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DETERMINATION OF STEROID HORMONES IN SOMES RIVER WATER BY SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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ABSTRACT. In this study, the occurrence of steroid hormones in Somes River was evaluated. The water samples were collected downstream of municipal sewage treatment plant of Cluj-Napoca, Romania. The used method was based on solid-phase microextraction (SPME), on-fiber silylation and, final, analysis by gas chromatography–mass spectrometry (GC-MS). The target compounds were estrone and beta-estradiol and were detected in wastewater effluent and river water. The values of target compounds in water samples were in the range of 0.010–0.080 µg/l.

Keywords: steroid hormones, SPME, MSTFA, GC-MS

INTRODUCTION

Humans and animals produce hormones, constantly excreted into the environment. The steroids of major concern are estrone and β-estradiol, since they exert their physiological effects at lower concentrations than other steroids [1, 2]. Estrone and β-estradiol cause reproductive disorders and abnormal development in wildlife and reduced fertility in human males, problems that may be caused by so-called endocrine disrupting chemicals (EDCs) released anthropogenically into the environment [3, 4].

Natural steroids, like estrone and β-estradiol are released into the aquatic environment through discharges from sewage treatment [5, 6, 7].

The purpose of this paper is to determine two natural steroid hormones in Somes River water samples using a sensitive and rapid technique. The method employs the solid-phase microextraction (SPME) by direct immersion in aqueous samples, with on-fiber silylation to separate the target compounds from the samples, followed by simultaneous determinations of the silylated

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derivatives by GC–MS. N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used as derivatization reagent to enhance selectivity and sensitivity [8, 9]. The chemical structures of estrone and β-estradiol are shown in Figure 1.

Due to the very low concentration (ng/l) of estrogenic compounds in the aqueous environment, sensitive and reliable methods are required for their determination [10, 11].

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 derivatised to produce less polar compounds [12, 13].

Until this study, we did not find any information about hormones levels in Somes River water obtained using SPME-GC/MS method.

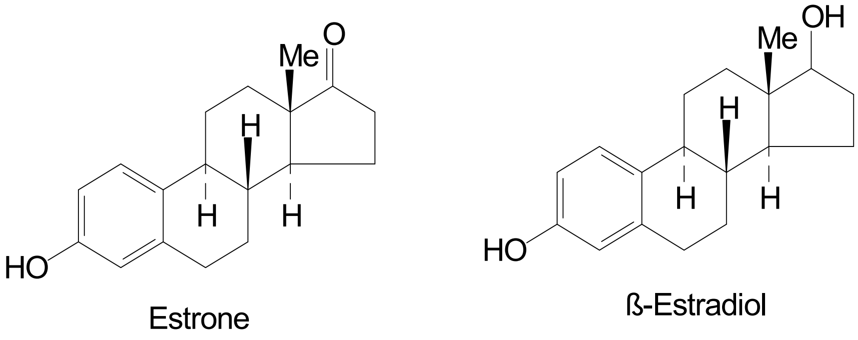

Figure 1. Structures of the steroid hormones

EXPERIMENTAL SECTION

Study area and sampling

The water samples were collected downstream of 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"
3	46°48'54,65"	23°44'56,61"

The study area comprising the three sampling points along the Somes River, downstream of sewage water treatment plant of Cluj-Napoca city is shown in Figure 2.

The samples collected in sampling point 1 point contained effluent sewage water after filtration, and the samples collected in sampling points 2 and 3 contained river water, downstream the treatment plant, approximately 3.5 km and 10 km, respectively.

Water samples were collected in triplicate, in August 2009 and were collected in pre-cleaned amber-glass bottles. 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 to extraction.

Figure 2. Study area (source Google Earth)

Chemicals

Methanol HPLC-grade was 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 [8].

Hydrochloric acid was used to adjust the pH of the sample at value 5, in order to increase the extraction efficiency of the analytes [8].

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

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.

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 [8].

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 [11].

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 [8, 9].

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 25 °C [8, 9].

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 mLmin^{-1}. The GC column temperature program is shown in Table 2.

Table 2. GC column temperature program

Step	Ramp, °C/min	Temperature, °C	Holding time, min
1		90	2
2	30	180	0
3	10	240	0
4	3	270	0
5	15	300	2

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

RESULTS AND DISCUSSION

The SIM chromatograms of the steroid hormones after silylated derivatization are shown in Figures 3-4. The ions monitored for each compound are listed in Table 3. Both target compounds in this work contained hydroxyl-group (Figure 1).

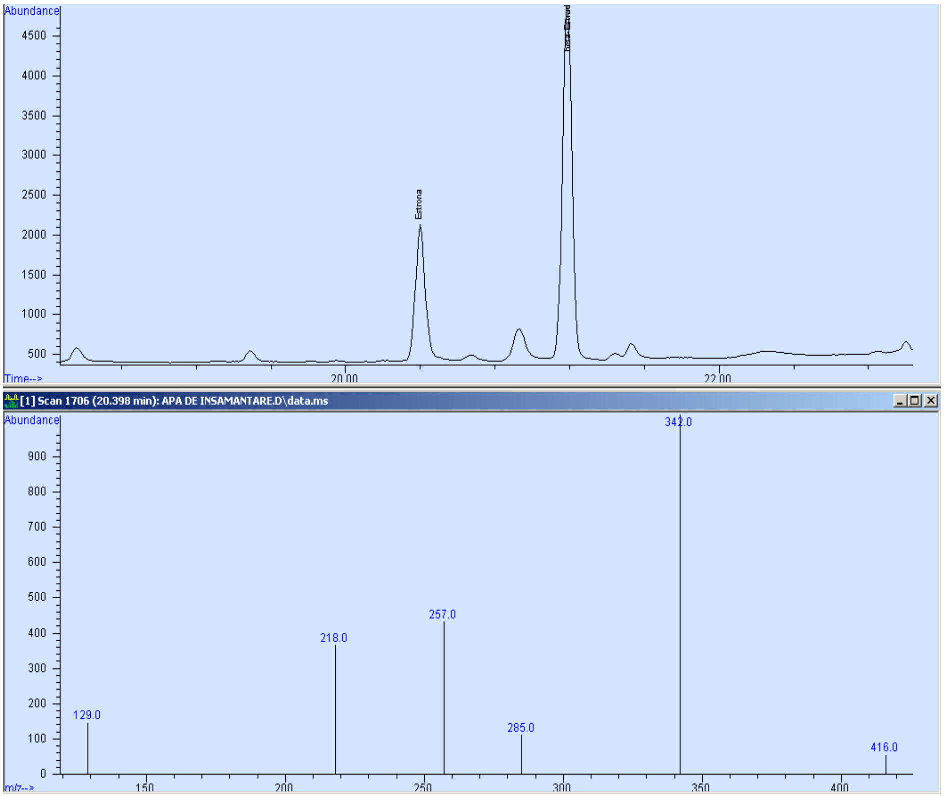

Figure 3. The SIM chromatogram of target compounds from waste water effluent (and the ions for the quantitative and qualitative analysis of silylation derivatives of estrone)

Estrone contained one hydroxyl group, the mono-TMSi derivatives were formed, and ß-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 ß-estradiol containing bis-hydroxi groups, the molecular ion at *m/z* 416 was show in the mass of derivative for ß-estradiol, indicating silylation of both hydroxyl groups. The ions monitored for estrone and ß-estradiol are listed in Table 3.

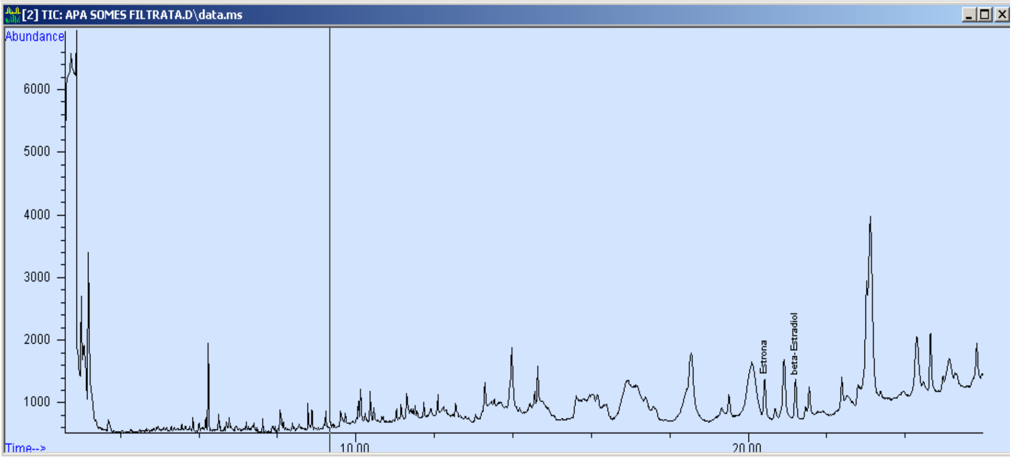

Figure 4. The SIM chromatogram of target compounds from river water (sampling point 2)

Table 3. Ions for the quantitative and qualitative analysis of silylation derivatives of target compounds

Compounds	Molecular mass	Ret. time (min)	Quantitative ions	Qualitative ions
Estrone	270	20.550	342	218, 257
ß-estradiol	272	21.368	416	129, 285

The relative standard deviations (RSD) for the target compounds were 10.2 and 11.5% for estrone and ß-estradiol, respectively, 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 DL for estrone and ß-estradiol were 0.013 and 0.008, respectively.

Highest concentrations of both estrogens were found in sampling point 1, the concentrations decreased after waste water treatment plant, along the Somes River. The obtained concentrations are shown in Table 4.

Table 4 The concentrations of estrogenic compounds in river water obtained by SPME GC-MS

Sampling point	Concentration estrone, µg/l	Concentration ß-estradiol, µg/l
1	0.056	0.080
2	0.032	0.040
3	0.020	< DL

The optimizations of reactions conditions (temperature, pH, derivatization time, extraction time) were selected according to other studies [8, 9].

Beta-estradiol was not detected in sampling point 3, suggesting that the concentration of this estrogen compound decreased due to the dilution effect.

The concentrations of this hormones obtained in the present study were higher than those obtained by Yang et al. (2006) in water samples collected from a pond in Sanjiao district (China), where the concentrations for estrone and ß-estradiol were 0.18 and 0.10 μg/l, respectively [8].

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.

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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