

Analysis of Multiple Endocrine Disruptors in Environmental Waters via Wide-Spectrum Solid-Phase Extraction and Dual-Polarity Ionization LC-Ion Trap-MS/MS

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An analytical method for the determination of 35 endocrine disrupting chemicals (EDCs) present in the aquatic environment was developed and validated. The procedure includes an off-line solid-phase extraction of 500-mL water samples using wide-spectrum polymer packing material combined with two LC-ESI-MS/MS runs, in negative and positive ionization modes. Limits of quantitation were established between 0.1 and 20.0 ng/L. Satisfactory recoveries were obtained ranging from 80.1 to 110.2%. Calibration, using deuterated internal standardization, was performed by linear regression analysis. Linearity ($R^2 > 0.99$) was demonstrated over individually specified ranges using seven calibration points for each analyte. Intrabatch and interbatch precision, as well as accuracy ($n = 5$), were investigated at low, medium, and high concentrations. Precision for all compounds, expressed as the RSD, proved to be less than 17.8 and 20.0%, respectively, for intra- and interbatch. Accuracy, expressed as the mean recovery, was between 83.1 and 108.4% at all concentrations. Stability experiments showed no significant loss or deterioration for any of the analytes. Finally, the method was applied on real samples.

Disruptions of the hormone system ascribed to the exposure of particular compounds in the environment have been reported for several years. Endocrine disrupting chemicals (EDCs) have been shown to have a profound influence on the reproduction of some species and their offspring. Wildlife examples include the occurrence of reproductive and developmental disruptions in snails, fish, piscivorous birds, alligators, and sea mammals.^{1–3} Effects on human health are still controversial. Various reports suggest possible involvement of EDCs in lower sperm counts, undescended testicles, early puberty, and thyroid dysfunction.^{4–7}

The range of substances reported to cause endocrine disruption is diverse and includes both natural and synthetic chemicals. The structural diversity within this amalgam of chemicals is not surprising, bearing in mind the complexity of endocrine systems. Renowned examples include polychlorinated biphenyls, dioxins, phthalates, a variety of pesticides and surfactants, and some pharmaceuticals, many of them ubiquitous in the environment.⁸ Some of these compounds exhibit very strong estrogenic potency and may lead to detrimental effects on organisms even at very low concentrations.⁹

The intensified awareness of the presence of EDCs in the environment has led to an increased interest in the trace analysis of such chemicals.^{10–13} Hitherto the technique most commonly applied for the environmental analysis of EDCs has been GC/MS.^{14–19} However, many compounds are not amenable to analysis by GC as a result of their thermal instability and polarity. To produce more volatile products, some analytes require derivatization. This additional step is often time-consuming and may lead to substantial analyte loss and, thus, decreased sensitivity. Furthermore, most derivatization steps are selective for a group of target analytes, discriminating other compounds of interest, simultaneously present in the sample.

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More recently, liquid chromatography coupled to mass spectrometry (LC-MS) has become a standard in environmental analysis. The advent of the robust atmospheric pressure ionization sources, which allow for the soft ionization of a wide range of substances, has led to an increasing number of LC-MS applications in the environmental area.

Numerous instrument combinations have been applied for the LC-MS analysis of EDCs in water samples.²⁰⁻²⁷ Coupled to single quadrupole, triple quadrupole, and ion trap LC-MS instruments, both pneumatically assisted electrospray (ESI) and atmospheric pressure chemical ionization have been used in either ionization mode. Although it is generally accepted that single quadrupole mass spectrometers show less specificity due to the limitation of single-stage MS analysis, the contribution of such instruments in environmental water analysis is noteworthy. Presumably, the cost of the instrument as compared to a tandem MS instrument is a decisive factor in this issue. Nonetheless, it is the authors' opinion that even when applying in-source collision-induced dissociation, the degree of confidence in the identification of compounds using a single MS instrument is insufficient for environmental analysis. For this purpose, tandem mass spectrometry (MS/MS), with ion trap or triple quadrupole MS, is of greater interest. Generating full-scan mass spectra of fragmented precursor ions, LC-MS/MS provides a selective tool for identifying trace amounts of unknown compounds in the environment. Additionally, tandem MS methods generally produce lower limits of detection as compared to single MS methods. Despite lower analytical responses, LC-MS/MS generates higher and more stable S/N ratios as a result of the specific fragmentation of isolated precursor ions and elimination of background noise. In general, triple quadrupole mass spectrometers usually offer better sensitivity. However, due to the cost-prohibitive character of the latter instruments, ion trap configurations are often preferred in routine analysis. One of the aims of the presented work was to evaluate the applicability of an ion trap LC-MS instrument for EDC trace analysis in environmental waters.

Although LC-MS is highly selective, sample preparation to remove possible interferences and preconcentrate the analytes of interest is still necessary. Classical approaches for environmental sample preparation such as solvent sublimation, steam distillation, and liquid extraction methods have been replaced by more efficient solid-phase extraction (SPE) and solid-phase microextraction techniques.²⁸ Nowadays, disks and disposable SPE cartridges are frequently used to isolate and concentrate EDCs from aqueous environmental samples. In many papers, octadecyl (C18) bonded silica is preferred as SPE material in the extraction of EDCs.²⁹⁻³² Also, graphitized carbon black cartridges^{21,33,34} and

immunoaffinity columns³⁵ have been employed for this purpose. Recently, the use of polymeric packing material is gradually increasing.^{20,36} Polymer sorbents exhibit multiple retention characteristics and higher binding capacities as compared to silica-based packing material. Therefore, multiple compounds can be extracted simultaneously. However, as a result of this wide captivity spectrum, together with target analytes, residual interferences can be coextracted also.

EDCs have been the topic of many analytical studies, yet few have attempted to combine several groups of EDCs in one analysis. This study focuses on 35 suspected EDCs known to be present in the aquatic environment. The analytes can be divided into seven groups: natural steroids, synthetic steroids, alkylphenols, diphenolalkanes, parabens, triazines, and carbamates. Another aim of this work was to develop an LC-ion trap-MS/MS method combined with off-line solid-phase extraction that could simultaneously analyze this mixture of 35 EDCs. To the best of our knowledge, this is the first method for the simultaneous analysis of this unique mixture, combining positive (PI) and negative (NI) ionization LC-MS/MS.

EXPERIMENTAL SECTION

Instrumentation. The HPLC system consisted of an G1313A autosampler with a 100- μ L loop and a G1311A quaternary pump, both from Agilent (Palo Alto, CA). Detection was carried out using a G1315B diode-array UV-visible detector, coupled in series with an 1100 LC-MSD Trap VL mass-selective detector from Agilent, equipped with an electrospray interface. Data were acquired using HP ChemStation for LC 3D System (software version A. 09.01). Quantitative data processing was performed by Quantanalysis 1.4 software. Evaporation under nitrogen was conducted in a TurboVap LV evaporator from Zymark (Hopkinton, MA).

Chemicals and Reagents. 17 β -Estradiol, estrone, estriol, 17 α -ethynylestradiol, and diethylstilbestrol (DES) were a kind gift from Professor Van den Bossche of the Laboratory for Pharmaceutical Chemistry and Drug Analysis (Ghent University, Belgium). 4-*tert*-Octylphenol, 4-octylphenol, 4-*tert*-butylphenol, 4-*sec*-butylphenol, 4-*tert*-amylphenol, 4-*n*-nonylphenol, 4-cumylphenol, bisphenol A, bisphenol F, methylparaben, ethylparaben, propylparaben, and benzylparaben were purchased from Sigma-Aldrich Chemicals (Bornem, Belgium). Atrazine-desisopropyl, atrazine-desethyl, hexazinone, simazine, cyanazine, metribuzine, desmetryn, atrazine, ametryn, sebutylazine, terbutylazine, propazine, prometryn, terbutryn, methomyl, aldicarb, and pyrimicarb were obtained from Dr. Ehrerstorfer (Augsburg, Germany). Ammonium formate was purchased from Sigma-Aldrich Chemicals. Water, methanol, acetonitrile, 2-propanol, and methyl *tert*-butyl ether (MTBE) were all of HPLC grade (Merck-Eurolab, Leuven, Belgium).

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Stock standard solutions of 1 g/L were prepared in methanol and stored in the dark at -20°C until use. Under these conditions, the solutions proved stable for more than 6 months. By diluting each stock solution with a mixture of water–acetonitrile (80:20, v/v), appropriate working solutions were obtained (500 ng/L–5 mg/L). The working solutions were prepared monthly and stored at 4°C .

Stable isotope-labeled standards 17α -ethynylestradiol-2,4,16, $-16-d_4$, bisphenol A- d_6 , and estrone-2,4,16, $16-d_4$ were purchased from C/D/N Isotopes (Quebec, Canada), diethylstilbestrol-ring-3,3',5,5'-diethyl-1,1,1',1'- d_8 was from Cambridge Isotope Laboratories, Inc. (Andover, MA), terbutryn- d_5 , simazine- d_{10} , terbutylazine- d_5 , 4-*n*-nonylphenol- d_8 , atrazine-desethyl- d_6 , sebuthylazine- d_5 , prometryn- d_6 , atrazine- d_5 , atrazine-desisopropyl- d_5 , and propazine- d_6 were from Dr. Ehrerstorfer (Augsburg, Germany), and 17β -estradiol-2,4- d_2 was obtained from Sigma-Aldrich Chemicals. Internal standard solutions were prepared in methanol at a concentration of 100 $\mu\text{g/L}$.

Water Samples. Four river water samples, three industrial effluents, and two wastewater treatment plant effluents (WWTP) were collected (500 mL) using stainless steel and Teflon bottles. Particulate matter was filtered onto a combination of a precombusted GF/F (0.7 μm nominal) glass fiber filter and a membrane filter (0.45 μm nominal) (Whatman, Maidstone, U.K.) and subsequently washed with 5 mL of methanol. The filtered samples were spiked with labeled standards (final concentration, 25 ng/L). Twenty-five milliliters of methanol was added as a wetting agent to avoid adsorption to the glass, and the water samples were stored at 4°C in dark glass bottles until extraction.

Sample Preparation. A SPE procedure for the isolation and preconcentration of the target endocrine disruptors from water was developed. An Oasis HLB (6 mL, 200 mg) (Waters, Milford, MA) column was installed on a vacuum manifold (VWR International, Leuven, Belgium) and preconditioned with 6 mL of 2-propanol–MTBE (10:90, v/v), 6 mL of methanol, and 6 mL of water. Thereafter, the aqueous samples (500 mL) were allowed to pass through the column at a rate of ~ 10 mL/min. The column was rinsed with 3 mL of a mixture of water and methanol (70:30, v/v), 3 mL of water, and 3 mL of 2% ammonia–methanol (90:10, v/v, pH 11.5). After the column was dried under vacuum for 30 min, the analytes were eluted with two 3-mL portions of 2-propanol–MTBE (10:90, v/v). The eluate was evaporated to dryness under a gentle stream of nitrogen. The dry residue was redissolved in 200 μL of water–acetonitrile (80:20, v/v), vortex mixed for 15 s, and transferred to an autosampler vial. Two subsequent injections were done in negative and positive ionization modes (50 and 10 μL , respectively). The recovery at low, medium, and high concentrations was calculated by comparing peak area ratios of standards (spiked pre- and postextraction) to the internal standards added after sample preparation ($n = 5$).

Chromatographic Conditions. HPLC separation was achieved on a C18 Luna column (100 \times 2 mm, 3 μm) fitted with a guard column with the same stationary phase (4 \times 2 mm, particle size 3 μm) (Phenomenex, Torrance, CA). Eluent flow rate was set at 200 $\mu\text{L}/\text{min}$, and the column was kept at ambient temperature. A switch valve was used to divert the column effluent to waste 2 min prior to and 1 min following elution of the analytes. Two gradient elution programs were applied with (A) 1 mM ammonium

formate in a mixture of water and acetonitrile (80:20, v/v) and (B) acetonitrile. For NI analysis, a linear gradient from 10 to 80% B in ran for 20 min and from 80 to 90% in 7 min, followed by return to starting conditions in 1 min. A 9-min equilibration time was incorporated between runs resulting in a total analysis time of 37 min. For PI analysis, it was isocratic for 2 min at 0% B, and then a linear gradient from 0 to 25% in 3 min, from 25 to 50% in 15 min, and from 50 to 75% in 5 min. Initial gradient conditions were reestablished in 2 min, and the column was equilibrated for 8 min. Here, the total analysis time was 35 min.

Mass Spectrometry. In series of continuous-infusion experiments, detection parameters were optimized for each analyte by evaluating signal intensity and fragmentation. Analytes were divided in two groups according ionization polarity (negative and positive ionization). The following optimized ESI parameters were applied: drying gas flow rate, 8 L/min; drying gas temperature, 350°C ; nebulizing gas pressure 30 psi; capillary voltage (NI/PI), + 3861/–3811 V. The electron multiplier and dynode voltages were set at 1740 V and 7.0 kV, respectively.

The signal in NI and PI was monitored in multiple reaction mode (MRM): for each analyte, the ionized species was isolated and fragmented. The fragmentation amplitude (V) excites the precursor ions, which in turn take up energy from the dipolar field and begin to collide with the helium background gas, leading to dissociation. The full-scan mass spectrum of product ions was recorded and specific product ions were selected to produce mass-extracted chromatograms. Different mass detector conditions, specific for the different analytes, were applied in consecutive time segments corresponding to the elution window of the different analytes. Seven time segments in NI and six in PI were incorporated (Figure 1). A complete overview of the MS/MS transitions, fragmentation amplitude, retention time, and corresponding segment for each analyte is given in Table 1.

Quantitation. Seven-point calibration curves were performed daily over the linear range of the instrument, determined for each analyte (Table 2). Calibration curve samples were treated in a way similar to the unknowns and quality control samples at low, medium, and high concentrations. The latter were prepared from fortified surface waters and dispersed among the samples in each analytical sequence. The concentration of the target analytes was calculated from daily regression lines. The use of stable isotope-labeled internal standards served as an automatic correction for losses of analytes during extraction or sample preparation, as well as for variations in instrument response from injection to injection. In the absence of commercially available stable isotopes for all compounds, the closest eluting deuterated compound was assigned as the internal standard (Table 2).

Method Validation. The method was validated by the following set of parameters: selectivity, sensitivity, linearity, precision, accuracy, and stability.

Sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ). LOD was defined as the lowest concentration with a signal-to-noise ratio of at least 3, and LOQ was set as the lowest concentration with a signal-to-noise ratio of at least 10. Both LOD and LOQ values were determined empirically using consecutive dilutions of spiked surface water.

Calibration curves were constructed by plotting peak area ratios between the analytes of interest and their corresponding

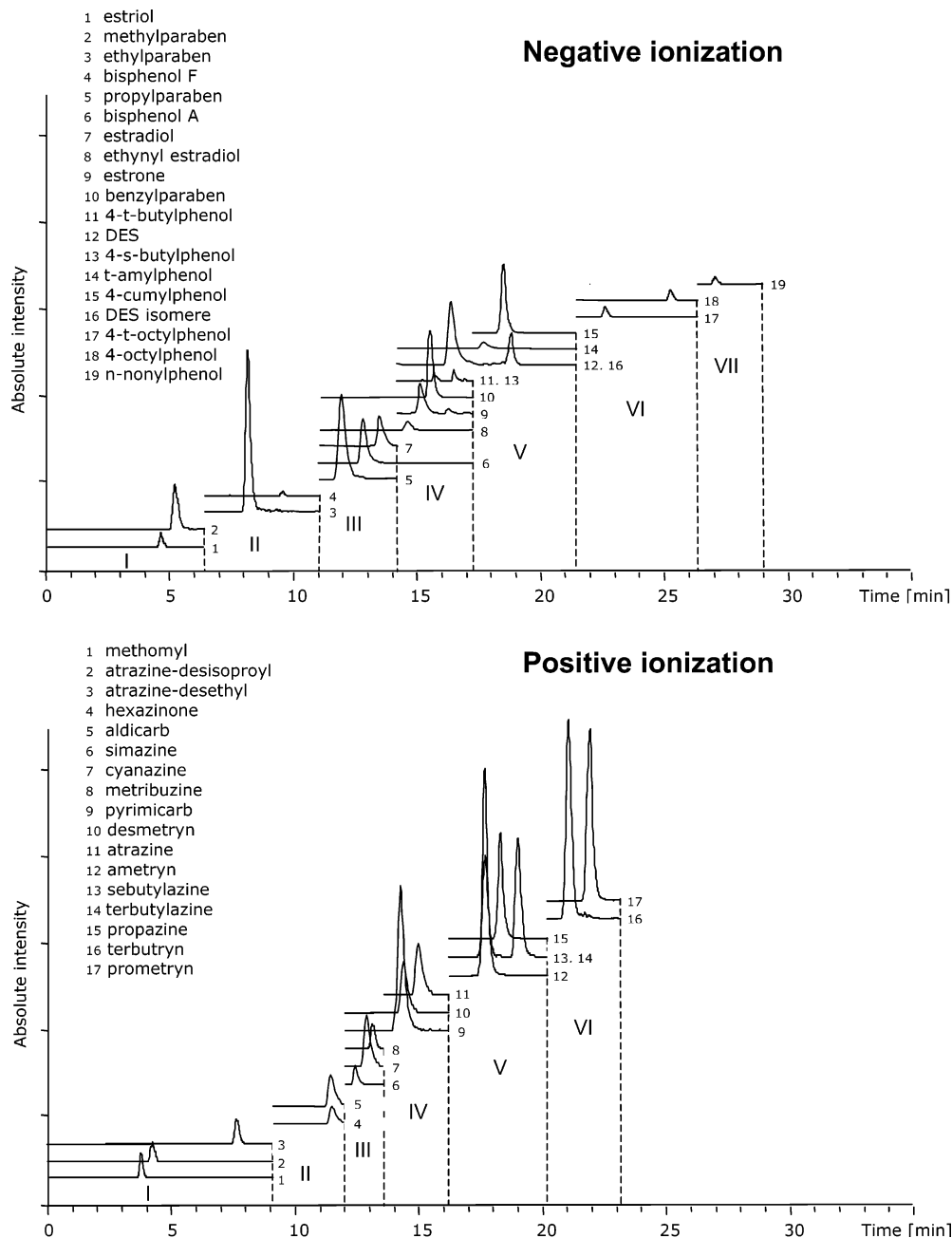


Figure 1. MRM chromatograms in negative and positive ionization of the 35 target EDCs (500 $\mu\text{g/L}$). Roman numbers indicate the corresponding time segments.

labeled internal standards, added to the samples prior to extraction, against the concentration ratios. A weighted ($1/x$) linear regression was used in an effort to account for data heteroscedasticity.

Precision, accuracy, and stability were evaluated at three concentration levels equally distributed over the linear range: low, medium, and high. The following specific concentrations were used for the compounds detected in NI, 10, 40, and 400 ng/L, with the exception of *n*-nonylphenol where, due to a lower sensitivity, validation levels were set at 40, 400, and 2000 ng/L. Low, medium, and high concentration levels for PI were established at 4, 40, and 400 ng/L. Blank surface water was

used as a representative matrix for the whole validation procedure.

Intrabatch precision was evaluated by analyzing five samples, spiked with the standard mixture on the same day. Interbatch precision was assessed by measuring five replicates per concentration level, on four consecutive days ($n = 20$). Accuracy was determined by comparison of the mean result for five analyses to the nominal concentration value.

Analyte stability was assessed at five stages during sample preparation: after loading on the SPE column, after elution of the SPE column, after evaporation, and after reconstitution in the LC eluent. Short-term stability was assessed by storing samples at

Table 1. LC-ESI-MS/MS Method Parameters

compound	MRM transition (<i>m/z</i>) ^a	fragmentation amplitude (V)	Rt (min)	segment
Negative Ionization				
estriol	287 > 145, 159, 171	1.05	4.9	I
methylparaben	151 > 136	1.30	5.1	I
ethylparaben	165 > 137	1.05	8.3	II
bisphenol F	199 > 123, 157, 171, 181, 199	1.10	9.8	II
propylparaben	179 > 137	1.05	12.0	III
bisphenol A	227 > 212	1.00	12.9	III, IV
bisphenol A- <i>d</i> ₆	233 > 215	1.00	12.9	III
estradiol	271 > 145, 158, 183, 225, 251, 253	1.40	13.3	III
estradiol- <i>d</i> ₂	273 > 145, 185	1.40	13.2	III
17 α -ethynylestradiol	295 > 185, 267	1.00	14.8	III, IV
17 α -ethynylestradiol- <i>d</i> ₄	299 > 269	1.00	14.8	III, IV
estrone	269 > 145, 159, 183, 225, 251, 253	1.40	15.1	IV
estrone- <i>d</i> ₄	273 > 147, 187	1.40	15.0	IV
benzylparaben	227 > 136	1.00	15.3	III, IV
4- <i>tert</i> -butylphenol	149 > 120, 133, 134	1.40	15.7	IV
DES	267 > 223, 238, 251	1.35	16.3	IV, V
DES- <i>d</i> ₈	275 > 229, 244, 259	1.35	16.3	IV
4- <i>sec</i> -butylphenol	149 > 120, 133, 134	1.40	16.5	IV
<i>tert</i> -amylphenol	163 > 133, 134, 147, 163	1.00	17.8	IV, V
4-cumylphenol	211 > 196	1.20	18.5	V
DES isomere	267 > 223, 238, 251	1.35	18.9	IV, V
4- <i>tert</i> -octylphenol	205 > 133	1.05	22.6	VI
4-octylphenol	205 > 106	1.05	25.2	VI
<i>n</i> -nonylphenol- <i>d</i> ₈	227 > 112	1.10	26.9	VII
<i>n</i> -nonylphenol	227 > 106, 215	1.10	27.0	VII
Positive Ionization				
methomyl	185 > 128	0.70	3.7	I
atrazine-desisopropyl	174 > 146, 132	1.13	4.0	I
atrazine-desisopropyl- <i>d</i> ₅	179 > 137	1.13	4.0	I
atrazine-desethyl	188 > 146, 158	1.00	7.4	I
atrazine-desethyl- <i>d</i> ₆	194 > 147	1.00	7.3	I
hexazinone	253 > 171	0.90	11.6	II
aldicarb	213 > 116	1.00	11.6	II
simazine	202 > 124, 132, 174	1.10	12.3	III
simazine- <i>d</i> ₁₀	212 > 134, 184	1.10	12.3	III
cyanazine	241 > 214	0.83	12.8	III
metribuzine	215 > 187	0.85	13.0	III
pyrimicarb	239 > 182, 195, 239	0.95	14.3	III, IV
desmetryn	214 > 172	0.85	14.4	III, IV
atrazine	216 > 174	1.00	15.0	IV
atrazine- <i>d</i> ₅	221 > 174, 179	1.00	14.9	IV
ametryn	228 > 158, 186	1.00	17.7	V
sebutylazine	230 > 174, 187	0.90	17.7	V
sebutylazine- <i>d</i> ₅	235 > 147, 179	0.90	17.7	V
propazine	230 > 146, 188	0.90	18.1	V
propazine- <i>d</i> ₆	236 > 147, 189, 194	0.90	18.1	V
terbutylazine	230 > 174	0.90	19.0	V
terbutylazine- <i>d</i> ₅	235 > 179	0.90	19.0	V
terbutryn	242 > 158, 200	1.05	21.2	VI
terbutryn- <i>d</i> ₅	247 > 159, 201, 206	1.05	21.2	VI
prometryn	242 > 186	1.05	21.9	VI
prometryn- <i>d</i> ₆	248 > 191	1.05	21.8	VI

^a MRM-selected product ions in italics.

room temperature and 4 °C for 24 h. For long-term stability, samples were stored at 4 °C for 7 days.

Safety Considerations. The method demands no specific safety precautions. General guidelines for work with organic solvents, acids, and bases have to be respected.

RESULTS AND DISCUSSION

Method Development. Recent trends in environmental analyses show a great demand for multicomponent assessment. In an attempt to analyze a broad range of EDCs simultaneously, a general extraction procedure was evaluated. During preliminary

experiments, several extraction procedures, including liquid extraction and SPEs with different packing materials, were tested. Fortified surface water was used to account for problems of matrix effect on extraction and ionization efficiency. Prior to extraction, samples were filtered through a glass fiber filter, entrapping large particulate matter, and a cellulose filter, incorporated to detain microinterferences. This filtration step was especially necessary in the case of waters with high levels of suspended solids or turbidity, to prevent clogging of the solid-phase columns. Five milliliters of methanol was used to elute possible adsorbed analytes. The polymer packing of Oasis HLB, [poly(divinylben-

Table 2. LODs, LOQs, and Calibration Results

compound	internal standard	LOD (ng/L)	LOQ (ng/L)	equation	R^2	linear dynamic range (ng/L)
Negative Ionization						
estriol	estradiol- d_2	2.0	4.0	$Y = 0.0168X + 0.0159$	0.996	4.0–500
methylparaben	estradiol- d_2	0.2	1.0	$Y = 0.0134X + 0.0103$	0.998	1.0–500
ethylparaben	estradiol- d_2	0.5	2.0	$Y = 0.0028X + 0.0021$	0.998	2.0–500
bisphenol F	bisphenol A- d_6	5.0	10.0	$Y = 0.0085X + 0.0113$	0.996	10.0–1000
propylparaben	estradiol- d_2	0.2	1.0	$Y = 0.0109X + 0.0109$	0.999	1.0–500
bisphenol A	bisphenol A- d_6	0.5	2.0	$Y = 0.0330X + 0.0068$	0.997	2.0–500
estradiol	estradiol- d_2	1.0	4.0	$Y = 0.0205X + 0.0012$	0.996	4.0–500
17 α -ethynylestradiol	17 α -ethynylestradiol- d_4	0.5	2.0	$Y = 0.0054X + 0.0037$	0.998	2.0–500
estrone	estrone- d_4	1.0	4.0	$Y = 0.0098X + 0.0002$	0.995	4.0–500
benzylparaben	estradiol- d_2	0.5	2.0	$Y = 0.0102X + 0.0054$	0.999	2.0–500
4- <i>tert</i> -butylphenol	bisphenol A- d_6	5.0	10.0	$Y = 0.0052X + 0.0129$	0.995	10.0–500
DES	DES- d_8	1.0	4.0	$Y = 0.0138X + 0.0002$	0.998	4.0–500
4- <i>sec</i> -butylphenol	bisphenol A- d_6	5.0	10.0	$Y = 0.0081X + 0.0113$	0.995	10.0–1000
<i>tert</i> -amylphenol	bisphenol A- d_6	5.0	10.0	$Y = 0.0101X + 0.0071$	0.997	10.0–1000
4-cumylphenol	estrone- d_4	0.2	1.0	$Y = 0.0054X + 0.0008$	0.995	1.0–500
DES isomere	DES- d_8	2.0	4.0	$Y = 0.0183X + 0.0009$	0.996	4.0–500
4- <i>tert</i> -octylphenol	DES- d_8	1.0	4.0	$Y = 0.0061X + 0.0042$	0.995	4.0–500
4-octylphenol	DES- d_8	5.0	10.0	$Y = 0.0075X + 0.0071$	0.995	10.0–1000
<i>n</i> -nonylphenol	4- <i>n</i> -nonylphenol- d_8	20.0	40.0	$Y = 0.0084X + 0.0107$	0.995	40–2000
Positive Ionization						
methomyl	atrazine-desisopropyl- d_5	0.2	1.0	$Y = 0.0069X + 0.0099$	0.997	1.0–500
atrazine-desisopropyl	atrazine-desisopropyl- d_5	0.5	2.0	$Y = 0.0109X + 0.0068$	0.995	2.0–500
atrazine-desethyl	atrazine-desethyl- d_6	0.5	2.0	$Y = 0.0096X + 0.0007$	0.996	2.0–500
hexazinone	simazine- d_{10}	0.5	2.0	$Y = 0.0056X + 0.0064$	0.997	2.0–500
aldicarb	simazine- d_{10}	2.0	4.0	$Y = 0.0079X + 0.0112$	0.998	4.0–500
simazine	simazine- d_{10}	0.5	2.0	$Y = 0.0059X + 0.0083$	0.997	2.0–500
cyanazine	simazine- d_{10}	0.5	2.0	$Y = 0.0052X + 0.0130$	0.998	2.0–500
metribuzine	simazine- d_{10}	1.0	4.0	$Y = 0.0097X + 0.0027$	0.999	4.0–500
pyrimicarb	atrazine- d_5	0.2	1.0	$Y = 0.0105X + 0.0010$	0.996	1.0–500
desmetryn	atrazine- d_5	0.1	1.0	$Y = 0.0070X + 0.0086$	0.999	1.0–500
atrazine	atrazine- d_5	0.2	1.0	$Y = 0.0106X + 0.0023$	0.998	1.0–500
ametryn	sebutylazine- d_5	0.2	1.0	$Y = 0.0118X + 0.0033$	0.998	1.0–500
sebutylazine	sebutylazine- d_5	0.1	1.0	$Y = 0.0127X + 0.0082$	0.999	1.0–500
propazine	propazine- d_6	0.1	1.0	$Y = 0.0891X + 0.0012$	0.999	1.0–500
terbutylazine	terbutylazine- d_5	0.2	1.0	$Y = 0.0049X + 0.0005$	0.997	1.0–500
terbutryn	terbutryn- d_5	0.2	1.0	$Y = 0.0073X + 0.0017$	0.998	1.0–500
prometryn	prometryn- d_6	0.2	1.0	$Y = 0.0057X + 0.0020$	0.999	1.0–500

zene-*co-N*-vinylpyrrolidone)] demonstrated the best results. Oasis HLB columns exhibit both hydrophilic and lipophilic retention characteristics and served as an excellent sorbent to retain a wide range of structurally different compounds. Recoveries ranged from 80.1 to 110.2% and are reported in Table 3.

The above-mentioned SPE procedure ensured the simultaneous extraction of all our components of interest. However, because of the difference in ionization behavior of the 35 EDCs (negative and positive ionization) a combined MS/MS method was not feasible. It was decided to divide the analytes of interest into two groups for LC–MS/MS analysis. Compounds detected in negative ionization mode included the natural and synthetic steroids, alkylphenols, diphenolalkanes, and parabens. Triazines and carbamates were analyzed in positive mode. The final method included a single wide-spectrum extraction procedure in combination with two separate selective LC–MS/MS runs. Figure 1 shows the MRM chromatograms of the 35 target analytes, recorded in negative and positive ionization modes.

Validation. *Selectivity.* Various types of environmental water samples were collected and analyzed to evaluate matrix impact. It was seen (data not shown) that the influence of sample origin on the total analysis was negligible. This was for the greater part a result of the effective sample cleanup. No interfering peaks were

detected in the MRM chromatograms. Additionally, selectivity was accomplished due to the unique combination of retention time and highly selective MS/MS detection.

Sensitivity. The complete method was evaluated according to the criteria described in the Experimental Section. Table 2 lists the LODs and LOQs obtained for all analytes. As can be seen, a clear difference in sensitivity was noticed for NI and PI. LODs in NI ranged from 0.2 to 20 ng/L, while in PI, LODs were established between 0.1 and 2 ng/L. The LOQ was set as the lowest point of the calibration graphs. The sensitivity of the method is comparable with some of the previously reported methods describing the analysis of individual groups of EDCs.^{21–27,35,36} Eventual differences in sensitivity between the presented method and the latter can be related to the type of instrument used and to the fact that for multicomponent methods often compromises have to be made. However, it is the authors' opinion that the detection limits obtained in the present work meet the needs for routine EDC analysis.

Linearity. Calibration curves were linear over the specified ranges (Table 2). Correlation coefficients (R^2 , weighting factor $1/x$) of 0.995 or higher were obtained. During initial calibration experiments, with 50 μ L as injected volume, the linear dynamic range in PI was found to be too low, related to the expected

Table 3. Recovery and Precision Data

analyte	precision (% RSD)								
	recovery ^a (%, <i>n</i> = 5)			intrabatch precision ^a (<i>n</i> = 5)			interbatch precision ^a (<i>n</i> = 20)		
	10	40	400	10	40	400	10	40	400
Negative Ionization									
estriol	83.7	87.5	86.5	12.8	8.6	5.3	18.7	14.6	9.6
methylparaben	99.3	92.7	95.7	6.2	4.4	3.8	12.2	8.4	4.3
ethylparaben	91.1	92.6	99.8	6.7	5.2	5.2	13.5	8.0	6.7
bisphenol F	107.1	100.9	99.8	10.6	8.3	9.0	18.7	12.9	8.3
propylparaben	91.5	99.5	104.8	6.0	5.4	3.6	13.7	6.2	5.0
bisphenol A	106.3	96.6	91.1	11.8	6.7	5.8	15.1	8.8	5.8
estradiol	95.6	97.1	99.2	10.0	7.5	5.6	16.0	8.1	6.8
17 α -ethynylestradiol	89.5	95.5	96.1	7.9	4.1	3.9	13.3	7.7	7.2
estrone	99.5	97.3	98.7	8.9	6.9	4.6	14.5	8.6	10.5
benzylparaben	93.7	98.7	93.2	7.7	4.2	4.0	11.8	4.6	4.9
4- <i>tert</i> -butylphenol	82.9	88.5	87.5	14.6	9.3	7.0	19.8	14.6	11.5
DES	95.7	95.1	101.7	10.1	9.3	6.7	14.6	7.0	7.2
4- <i>sec</i> -butylphenol	85.7	81.5	84.5	14.5	8.3	6.3	18.4	13.3	10.3
<i>tert</i> -amylphenol	99.0	93.8	96.7	8.9	7.1	5.9	17.9	9.3	10.3
4-cumylphenol	110.2	99.5	100.5	11.4	10.1	6.7	18.2	14.1	10.0
DES isomere	93.8	95.6	101.3	9.2	7.8	5.5	15.5	10.3	9.0
4- <i>tert</i> -octylphenol	87.9	89.9	93.1	9.0	6.3	6.6	16.4	8.2	6.6
4-octylphenol	81.6	88.1	89.2	12.2	9.6	7.3	18.6	12.7	10.1
<i>n</i> -nonylphenol ^b	80.1	82.7	86.8	17.8	12.4	10.0	20.0	17.3	14.6

analyte	precision (% RSD)								
	recovery ^a (%, <i>n</i> = 5)			intrabatch precision ^a (<i>n</i> = 5)			interbatch precision ^a (<i>n</i> = 20)		
	4	40	400	4	40	400	4	40	400
Positive Ionization									
methomyl	90.6	93.6	93.6	7.4	4.1	3.8	11.8	6.7	4.6
atrazine-desisopropyl	100.8	98.4	96.0	8.4	5.7	5.7	13.1	8.4	6.4
atrazine-desethyl	108.7	97.5	95.3	8.9	7.1	5.0	12.7	7.0	7.6
hexazinone	94.6	91.7	96.0	7.0	5.1	5.6	13.9	7.5	5.0
aldicarb	87.0	92.2	92.9	8.5	8.0	6.0	16.1	8.7	6.1
simazine	93.3	95.4	101.7	6.5	3.8	3.5	14.7	7.3	5.6
cyanazine	87.1	95.7	95.9	6.9	4.2	6.6	15.2	8.9	6.2
metribuzine	93.3	100.4	98.5	8.2	6.6	6.5	14.4	9.0	6.3
pyrimicarb	95.0	92.9	97.4	10.2	8.6	6.7	13.3	7.7	5.1
desmetryn	97.9	98.3	101.7	6.9	6.1	3.5	11.8	5.8	4.3
atrazine	97.2	98.6	105.7	5.0	3.7	3.0	11.3	6.0	5.2
ametryn	90.3	96.5	98.2	4.5	4.0	4.1	12.4	4.7	5.4
sebutylazine	97.8	102.6	98.4	5.1	4.9	4.1	12.9	6.4	5.6
propazine	107.3	100.1	100.4	3.9	3.1	3.2	10.8	5.7	3.6
terbutylazine	99.9	97.5	98.4	3.4	3.0	3.2	11.1	4.8	5.1
terbutryn	98.8	101.1	102.2	4.1	3.6	4.3	12.0	5.9	4.6
prometryn	102.1	97.8	97.7	3.9	3.5	3.1	10.3	5.0	4.5

^a Nanograms per liter. ^b Validation levels: 40, 400, and 2000 ng/L.

concentrations in real-time water samples. It was decided to decrease the injection volume to 10 μ L in order to shift the linear range up by a factor of 5.

Precision and Accuracy. Intra- and interbatch precision were thoroughly investigated at the aforementioned levels (Table 3). Intrabatch precision for all compounds, expressed as RSD, proved to be equal to or lower than 14.6%, with the exception of *n*-nonylphenol (17.8%), while interbatch precision was maximum 20.0% for all compounds. Accuracy, calculated as the mean recovery, was between 83.1 and 108.4% at all concentrations (data not shown).

Stability. Analyte recoveries in the stability experiments were within the variability range obtained for precision and accuracy.

No significant loss or deterioration for any of the compounds of interest was observed (data not shown).

Application. To demonstrate the feasibility of the presented LC-MS/MS method, a number of real samples were analyzed. Environmental waters from different origins were sampled, including river water and industrial and WWTP effluents. Table 4 lists the concentration of several pollutants in three representative samples. As much as 24 different substances could be identified. As can be seen, the presence of natural estrogens could be demonstrated in river water and industrial effluents. Especially in river water, the detected concentration of estrone (21.7 ng/L) was alarming, taking into account its estrogenic potency.⁹ Furthermore, although its estrogenic character is much lower,⁹ the

Table 4. Levels (ng/L) of Some EDCs Found in Three Representative Water Samples

analyte	river water	WWTP	industrial effluent
Negative Ionization			
methylparaben	85.1	2.1	3.9
ethylparaben	53.8		
propylparaben	78.4	3.1	6.1
bisphenol A		8.5	5.8
estradiol	<LOQ		
estrone	21.7		<LOQ
4- <i>sec</i> -butylphenol	34.8		33.8
amyphenol			17.3
4- <i>tert</i> -octylphenol		4.8	15.3
4-octylphenol	50.1	15.9	33.6
Positive Ionization			
atrazine-desisopropyl			12.1
atrazine-desethyl	33.8	15.7	
hexazinone		2.4	7.2
simazine	56.9	15.1	35.1
cyanazine			76.4
metribuzine			4.8
pyrimicarb	34.3	5.7	27.3
atrazine	64.6	78.2	
ametryn		5.8	
sebutylazine		2.5	
propazine		4.4	
terbutylazine		3.7	3.7
terbutryn		5.6	
prometryn	24.5	8.9	

presence of parabens (up to 78.4 ng/L in river water) was noteworthy. Parabens are mainly used as preservatives in cosmetics and as antibacterial agents in some toothpastes. Very often these products contain two or more parabens as part of a preservative system, which could be confirmed from the results in Table 4. Practically all samples were characterized by the presence of alkylphenols (4-*sec*-butylphenol, amyphenol, 4-*tert*-octylphenol, 4-octylphenol) and the typical diphenolalkane, bisphenol A. Next to the ubiquitous occurrence of triazine pesticides,

(37) EEC Drinking water guideline, 80, 779, EEC; EEC L229/11-29; EEC: Brussels, 30 August 1980.

the presence of pyrimicarb was demonstrated. Pesticide levels in all samples analyzed, however, rarely exceeded the maximum allowable individual concentration of 100 ng/L imposed by the EU.³⁷

From these results, it can be concluded that the LC-ion trap-MS/MS method developed permits supporting large-scale monitoring studies with unequivocal identification and quantification of a wide range of relevant EDC target analytes.

CONCLUSION

The combination of a wide-spectrum SPE combined with two selective LC-ion trap-MS/MS analyses proved to be an efficient, precise, and sensitive method for the simultaneous detection of 35 EDCs in water samples. Negative and positive ionization analyses were separated to broaden the range of target analytes. Losses of analytes during extraction and possible influence of the matrix on ionization efficiency were compensated for by stable isotope-labeled standards. The method fulfilled analytical validation criteria. Sensitivity and linear dynamic range were relevant to environmental water analyses, and precise and accurate measurement of the compounds of interest in surface water samples was achieved. Future work will imply the presented LC-MS/MS method to support environmental studies assessing the total EDC burden.

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