

## Area 6.4 • Monitoring and Fate of Persistent Chemicals

## Research Article

## Determination of Phenolic and Steroid Endocrine Disrupting Compounds in Environmental Matrices\*

Anastasia Arditoglou and Dimitra Voutsas\*\*

Aristotle University of Thessaloniki, Chemistry Department, Environmental Pollution Control Laboratory, 54124 Thessaloniki, Greece

\*\* Corresponding author (dvoutsas@chem.auth.gr)

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## Abstract

**Background, aim and scope.** Many pollutants have received significant attention due to their potential estrogenic effect and are classified as endocrine disrupting compounds (EDCs). EDCs comprise many classes of organic compounds. The development or optimization of analytical protocols for the simultaneous determination of EDCs in environmental samples is an analytical challenge because these compounds exhibit different physico-chemical characteristics, they occur in the aquatic environment in relatively low concentrations and, furthermore, environmental samples are considered as complex matrices.

The aim of this study is the development of analytical methods for the simultaneous determination of phenolic and steroid EDCs in aqueous and solid samples. The target compounds are 4-nonylphenol, 4-octylphenol, their ethoxylate oligomers (mono- and di-ethoxylates of nonylphenol and octylphenol), bisphenol A, the estrogens (estriol, estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -estradiol) and the synthetic steroids (mestranol and 17 $\alpha$ -ethynylestradiol).

**Materials and Methods.** Solid phase extraction employing Oasis HLB cartridges and different elution solvents was used for the recovery studies of the target compounds from various types of water samples (ultrapure water, artificial seawater, river water and seawater). Ultrasonic assisted extraction was applied for the recovery of the target EDCs from the solid samples. The recoveries were assessed using various solvents for the extraction and the elution of EDCs from different SPE cartridges used for clean up. Gas chromatography-mass spectrometry after derivatization with N,O-bis(trimethylsilyl)-trifluoroacetamide was employed for the determination of these compounds.

**Results and Discussion.** The recovery rates of three elution solvents (methanol, acetone and ethylacetate) for the extraction of target EDCs from artificial seawater were assessed after preconcentration on SPE cartridges. Acetone showed better recoveries and was further tested for its extraction efficiency in different water types (river water, seawater). Ultrasonic assisted extraction was used for the recovery of target EDCs from solid matrices. Acetone, methanol, mixture of acetone-methanol (1:1) and ethylacetate were used as extraction solvents. Ethylacetate and the mixture of acetone-methanol (1:1) exhibited better extraction efficiencies. An additional clean up step was necessary

for sediment samples. Different SPE cartridges were employed for clean up of the extracts (Oasis HLB, C18, Florisil, silica, combination of silica and alumina). Florisil cartridges were finally used. The proposed methods were further validated on the determination of target EDCs in field collected samples (river water, seawater, wastewater, total suspended solids and sediments) from the major area of Thessaloniki, Greece.

**Conclusions.** Efficient and accurate integrated methods for the simultaneous determination of alkylphenols (nonylphenol, octylphenol), their ethoxylate oligomers (mono- and di-ethoxylate of nonylphenol and octylphenol), bisphenol A and steroids (estriol, estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -estradiol, mestranol and 17 $\alpha$ -ethynylestradiol) in aqueous and solid samples were developed. The proposed methods were applied for the determination of the target compounds in representative environmental samples in the area of Thessaloniki, Northern Greece.

**Recommendations and Perspectives.** This study confirms the occurrence of selected EDCs in inland and marine waters in the area of Thessaloniki, Northern Greece. Since there is no previous data on the occurrence of the target EDCs in the major area, an extended survey is in progress to evaluate the occurrence and fate of these compounds.

**Keywords:** Alkylphenols; analytical method development; endocrine disrupting compounds; estrogens; nonylphenol; solid phase extraction; sediment; total suspended solids; ultrasonication; water

## Introduction

A wide variety of pollutants have received significant attention due to their potential estrogenic effect and are classified as endocrine disrupting compounds (EDCs) (European Commission 1997, US EPA 1997). EDCs comprise many classes of organic compounds such as alkylphenols, alkylphenol ethoxylates, polychlorinated biphenyls, selected pesticides, bisphenol A, polybrominated compounds, steroid sex hormones and phthalates (Sonnenschein and Soto 1998, Metzler and Pfeiffer 2001). Domestic and industrial wastewaters are significant sources of EDCs to the receiving surface, coastal waters and regional environments (Ahel et al. 1994, Ahel et al. 1996, Ying et al. 2002a,b, Vethaak et al. 2005, Voutsas et al. 2006, Zuccato et al. 2006).

The development or optimization of analytical protocols for the simultaneous determination of phenolic and steroid EDCs

\* **ESS-Submission Editor:** Prof. Dr. Thomas Braunbeck  
(Braunbeck@zoo.uni-heidelberg.de)

in environmental samples is an analytical challenge because these compounds exhibit different physicochemical characteristics (i.e. wide range of log  $K_{ow}$  and solubility values ranging from 2.81–4.67 and 0.3 to 120 mg/l. respectively), they occur in the aquatic environment in relatively low concentrations and, furthermore, environmental samples are considered as complex matrices. The general analytical scheme usually comprises isolation of target compounds from samples through extraction, clean up steps and determination by employing an analytical technique, mainly liquid or gas chromatography coupled to mass spectrometry (GC-MS, GC-MS/MS, LC-MS, LC-MS/MS) (Jeannot et al. 2002, Petrovic et al. 2002). Different extraction techniques have been proposed for the isolation of target compounds from environmental samples. Liquid–liquid extraction (Soliman et al. 2004), solid phase extraction (Liu et al. 2004a, Jeannot et al. 2002, Laganá et al. 2004, Voutsas et al. 2006), and recently solid phase microextraction (Kawaguchi et al. 2004, Basheer et al. 2005) have been employed in water samples (river water, groundwater, surface water, wastewater). The extraction procedures of EDCs from solids (soils, biosolids, sediments, suspended solids, sludges) comprise Soxhlet extraction (Gibson et al. 2005, Peng et al. 2006), microwave assisted extraction (Liu et al. 2004b, Bartolomé et al. 2005), pressurized liquid extraction (Petrovic et al. 2003, Andreu et al. 2007), supercritical fluid extraction (Long et al. 1998), accelerated solvent extraction (Fiedler et al. 2006, Noppe et al. 2007), ultrasonic assisted extraction (Peng et al. 2006, Núñez et al. 2007) and steam distillation (Lye et al. 1999). Although there are many methods proposed for the determination of phenolic and steroid EDCs separately, there are few studies on the simultaneous determination of selected phenolic and steroid EDCs by GC-MS or GC-MS/MS (Jeannot et al. 2002, Stachel et al. 2003, Liu et al. 2004a,b).

In this study, target EDCs include 4-nonylphenol and 4-octylphenol, their ethoxylate oligomers, bisphenol A and selected steroids. Alkylphenols (APs) and alkylphenol ethoxylates (APEOs) are used in industrial, agricultural and household applications as detergents, emulsifiers, wetting agents, dispersants or solubilisers (Knepper and Berna, 2003). The potential adverse effects of APEOs and APs to human and organisms resulted in a reduction on their use in several countries either through voluntary replacement or by legal regulations. Since January 2005, there has been a restriction in Europe on the sale and use of products that contain more than 0.1% of 4-nonylphenol ethoxylates (NPEOs) or 4-nonylphenols (NPs) (European Commission 2003). According to recent legislation, nonylphenols are identified as priority hazardous substances, whereas octylphenols are subject to a review for inclusion in this category (European Commission 2001). Bisphenol A is used in the production of polycarbonate and epoxy resins that account for almost 96% of the worldwide production. Other applications include its use as a stabilizing agent in plastics, as an antioxidant in tire production, as a basic chemical in the production of certain flame retardants (Staples et al. 1998, Fürhacker et al. 2000). Natural or synthetic steroids are excreted by mammals and, eventually, they occur in domestic effluents and in livestock waste (Ying et al. 2002b).

The aim of this study is the development of analytical methods for the simultaneous determination of 4-nonylphenol, 4-octylphenol, their ethoxylate oligomers (mono- and diethoxylates of nonylphenol and octylphenol), bisphenol A, the estrogens (estriol, estrone,  $17\beta$ -estradiol,  $17\alpha$ -estradiol) and the synthetic steroids (mestranol and  $17\alpha$ -ethynylestradiol) in aqueous and solid samples. The analytical protocol for aqueous samples includes solid phase extraction, derivatization and determination by GC-MS. The analytical protocol for solid samples includes ultrasonic extraction, clean up, derivatization and determination by GC-MS. The proposed methods were applied for the determination of the target compounds in seawater, river water, wastewater, total suspended solids and marine sediments in the area of Thessaloniki, Northern Greece.

## 1 Experimental

### 1.1 Reagents and materials

The standards  $17\alpha$ -estradiol,  $17\beta$ -estradiol,  $17\beta$ -estradiol- $d_2$  were purchased from Sigma (Dorset, UK), while estriol, estrone, mestranol,  $17\alpha$ -ethynylestradiol were supplied by Riedel de Haën (Seelze, Germany). Igepal CA-210 (a mixture of octylphenol mono- and di-ethoxylate), Igepal CO-210 (a mixture of nonylphenol mono- and di-ethoxylate) and bisphenol A- $d_{16}$  were purchased from Aldrich (Dorset, UK), 4-n-octylphenol from Supelco (Bellefonte, PA, USA), 4-n-butylphenol from Greyhound Chem Service (Merseyside, UK), bisphenol A from Cerilant (Austin, TX), 4-t-octylphenol, 4-nonylphenol and N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) from Fluka (Buchs, Switzerland). Individual stock solutions of the studied compounds were prepared in methanol and stored in amber glass vials at  $-18^\circ\text{C}$ . Two standard solutions were prepared by dilution of the stock solutions at a concentration of 1 ng/ $\mu\text{l}$  and were stored at  $-18^\circ\text{C}$  prior to use: the first standard contained all the studied EDCs and the second standard contained the compounds used as internal standards 4nBP, 4nNP, BPA- $d_{16}$ ,  $\beta\text{E}2$ - $d_2$ . The working standards contained the target compounds at different concentrations and the internal standards at a constant, absolute concentration of 100 ng for each compound. Glass fiber filters (GF/F) were supplied by Whatman (Middlesex, UK). Oasis HLB extraction cartridges (200 mg, 6 ml) were purchased from Waters (Milford, MA, USA). Silica gel (500 mg, 3 ml), C18 (1,000 mg, 6 ml) cartridges were from J.T. Baker (Deventer, Netherlands), while Florisil (500 mg, 4 ml) and aminopropyl (500 mg, 4 ml) cartridges were from Alltech (Alltech, UK). Acetone Pestanal, isooctane Pestanal, ethyl acetate Chromasolv and dichloromethane were from Riedel de Haën (Seelze, Germany). Methanol Lichrosolv and acetonitrile Lichrosolv were from Merck (Darmstadt, Germany). Double distilled tap water (Autostill, Jencons) was used for the preparation of ultrapure water at the laboratory (Purite Still Plus, Jencons). Artificial seawater was prepared according to standard procedure (Eaton et al. 1995). Quartz was obtained from Merck (Darmstadt, Germany) and copper granules from Riedel de Haën (Seelze, Germany).

## 1.2 Sample preparation

**Aqueous samples.** Samples (1,000 ml) were filtered through 0.7  $\mu\text{m}$  glass fiber filters and spiked with internal standards at an absolute amount of 100 ng. Solid phase extraction (SPE) utilizing Oasis HLB cartridges was employed for the isolation of target EDCs. The cartridges were placed on a vacuum manifold and conditioned sequentially with 5 ml of acetone, 5 ml of methanol and 3 x 5 ml of ultrapure water at a flow rate of 1 ml/min. Then, samples were percolated through the cartridges at a flow rate of 8 ml/min. The cartridges were dried under vacuum and EDCs were eluted with 10 ml of a solvent at a flow rate of 1 ml/min. Three solvents (acetone, methanol and ethyl acetate) were tested for their extraction efficiency. The extracts were reduced to 0.5 ml with a gentle stream of nitrogen and submitted directly to the derivatization procedure. Recovery studies were conducted on different water types (ultrapure water, artificial seawater, river water, field seawater) spiked with target compounds at two concentration levels (100, 200 ng/l).

**Total suspended solids.** Total suspended solids (TSS) were collected by filtration of aqueous samples on preweighed glass fiber filters (GF/F). Loaded filters were air dried and TSS were determined gravimetrically. Loaded filters spiked with internal standards at absolute amounts of 100 ng and with methanolic solutions of target EDCs at two levels (100–300 ng) were left until methanol was evaporated. Then, extraction took place in an ultrasonic bath with 20 ml of solvent for 20 min, in triplicate. Four different solvents, ethyl acetate, methanol, acetone, and a mixture of acetone:methanol (1:1) were tested for their extraction efficiency. The extracts were reduced to 0.5 ml by rotary evaporation and submitted directly to the derivatization procedure.

**Sediments.** Samples of freeze-dried marine sediment (0.5 g) were spiked with internal standards at an absolute amount of 100 ng and with methanolic solutions of target EDCs at two concentration levels (200 and 300 ng). The samples were vortex stirred to ensure sufficient contact of the methanolic solution with the solid matrix and were left until methanol was evaporated. Copper granules were added for the removal of sulfur. Then, the samples were ultrasonically extracted in triplicate with 20 ml of solvent for 20 min. A clean up step was necessary. For this reason, different SPE cartridges (Oasis HLB, Florisil, C18, Silica, Alumina-Silica) were tested. The clean extracts were reduced to 0.5 ml by rotary evaporation and submitted directly to the derivatization procedure.

## 1.3 Derivatization

The working standards and the final sample extracts were transferred to autosampler vials and evaporated to dryness with a gentle stream of nitrogen. After addition of 100  $\mu\text{l}$  of BSTFA, the vials were heated at 70°C for 60 min. The silylated derivatives were analyzed by GC-MS.

## 1.4 Analytical determination

GC-MS analysis was performed using a gas chromatograph (Trace GC ultra, Thermo Finnigan Electron Corporation)

coupled with an ion trap mass spectrometer (Polaris Q, Thermo Finnigan) and an autosampler (AI 3000, Thermo Finnigan Electron Corporation). An Rtx-5MS Crossbond 5% diphenyl-95% dimethyl polysiloxane capillary column (30 m length, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) with a capillary precolumn Rtx – 5MS (7 m length, 0.32 mm i.d) from Thames Restek UK Ltd was employed. Helium carrier gas (99.99% purity) was maintained at a constant rate of 1.5 ml/min. The GC column temperature program was as follows: initial temperature 60°C for 1.5 min, from 60°C to 180°C at a rate of 20°C/min then to 250°C at 5°C/min and finally to 300°C at 20°C/min. Final temperature was maintained for 6 min. Sample injection (2  $\mu\text{l}$ ) was in splitless mode and the temperature at the injector was 280°C. The MS conditions were as follows: electron ionization (EI) mode at ionization energy of 70 eV, emission current of 250  $\mu\text{A}$ , ion source and transfer line temperature at 200°C and at 300°C, respectively. A selected ion monitoring (SIM) method was employed after a solvent delay of an initial 7.5 minutes. Under these conditions, the following compounds were determined: 4-n-butylphenol (4nBP), 4-t-octylphenol (4tOP), 4-nonylphenol (NP), 4-n-octylphenol (OP), 4-n-nonylphenol (4nNP), 4-octylphenol monoethoxylate (OP1EO), 4-nonylphenol monoethoxylate (NP1EO), bisphenol A- $\text{d}_{16}$  (BPA- $\text{d}_{16}$ ), bisphenol A (BPA), 4-octylphenol diethoxylate (OP2EO), 4-nonylphenol diethoxylate (NP2EO), estrone (E1), 17 $\alpha$ -estradiol ( $\alpha\text{E2}$ ), 17 $\beta$ -estradiol ( $\beta\text{E2}$ ), 17 $\beta$ -estradiol- $\text{d}_2$  ( $\beta\text{E2-d}_2$ ), mestranol (MeEE2), 17 $\alpha$ -ethynylestradiol (EE2) and estriol (E3). A representative chromatogram is shown in Fig. 1.

## 1.5 Identification – Quantification

The silylated derivatives of the studied EDCs were identified by means of matching their retention times with those of calibration standards and by the ratio of target ions (Table 1). The ratio between the selected ions has to be the same in both the sample and the standard. The criteria reported in the Commission Decision (2002/657/EC) concerning the performance of analytical methods were used for the maximum permitted tolerances of the relative ion intensities (European Commission 2002). Generally, the tolerances were  $\pm 10\%$  for ions with a relative intensity  $>50\%$  of the base peak,  $\pm 15\%$  for ions with a relative intensity of 20–50%,  $\pm 20\%$  for ions with a relative intensity of 10–20% and  $\pm 50\%$  for ions with a relative intensity of  $<10\%$ . Nine peaks were obtained for the derivatized NP, eight peaks for NP1EO and NP2EO, and one peak for the other compounds. Seven calibration standard solutions were used to calculate response factors for each compound relative to the internal standard. Quantification of OP, OP1EO, OP2EO was carried out by calculating the relative response factors based on the area of the internal standard 4nNP. NP, NP1EO, NP2EO were quantified comparing the integrated peak area of the summed selected ions with the peak area of 4nNP. For the quantification, the ion traces with the smallest interferences were used. BPA was quantified by calculating the relative response factors based on the area of the internal standard BPA- $\text{d}_{16}$ . Steroids were quantified by calculating the relative response factors based on the area of the internal standard E2- $\text{d}_2$ .

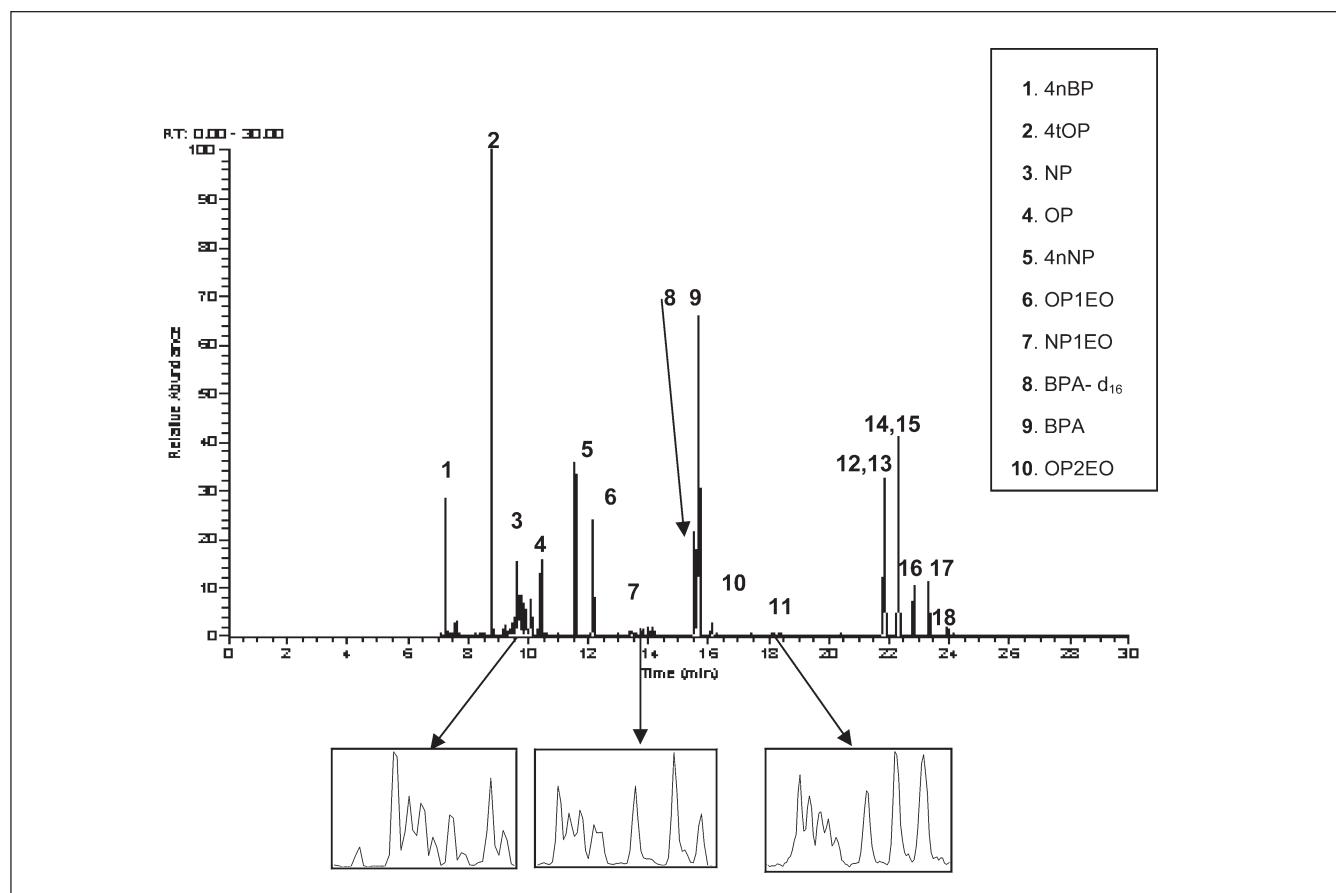


Fig. 1: Chromatogram of standard mixture of the examined compounds. Chromatographic conditions as described in the text

Table 1: Identification and quantification of the silylated derivatives of the target EDCs

Compound	Abbreviation	Molecular mass	Retention time (min)	Target ions (abundance)		Quantitation ion
4-n-butylphenol	4nBP*	150.22	7.54	179.1 (100)	222.1 (47.0)	179.1
4-t-octylphenol	4tOP	206.33	9.25	207.1 (100)	208.2 (20.7)	207.1
4-nonylphenol	NP	220.35	9.96–10.72	179.1 (43.3)	193.1 (42.3)	Sum of 9 peaks
				207.1 (100)	221.1 (46.2)	
4-n-octylphenol	OP	206.33	11.01	207.1 (11.8)	278.1 (100)	278.1
4-n-nonylphenol	4nNP*	220.35	12.22	179.1 (100)	180.2 (17.3)	179.1
4-octylphenol monoethoxylate	OP1EO	250.38	12.84	251.1 (100)	252.1 (8.0)	251.1
				251.1 (100)	265.1 (55.4)	
4-nonylphenol monoethoxylate	NP1EO	264.41	14.09–14.80	251 (100)	265.1 (55.4)	Sum of 8 peaks
Bisphenol A-d <sub>16</sub>	BPA-d <sub>16</sub> *	244.38	16.36	197.2 (8.2)	368.3 (100)	368.3
Bisphenol A	BPA	228.29	16.46	357.2 (100)	358.2 (38.3)	357.2
4-octylphenol diethoxylate	OP2EO	294.44	16.95	295.1 (100)	296.2 (11.7)	295.1
4-nonylphenol diethoxylate	NP2EO	308.47	18.3–18.57	295 (100)	308.9 (70.2)	Sum of 8 peaks
				295 (100)	308.9 (70.2)	
Estrone	E1	270.37	22.52	218.2 (20.7)	257.2 (5.4)	342.1
				342.1 (100)		
17 $\alpha$ -estradiol	$\alpha$ E2	272.39	22.52	285.2 (100)	326.2 (2.6)	285.2
17 $\beta$ -estradiol	$\beta$ E2	272.39	22.94	285.2 (100)	326.2 (4.6)	285.2
17 $\beta$ -estradiol-d <sub>2</sub>	$\beta$ E2-d <sub>2</sub> *	274.39	22.94	287.2 (100)	418.2 (42.1)	287.2
Mestranol	MeEE2	310.43	23.37	227.1 (59.3)	367.2 (100)	367.2
17 $\alpha$ -ethynyl- estradiol	EE2	296.41	23.82	232.3 (39.1)	425.3 (100)	425.3
Estriol	E3	288.39	24.41	324.2 (100)	386.2 (2.3)	324.2
				414.2 (11.7)		

\* Internal standard



### 1.6 Quality control

Linearity of the method was tested with working standards at seven concentration levels (10–250 ng). A linear fit with high correlation coefficient was obtained for the studied compounds (Table 2). The instrumental repeatability, as relative standard deviation of seven replicates of the lowest working standard, ranged from 0.440 to 8.18%. The instrumental detection limits (IDLs) were calculated from the standard deviation of seven replicates of the lowest working standard by the equation  $IDL = t_{(n-1, 1-\alpha=0.99)} \times SD$ , where  $t_{(n-1, 1-\alpha=0.99)}$  is the Student's value appropriate for a 99% confidence level and SD is the standard deviation estimate with  $n - 1$  degrees of freedom (US EPA, code 40). The IDLs for the studied compounds are shown in Table 2. The method detection limits (MDLs) were evaluated by seven replicates of clean samples spiked with all target compounds at a concentration five times the estimated method detection limit. Clean samples refer to ultra pure water for aqueous samples and quartz for solid samples. After extraction, derivatization, GC-MS analysis and quantification, the standard deviation (SD) of seven replicates for each compound was calculated and, subsequently, the MDL by way of the above mentioned equation. The quantitation limits (LOQs) were calculated to be ten times the standard deviation.

## 2 Results and Discussion

### 2.1 Aqueous samples

Oasis HLB cartridges were tested for their extraction efficiency of fourteen compounds (4tOP, NP, OP, OP1EO, OP2EO, NP1EO, NP2EO, BPA, E1,  $\alpha$ E2,  $\beta$ E2, MeEE2, EE2, E3) from artificial seawater. The average recoveries of the target compounds employing three different elution solvents, methanol, acetone and ethyl acetate, are presented in Table 3. Acetone gave better recoveries for all EDCs ranging from 54 to 118% for phenolic and 41 to 105% for steroid EDCs.

To further validate the proposed method, recovery tests were carried out using field water samples with different physico-chemical characteristics spiked with target compounds at two concentration levels (100 and 200 ng/l). The recoveries

**Table 3:** Recoveries (mean  $\pm$  SD) of phenolic and steroid EDCs from artificial seawater employing Oasis HLB extraction cartridges and different elution solvents (spike levels were at 200–300 ng of each compound,  $n=4$ )

Compound	Acetone	Methanol	Ethyl acetate
<b>Phenolic</b>			
4tOP	92 $\pm$ 7	41 $\pm$ 5	48 $\pm$ 9
NP	118 $\pm$ 14	132 $\pm$ 20	47 $\pm$ 10
OP	54 $\pm$ 14	*	*
OP1EO	106 $\pm$ 19	127 $\pm$ 2	51 $\pm$ 38
NP1EO	94 $\pm$ 24	95 $\pm$ 27	49 $\pm$ 11
BPA	57 $\pm$ 9	56 $\pm$ 35	53 $\pm$ 25
OP2EO	59 $\pm$ 31	21 $\pm$ 9	21 $\pm$ 9
NP2EO	114 $\pm$ 22	*	*
<b>Steroid</b>			
E1	88 $\pm$ 30	35 $\pm$ 16	37 $\pm$ 15
$\alpha$ E2	105 $\pm$ 21	103 $\pm$ 13	90 $\pm$ 25
$\beta$ E2	96 $\pm$ 12	70 $\pm$ 5	69 $\pm$ 38
MeEE2	58 $\pm$ 23	43 $\pm$ 12	35 $\pm$ 18
EE2	43 $\pm$ 19	55 $\pm$ 30	15 $\pm$ 2
E3	41 $\pm$ 14	21 $\pm$ 11	14 $\pm$ 5
* not eluted			

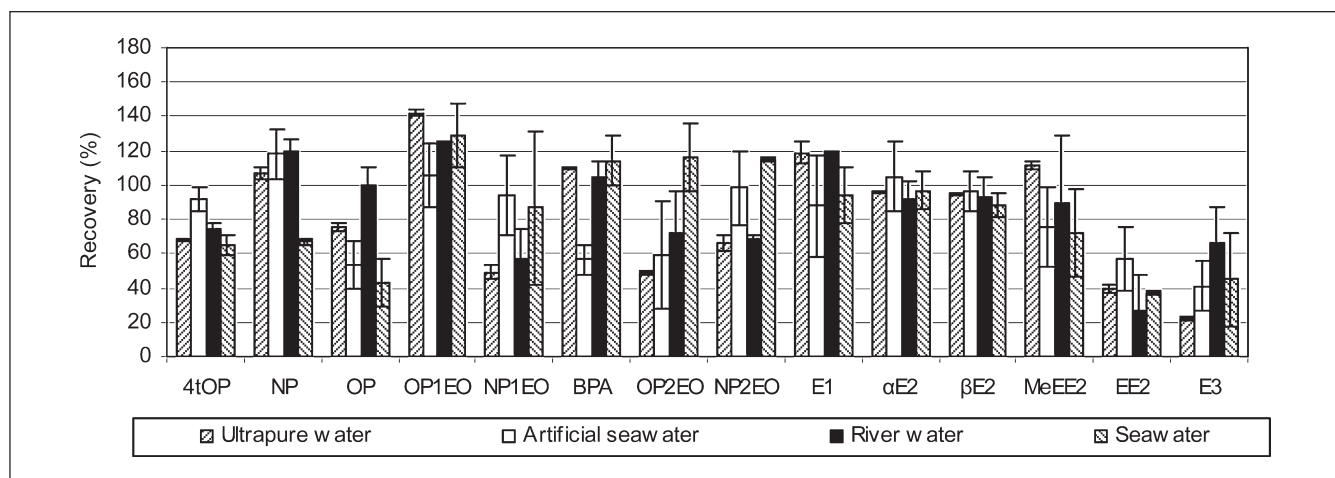
of the examined compounds from different water types (ultrapure water, artificial seawater, river water and seawater) are illustrated in Fig. 2. Although there are some differences among water types, it is hard to see any trend. For this reason, the mean absolute deviations (MADs) for each water type were calculated as follows:

$$MAD = \frac{\sum^n |x_i - 100\%|}{n}, \text{ where } x_i \text{ is the \% recovery of compound } i \text{ and } n \text{ is the number of compounds studied.}$$

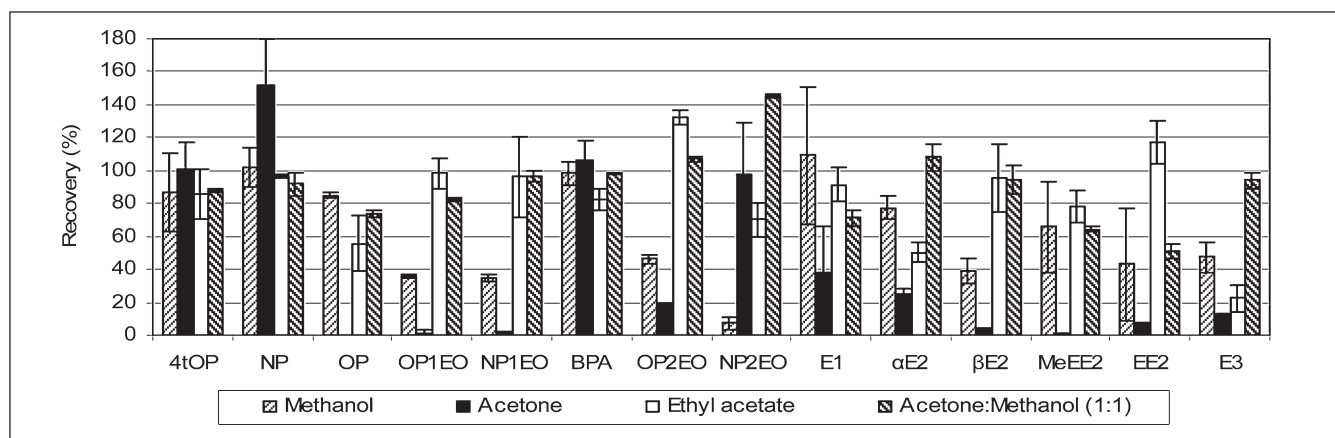
MAD gives an estimation of the accuracy and variability of a method particularly when summarizing data from a number of compounds. Similar MAD values were obtained for all water types (35.9 $\pm$ 4.3 for ultrapure water, 32.4 $\pm$ 2.8 for artificial seawater, 35.8 $\pm$ 0.4 for seawater and 33.3 $\pm$ 8.7 for river water), with no significant difference ( $p=0.05$ ), suggesting that the recovery of EDCs was not affected by water type.

**Table 2:** Quality control parameters for the proposed methods

Compound	Linearity			Instrumental		Water		Sediment	
	R <sup>2</sup>	RSD	IDL (ng/ $\mu$ l)	MDL (ng/l)	LOQ (ng/l)	MDL (ng/g)	LOQ (ng/g)		
4tOP	0.9870	0.44	0.0111	1.50	4.77	5.97	19.0		
NP	0.9980	2.70	0.040	12.3	38	13.3	42		
OP	0.9962	2.20	0.0341	7.7	24.3	11.7	35.0		
OP1EO	0.9979	2.40	0.0368	5.6	17.7	4.7	15.0		
NP1EO	0.9913	2.21	0.0327	14.1	45	4.2	13.5		
BPA	0.9994	2.33	0.0422	2.30	7.2	4.93	15.7		
OP2EO	0.9813	4.19	0.056	3.50	11.1	5.9	18.8		
NP2EO	0.9862	5.01	0.044	13.9	45	13.2	42		
E1	0.9971	3.96	0.058	11.5	37	9.5	30		
$\alpha$ E2	0.9965	3.50	0.051	2.02	6.4	5.3	16.8		
$\beta$ E2	0.9963	2.71	0.0364	1.91	6.1	4.5	14.4		
MeEE2	0.9999	3.01	0.045	7.2	23.0	10.4	33		
EE2	0.9976	1.54	0.0221	6.6	21.1	5.3	16.9		
E3	0.9932	8.18	0.066	3.0	9.5	8.6	27		



**Fig. 2:** Recoveries (mean  $\pm$  SD) of the target EDCs from different water types (SPE through Oasis HLB cartridges using acetone as elution solvent, spike levels 100, 200 ng of each compound,  $n=4$ )



**Fig. 3:** Recoveries (mean  $\pm$  SD) of the target EDCs from TSS employing ultrasonic extraction and different solvents (spike levels 100–300 ng for each compound,  $n=4$ )

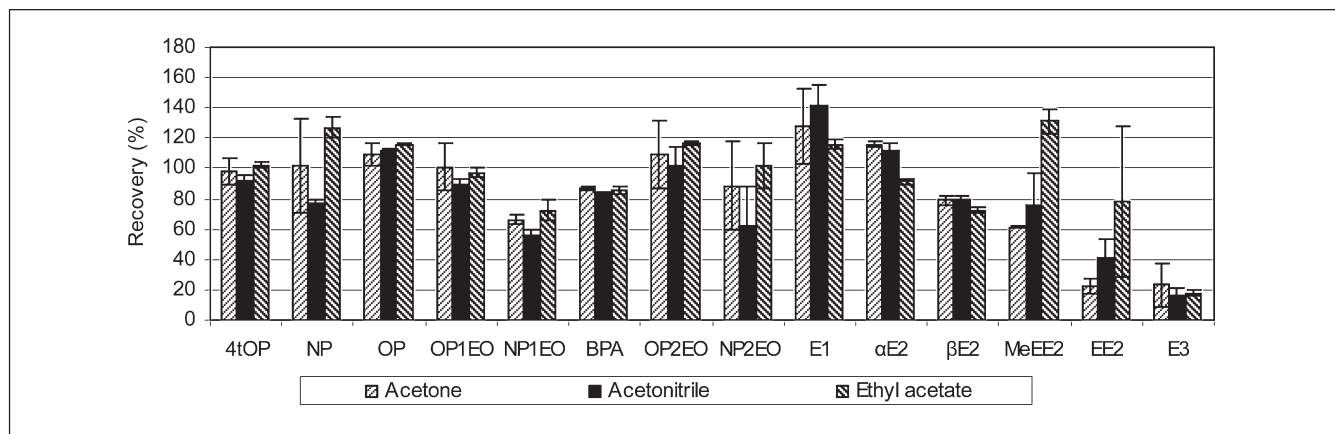
## 2.2 Total Suspended Solids

Ultrasonication was employed for the extraction of EDCs. The recovery efficiencies of four different solvents were tested. The obtained results are shown in Fig. 3. Methanol and acetone gave satisfactory results only for selected compounds such as 4tOP or BPA, whereas low recoveries were found for the other EDCs. Ethyl acetate and the mixture of acetone:methanol (1:1) exhibited better extraction efficiencies with recoveries ranging from 50% for  $\alpha$ E2 to 132% for OP2EO and from 51% for EE2 to 145% for NP2EO, respectively.

## 2.3 Sediments

Ethyl acetate and the mixture of acetone:methanol (1:1) were used for the extraction of EDCs from sediments employing ultrasonication. Dark colored and colloidal extracts were obtained probably due to interferences from the sediment's characteristics (i.e., organic content). Thus, a clean up step was necessary. Different SPE cartridges were employed for clean up of the extracts (Oasis HLB, C18, Florisil, silica, combination of silica and alumina). In most cases, the clean up procedure was not effective enough and the determination could not be performed. The method that gave the

clearer extracts combines extraction in ultrasonic bath with a mixture of methanol:acetone (1:1) for 20 min in triplicate, evaporation, solvent exchange into isooctane and clean up through Florisil cartridges. Three different solvents (acetone, acetonitrile and ethyl acetate) were tested for the elution of target compounds from Florisil cartridges. The overall recoveries of the analytical method are shown in Fig. 4. Acetone and ethyl acetate as elution solvents showed similar extraction efficiency giving better recoveries compared to acetonitrile. E3 and EE2 showed the lowest recoveries from sediments and from water samples. Low recoveries for E3 were also reported by other investigators. Peng et al. (2006) reported 27% recovery of E3 from sediments by employing ultrasonic extraction. Soliman et al. (2004) found 15% recovery of E3 from waters by employing on-line continuous liquid-liquid extraction. This behavior could be attributed to the more polar character of this compound ( $\log K_{ow} = 2.81$ ) compared to the other target steroid compounds ( $\log K_{ow} = 3.43\text{--}4.67$ ). The low recovery of EE2 has also been observed by other investigators and could be attributed to the derivatization procedure, since it is possibly a partial conversion of trimethylsilyl derivatives of EE2 to their respective E1 derivatives (Zhang and Zuo 2005, Shareef et al. 2006).



**Fig. 4:** Overall recoveries (mean ± SD) of EDCs from sediments employing ultrasonic extraction, clean up through Florisil and elution with different solvents (spike levels 200–300 ng of each compound, n=4)

**2.4 Environmental levels**

The proposed methods were applied for the determination of the target EDCs in the area of Thessaloniki, Northern Greece. The occurrence of these EDCs in the major area is examined for the first time. Samples were collected from Thermaikos Gulf (seawater, TSS, sediments), Loudias River (water, TSS) and from a canal (water, TSS). These samples could be considered as representative of the study area. The concentrations of EDCs are shown in Table 4. Alkylphenols (NP and 4tOP), their ethoxylate oligomers (NP1EO, NP2EO, OP1EO, OP2EO) and bisphenol A were determined in the examined samples, whereas steroids were not detected. The higher concentrations of all the phenolic compounds were determined in a canal which receives runoff water as well as

treated and raw wastewaters. Lower concentrations were found in river water and seawater with nonylphenol being the most abundant among the phenolic compounds. The results for phenolic EDCs are comparable to those reported in other studies for coastal and river samples. For example, the concentrations of nonylphenol which is considered as a priority pollutant, were in the range of <2.6–211 ng/l in a Venice coastal lagoon (Italy), nd-210 ng/l in Catalonia (Spain), <11–1700 ng/l in the Dutch coastal zone (Netherlands) and 20.2–269 ng/l in Jiaozhou Bay (China) (Fu et al. 2007, Pojana et al. 2007, González et al. 2004, Jonkers et al. 2005). A wide range in concentrations of nonylphenol has been reported for rivers, the higher values being measured at sites directly impacted by municipal and industrial

**Table 4:** Concentrations of the studied EDCs in the area of Thessaloniki, Northern Greece

Compound	Thermaikos Gulf (coastal sample)			Loudias River (river sample)		Canal (runoff/wastewater)	
	Seawater (ng/l)	TSS (ng/g)	Sediment (ng/g)	Water (ng/l)	TSS (ng/g)	Water (ng/l)	TSS (ng/g)
4tOP	18.2	98	8.0 (E)	13.4	274	85	181
NP	112	571	266	277	1,941	1,990	11,430
OP	n.d.	n.d.	n.d.	n.d.	48.6	n.d.	n.d.
OP1EO	n.d.	n.d.	n.d.	16.3 (E)	323	19.5	112
NP1EO	113	915	137	147	10,780	720	16,200
BPA	15.1	33.1	17.0	138	56	380	1,450
OP2EO	11.2	77	12.7(E)	33.5	540	100	166
NP2EO	50	1358	94	29 (E)	5,160	43,860	35,835
E1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
αE2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
βE2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MeEE2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EE2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected (<MDL)  
E: estimation (MDL<estimated value<LOQ)

wastewaters. Concentrations of nonylphenol ranged between 68–326 in Glatt River, Switzerland, <50–6,300 ng/l in rivers in Netherlands, 269–1,190 in rivers in USA, <150–37,300 in various rivers in Spain, 80–1,080 ng/L in Tamagawa River, Japan (Voutsas et al. 2006, Vethaak et al. 2002, Snyder et al. 1999, Céspedes et al. 2005, Isobe et al. 2001).

In the major area of Thessaloniki, various urban, industrial and agricultural activities take place. The domestic and industrial wastewater produced, as well as surface runoff, end up in Thermaikos Gulf either directly or through rivers and streams and could possibly be sources of EDCs in the coastal environment. Since there is no previous study on the occurrence of the target EDCs in the major area and in the coastal environment, an extended survey is in progress.

### 3 Conclusions

Efficient and accurate integrated methods for the simultaneous determination of alkylphenols (nonylphenol, octylphenol) and their ethoxylate oligomers (mono- and diethoxylate of nonylphenol and octylphenol, bisphenol A and steroids (estriol, estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -estradiol, mestranol and 17 $\alpha$ -ethynylestradiol) in aqueous and solid samples were developed.

The analytical protocol for water samples is based on an SPE/GC-MS method. The recoveries of target compounds were assessed using Oasis HLB extraction cartridges and different elution solvents. The analytical protocol for solid samples included ultrasonication, clean up in the case of sediments and GC-MS determination. The extraction recoveries of target compounds were assessed using different extraction solvents, different cartridges for clean up and different elution solvents. A derivatization step was carried out to enhance selectivity and sensitivity of the analysis.

The proposed methods were applied for the determination of the target compounds in river and coastal samples from the major area of Thessaloniki, Northern Greece.

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