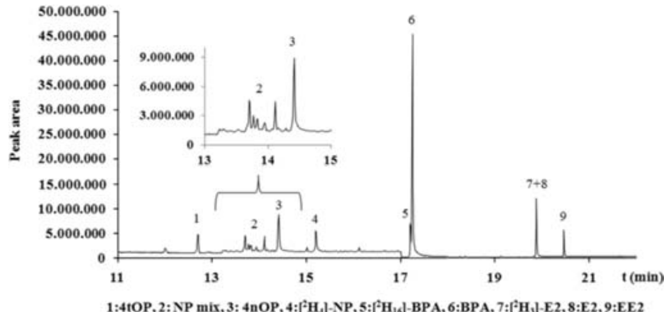


Fig. 3. Chromatogram obtained by GC–MS of the target analytes at the concentration of 150 ng/L (4tOP, 4nOP, E2 and EE2) and 300 ng/L (NP mix and BPA) concentration levels under the optimum conditions (150 mL sample, 10% of NaCl, 12 h of extraction time and 16 min of desorption time).

Finally in Figs. 3 and 4 we can see the chromatograms obtained for the analytes of interest at 150 ng/L (4tOP, 4nOP, E2 and EE2) and 300 ng/L (NP mix and BPA) concentration levels under the optimum conditions (150 mL sample, 10% of NaCl, overnight at 1200 rpm and subsequent desorption with 300 μL of organic solvent for 16 min using ultrasonic bath) in the SIM mode using GC–MS and dynamic MRM mode using LC–MS/MS.

3.3. Performance of the analytical methods: PES-silylation-GC–MS vs. PES-LC–MS/MS

The LC–MS/MS quantification of non derivatized extracts was performed with external standard calibration approach; i.e., a set of standards containing target compounds at concentrations ranging from LOD to 215 ng/mL for 4tOP, 4nOP, E2 and EE2 and from LOD to 430 for NP mix and BPA. Isotopically labeled compounds were used as surrogates (see Table 2).

On the other hand, procedural calibration curves were required to get accurate results for the derivatized compounds quantified using GC–MS. In this case, the calibration curves were tested by spiking Milli-Q water with different amounts of standards (from 15 to 250 ng/L for 4tOP, 4nOP, E2 and EE2 and from 30 to 500 ng/L for BPA and NP mix).

In the case of PES-LC–MS/MS methodology, good linearity was attained for all the target compounds obtaining coefficients of determination (r^2) higher than 0.99 except for NP mix (r^2: 0.61). Good linearity was also obtained by means of PES-silylation-GC–MS method for all the target compounds ($r^2>0.99$) except for NP mix ($r^2=0.91$), but even better than the obtained with LC–MS/MS.

In Table 3 we have collected the figures of merit of the two analytical methods based on PES extraction.

The repeatability of the methods was assessed in terms of relative standard deviation (RSD %) using Milli-Q water samples spiked at 100 ng/L for 4tOP, 4nOP, E2 and EE2; and 200 ng/L for BPA and NP mix, for three replicates analyzed within a day. As it is summarized in Table 3, good precision values were obtained for all target compounds using both methods, i.e., PES-silylation-GC–MS method (RSD < 12%) and PES-LC–MS/MS method (RSD < 16%), except for NP mix analyzed using LC–MS/MS (RSD < 45%), which restricts the applicability of this method.

Extraction efficiency was calculated for 150 mL of Milli-Q water samples spiked at two concentration levels and using both analytical approaches; i.e., at 100 ng/L and at 200 ng/L for 4tOP, 4nOP, E2 and EE2 and at 200 ng/L and at 400 ng/L for BPA and NP mix, respectively. Extraction efficiency was calculated by comparing the spiked concentration with the concentration obtained from

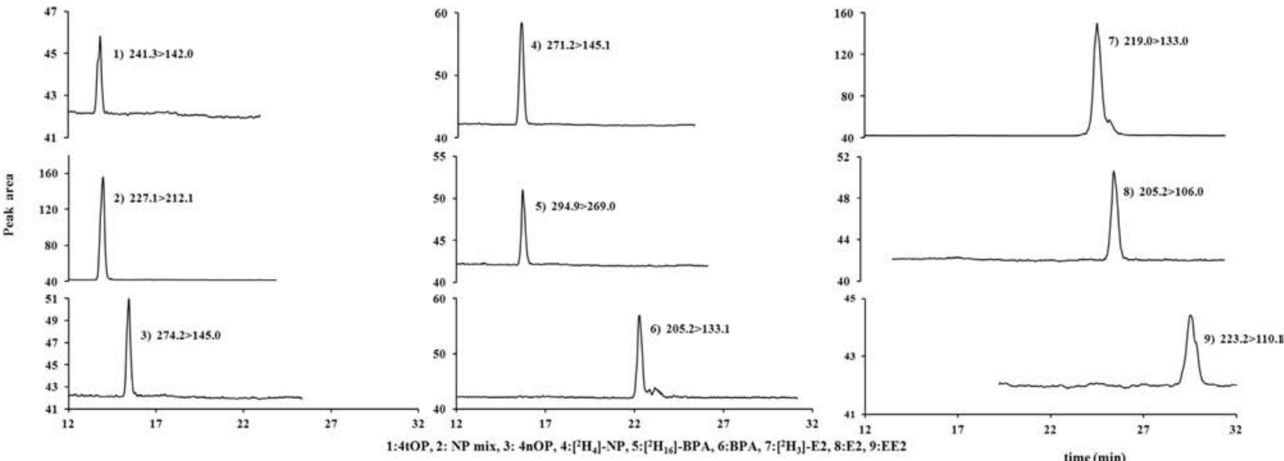


Fig. 4. Chromatogram obtained by LC–MS/MS of the target analytes at the concentration of 150 ng/L (4tOP, 4nOP, E2 and EE2) and 300 ng/L (NP mix and BPA) concentration levels under the optimum conditions (150 mL sample, 10% of NaCl, 12 h of extraction time and 16 min of desorption time).

Table 3
Data obtained for method validation in terms of extraction efficiency (%) ($n=3$), apparent recovery (%) ($n=3$), RSD (%) ($n=3$), limits of detection (LODs) and Method Detection Limits (MDLs, ng/L) ($n=7$, 99%) for effluent WWTP and estuarine water samples using both methodologies, i.e., PES-LC–MS/MS and PES-silylation-GC–MS.

Analyte	Extraction efficiency % ($n=3$) (LC–MS/MS)	Apparent recovery % ($n=3$)		RSD % ($n=3$)		LOD (ng/L) ($n=3$)		MDL (ng/L) ($n=7$, 99%) effluent WWTP		MDL (ng/L) ($n=7$, 99%) estuarine	
	LC–MS/MS	LC–MS/MS	GC–MS	LC–MS/MS	GC–MS	LC–MS/MS	GC–MS	LC–MS/MS	GC–MS	LC–MS/MS	GC–MS
4tOP	55[b]	118[a]/114[b]	102[a]/82[b]	9[a]/14[b]	1[a]/26[b]	5	22	61	6	37	15
NP mix	52[b]	109[a]/122[b]	94[a]/68[b]	45[a]/39[b]	12[a]/9[b]	42	76	221	34	93	70
4nOP	54[b]	113[a]/115[b]	99[a]/84[b]	8[a]/10[b]	11[a]/7[b]	1	8	29	10	72	15
BPA	50[b]	94[a]/89[b]	101[a]/89[b]	8[a]/8[b]	13[a]/2[b]	11	20	64	23	86	18
E2	38[b]	104[a]/108[b]	101[a]/92[b]	16[a]/5[b]	6[a]/1[b]	1	2	92	21	66	40
EE2	31[b]	81[a]/90[b]	103[a]/83[b]	10[a]/1[b]	7[a]/8[b]	14	25	169	55	121	89

[a] Low level (100 ng/L for 4tOP, 4nOP, E2 and EE2 and at 200 ng/L for BPA and NP mix).
[b] High level (250 ng/L for 4tOP, 4nOP, E2 and EE2 and at 450 ng/L for BPA and NP mix).

external standard calibration curve without using surrogates. The obtained results were statistically comparable at 95% of confidence level for the two tested detection methods, thus only the values obtained for LC–MS/MS are shown in Table 3. Briefly, the obtained extraction efficiencies were in the range of 27–56%, which were in good agreement with other works found in the literature in which these analytes were extracted using other microextraction approaches [27,40,41].

The accuracy of the results was estimated using 150 mL of Milli-Q water samples (by triplicate) spiked at 100 ng/L for 4tOP, 4nOP, E2 and EE2 and at 200 ng/L for BPA and NP mix (low level) and 250 ng/L for 4tOP, 4nOP, E2 and EE2 and at 450 ng/L for BPA and NP mix (high level) that were submitted to the whole extraction process under optimal conditions.

Two different approaches were evaluated for the apparent recovery calculation. On the one hand, a procedural calibration curve with the fortified quantity was built (i.e., calibration curve built with fortified water samples submitted to the whole analytical procedure) and on the other hand, the quantification was performed using internal standard calibration method (i.e., calibration curve built with standards and concentrations corrected with the corresponding surrogate).

In the case of derivatized compounds analyzed by GC–MS, the use of external calibration approach did not provide satisfactory recoveries (90–241%) and it was automatically discarded. On the contrary, good accuracies were obtained using the procedural calibration approach, being the apparent recoveries between 94–103% and 68–92% in the case of low and high fortified concentration levels, respectively for all the studied compounds (see Table 3).

Regarding to the underivatized compounds detected by LC–MS/MS, adequate apparent recoveries; 93–115% for all the analytes, except for NP mix (37%) were obtained after the use of a procedural calibration and the quantification performed using external standard calibration method also provided satisfactory results in terms of apparent recoveries (81–118% and 89–122% in the case of low and high spiked levels, respectively) for all target compounds (see Table 3).

Finally, the limits of detection (LODs) were calculated using blank samples (150 mL Milli-Q water samples), being between 2 and 76 ng/L and between 1 and 42 ng/L for PES-silylation-GC–MS and PES-LC–MS/MS methods, respectively. These values were similar to those found in the literature for APs, BPA and hormones using different extraction approaches and MS detection (see Table 4). Better LOD values compared with some studies using other detection techniques like ultraviolet (UV), were obtained in the present work (see Table 4).

Afterwards, method detection limits (MDLs) were also determined spiking wastewater and estuarine waters at the corresponding LOD of each target compound as indicated in the guidance for MDL calculation proposed by U.S. Environmental Protection Agency (U.S.E.P.A 2013) [48]. For the extracts analyzed by GC–MS, the samples were spiked at 25 ng/L (4tOP and 4nOP), 50 ng/L (BPA) and 200 ng/L (NP mix, E2 and EE2) and for the extracts analyzed by LC–MS/MS, the water samples were fortified at 50 ng/L (4tOP, 4nOP and E2) and 200 ng/L (NP mix and EE2). The signals obtained for non spiked samples were subtracted to spiked samples. Finally, MDLs were calculated at 99% of confident level for 7 samples following the recommendations ($MDL_{99}=t_{99,7}\times s$, where s is the standard deviation). The values obtained were in the range of 6–89 ng/L and 29–221 ng/L for PES-*silylation*-GC–MS and PES-LC–MS/MS, respectively (see Table 3).

Table 4
Review of limits of detection (LODs) and quantification (LOQs) found in the literature by means of different microextraction techniques and those obtained in this work for the determination of alkylphenols, BPA and hormones in aqueous samples.

Analyte	Microextraction	Analysis	Derivatization	LOD (ng/L)	LOQ (ng/L)	Matrix	Refs.
NP mix	MEPS	LVI-GC–MS	No	96	151	MilliQ water	[38]
	PES	GC–MS	Yes	76	154	MilliQ water	In this work
	PES	LC–MS/MS	No	42	63	MilliQ water	In this work
4nOP	LPME	GC–MS	Yes	10	24	MilliQ water	[42]
	PES	GC–MS	Yes	8	22	MilliQ water	In this work
	PES	LC–MS/MS	No	1	3	MilliQ water	In this work
4tOP	HF-LLLME	HPLC-UV	No	1460	4900	MilliQ water	[43]
	PES	GC–MS	Yes	22	35	MilliQ water	In this work
	PES	LC–MS/MS	No	5	11	MilliQ water	In this work
BPA	MEPS	LVI-GC–MS	No	177	293	MilliQ water	[38]
	LPME	GC–MS	Yes	14	24	MilliQ water	[42]
	DLLME	HPLC-UV	No	700	–	MilliQ water	[44]
	SBSE	GC–MS	Yes	5	20	River water	[45]
	SBSE	GC–MS	No	500	2000	River water	[45]
	LPME	GC–MS	Yes	2	10	River water	[46]
	LPME	GC–MS	No	200	1000	River water	[46]
	HF-LLLME	HPLC-UV	No	55	180	MilliQ water	[43]
	PES	GC–MS	Yes	20	34	MilliQ water	In this work
	PES	LC–MS/MS	No	11	17	MilliQ water	In this work
E2	SPME	GC–MS	Yes	7	22	Pure water	[47]
	SPME	GC–MS	Yes	9	32	River water	[47]
	HF-LLLME	HPLC-UV	No	660	2200	MilliQ water	[43]
	PES	GC–MS	Yes	2	2	MilliQ water	In this work
	PES	LC–MS/MS	No	1	2	MilliQ water	In this work
EE2	MEPS	LVI-GC–MS	No	125	136	MilliQ water	[38]
	HF-LLLME	HPLC-UV	No	550	1800	MilliQ water	[43]
	PES	GC–MS	Yes	25	26	MilliQ water	In this work
	PES	LC–MS/MS	No	14	18	MilliQ water	In this work

Abbreviations: MEPS (microextraction by packet sorbents), LPME (liquid phase microextraction), HF-LLLME (hollow fiber liquid–liquid–liquid microextraction), DLLME (dispersive liquid–liquid microextraction), SBSE (stir bar sorptive extraction), GC–MS (gas chromatography–mass spectrometry), LVI-GC–MS (large volume injection-gas chromatography–mass spectrometry), HPLC–UV (high pressure liquid chromatography-ultraviolet), SPME (solid phase micro extraction).

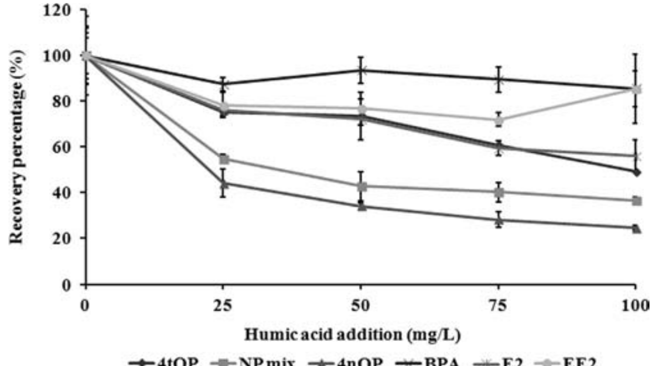


Fig. 5. Comparison of the recovery percentages (concentration 500 ng/L) obtained for the target compounds in Milli-Q water at different concentrations (mg/L) of humic acids with no correction of the signals with the corresponding surrogate.

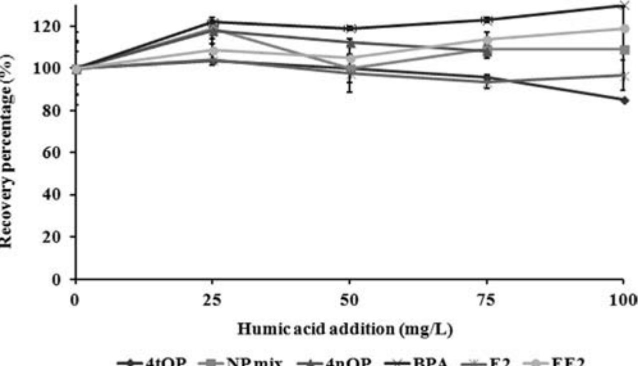


Fig. 6. Comparison of the recovery percentages (concentration 500 ng/L) obtained for the target compounds in Milli-Q water at different concentrations (mg/L) of humic acids after the correction of the signals with the corresponding surrogate.

3.4. Matrix effect evaluation and application of the developed methods to real samples analysis

Organic matter can reduce the amount of extractable organic compounds and/or interfere in the analysis of target analytes. The influence of organic matter on the extraction efficiency of them using PES polymers was simulated with Milli-Q water samples in the presence of humic substances. Hence, the recoveries of the analytes (500 ng/L each) in Milli-Q water spiked at different concentrations of humic acids (0, 25, 50, 75 and 100 mg/L) were determined for the PES-silylation-GC–MS procedure.

Figs. 5 and 6 show the recoveries obtained at different concentrations of humic acids for the analytes studied without and with surrogate corrections, respectively. For BPA and EE2 similar recoveries were obtained in presence of low and higher humic acid concentrations. This means that the presence of organic matter either has not any influence or has little influence on the amount of analyte detected and indicated no tendency to interact with the analytes prior to extraction. For the rest of the analytes, a decrease of the extraction efficiency was observed, since the recoveries decreased up to a 20–40%. However, the use of surrogates allowed correcting the effect created by the presence of humic substances in the samples, so accurate results were guaranteed (see Fig. 6).

The optimized extraction and detection methods were applied to estuarine water and WWTP effluent water samples. The average concentration ($n=3$) obtained for all the analytes with their corresponding uncertainties at 95% of confidence level are shown in Table 5. Regarding to the concentration found at environmental waters (wastewater and estuarine), NP mix and BPA were the only

Table 5
Concentrations found ($n=3$, 95%) at ng/L for the target analytes in WWTP effluent and estuarine water samples using PES-LC–MS/M-S and PES-silylation-GC–MS methodologies.

Analyte	WWTP effluent		Estuarine	
	LC–MS/MS	GC–MS	LC–MS/MS	GC–MS
4tOP	< MDL	< MDL	< MDL	< MDL
NP mix	81 ± 17	99 ± 19	99 ± 2	83 ± 2
4nOP	< MDL	< MDL	< MDL	< MDL
BPA	92 ± 4	88 ± 16	388 ± 3	347 ± 1
E2	< MDL	< MDL	< MDL	< MDL
EE2	< MDL	< MDL	< MDL	< MDL

target compounds detected at ng/L level (see Table 5). In the case of NP mix, similar concentrations were obtained in both types of water samples whereas a higher concentration of BPA was found in estuarine water samples.

4. Conclusions

Extraction using PES tubes has been proved to be a cheap, simple and precise alternative for the extraction of APs, hormones and BPA from WWTP effluents and estuary water samples using sample volumes of 150 mL. Small solvent volume consumption (300 μL) and low overall cost of the present method taking into account the reduced cost of the polymer (c.a. 0.05 €/unit), together with the scarcely affection of the yield in the whole procedure by the type of water are the main advantages of the present methodologies. It could be also underlined that the extraction procedure is carried out overnight with samples being simultaneously concentrated in a 15 positions magnetic stirring plate.

Comparable results in terms of precision were obtained by PES-silylation-GC–MS and PES-LC–MS/MS for all the analytes except for NP mix (45%). Although, better MDLs (6–55 ng/L and 15–89 ng/L for WWTP and estuarine, respectively) were obtained in the case of PES-silylation-GC–MS protocol, PES-LC–MS/MS method provided a shorter analysis time (no need of derivatization) and an external calibration approach was enough for the accurate quantification of the target compounds. However, LC–MS/MS was not able to measure NP mix with good quality (in terms of precision) and as a consequence, LC–MS/MS could not be employed in order to analyze NP mix at the low concentration levels considered in the present work. In this case, even if the sample preparation is a bit more tedious, GC–MS analysis may be used.

The good MDLs, linearity and repeatability together with the simplicity, reduced costs and automation possibilities makes this technique an adequate tool for quality routine analysis of these compounds in a wide range of different aqueous samples.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.11.015.

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