Comparison of DI-SPME-GC-MS and SPE-GC-MS with Derivatization for Analysis of Steroid Hormones in River Water

MIRELA MICLEAN[1]*, LACRIMIOARA SENILA[1], CECILIA ROMAN[1], ADRIANA GOG[1], ERIKA LEVEI[1], IOAN GROZA[2]
[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Str., 400293, Cluj-Napoca, Romania
[2]University of Agricultural Science and Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania

In this study two extraction methods were performed for analysis of estrone and beta-estradiol in wastewater effluent and Somes River water: solid-phase microextraction (SPME) and solid phase extraction (SPE), both followed by gas chromatography - mass spectrometry (GC-MS). The target compounds were detected in water samples collected downstream of municipal sewage treatment plant of Cluj-Napoca, Romania. The results obtained by SPME and SPE methods for analyzed samples were of the same order of magnitude.

Keywords: steroid hormones, SPME, SPE, MSTFA, GC-MS, Somes River

Endocrine disrupting compounds (EDCs), like estrone and β-estradiol, could elicit harmful effects on humans, wildlife and aquatic organisms [1, 2]. They are released anthropogenically into the aquatic environment through discharges from sewage treatment plants [3-7].

It is important to determine the steroid hormones in the environment due to the input of treated wastewater directly into surface waters. Several studies have identified the natural steroids estrone and β-estradiol as the most potent estrogenic compounds in treated municipal sewage[8].

The purpose of this paper is to compare two different extraction methods for analysis of natural steroid hormones (estrone and β-estradiol) in Somes River water samples: 1. solid-phase microextraction (SPME) by direct immersion in aqueous samples, with on-fiber silylation to separate the target compounds from the samples; 2. solid phase extraction (SPE), followed by silylation. The silylated derivatives were simultaneous determinated by GC–MS. N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used as derivatization reagent to enhance selectivity and sensitivity [9, 10].

The chemical structures of estrone and β-estradiol are shown in figure 1.

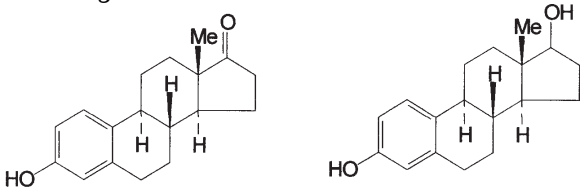

Fig. 1. Structures of the steroid hormones

Due to the very low concentration (ng/L) of estrogenic compounds in the aqueous environment, sensitive and reliable methods are required for their determination [11, 12].

The most used analytical technique for estrogen detection and quantification is gas chromatography coupled to mass spectrometry (GC–MS), tandem mass spectrometry (GC–MS–MS), liquid chromatography coupled to mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS–MS). The drawback of GC-MS technique is the use of derivatization step prior to chromatographic analysis. The target compounds need to be derivatized to produce less polar compounds [13, 14].

* *email: icia@icia.ro*

Experimental part

Study area and sampling

The water samples were collected downstream the municipal sewage treatment plant, which collect and filter the urban residues of Cluj-Napoca, a city with approximate 400000 inhabitants. The geographic coordinates of sampling points are shown in table 1.

Table 1
GEOGRAPHIC COORDINATES OF SAMPLING POINTS

Sampling point	Latitude, N	Longitude, E
1	46°47'29,03"	23°41'7,53"
2	46°47'37,03"	23°43'10,60"

The sample collected in sampling point 1 point contained effluent sewage water after filtration, and the samples collected in sampling points 2 contained river water, downstream the treatment plant, approximately 3.5 km away.

Water samples (in triplicate) were collected in pre-cleaned amber-glass bottles, in September 2009. Samples were stored at 4°C until filtration and extraction.

Filtered sewage water was filtered again in the laboratory through a 1μm glass fibre filter (Whatman, Mainstone, UK) prior the extraction.

Materials and reagents

Methanol HPLC-grade, hexane, ethyl acetate were purchased from Merck (Darmstadt, Germany). Steroid hormones: estrone (99 %) and ß-estradiol (98 %) were supplied by Sigma–Aldrich. Sodium chloride (NaCl, 99%) and hydrochloric acid (HCl, 37%) were obtained from Merck (Darmstadt, Germany). The derivatization agents N-Methyl-N-(trimethylsilyl) trifluoro acetamide (MSTFA) were purchased from Sigma–Aldrich.

Sodium chloride was used to decrease the solubility of organic compounds in water. A concentration level of 100 g/L NaCl was selected, according to other studies [9].

Hydrochloric acid was used to adjust the *p*H of the sample at value 5, in order to increase the extraction efficiency of the analytes [9].

Stock standard solutions of estrone and β-estradiol (1 mg/mL) were prepared in methanol and stored at -18 °C in

Table 2
IONS FOR QUANTITATIVE AND QUALITATIVE ANALYSIS OF SILYLATED DERIVATIVES OF STEROID COMPOUNDS

Compounds	CAS no.	Molecular mass	Retention time (min.)	Quantitative ions (*m/z*)	Qualitative ions
Estrone	53-16-7	270	20.550	342	257, 218
ß-estradiol	50-28-2	272	21.368	416	129, 285,326

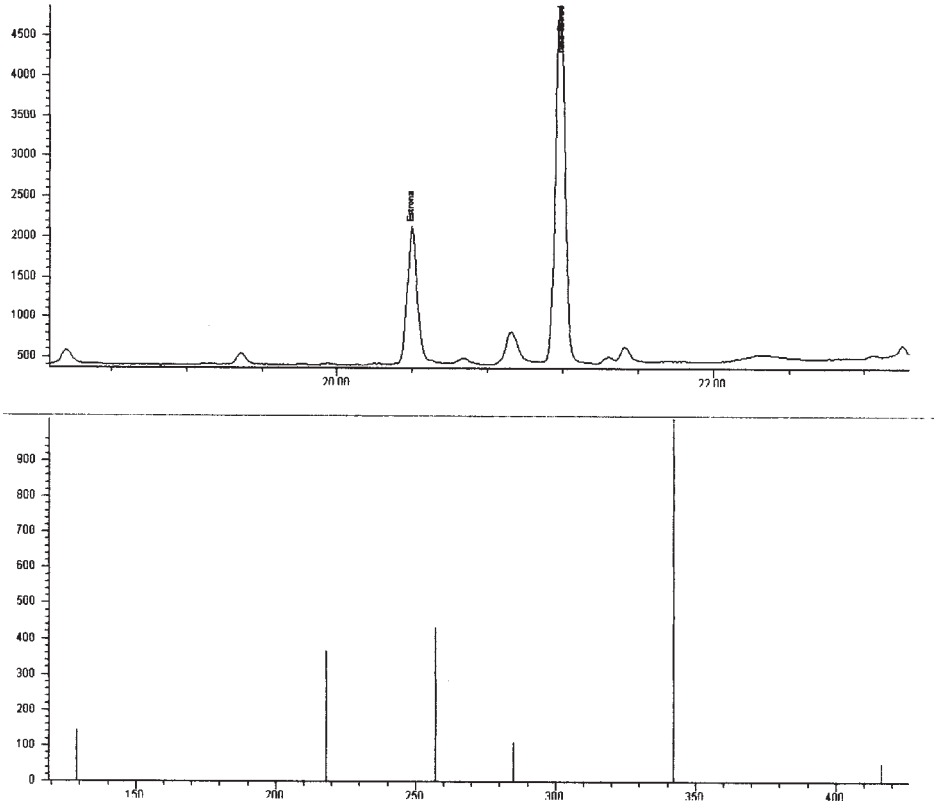

Fig.2. SIM chromatogram of target compounds from waste water effluent (and the ions for the quantitative and qualitative analysis of silylation derivatives of estrone)

dark. Working solutions were prepared by appropriate dilution of the stock standard solutions with ultrapure water and were stored at 4°C in dark. Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

For the SPME extraction a manual fiber holder Supelco Inc. (Bellefonte, PA, USA) with an 85 µm polyacrylate (PA) fiber Supelco Inc. (Bellefonte, PA, USA) were used. After every analysis the fiber was conditioned in the GC inlet for 2 h at 300°C [9]. Cartridges for SPE, Strata-X 6cc, were from Phenomenex (Torrace, CA, USA) acquisitioned.

Instrumentation

A gas chromatograph 6890N (Agilent Technologies) coupled with a mass spectrometer 5973N MSD (Agilent Technologies) and a capillary column HP-5 MS (30 m×0.25 mm×0.25 µm) were used to analyze the steroid hormones.

SPE extractions were performed on the Visiprep DL Vacuum Manifold for 12 samples from Supelco Inc. (Bellefonte, PA, USA).

Extracts were dried on a Laborota 4010 (Heidolph, Germany) rotavapor using a Ilmvac Vacuum pump (Germany).

Direct SPME extraction and headspace derivatization

Solid phase microextraction (SPME) is a unique sample preparation technique that requires no solvents or complicated apparatus. It can concentrate volatile and nonvolatile compounds (in liquids or gaseous samples), for subsequent analysis by GC or HPLC. Because analytes are concentrated on the fiber, and are rapidly delivered to the column, minimum detection limits are improved and resolution is maintained [12].

A volume of 18 mL sample, 1.8 g NaCl and a magnetic stirring bar for sample homogenization were put in a 20 mL sampler vial sealed with septa. The needle of the manual fiber holder pierced the septa, the PA fiber was released into the water sample and the extraction was performed at 120 min, and the temperature at 45°C [9, 10].

After SPME, the analytes were derivatized using the headspace derivatization technique, by exposing the fiber to the vapor of 100 µL MSTFA, in a sampler vial sealed with a septum, for 60 min at 45 °C [9, 10].

SPE and derivatization

Cartridges, prior to extractions were treated with 5 mL hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL ultrapure water. Water samples (0.5 L) were filtered and adjusted to *p*H 4 and extractions were performed with a rate of 4-5 mL/min. Cartridges were dried by vacuum, and elutions were carried out with 5 mL hexane, 5 mL ethyl acetate, and 14 mL methanol. The unified eluents were evaporated to dryness using the rotary evaporator at 40 °C [15].

To the obtained extract were added 100 µL MSTFA for derivatization, for 60 min at 25°C, on a water bath.

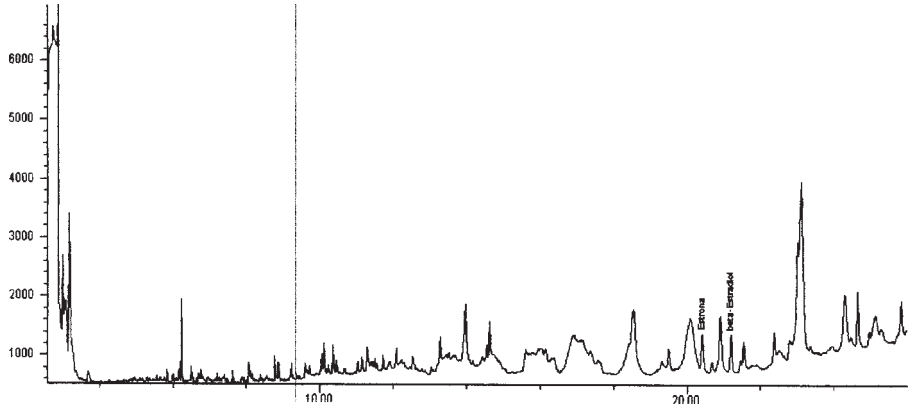

Fig.3. SIM chromatogram of target compounds from river water (sampling point 2)

Sampling point	Concentration estrone, μg/l		Concentration ß-estradiol, μg/l	
	SPME	SPE	SPME	SPE
1	0.061	0.063	0.086	0.083
2	0.038	0.032	0.055	ND

ND=not detected

Table 3
CONCENTRATIONS OF ESTROGENIC COMPOUNDS IN RIVER WATER OBTAINED BY SPME GC-MS

GC-MS analysis

For quantitative determination, the MS system was operated in SIM mode. The injector was equipped with a 4 mm-I.D. glass liner. The carrier gas was helium at constant flow rate of 1.0 mL/min. The GC column temperature program applied was as follows: the initial oven temperature was set at 90 °C, held for 2 min, from 90 to 180°C via a ramp of 30°C/min, 180 to 240°C at a ramp of 10°C/min and 240 to 270°C at 3°C/min, 270 to 300°C at 15°C/min and maintained at 300°C for 2 min. The PA fiber was manually injected into the injector heated at 290°C, and 1μL from the derivatized sample extracted by SPE was injected into the injector.

The identification of steroid hormones was based on the standard mass spectra of the MS spectral library.

Results and discussions

Estrone contains mono-hydroxyl group, the mono-TMSi was formed, β-estradiol contains bis-hydroxyl groups, the bis- TMSi was formed. The quantitative ions for estrone and β-estradiol were evidenced by the presence of *m/z* 342 and 416, respectively.

The ions monitored for each compound are listed in table 2.

The SIM chromatograms of the steroid hormones after silylated derivatization are shown in figures 2-3. The ions monitored for each compound are listed in table 2. Both target compounds in this work contained hydroxyl-group.

Estrone contained one hydroxyl group, the mono-TMSi derivatives were formed, and β-estradiol contained two hydroxyl groups, the *bis*- TMSi derivatives were formed. The mono derivates for estrone were evidenced by the presence of *m/z* 342, 218 and 257, respectively. For β-estradiol containing bis-hydroxi groups, the molecular ion at *m/z* 416 was show in the mass of derivative for β-estradiol, indicating silylation of both hydroxyl groups.

The relative standard deviations (RSD) for the target compounds were 10.6 and 11.6% for estrone and β-estradiol, respectively, using SPME procedure, and 13.5 and 11.2% for estrone and β-estradiol, respectively, using SPE procedure, showing good reproducibility for the analytes. The RDSs were calculated for 6 replicates of a water sample. The limit of detection (DL), defined as the concentration that corresponds to three times the standard deviation of blanks, was measured by integrating blank peak area for each compound in 10 independent analyses with ultrapure water as blank [8]. The obtained detection limits for estrone and β-estradiol were 0.015 and 0.008 μg/L respectively, using SPME procedure, and 0.020 and 0.110 μg/L, for estrone and β-estradiol respectively, using SPE technique.

The highest concentrations of both estrogens were found in sampling point 1, the concentrations decreased after waste water treatment plant, in sampling point 2, suggesting that the concentration of these estrogen compounds decreased due to the dilution effect. The obtained concentrations are shown in table 3.

The optimizations of reactions conditions (temperature, *p*H, derivatization time, extraction time) were selected according to other studies [9, 10].

The concentrations of these hormones obtained in the present study were higher than those obtained by Yang et all [9, 10].

Extraction of estrogens from Somes River by SPME with on-fiber silylation with MSTFA is a simple and fast analytical method, environmental friendly and capable to analyze small sample volume.

In Romania, several studies were previously performed regarding the wastewater and surface water for chemical and / or biological treatments [16-19].

Conclusions

Exposure of aquatic organisms to steroid hormones is an important concern due to the possible harmful effect. Discharges of municipal sewage in Somes River at Cluj-Napoca are the primary sources of estrogenic steroids. In this study, the analysis of estrogenic steroids in treated sewage, after dilution in Somes River was investigated. The results indicated the presence of natural estrogens, estrone and β-estradiol in water samples. The concentrations of both estrogens decreased along the

Somes River, downstream from waste water treatment plant.

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