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Matrix effect during the membrane-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry for the determination of a variety of endocrine disrupting compounds in wastewater

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ABSTRACT

Membrane-assisted solvent extraction (MASE) coupled to liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) was studied for the determination of a variety of emerging and priority compounds in wastewater. Among the target analytes studied certain hormones (estrone (E1), 17β-estradiol (E2), androsterone (ADT), 17α-ethynyl estradiol (EE2), diethylstilbestrol (DES), equilin (EQ), testosterone (TT), mestranol (MeEE2), 19-norethisterone (NT), progesterone (PG) and equilenin (EQN)), alkylphenols (APs) (4-*tert*-octylphenol (4*t*OP), nonylphenol technical mixture (NPs) and 4*n*-octylphenol (4*n*OP)) and BPA were included. The work was primarily focused in the LC–MS/MS detection step, both in terms of variable optimization and with respect to the matrix effect study. Both, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were assessed both in the negative and positive mode, including the optimization of MS/MS operating conditions. The best results were obtained, in most of the cases, for ESI using 0.05% ammonium hydroxide as buffer solution in the mobile phase, composed with methanol and water. Under optimum detection conditions, matrix effect during the detection step was thoroughly studied. Dilution, correction with deuterated analogues and clean-up of the extracts were evaluated for matrix effect correction. Clean-up with Florisil together with correction with deuterated analogues provided the most satisfactory results, with apparent recoveries in the 57–136% range and method detection limits in the low ng L^{-1} level for most of the analytes. For further validation of the method, two separated extraction procedures, the above mentioned MASE, and conventional solid phase extraction (SPE) were compared during the analysis of real samples and comparable results were successfully obtained for E1, E2, EE2, DES, NT, TT, EQ, PG, BPA, ADT, 4*n*OP, 4*t*OP, NPs and EQN.

1. Introduction

Endocrine disrupting compounds (EDCs) have become a public health concern in modern times because of their detrimental effects on the human endocrine system [1,2]. Although natural and synthetic EDCs can be degraded biologically, they cannot be completely removed in wastewater treatment plants (WWTPs). In fact, they are often detected in WWTP effluents and discharged into surface waters. Therefore, an elevated concern over the safety and quality of natural or treated water bodies has been generated and the need for water testing by water utilities and regulatory agencies has increased [3–5].

EDCs include natural estrogens, such as estrone (E1) and 17β-estradiol (E2), natural androgens, such as testosterone (TT), and androsterone (ADT), artificial synthetic estrogens or androgens such as 17α-ethynylestradiol (EE2), as well as other industrial compounds such as bisphenol A (BPA) and alkylphenols (APs).

In order to evaluate the removal efficiency of specific estrogenic compounds from water samples, and due to the low concentrations present in real samples, there is a huge need for the development of

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cost-effective and user-friendly multiresidue strategies, as well as highly sensitive and selective methods, for a wide range of emerging pollutants such as EDCs in water samples. The existence of complex interfering components in the aquatic and biological sample matrices requires the development of effective strategies in order to avoid matrix effect.

Nowadays, analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) offer a tool to identify and quantify compounds of medium to high polarity in different water bodies and in solid matrices [6–9]. However, a serious drawback of LC–MS/MS methods is its susceptibility to matrix effects, i.e. the signal suppression or enhancement by matrix compounds entering the ion source at the same time than the target analytes [10]. It has been reported that atmospheric pressure chemical ionization (APCI) is generally less sensitive to matrix effects than the more commonly used electrospray ionization (ESI). Although LC–MS/MS has been proved to be a more versatile technique applicable to a full range of contaminants, GC–MS is still a more readily available technique in many laboratories. However, when GC–MS is performed, the analysis of underivatized polar compounds is restricted, being necessary a previous derivatization step.

In the last years, microextraction techniques have become popular as sample preparation techniques for water analysis due to the small amounts of solvent (μL range) and sample (mL range) required. Concerning preconcentration and clean-up strategies, the application of sorptive techniques and the use of membranes are becoming promising solvent free methods. Two types of techniques can be distinguished between membrane based liquid–liquid extraction depending on the characteristics of the membrane: (i) supported liquid membrane (SLM) and microporous membrane liquid–liquid extraction (MMLLE), which use porous membranes, and (ii) membrane assisted solvent extraction (MASE) that uses non-porous membranes [11].

MASE is carried out using a non-porous membrane as interface between the sample (donor phase) and the organic solvent (acceptor phase). The organic analytes in the aqueous phase are dissolved in the membrane material and diffuse through the polymer into the acceptor phase [12]. The membrane is not only considered as a barrier for particles and macromolecules, but it can also provide selectivity in terms of permeation and transport through the membrane by choosing appropriate membrane material and organic acceptor phase [13,14]. Thanks to the use of non-porous membranes, miscible and non-miscible water solvents can also be used. Although MASE can be coupled to both LC and GC, due to the common use of non polar solvents as acceptor phases, MASE has been typically combined with GC [15–17]. However, MASE has also been successfully applied to the analysis of moderately polar compounds in combination with LC–MS/MS [18–20].

The aim of the present work was the analysis of certain hormones (E1, E2, ADT, TT, EQ, DES, EE2, MeEE2, NT, PG and EQN), APs and BPA, which are included as priority or emerging pollutants in the European Water Framework Directive (WFD 2000/60/EC). With this purpose, previously optimized MASE extraction [21] was coupled to LC–MS/MS, including some hormones (NT, TT and PG), which had not been studied in the previous work. Different alternatives were studied in order to avoid or minimize matrix effect during the detection in LC–MS/MS, including sample dilution, the use of deuterated analogues and clean-up of sample extract. Developed MASE–LC–MS/MS method was applied to samples from two local WWTPs of Biscay (north of Spain). The results were compared with classical SPE and both extraction techniques followed by LC–MS/MS were applied to samples from two local WWTPs of Biscay (north of Spain).

2. Experimental

2.1. Reagents and material

The solid reagents 4*n*-octylphenol (4*n*OP, 99%), BPA (99+ %), DES (99.9%), ADT (98.2%), EQ (97.6%), TT (99.9%), NT (99.5%), PG (99.6%), 17β-estradiol-d_3 ([$^2H_3$]-E2, 98%), nonylphenol-d_4 ([$^2H_4$]-NP, 97%), progesterone-d_9 ([$^2H_9$]-PG, 98%) and equilin-d_4 ([$^2H_4$]-EQ, 98%) were purchased from Sigma Aldrich (Steinheim, Germany), while nonylphenol technical mixture (NPs, 94%), E1 (99.5%), MeEE2 (99.4%), EE2 (99.4%) and E2 (99.7%) were obtained from Riedel-de HaënSeelze (Steinheim, Germany). 4-*Tert*-octylphenol (4*t*OP, 99.4%) and bisphenol A-d_{16} ([$^2H_{16}$]-BPA) were obtained from Supelco. In the case of EQN individual solution at 100 μg mL^{-1} in ACN was also purchased from Sigma Aldrich. Individual stock solutions for each target compound, as well as the deuterated analogues, were dissolved to prepare 5000 mg L^{-1} solutions in anhydrous methanol (99.9%, Alfa-Aesar, Karlsruhe, Germany). All the standards and stock solutions were stored at −20 °C.

Sodium chloride (NaCl, Merck, Darmstadt, Germany) and HPLC-grade methanol (MeOH) (LabScan, Dublin, Ireland) were used for matrix modification experiments. NaCl was ultrasonically cleaned with methylene chloride (DCM) (HPLC grade, LabScan) and dried at 150 °C before use. Ethyl acetate (EtOAc) and isooctane, both HPLC grade, were also purchased from LabScan.

Ultra-pure water was obtained using a Milli-Q water purification system (<0.05 μS/cm, Milli-Q model 185, Millipore, Bedford, MA, USA). Acetonitrile (ACN) (Romil-UpS, Waterbeach, Cambridge, UK) and MeOH (Romil-UpS) were used as organic modifiers or mobile phase eluents. Acetic acid (HOAc, Merck), hydrochloric acid (HCl, 36%, Merck,), ammonia (25% as NH_4OH, Panreac, Reixac, Barcelona, Spain) and ammonium acetate (NH_4OAc, Sigma Aldrich, Steinheim, Germany) were used for mobile phase modifications.

125 μm (125 mm diameter), 11 μm (70 mm diameter) and 0.45 μm (90 mm diameter) cellulose filters were purchased from Whatman (Maidstone, UK). Extracts were filtered before analysis with acrodisc syringe (13 mm diameter, 0.2 μm pore size) filters (GHP, PTFE, Nylon, PVDF, PES, HT Tuffryn or polysulfone) obtained from Pall Life Sciences (USA).

Home-made low density polyethylene (LDPE) membranes (40 mm length, 6 mm i.d. and 0.02 mm thickness) were cleaned in chloroform (HPLC-grade, Labscan) overnight and kept in clean chloroform until use.

200 mg Bond Elut Plexa cartridges were purchased from Agilent Technologies (USA) and 1 g Florisil cartridges from Supelco (Walton-on-Thames, UK). The SPE step was performed with a Visiprep SPE manifold from Supelco.

2.2. Sampling

Samples were collected at the WWTPs located in Galindo and Gernika (Basque Country, north of Spain).

Samples were collected in pre-washed amber bottles and carried to the laboratory in cooled boxes. Samples were filtered through 0.45 μm cellulose filters (influent samples were prefiltered with 125 μm and 11 μm cellulose filters) and kept in the fridge at 4 °C before analysis, which was performed within 48 h.

2.3. Membrane-assisted solvent extraction methodology

The optimization of MASE extraction step for the target analytes is fully described elsewhere [21]. Briefly, 130-mL aliquot of the filtered sample together with 26 g of NaCl was introduced in a 150-mL headspace-vial and membranes were attached to a metal funnel and fixed with Teflon rings (Gerstel, Mülheim an der Rhur,

Germany). The membrane was filled with 800 μL of chloroform, the funnel was suspended in the bottleneck and the vials were closed with PTFE septa and aluminium crimp caps. The vials were stirred at 750 rpm at room temperature for 90 min. Once the extraction step was over, the organic phase was transferred to a 2 mL amber vial, evaporated to dryness under a gentle stream of nitrogen in a Turbovap LV Evaporator (Zymark, Hopkinton, USA) and re-dissolved in 100 μL of isooctane (when the clean-up step was applied) or 100 μL MeOH (when no clean-up was applied).

2.4. Solid phase extraction

SPE was carried out according to a method previously optimized in our group [22] with some modifications. Briefly, a 250 mL aliquot of wastewater was passed at 3 mL min^{-1} through a 200 mg Bond Elut Plexa 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 loading, 5 mL of Milli-Q:MeOH (95:5, v/v) mixture was added with cleaning purposes and, then, the cartridge was dried for 1 h under vacuum. Finally, the analytes were eluted using 8 mL of EtOAc, submitted to evaporation and re-dissolved in 100 μL of isooctane before Florisil clean-up.

2.5. Clean-up of the extracts

The MASE and SPE extracts were submitted to a clean-up step according to the procedure established by Guitart et al. [23]. Briefly, the isooctane extracts were loaded onto 1-g Florisil cartridges previously activated with 5 mL of *n*-hexane. The analytes were eluted with 8 mL of the mixture DCM:EtOAc:MeOH (40:40:20, v/v) and submitted to evaporation as described above. Finally, the residue was re-dissolved in 100 μL of MeOH and filtered through 0.2 μm syringe PTFE microfilters.

2.6. Liquid chromatography tandem mass spectrometry with triple quadrupole (LC–MS/MS) detection

Samples were 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 both ESI and APCI sources (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 selected reaction monitoring (SRM) mode. High purity nitrogen gas (99.999%) was used as nebulizer, drying and collision gas. MS/MS ionization parameters were set as follows: a N_2 flow rate of 11 L min^{-1}, a capillary voltage of 4000 V, a nebulizer pressure of 52 psi (358.5 kPa) and a source temperature of 325 °C.

Fragmentor electric voltage and collision energy were optimized, both for ESI and APCI sources, in the 81–215 V and 5–50 eV ranges (both negative and positive voltages), respectively, by injection of individual compounds.

Different mobile phases (MeOH:water, MeOH:water acidified with 0.1% HOAc, MeOH:water with 1 mM, 3 mM, and 5 mM NH_4OAc, and MeOH:water with 0.05% NH_4OH) were tested in order to improve the ionization of the target analytes.

Separation of analytes was carried out using two different analytical columns: an Agilent Zorbax Extend-C18 (2.1 mm, 50 mm, 1.8 μm) column (pH range 2.0–11.5) and an Agilent Zorbax SB-C18 (2.1 mm, 50 mm, 1.8 μm) column (pH range 1.0–8.0). In all the cases an UHPLC Zorbax Eclipse XDB-C18 pre-column (2.1 mm, 5 mm, 1.8 μm) was used. The column temperature was set to 35 °C for Agilent Zorbax Extend-C18 column and at 45 °C in the case of Agilent Zorbax SB-C18 column. The injection volume was set at 10 μL and the flow rate at 0.2 mL min^{-1}.

Under optimized conditions a binary mixture consisting of Milli-Q containing 0.05% NH_4OH (eluent A) and MeOH containing 0.05% NH_4OH (eluent B) were used for gradient separation of target analytes. Linear gradient for both ionization modes was as follows: 30% B maintained for 4 min, increased to 60% B in 3 min and to 80% B in 10 min, where it was maintained constant for 15 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 (version 05.02) was used for data treatment.

3. Results and discussion

3.1. Optimization of LC–MS/MS

Although most studies [24,25] determined satisfactory results with ESI coupled with LC–MS/MS, the suitability of APCI deserve further exploration since APCI is less susceptible to matrix effects. In this sense, ESI and APCI interfaces were both assessed for LC–MS/MS detection as an alternative to GC–MS following silylation [21].

Thus, MS/MS operating conditions for both ESI and APCI in the positive and the negative ionization modes were optimized. For this purpose, two precursor-product ion transitions were studied for each target analyte, and also, fragmentor electric voltage and collision energy in the 80–180 V and 5–45 eV ranges, respectively, were optimized (data not shown). The optimum values obtained using the two ionization modes for the target analytes are described in Table S1 in the Supplementary material. The effect of the pH of the mobile phase in both APCI and ESI ionization was also tested. In a first approach and using the SB-C18 column (pH range 1.0–8.0) three different mobile phase compositions were tested: pure water:MeOH, water:MeOH with %0.1 HOAc and water:MeOH with 1 mM NH_4OAc. The results obtained are included in Fig. 1(a) and (b) for ESI and APCI, respectively. While NH_4OAc gave the best results for ESI, pure MeOH:water mixture provided the best results for APCI. In the case of ESI different NH_4OAc concentrations (0 mM, 1 mM, 3 mM and 5 mM) were further tested. According to the results 1 mM NH_4OAc provided the best results.

As recent results in the literature have shown that more basic mobile phases (pH = 10.5) provided better sensitivity for APs [26], a further study of the effect of the pH was performed but using the Agilent Zorbax Extend-C18 column (pH range 2.0–11.5). The best sensitivity results were obtained, when NH_4OH was added, thus, in the case of ESI, this pH (pH = 10.5) was selected as optimum. The results of the comparative study of different mobile phases for ESI are summarized in Fig. 1(b).

Under optimized conditions for both APCI and ESI, instrumental limits of detection (LODs) were estimated and included in Table 1. LODs were estimated using a signal to noise ratio (S/N) of 3. As can be observed the best instrumental LODs were obtained using ESI, except for MeEE2. In the case of EE2 and NPs similar LODs were obtained using both ionization sources. MeEE2 was discarded from further optimization due to the high instrumental LODs obtained using ESI as ionization source.

In addition, the enrichment factors (EF) were also calculated as the ratio of concentration of analytes in organic phase (C_o) and concentration of analyte in aqueous phase (C_w) and ranged from 91 to 832 for all the compounds (see Table 1).

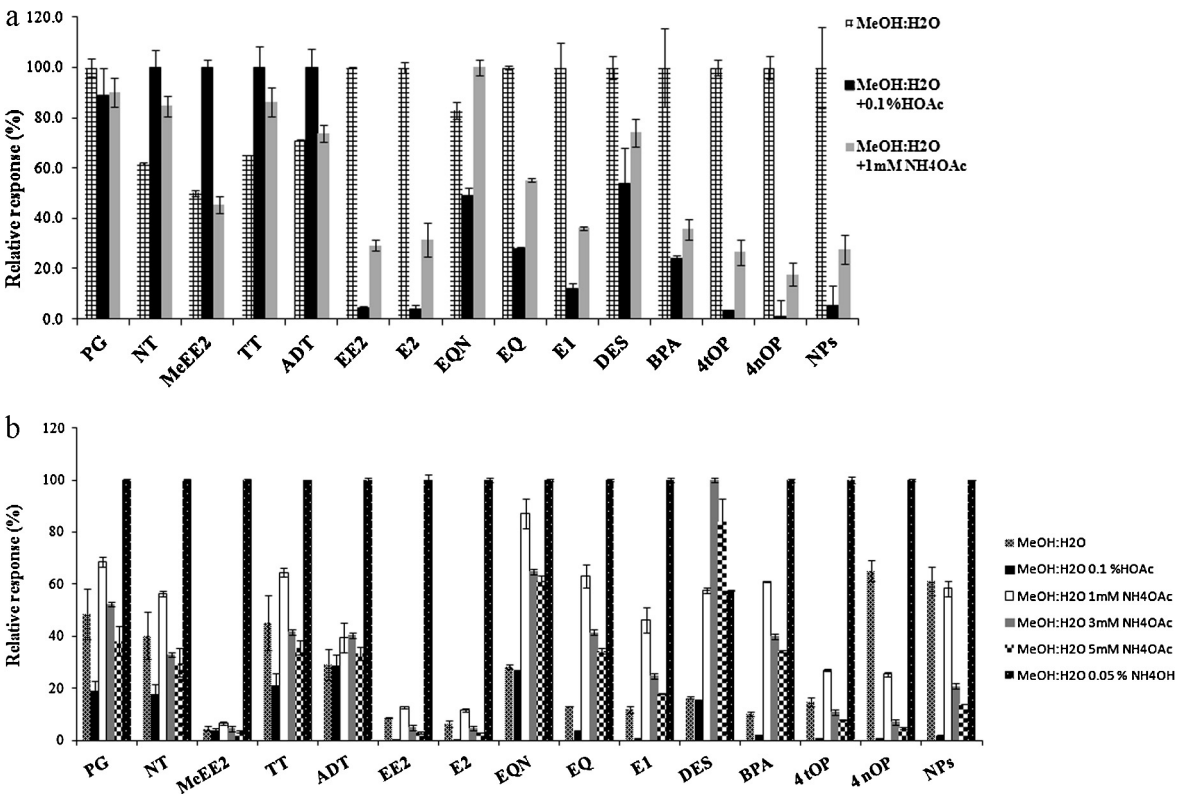

Fig. 1. Effect of the pH of the mobile phase in APCI ($n = 3$) (a) and effect of the pH of the mobile phase in ESI ($n = 3$) (b).

3.2. Matrix effect and method validation for MASE coupled to LC–(ESI)–MS/MS

Apparent recoveries of MASE coupled to LC–(ESI)–MS/MS without further extract clean-up were calculated for Milli-Q water samples spiked at 1000 ng L^{-1}.

As can be observed from the data included in Table 2, satisfactory results were obtained. However, since a strong matrix effect was observed in our previous work when MASE followed by LVI-PTV-GC–MS was applied for wastewater samples [21], signal suppression or enhancement was also evaluated for LC–(ESI)–MS/MS when MASE is applied as extraction technique to wastewater samples. In that previous work of our research group, matrix effect was observed for the whole analytical process, which included extraction, derivatization and GC–MS analysis. In the present work matrix effect was studied for the detection step. Sample dilution (1:5 dilution), the use of deuterated analogues and the addition of a clean-up step were studied as different alternatives for matrix effect correction during the LC–ESI–MS/MS analysis.

Influent and effluent samples, diluted and not diluted, without and with correction with the corresponding deuterated analogue from WWTPs of Galindo (GAL) and Gernika (GER) (Basque Country, Spain) were extracted under optimized MASE conditions in triplicate. Sample extracts were spiked (equivalent to 1000 ng L^{-1} in the water solution) in order to determinate the possible matrix

Table 1
Enrichment factors, instrumental detection limits (LOD, ng mL^{-1}) and linearity for LC–APCI–MS/MS and LC–ESI–MS/MS. The deuterated analogue used for correction of each analyte is indicated.

Analyte	Deuterated analogue used for correction	Enrichment factor	LC–(APCI)–MS/MS		LC–(ESI)–MS/MS	
			Linearity (LOQ-1000 ng mL^{-1})	LODs (ng mL^{-1})	Linearity (LOQ-1000 ng mL^{-1})	LODs (ng mL^{-1})
4*n*OP	[$^2H_4$]-NP	468	0.9974	33	0.9998	6
4*t*OP	[$^2H_4$]-NP	260	0.9994	61	0.9988	3
NPs	[$^2H_4$]-NP	832	0.9997	32	0.9834	30
BPA	[$^2H_{16}$]-BPA	104	0.9987	15	0.9996	1
ADT	[$^2H_3$]-E2	299	0.9767	6	0.9995	1
DES	[$^2H_3$]-E2	156	0.9994	27	0.9997	3
E1	[$^2H_4$]-EQ	234	0.9899	5	0.9993	1
EQ	[$^2H_4$]-EQ	377	0.9993	54	0.9999	1
TT	[$^2H_9$]-PG	338	0.9967	13	0.9999	0.05
E2	[$^2H_3$]-E2	182	0.9988	16	0.9993	3
NT	[$^2H_9$]-PG	312	0.9975	11	0.9997	0.1
EQN	[$^2H_4$]-EQ	260	0.9992	71	0.9997	0.5
PG	[$^2H_9$]-PG	364	0.9951	5	0.9997	0.05
EE2	[$^2H_3$]-E2	91	0.9966	10	0.9969	12
MeEE2	[$^2H_3$]-E2	169	0.9953	16	0.9946	100

Table 2
Detection limits of the method (DLM, ng L^{-1}) for MASE–LC–ESI–MS/MS and SPE–LC–ESI–MS/MS and relative standard deviations (RSD) and average recoveries ($n = 3$) (corrected and non-corrected) for MASE–ESI–LC–MS/MS method in Milli-Q and wastewater from Galindo and Gernika, in December 2013 campaign.

Analyte	DLM (ng L^{-1})		Recovery (%)								
			Milli-Q	Galindo WWTP				Gernika WWTP			
				Influent		Effluent		Influent		Effluent	
	MASE	SPE		Non-corrected	Corrected	Non-corrected	Corrected	Non-corrected	Corrected	Non-corrected	Corrected
4OP	23	4	101 ± 6	58 ± 5	74 ± 3	82 ± 29	129 ± 37	43 ± 3	99 ± 5	51 ± 7	109 ± 22
4*t*OP	50	5	86 ± 11	172 ± 25	136 ± 6	124 ± 2	132 ± 20	82 ± 4	90 ± 2	86 ± 2	95 ± 1
NPs	100	28	93 ± 13	108 ± 16	86 ± 19	103 ± 38	109 ± 16	87 ± 17	56 ± 10	104 ± 38	108 ± 8
BPA	15	0.9	112 ± 10	115 ± 5	104 ± 14	100 ± 3	299 ± 25	107 ± 7	80 ± 9	138 ± 7	137 ± 15
ADT	17	2	116 ± 3	119 ± 11	85 ± 10	117 ± 3	167 ± 46	153 ± 4	108 ± 9	116 ± 6	90 ± 15
DES	9	0.7	106 ± 1	93 ± 11	116 ± 6	83 ± 2	237 ± 40	140 ± 17	147 ± 25	142 ± 10	158 ± 19
E1	10	1	97 ± 9	87 ± 7	88 ± 6	70 ± 11	120 ± 5	116 ± 10	92 ± 12	103 ± 13	84 ± 12
EQ	16	2	99 ± 15	90 ± 15	113 ± 10	60 ± 3	115 ± 16	109 ± 4	108 ± 11	98 ± 12	100 ± 11
TT	0.6	0.1	107 ± 3	87 ± 1	118 ± 15	65 ± 11	115 ± 23	120 ± 6	128 ± 19	103 ± 13	130 ± 7
E2	3	0.2	107 ± 15	123 ± 16	96 ± 10	64 ± 22	98 ± 26	136 ± 1	103 ± 9	131 ± 23	115 ± 16
NT	1	0.2	96 ± 14	85 ± 3	118 ± 22	65 ± 5	102 ± 19	124 ± 5	134 ± 23	102 ± 11	133 ± 1
EQN	6	1	103 ± 15	105 ± 27	103 ± 12	70 ± 15	99 ± 22	97 ± 8	95 ± 15	83 ± 6	86 ± 10
PG	0.4	0.1	100 ± 14	36 ± 10	114 ± 22	69 ± 11	105 ± 5	86 ± 5	99 ± 8	36 ± 4	105 ± 2
EE2	100	4	97 ± 6	118 ± 5	93 ± 3	123 ± 7	143 ± 49	101 ± 11	64 ± 5	58 ± 12	44 ± 10

effect during the detection step. Non-spiked samples were also processed in order to subtract the signal from the spiked extracts. After extraction, the chloroform extract of both spiked and non spiked samples were evaporated to dryness and re-dissolved in 100 μL of MeOH. The signals obtained, after subtraction of the signals of non-spiked samples, were compared with chloroform extracts spiked at the same level and submitted to the same evaporation process, re-dissolution in MeOH and LC–(ESI)–MS/MS analysis. Fig. 2(a–d) show the signal ratio for extracts spiked after extraction vs chloroform spiked at the same level, without and with correction with the corresponding deuterated analogue, diluted and non diluted for influent and effluent samples from GER and GAL, respectively.

In the case of Gernika influent and effluent samples, a strong matrix effect was observed for diluted and non diluted samples when no correction was performed for most of the analytes (EE2, E2, EQN, EQ. E1, DES or BPA among others), while the signal enhancement observed was corrected using the corresponding deuterated analogues, except for NPs and 4*n*OP in the non diluted effluent samples. However, in the case of GAL samples, the use of deuterated analogues only corrected for matrix effect after dilution of the sample, and not in all the cases (NPs and 4*n*OP in influent samples). In order to understand the differences observed for the samples from the two WWPTs it should be highlighted that, while Galindo is the largest WWTP in Biscay (Basque Country, Spain) with an average wastewater flow of 350 000 m^3 per day and 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 for a much lower population (www.accion-agua.es).

It could be concluded that dilution of the sample together with the use of deuterated could correct for matrix effect in the detection step in most of the cases. However, dilution of the sample (1:5 dilution) increased the detection limits of the method (DLMs) for real samples and, therefore, sample clean-up was studied as alternative to compensate matrix effect in the detection step without dilution of the sample. Therefore, similar experiments were performed for different wastewater samples, but after a clean-up of the extract. In this case, water samples were spiked at 250 ng L^{-1}, extracted, evaporated and submitted to a clean-up step using Florisil. Non-spiked samples were also processed for blank subtraction and the results are included in Table 2. For most of the analytes, even non-corrected recoveries were satisfactory. Only the corrected values for DES and ADT were not satisfactory. Different surrogates were tested for the correction of DES and among the deuterated analogues available and, although [$^2H_3$]-E2 provided good results in some samples, correction was not always satisfactory. It should be highlighted that all the surrogates should be added at concentrations of at least at 500 ng L^{-1} due to the lower sensitivity obtained for the deuterated analogues compared with the non deuterated target compounds. Therefore, it could be concluded that the clean-up of the extract reduced matrix effect during the detection step, guaranteeing quantitative results even without correction in most of the cases.

Detection limits of the method (DLMs) were calculated as three times the *S*/*N* of spiked Milli-Q samples [27–30], estimated by extrapolating results from the lower levels of the calibration curve. For compounds as BPA, ADT, DES, E1, EQ, 4*n*OP, EQN and E2 Milli-Q water was spiked at 20 ng L^{-1}, NT, PG and TT at 2 ng L^{-1}, 4*t*OP at 50 ng L^{-1} and finally, NPs and EE2 at 100 ng L^{-1}. Results are included in Table 2. Compared with the DLMs values previously obtained when MASE extracts were derivatized and analyzed by means of LVI-PTV-GC–MS [21], it should be highlighted that in the present work TT, PG and NT could be included, while MeEE2 was discarded since ESI was chosen as ionization source. The DLM values obtained for NPs and EE2 are worse but probably better values would be obtained in APCI. For the rest of the analytes similar or better DLM values were obtained in the present work, which avoids the derivatization step necessary for the analysis of the target compounds by means of LVI-PTV–GC–MS. It should be kept in mind that silylation reaction used for derivatization can introduce loss of analytes through evaporation and re-suspension steps, contamination of samples during work-up, and the interference of water in the reaction system, since silylating reagents and the resulting derivatives are extremely sensitive to the presence of water [31–33]. The DLM values obtained are lower than the environmental quality standards (EQS) established for the annual average values (AA-EQS) in inland surface waters (300 ng L^{-1} for linear NP and 100 ng L^{-1} for 4*t*OP) and other surface waters (300 ng L^{-1} for linear NP and 10 ng L^{-1} for 4*t*OP) by the European Water Framework Directive (WFD) (http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF).

It should be highlighted that while DLMs for NP in the present work were calculated for the NP mixture, the EQS values of the WFD are established for the linear NP and, therefore, an overestimation of the DLM values is expected for linear NP.

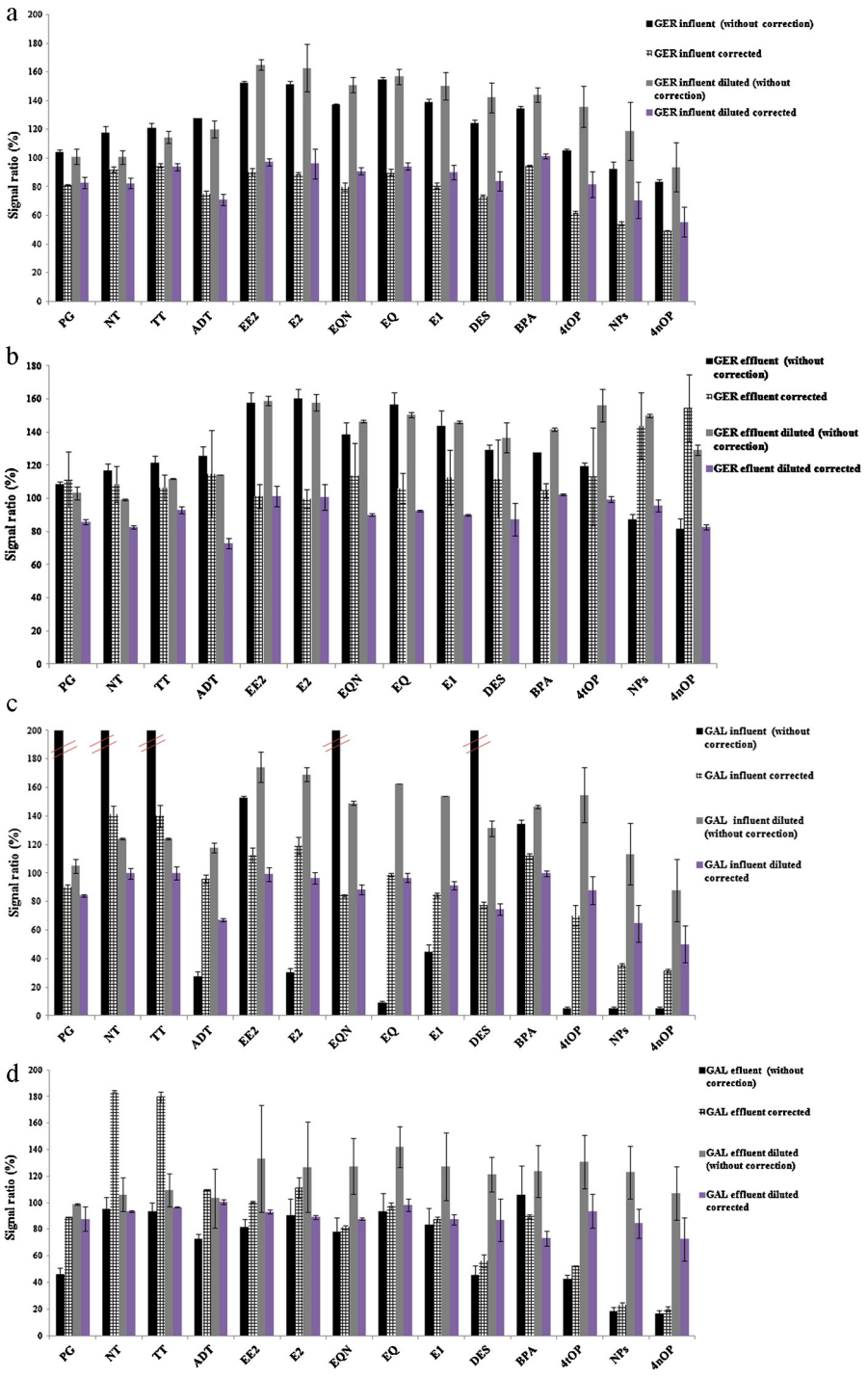

Fig. 2. (a). Summary of signal ratio values for Gernika influent samples, diluted and non diluted, with and without correction with the corresponding deuterated analogue. (b) Summary of signal ratio values for Gernika effluent samples, diluted and non diluted, with and without correction with the corresponding deuterated analogue. (c) Summary of signal ratio values for Galindo influent samples, diluted and non diluted, with and without correction with the corresponding deuterated analogue. (d) Summary of signal ratio values for Galindo effluent samples, diluted and non diluted, with and without correction with the corresponding deuterated analogue.

3.3. Comparison with SPE and application of the developed method to real samples

The developed MASE–LC–MS/MS method was applied for the analysis of wastewater samples from the local WWTPs. The results obtained are summarized in Table 3. In terms of pollution levels, the concentrations found in this study for target EDCs are in agreement with those published by other authors in similar environmental water samples [22,25,33–37].

Comparison with previously optimized and validated SPE [22] method was done with samples from Gernika and Galindo influent in campaign B (see Table 3). According to one factor analysis of variance (ANOVA), results obtained with both procedures were in good agreement.

Although it is true that considerably better DLMs were obtained when SPE is applied as extraction technique (see Table 2), due to the higher volume loaded (250 mL) and quantitative extraction efficiencies [22], MASE is better in terms of organic solvent

Table 3
Concentrations (ng L^{-1}) found ($n = 3$) in influent and effluents from Gernika and Galindo WWTPs ($n = 95\%$ of confidence level) in campaigns A and B. Both SPE and MASE were applied in campaign B.

Analyte	Campaign A				Campaign B			
	Gernika WWTP		Galindo WWTP		Gernika influent		Galindo influent	
	Influent	Effluent	Influent	Effluent	SPE	MASE	SPE	MASE
4OP	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
4*t*OP	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
NPs	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
BPA	457 ± 100	344 ± 16	428 ± 61	202 ± 22	77 ± 6	74 ± 10	408 ± 5	398 ± 35
ADT	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
DES	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	5 ± 1	≤DLM
E1	≤DLM[a]	≤DLM[a]	≤DLM[a]	≤DLM[a]	13 ± 3	≤DLM[a]	43 ± 3	38 ± 2
EQ	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
TT	≤DLM	≤DLM	11 ± 1	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
E2	84 ± 4	31 ± 6	25 ± 3	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
NT	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
EQN	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM
PG	≤DLM	≤DLM	22 ± 1	≤DLM	≤DLM	≤DLM	18 ± 2	19 ± 2
EE2	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM	≤DLM

[a] These values are higher than calculated DLMs, but lower than the limits of quantification of the method.

consumption, simplicity of the laboratory procedure, cost and time. It should be highlighted that in terms of cost, while membranes are built using freezer bags bought in a local supermarket, a box containing 200 mg Bond Elut Plexa cartridges suppose a cost of 120 euros approximately. Concerning time, loading sample and drying cartridge takes more than 3 h when SPE is employed, more than the hour and a half necessary for MASE extraction. In connection with the organic solvent volume consumption and, consequently, with the contribution to green chemistry, SPE requires 8 mL of EtOAc only for the elution step, while MASE extraction only supposes a consumption of 800 μL of chloroform. Therefore, we consider MASE as an interesting alternative to SPE in the analysis of wastewater.

4. Conclusions

MASE coupled to LC–ESI–MS/MS was applied for the determination of certain EDCs in wastewater samples. Optimization of LC–MS/MS parameters was carried out, studying both ESI and APCI interfaces. ESI was chosen as the best alternative for the analysis of most of the target analytes, except for MeEE2. NPs and EE2 should also be tested by APCI. Matrix effect during the detection was observed and different alternatives were applied in order to correct this effect. Results showed that a clean-up using Florisil cartridges compensates matrix effect, not only during the extraction step but also during the separation/detection step in LC–ESI–MS/MS and it was considered a better approach than dilution of the sample. When the clean-up was applied, correction with expensive deuterated analogues was not even necessary for some of the target analytes. Satisfactory results were obtained when MASE was compared with SPE and the developed method was applied for the analysis of wastewater samples in different campaigns. Thus, it can be concluded that, in spite of providing less sensitivity, in terms of simplicity, cost, time and contribution to green chemistry, MASE is an interesting alternative to more traditional SPE methodology in order to analyse EDCs in wastewater.

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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.2014.06.051.

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