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Determination of weakly acidic endocrine-disrupting compounds by liquid chromatography–mass spectrometry with post-column base addition

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Abstract

A sensitive analytical method based on liquid chromatography–electrospray ionisation mass spectrometry (LC–ESI-MS) has been developed for the determination of seven endocrine-disrupting compounds: 4-*n*-nonylphenol (NP), 4-*tert*-butylphenol (*t*-BP), bisphenol A (BPA), 2,4-dichlorophenol (DCP), 2,4,5-trichlorophenol (TCP), pentachlorophenol (PCP) and 4-*tert*-butylbenzoic acid (BBA) in water samples. To achieve a good LC separation, acidification of the LC mobile phase was necessary, but this led to MS signal suppression for the less acidic compounds. In order to enhance the sensitivity for these analytes, post-column addition of different bases such as ammonia, trimethylamine, and 1,8-diazabicyclo-(5,4,0)undec-7-en (DBU) was evaluated. The post-column addition of base is proposed here to raise effluent pH, helping in the ionisation process of the compounds with higher pK_a values (*t*-BP, BPA, DCP and NP). The use of DBU, diluted in MeOH, proved to be the most efficient post-column reagent for enhancing the MS signal. The signal-to-noise ratios for *t*-BP and NP increased by more than 200-fold and 35-fold, respectively, whereas for DCP and BPA an increase of about 10-fold was achieved. This strategy permitted direct determination of the seven compounds at low ppb levels. For application to real water samples, an extraction and preconcentration step using the solid-phase extraction (SPE) technique was carried out. The applicability of three solid-phase materials—Bond Elut C_{18}, and two polymeric sorbents: LiChrolut EN and Oasis HLB—and the optimization of other SPE parameters such as the elution solvent and sample volume used, were studied in order to maximize extraction efficiency. Oasis HLB provided the best results, obtaining—with the proposed SPE procedure—satisfactory percentage recoveries for all compounds (70–110%) with the exception of NP, for which a recovery of 54% was achieved. Application of the whole method, SPE–LC–(ESI)-MS, to natural waters permitted low nanogram-per-liter determination of all seven compounds.

Keywords: Water analysis; Post-column base addition; Solid-phase extraction; Endocrine disruptors; Diazabicyclo-(5,4,0)undec-7-en

1. Introduction

Emerging evidence from wildlife and laboratory studies indicates that some synthetic chemicals may interfere with the endocrine system. This can occur in a broad variety of ways, including the imitation of hormones, blocking their action and accelerating their breakdown. Compounds identified as endocrine-disrupting chemicals (EDCs) are members of different groups of chemicals, including pesticides, certain polychlorinated biphenyls (PCBs), dioxins, furans, alkylphenols, synthetic steroids, and natural products.

A report from the European Commission [1] proposed a candidate list of 553 substances for further evaluation of their role in endocrine disruption. The analytes studied in the present work are included among these substances: bisphenol A (BPA), 4-*tert*-butylbenzoic acid (BBA), the alkylphenols: 4-nonylphenol (NP) and 4-*tert*-butylphenol (*t*-BP), and the chlorophenols 2,4-dichlorophenol (DCP), 2,4,5-trichlorophenol (TCP) and pentachlorophenol (PCP).

These compounds are used in industry or are produced as intermediates in certain industrial processes. Alkylphenols are mainly used to make alkylphenol ethoxylate surfactants (detergents) and can themselves be used as plasticisers in plastics. BPA is used in the production of epoxy resins and polycarbonate plastics. These plastics are used in much food

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and drink packaging applications, whilst the resins are commonly used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Chlorophenols are released as by-products in a number of chemical processes involving chlorine. Pentachlorophenol is still used in some countries, such as Canada, as a heavy-duty wood preservative, and 4-*tert*-butylbenzoic acid can be used as an intermediate in pharmaceuticals.

The globally increased concern about these compounds highlights the need to develop highly sensitive and specific analytical methods for their extraction and determination in environmental samples. Generally, GC–MS has been the technique most commonly employed for the environmental analysis of EDCs. However, derivatisation steps are required for more polar or non-volatile compounds [2–4]. This is why for some groups of EDCs the GC–MS methodology is partially replaced by LC–MS or LC–MS–MS. In recent years, LC–MS has become an important tool in environmental analyses due to the sensitivity and efficiency provided by the newer atmospheric pressure ionisation (API) interfaces such as electrospray (ESI) and atmospheric pressure chemical ionisation (APCI).

The EDCs chosen for study here are weakly acidic compounds and have been routinely separated by reversed-phase liquid chromatography (RPLC). For the more acidic compounds, chromatographic retention may become poor. To solve this limitation, the addition of formic or acetic acid has been used to enhance retention, but this suppresses the sensitivity of the compounds in MS detection [5]. Post-column addition of different bases has been reported to raise the effluent pH, facilitating the ionisation process [6–9]. The interfaces most widely used for the LC–MS analysis of EDCs in the aquatic environment are ESI and APCI. Some authors have reported ESI to be superior for the detection of the most acidic compounds, while for less acidic phenols, bisphenol A, and alkylphenol APCI has proved to be more sensitive [10–12]. However, the ESI interface is also frequently used for the analysis of alkylphenol compounds [13,14]. Due to the acidity of the compounds, the negative mode was applied with both sources.

Analyte enrichment is necessary to isolate the target compounds from the matrix and to achieve the required detection limits (DL). For preconcentration of EDCs from aqueous samples, solid-phase extraction (SPE) is considered to be the most appropriate technique and is preferred over conventional methods, such as liquid–liquid extraction (LLE). Generally, LLE is performed with dichloromethane or ethyl acetate after sample acidification, obtaining good recoveries and reproducibilities [15]. Octadecyl (C_{18})-bonded silica has been the SPE material most widely employed for the extraction of these compounds [15,16], although it is not suitable for the simultaneous preconcentration of the more polar analytes. In order to overcome the problem and extend the applicability to more compounds, graphitised carbon black (GCB) cartridges [17], polymeric phases [2,5,8,10,18] or specially modified polymeric phases [5,19] have been employed, showing high efficiency of extraction even for the most polar analytes. Another approach to increase analyte retention is to derivatize them prior to enrichment. This approach is achieved by direct acetylation of the acidic phenolic hydroxyl group of the aqueous phenols with acetic anhydride [20]. Solid-phase micro-extraction (SPME) with poly (acrylate)-coated fibre has also been used for these compounds in environmental water samples [21].

The aim of the present work was to develop a sensitive SPE–LC–MS method for the determination of seven EDCs in water samples. Post-column addition of a volatile strong base—1,8-diazabicyclo-(5,4,0)undec-7-en (DBU)—was performed to enhance sensitivity by ESI-(NI)-MS detection. To our knowledge, this is the first work in which DBU has been used as a base for this purpose. The suitability of this soluble organic amidine Lewis base, widely used in a variety of base-mediated organic transformations, was evaluated. A solid-phase extraction procedure for preconcentration of the analytes was optimised for water samples in order to attain low nanogram-per-liter levels in the determination of all seven compounds.

2. Experimental

2.1. Chemicals

The compounds studied were as follows: 4-*n*-nonylphenol, CAS RN [104-40-5]; 4-*tert*-butylphenol, CAS RN [98-54-4]; bisphenol A, CAS RN [80-05-7]; 2,4-dichlorophenol, CAS RN [120-83-2]; 2,4,5-trichlorophenol, CAS RN [95-95-4]; pentachlorophenol, CAS RN [87-86-5], obtained from Dr. Ehrenstorfer (Augsburg, Germany) and 4-*tert*-butylbenzoic acid, CAS RN [98-73-7], obtained from Aldrich. Stock solutions of each compound were prepared in acetonitrile at 500 mg L^{-1}.

The sorbents used for solid-phase extraction were silica-based bonded C_{18} cartridges (Bond Elut 500 mg, Varian) and polymeric cartridges (Oasis HLB 60 mg, Waters and LiChrolut EN 200 mg, Merck).

The organic solvents—acetonitrile (ACN), methanol (MeOH) and ethyl acetate (AcOET)—were of HPLC grade (Merck) and were used as received. The bases used for post-column addition studies were analytical reagent grade ammonia, 25% solution, which was used as received; 1,8-diazabicyclo-(5,4,0)undec-7-en, obtained from Aldrich, and distilled triethylamine, which were both used as 2 M solutions in methanol. Ultra-high-quality (UHQ) water was obtained with an Elgastat UHQ water purification system. All chemicals used for the preparation of the buffer electrolytes were of analytical reagent grade.

2.2. Instrumentation

LC–MS analysis was performed using a Waters System (Mildford, MA, USA), consisting of a Waters Alliance HT

2795 HPLC System connected to a Waters 2996 photodiode array (PDA) detector and a Waters ZQ 4000 quadrupole mass spectrometer system that can use either APCI or ESI interfaces. The LC–MS operation control and the data process was realised by a Masslynx software. The system allowed full-scan and SIM acquisition modes to be used simultaneously. The chromatographic system was equipped with an automatic injector, a vacuum membrane degasser, a quaternary pump and a column oven. The chromatographic column was a 100 mm × 2.1 mm XTerra MS C_{18} with 3.5 μm particle size (Waters).

Post-column addition of the DBU solution was made after the photodiode array detector, using the embedded syringe pump of the ZQ 4000, via a T-connection. The flow rate was 10 μL min^{-1}.

To convey the sample through the SPE cartridge, a Gilson minipuls 2 HP 4 peristaltic pump was used. Drying and conditioning of the cartridge were accomplished in a vacuum pump (Afa, Barcelona, Spain) coupled to a 20 place manifold for sample preparation (Varian, Harbor City, USA).

2.3. Procedures

2.3.1. Chromatographic conditions

The mobile phase consisted of methanol (solvent A) and 0.0025 M ammonium formate buffer adjusted to pH 3.1 with formic acid (solvent B). Below, the term *ammonium formate buffer* refers to this pH-adjusted ammonium formate buffer solution. Gradient elution was as follows: the mobile phase started with 55% of methanol, which was linearly increased to 100% in 10 min; then, for 5 min the percentage of methanol was kept constant and was returned to the initial conditions in 3 min. The column was equilibrated for 5 min.

Column temperature was thermostatted to 30 °C and the mobile phase flow-rate was 0.2 mL min^{-1}. The volume injected was 20 μL in preliminary studies and 50 μL when the whole method was applied.

2.3.2. Mass spectrometric conditions

The ESI interface in negative-ion mode (NI) was chosen for the quantitative determination of the compounds studied. The different operating parameters of the interface were optimised by direct injection of standard solutions of each compound (2 mg L^{-1}) in MeOH. The selected parameters were as follows: source temperature, 120 °C; cone temperature, 20 °C; desolvation temperature, 250 °C, and capillary voltage, 3000 V. Nitrogen was used as both the desolvation gas (410 L/h) and as the cone gas (50 L/h). Optimisation of the cone voltage was accomplished by scanning the voltage from 10 to 120 V with the full scan mode with a scan time of 0.1 s. A value of 40 V was selected.

2.3.3. Solid-phase extraction procedure

Extraction and preconcentration of the analytes were achieved with Oasis HLB, a co-polymer of poly(divinylbenzene–co-*N*-vinylpyrrolidone). The optimum method chosen involved a conditioning with 5 mL of ethyl acetate, then 5 mL of acetonitrile and, finally, 5 mL of UHQ water. Sample passage was carried out at a flow rate of 7 mL min^{-1} by means of a peristaltic pump. Preconcentration must be carried out under acidic conditions to avoid ionisation of the compounds in the sample, and hence 10 mM of ammonium formate buffer was added. Once the retention step had been completed, the cartridges were dried for 15 min under a vacuum of 10 mmHg (1 mmHg = 133.322 Pa). The compounds retained were eluted with 0.5 mL of acetonitrile and 3 mL of ethyl acetate. The organic phase obtained was carefully evaporated to dryness under a gentle stream of nitrogen at 30–35 °C, and the residue was reconstituted with the optimised injection medium (MeOH–ammonium formate buffer, 50:50, v/v).

3. Results and discussion

3.1. Determination of endocrine-disrupting compounds by LC–MS

3.1.1. Optimisation of the chromatographic separation

Different mobile phases were tested in order to optimise the separation and peak shapes of all the compounds studied. To achieve a good LC separation, acidification of the LC eluent was necessary to suppress the ionic moieties of the analytes [22]. Upon distancing the mobile phase pH away from the pK_a of the compounds, peak shape is improved because the analytes are in a single protonated form and are less likely to interact with the silanols on the silica surface, reducing tailing and retention.

Two organic solvents commonly used in reversed-phase LC—methanol and acetonitrile—were evaluated. Methanol gave better chromatographic separations due to its lower eluotropic strength, which permitted complete peak separation. Moreover, the MS signals for all the compounds assayed were higher for methanol than for acetonitrile probably due to the more favourable ionisation properties [17] of the former solvent.

The best separation was obtained using a mobile phase containing methanol as solvent A and ammonium formate buffer adjusted to pH 3.1 with formic acid as solvent B. A linear gradient from 55 to 100% of solvent A in 10 min and then 5 min at 100% afforded adequate resolution of these compounds in less than 13 min.

3.1.2. Optimisation of the MS detection

APCI and ESI interfaces were evaluated for the compounds under NI conditions. The ESI interface was chosen because of its higher sensitivity.

3.1.2.1. Influence of post-column addition of different bases. Upon using the previously optimised chromatographic mobile phase, ESI-NI-MS gave the best results for these compounds, although a poor response was obtained for BPA, *t*-BP,

NP and DCP. The acidic nature of all seven compounds studied makes them amenable to analysis by ESI-NI-MS because they are readily ionised in basic solution. Here, therefore, post-column addition of a base is proposed to enhance sensitivity because it allows the effluent pH to be raised, facilitating the ionisation process.

In this work, different volatile strong bases were tested; namely, ammonia (pK_a = 9.3), triethylamine (pK_a = 9.8) and DBU (pK_a = 12). The base solution was introduced after the photodiode array detector and prior to entering into the mass spectrometer interface, employing a T-connection.

Fig. 1 summarizes the results obtained by post-column addition of different base solutions. The behaviour of the target compounds can be explained in terms of their pK_a values. The weaker acidic compounds, such as *t*-BP (pK_a = 10.31), BPA (pK_a = 9.8), DCP (pK_a = 7.7) and 4-NP (pK_a = 7.2), gave a low response without base; an important increase in response was achieved with post-column addition. This can be explained in terms of the more favourable deprotonation with the increase in the pH of the mobile phase after base addition. Higher responses were observed for the strongest base, DBU, diluted 1:20 (v/v) in methanol although the use of a more concentrated DBU solution did not produce better results.

On the other hand, for compounds with lower pK_a values, BBA (pK_a = 4.4) and PCP (pK_a = 4.9), which were probably dissociated in the original mobile phase, an important decrease in response was observed after post-column addition of whichever base used.

For TCP, which has an intermediate pK_a value (pK_a = 6.72), an increase in signal response following the addition of trimethylamine was observed, but no later improvements were obtained with DBU.

For all the compounds, the sensitivity of detection was severely reduced at higher base concentrations. This behaviour is unexpected and is not clear. Wang and Budde [23] described this phenomenon for nitrogenous compounds with ESI in the positive-ion mode. Those authors considered that at elevated concentrations the free anion of the acid or base may inadvertently form ion-pairs with the anions of the analytes, which are non-ionic in solution, and thus the analytes are less effectively transferred into the mass spectrometer. Another possible explanation for this behaviour may be that high concentrations of base lead to ion suppression. As a major component, the base will be preferentially ionised and will therefore suppress, to some extent, the ionisation of the other trace compounds.

Table 1
Signal-to-noise ratios with and without post-column DBU base addition

	S/N		*B*/*A*
	Without base (*A*)	Post-column addition[a] (*B*)	
BPA	63	513	8
DCP	111	1364	12
t-BP	5	1171	234
NP	44	1562	35
TCP	2851	7362	3
BBA	2081	1244	0.6
PCP	5706	2841	0.5

[a] DBU base 2 M 1:20 in MeOH.

In further experiments, a solution of 2 M DBU diluted 1:20 (v/v) in methanol was used since this afforded a considerable enhancement of the response, allowing better detection of compounds for which ESI-NI-MS is less sensitive. Table 1 shows the signal-to noise (S/N) values without and with post-column addition of DBU. The signal-to-noise ratios for *t*-BP and NP increased more than 200- and 35-fold, respectively, whereas for DCP and BPA an increase of about 10-fold was achieved.

3.1.2.2. Mass spectrometric conditions. The optimisation of the ESI-MS conditions was made by direct injection of standard solutions of each compound (2 mg L^{-1}) in MeOH. The parameters selected are reported in Section 2. The full-scan

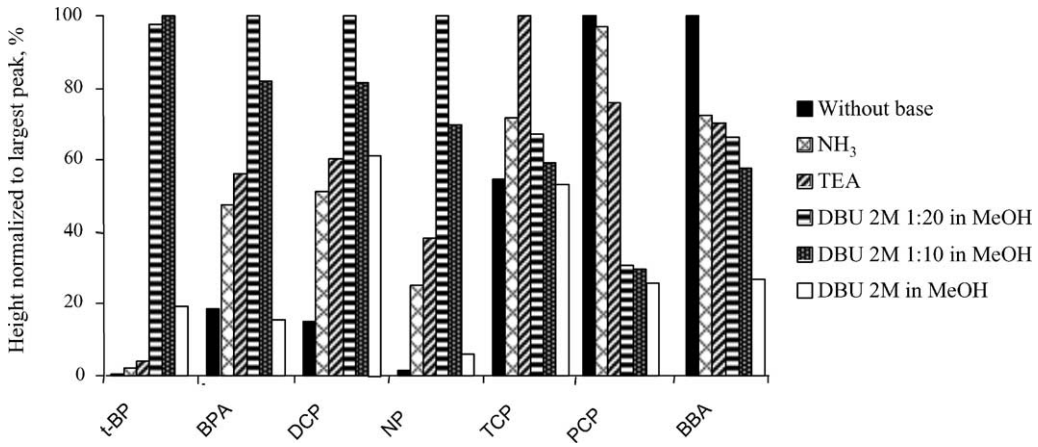

Fig. 1. Influence of post-column addition of different bases—ammonia (NH_3), triethylamine (TEA) and DBU—on the peak response height of the target compounds. Peak response height expressed as data normalized to the largest peak (%). Analyte identification: (BPA) bisphenol A, (DCP) 2,4-dichlorophenol, (*t*-BP) 4-*tert*-butylphenol, (BBA) 4-*tert*-butylbenzoic acid, (TCP) 2,4,5-trichlorophenol, (PCP) pentachlorophenol and (NP) 4-*n*-nonylphenol.

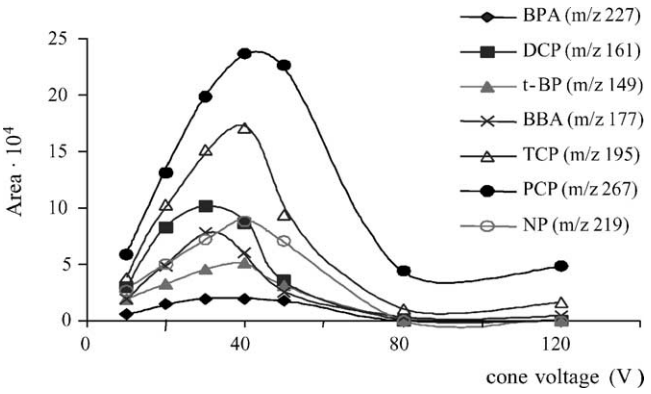

Fig. 2. Influence of cone voltage on the response by (ESI)-MS in NI mode. Analyte identification as in Fig. 1.

acquisition mode revealed that enough structural information could be obtained for the chlorophenols, due to their characteristic isotope ratio signals of the halide, and for BBA, for which an ion fragment with a mass of 133, resulting from the loss of the carboxylic group of the acid, is present. By contrast, for the alkyl phenols only the deprotonated molecule $[M - H]^-$ is present in the spectra. In order to obtain more structural information for these compounds, higher cone voltages were checked. The cone voltage was studied for each compound in the 10–120 V range (Fig. 2). No additional molecular fragment was obtained upon increasing voltage and hence a cone voltage of 40 V was chosen because this provided good sensitivity for most of the compounds studied. Quantification of the analytes was carried out under SIM acquisition mode using the deprotonated molecule. For chlorophenols the highest isotope peak of the deprotonated molecule was employed with the exception of pentachlorophenol, in which the isotope signal (*m*/*z* 267) was used because of the better peak morphology.

None of the signals shown by DBU interfered in the detection or quantification of the analytes. The full scan (*m*/*z* 50–400) spectrum of the DBU base using a cone voltage of 40 V presents peaks at *m*/*z* 169 (100), *m*/*z* 213 (70), *m*/*z* 112 (35), *m*/*z* 159 (30), *m*/*z* 88 (25) and *m*/*z* 59 (15), giving their relative abundances in brackets. The two main peaks at *m*/*z* 169 and *m*/*z* 213 probably result from $[M + OH]^-$ and $[M + HCO_3]^-$ adduct ion formation, respectively.

3.1.3. Analytical characteristics of the LC–MS method

Calibration model for the LC–MS method was constructed by injecting different standard solutions at concentrations ranging from 0.02 to 4 mg L^{-1} for MS detection under the SIM acquisition mode. Linear calibration graphs were found between peak areas and analyte concentration in the whole range studied, obtaining a relative standard deviation that never surpassed 18% ($n = 7$ at 0.02 mg L^{-1} level). The detection limits (S/N = 3) varied between 1 μg L^{-1} for TCP and PCP and 4 μg L^{-1} for BPA.

The DAD-UV detector connected in line to the LC system also provided a good linearity in the concentration range studied (0.25–4 mg L^{-1}). However, relative standard deviation values close to 30% were obtained for PCP and NP ($n = 7$ at 0.5 mg L^{-1}). The detection limits for DAD-UV detection (S/N = 3) varied between 40 μg L^{-1} for BPA and 330 μg L^{-1} for PCP.

3.2. Solid-phase extraction for endocrine-disrupting compounds

In order to obtain a more sensitive method for the quantification of these compounds, a study was performed using SPE as an extraction-preconcentration step prior to chromatographic determination. The applicability of three solid-phase materials was evaluated: C_{18} sorbent (Bond Elut 500 mg), and two polymeric sorbents—Oasis HLB, a co-polymer of poly(divinylbenzene–co-*N*-vinylpyrrolidone), and LiChrolut EN, a polymeric cartridge of styrene–divinylbenzene.

To accomplish this, 25 mL samples of ultra-high quality water spiked with 0.4 mg L^{-1} of each analyte were percolated through the different sorbents. To elute the analytes from the cartridges, 0.5 mL of acetonitrile and 3 mL of ethyl acetate were used. Prior to extraction, each SPE cartridge was conditioned by rinsing with ethyl acetate, acetonitrile and UHQ water (5 mL of each). The recoveries are shown in Table 2. Good recovery values were obtained for all the compounds studied when C_{18} (113–58%) and Oasis HLB (111–54%) were used. LiChrolut EN was the only phase exhibiting poor extraction efficiency with respect to most of the compounds. With this sorbent, the strongest acids—BBA and PCP—were not retained. This kind of behaviour has been described previously by Mol et al. [15], and it appears because at the pH of water these compounds are in their ionised form and breakthrough occurs. This was not the case when C_{18} or Oasis HLB cartridges were used. Even at pH 6—i.e., above the pK_a of both compounds—good recoveries were obtained. In the case of C_{18}, Mol et al. attributed this behaviour to selective interactions of those compounds with residual silanol groups of the stationary phase; in the case of Oasis, we consider that probably its hydrophilic–lipophilic balance (HLB) allowed the stationary phase to retain the ionised analytes more strongly than the silica-based reversed-phase sorbents. However, recoveries may be enhanced when analyte ionisation

Table 2
Recoveries (%) obtained after solid-phase extraction with different sorbents

	Recovery (%)		
	Bondelut C_{18}	Oasis HLB	LiChrolut EN
BPA	114	111	94
DCP	93	104	68
t-BP	104	102	70
BBA	69	80	ND
TCP	76	81	20
PCP	68	73	ND
NP	58	54	51

ND: not detected compound.
SPE conditions: 25 mL of UHQ water spiked at 0.4 mg L^{-1} and eluted with 0.5 mL of acetonitrile and 3 mL of ethyl acetate.

is suppressed. Therefore, Oasis HLB, as a SPE sorbent, and acidification of the water samples by the addition of 10 mM of ammonium formate buffer was used in later analysis to improve recoveries.

Elution solvents with higher eluting strength—acetone and dichloromethane—were also tested, but no improvement in recoveries was observed.

Direct LC–MS injection of the organic phase obtained in the extraction process afforded chromatographic peaks with frontal asymmetry. Therefore, evaporation of the extract and later re-dissolution of the residue in the optimised injection medium (MeOH–ammonium formate buffer, 50:50) was necessary. 500 μL was employed as the reconstituted volume. Some of the compounds studied, especially DCP and *t*-BP, are volatile analytes so special care was necessary in the evaporation step. Evaporation to dryness in a rotary evaporator at a temperature of 40 °C led to a certain loss of the most volatile compounds. For instance, about 82% of the DCP and 55% of the *t*-BP were lost and, for this evaporation step, respective relative standard deviations of 56% and 39% were obtained (for five replicates). In contrast, the use of gentle nitrogen evaporation at 30–35 °C proved to be a successful alternative, satisfactory percentage recoveries being achieved for all seven compounds and obtaining, for this step, precisions never surpassing 15% (expressed as the relative standard deviation of seven replicates).

In order to achieve higher enrichment factors, and hence improve the sensitivity of the method, a study was carried out to evaluate the possibility of increasing the volume of sample. However, breakthrough may occur when the volume to be preconcentrated is increased, especially in the case of highly polar analytes. Breakthrough in UHQ water was evaluated with volumes of sample ranging between 25 and 500 mL. Fig. 3 shows the recoveries for the Oasis HLB sorbent as a function of the preconcentrated sample volume of water matrix. From this figure, it can be concluded that the recoveries for all seven compounds are independent of the preconcentrated sample volume. Accordingly, a 500 mL water sample volume was selected as the preconcentration volume.

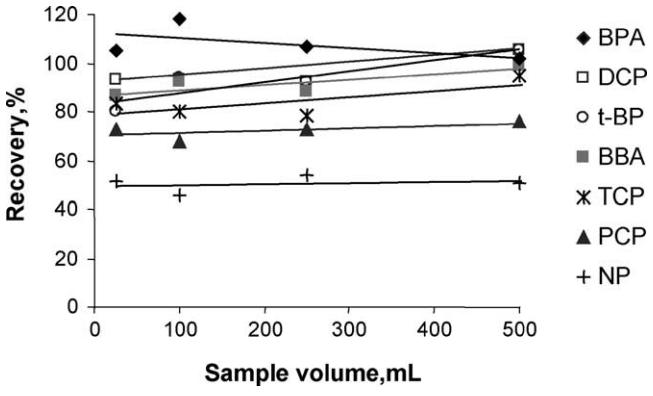

Fig. 3. Influence of the sample volume on the recovery (%). Sorbent: Oasis HLB; eluent: 0.5 mL acetonitrile and 3 mL of ethyl acetate. Analyte identification as in Fig. 1.

With the proposed SPE procedure, satisfactory percentage recoveries for all compounds were obtained (70–110%), except for NP, for which a recovery of 54% was achieved.

3.3. Analytical data for SPE–LC–(ESI)-MS method in natural water

In order to determine its analytical possibilities, the whole method was applied to tap-water samples. To decrease the detection limit of the method, a study was performed to evaluate the possibility of increasing the injection volume. An injection volume ranging from 20 to 50 μL was studied and no extra broadening of the peaks was observed, even at maximum value. Accordingly, 50 μL was chosen as the injection volume.

Samples of unspiked tap-water were analysed first, observing that two of the compounds studied, *t*-BP and BBA, were present at concentrations of 64 and 90 ng L^{-1}, respectively. In unspiked UHQ water, BBA was also observed at a similar concentration whereas *t*-BP was not present.

Table 3 shows the analytical characteristics of the method when 500 mL of tap-water sample was preconcentrated. It may be concluded that there is a good linear relationship between the analytical signal and the analyte concentration prior to preconcentration in the concentration range studied (25–1000 ng L^{-1}).

One-day precision, expressed as the relative standard deviation, did not surpass 16% for any of the seven compounds. These values can be considered highly acceptable if it is taken into account that the whole procedure comprises an SPE preconcentration step followed by re-dissolution of the evaporated extract.

The detection limits in tap water (S/N = 3) ranged from 7 ng L^{-1} for PCP to 25 ng L^{-1} for BPA. These values were compared with those obtained when the method detection limit (MDL) = *ts* criterion was used. In this expression, *s* is the standard deviation of the concentrations calculated for *n* samples with a concentration close the estimated detection limit, and *t* is Student's *t* for $n - 1$ degrees of freedom and a given level of significance [24]. The MDL values obtained were higher but similar to those obtained by the S/N = 3 criterion, except in the case of *t*-BP and BBA, for which MDL values of 27 and 60 ng L^{-1}, respectively, were calculated. Such higher values could be due to the high standard deviation obtained owing to their natural occurring presence in the tap-water matrix.

A reconstructed ion chromatogram obtained by ESI-MS in NI mode after SPE for a tap-water sample at 25 ng L^{-1} is shown in Fig. 4.

The detection limits for DCP, BBA, PCP and NP achieved in this work were similar to those obtained by the GC–MS method reported by Mol et al. [15], but a minor sample treatment was here performed.

The determination of DCP, TCP and PCP by a LC–ESI-MS method using dimethylamine (DMA) as a post-column base reagent has been proposed by Jáuregui et al. [7]. The

Table 3
Analytical characteristics for SPE–LC–(ESI)-MS method in tap water samples

	SPE–LC–(ESI)-MS						
	Ion (*m/z*)	Slope (U A/ng L^{-1})	Intercept (U A)	R^2	R.S.D.[a] (%)	MDL[b] (ng L^{-1})	DL[c] (ng L^{-1})
BPA	227	51 ± 2	$-0.6\pm1.1 \times 10^3$	0.999	16	21	25
DCP	161	223 ± 7	$(-0.2\pm3.1) \times 10^3$	0.999	9	12	8
t-BP	149	129 ± 3	$(0.3\pm1.3) \times 10^3$	1.000	10	27	11
BBA	177	182 ± 9	$(0.2\pm4.3) \times 10^3$	0.999	16	59	13
TCP	195	322 ± 12	$(0.4\pm5.5) \times 10^3$	0.999	9	14	12
PCP	267	256 ± 15	$(7.5\pm7.1) \times 10^3$	0.998	4	7	6
NP	219	124 ± 9	$(-3.0\pm4.1) \times 10^3$	0.998	15	12	9

[a] R.S.D., relative standard deviation for a concentration of 50 ng L^{-1} (n = 7).
[b] MDL = ts (method detection limit; t is Student's t-value for 7 − 1 degrees of freedom and a significance level of α = 0·05; and s is the standard deviation calculated with the same measurements employed for R.S.D.).
[c] DL, detection limit for a signal-to-noise ratio of 3.

detection limits obtained for tap water using that procedure were about one order of magnitude higher than those obtained with the present method, in which DBU was used as base reagent.

For BPA and NP, a method based on LC–MS–MS with ammonia post-column addition has been reported by Laganà et al. [8] where, the levels detected in the different water matrices were in the 3–0.2 ng L^{-1} range for BPA but higher DLs were achieved for NP (35–180 ng L^{-1}).

4. Conclusions

A sensitive method based on liquid chromatography–mass spectrometry for the determination of seven endocrine-disrupting compounds at low-nanogram per liter level has been developed. The post-column addition of a strong volatile base, DBU, proved to be an effective way to enhance the sensitivity of the least acidic compounds. As far as we are aware, this is the first description of the use of DBU for this purpose.

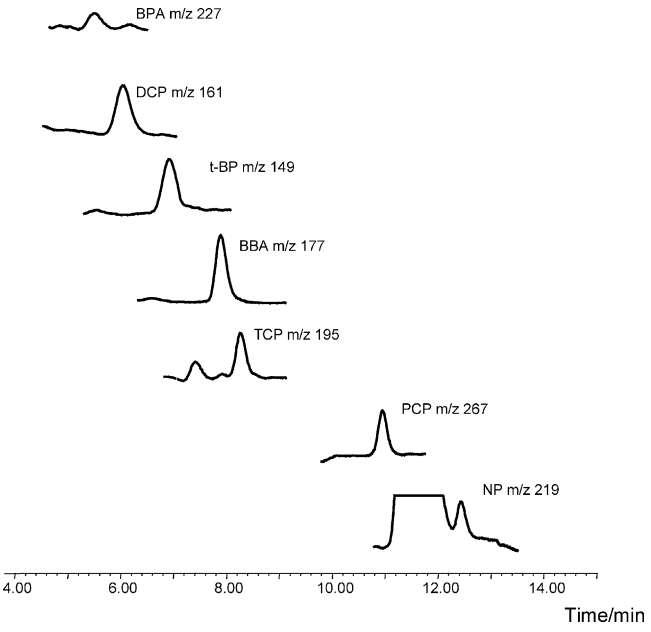

Fig. 4. Reconstructed ion chromatogram obtained by ESI-MS in NI mode after solid-phase extraction with Oasis HLB sorbent of 500 mL of a tap-water sample spiked at 25 ng L^{-1} level. For analyte identification, see Fig. 1.

Analyte enrichment from water samples was accomplished by solid-phase extraction with a polymeric sorbent: Oasis HLB. The parameters for SPE were optimised in order to maximize extraction efficiency.

Tap water was used to check the applicability of the proposed SPE–LC–(ESI)-MS method to the analysis of natural waters. Two of the analytes studied—4-*tert*-butylphenol and 4-*tert*-butylbenzoic acid—were found in unspiked tap water samples at a concentrations of 64 and 90 ng L^{-1}, respectively.

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