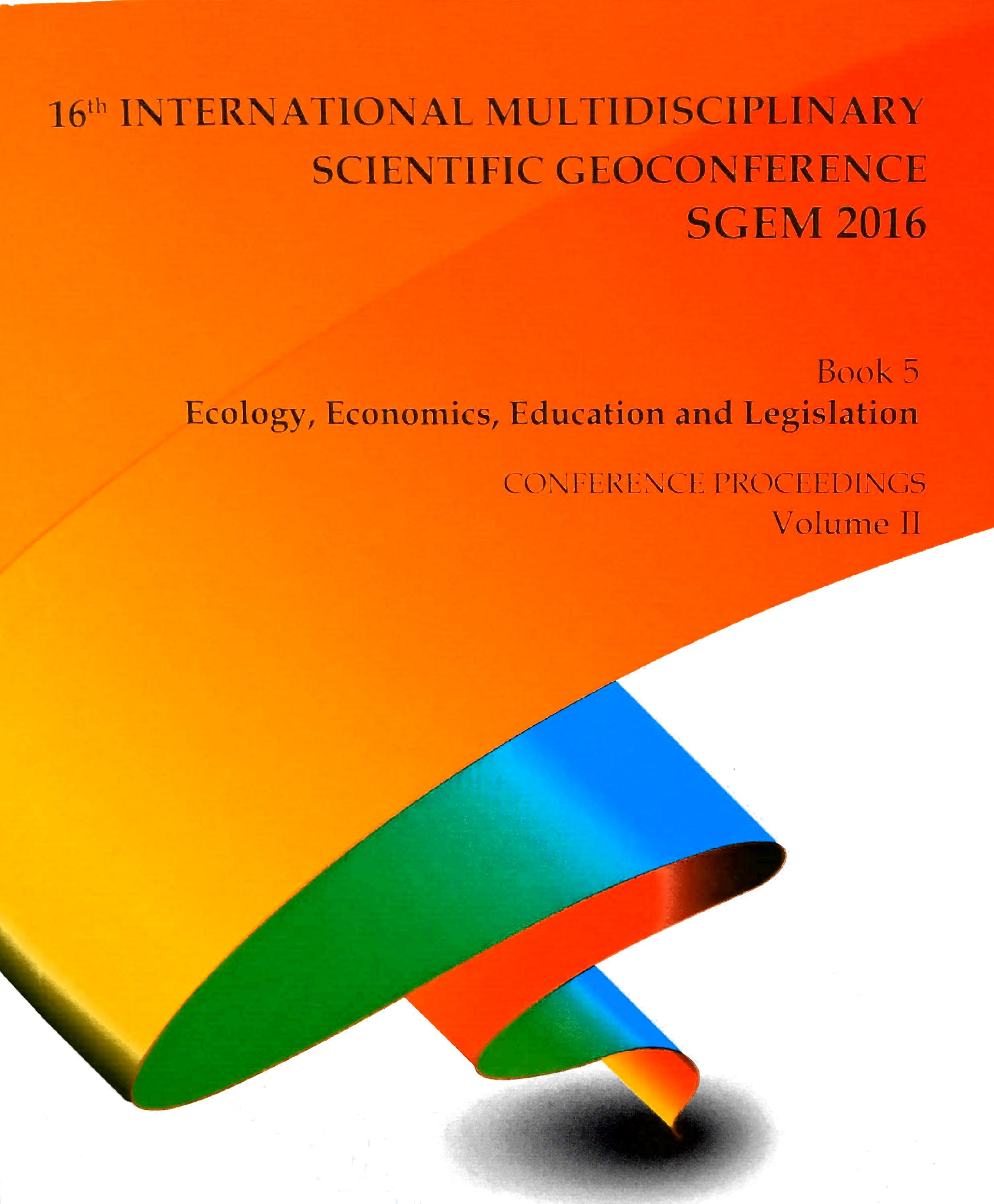
16th INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE
SGEM 2016

Book 5

Ecology, Economics, Education and Legislation

CONFERENCE PROCEEDINGS

Volume II



ECOLOGY &
ENVIRONMENTAL PROTECTION

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ECOLOGY AND ENVIRONMENTAL PROTECTION

30 June – 6 July, 2016
Albena, Bulgaria

DETERMINATION OF ETHINYLESTRADIOL AND NORGESTREL IN DRINKING WATER BY SOLID-PHASE MICROEXTRACTION

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ABSTRACT

At the present day, wide use and production of pharmaceuticals, household chemicals, pesticides and other organic pollutants threatens the favorable environmental conditions around the world. Organic pollutants, particularly endocrine disruptors are of large interest and have a negative impact on the endocrine system of living organisms. Particular danger is caused by such endocrine disruptors as steroid hormones, alkylphenols, organochlorine pesticides, phthalates. The main representatives of the group of endocrine disruptors are human induced and natural hormones - ethinylestradiol, estradiol. Existing methods for determination of endocrine disruptors in water bodies are based on chromatographic analysis combined with modern methods of sample preparation. Solid-phase microextraction is widely used in determination of phthalates, pesticides, alkylphenols in natural and waste waters. Development and application of the method of solid-phase microextraction in the study of steroid hormones such as ethinylestradiol relates to the difficult problems of analytical chemistry. The aim of this work was to develop the method for determination of ethinylestradiol in drinking water using solid-phase microextraction (SPME) and gas chromatography. This research describes the results of the method development for determination of ethinylestradiol in water bodies by solid-phase microextraction coupled with gas chromatography-mass spectrometry. Optimization was performed on the model samples of drinking water spiked with ethinylestradiol. Conducted research resulted in the optimized on-fiber SPME main parameters: extraction temperature - 80 °C and extraction time - 3 min. Developed parameters of SPME can be used for determination of the representatives of steroid hormones in the water bodies.

Keywords: endocrine disruptors, drinking water, solid-phase microextraction, gas chromatography, mass spectrometry.

INTRODUCTION

Endocrine disruptors are organic substances that negatively affect the function of the endocrine system and have adverse health effect on reproductive function of living organisms. These effects related to mimic and blocking of the action of natural hormones, such as ethinylestradiol, estradiol and hence biological functions. Thus, it leads to disruptions of growth and development [1-2]. These compounds can be the results of human pharmaceuticals consumption, using pesticides, household chemicals and industry wastewater [3]. The high hazard of getting into tap water and negative effect of the human induced and natural hormones make the need to develop analytical methods of determination of low concentration of these compounds in the environmental objects.

Different analytical methods have been proposed to determine these compounds in different matrixes [4-6]. Most of them, gas chromatography coupled with mass spectrometry was used for determination of endocrine disruptors, despite the fact that this technique is limited to the analysis of compounds with high molecular weight and low volatility of compounds. The extraction and pre-concentration methods of the analytes most frequently employed are liquid-liquid extraction, solid-phase extraction and solid-phase microextraction [7-9]. The founder of solid-phase microextraction method, Pawliszyn and his research group successfully applied this method for a wide range of organic compounds in water samples [10-11]. Also, use of an expensive organic solvents and adsorbents in the process of extraction of analytes by these methods make liquid-liquid extraction and solid-phase extraction costly and time-consuming.

Literature research on this problem showed that solid-phase microextraction method was successfully used for determination of endocrine disruptors, such as phthalates, pesticides and alkylphenols [12-13]. On the other hand, the solid-phase microextraction have not been used yet for determination of steroid hormones. Development of solid-phase microextraction method for extraction of endocrine disruptors as hormones and their determination in water samples relates to the actual problems of analytical chemistry.

The aim of this work was to develop a solid-phase microextraction method for determination of steroid hormones in water samples using gas chromatography with mass spectrometry. The main tasks of this research included the optimization of main parameters of solid-phase microextraction using a real drinking water samples, which were spiked with standards of steroid hormones (ethinylestradiol and norgestrel). Solid-phase microextraction parameters were optimized in on-fiber mode of extraction.

EXPERIMENTAL

Reagents and samples

Samples of drinking water were collected in Almaty, Kazakhstan. The samples were clean and free from any mechanical particles, thus these samples allowed the use on-fiber mode of the solid phase microextraction. For spiking, the standards of steroid hormones were used from "Rigevidon" (Gedeon Richter, Hungary) medicine, which was bought in a pharmacy market in Almaty. This medicine contains the widely used hormones – ethinylestradiol and norgestrel.

Sample preparation

One crushed tablet (0.03 mg of ethinylestradiol and 0.15 g of norgestrel) of hormone standard was transferred into 20-mL headspace vial (CTC Analytics AG, Switzerland) and dissolved in 13 mL of drinking water sample.

Solid-phase microextraction

Extraction was performed in on-fiber mode and stay on-line not for a long time in order to avoid damage of fiber coating. Two most important parameters of solid-phase microextraction method were experimentally optimized – extraction time and extraction temperature. To choose optimal value of temperature, extraction was performed at such temperatures as 50 and 80°C in order to measure and compare responses of hormones. Samples were incubated for 10 min and desorption was performed for 5 min. After choosing optimal extraction temperature for 2 analytes, such extraction times as 1, 3 and 5 minutes were checked. The fiber coating 100μm PDMS was used for extraction of hormones. This fiber has medium polar nature which is corresponds with polarity of the most wide range of steroid hormones. Analysis was performed in two replicates.

GC-MS parameters

SPME was performed on MultiPurpose Sampler (Gerstel, Germany) installed on the 7890A/5975C (Agilent, USA) GC-MS system equipped with a split/splitless injector. During the experiment after extraction, the fiber was introduced into the inlet of GC-MS heated to 250°C in splitless mode, and left there for 5 minutes until the next extraction. The gas chromatograph was equipped with 30 m x 250 μm, 0.25 μm film DB-35MS column at a constant flow rate of helium (>99.995%, Orenburg-Tehgas, Russia) of 1.0 mL/min. The oven temperature was programmed from 80°C (held for 1 min) to 200°C at a heating rate of 40°C/min to 300°C at a heating rate of 12.5°C/min, final temperature was held during 10 min. Retention time of ethinylestradiol at such conditions was 14.9 min, norgestrel - 15.4 min. Temperatures of MS source, quadrupole and interface were set to 280, 150 and 240 °C, respectively. Detection was performed in scan mode (m/z 34-300).

RESULTS AND DISCUSSION

Effect of extraction temperature

Experiment on selection of optimal extraction temperature is important to conduct in order to increase extraction efficiency of hormones from water samples. The results of the experiment showed that norgestrel extracted with higher efficiency (3-fold) in on-fiber mode at 80°C (Fig. 1). This evidence can be explained by the fact that diffusion of the molecules in analytes is accelerated in sample volume, thereby increasing the possibility of extraction of these compounds in on-fiber mode. At the same time response of ethinylestradiol stayed almost unchanged (Fig. 2). Therefore, extraction temperature 80°C was selected as optimal.

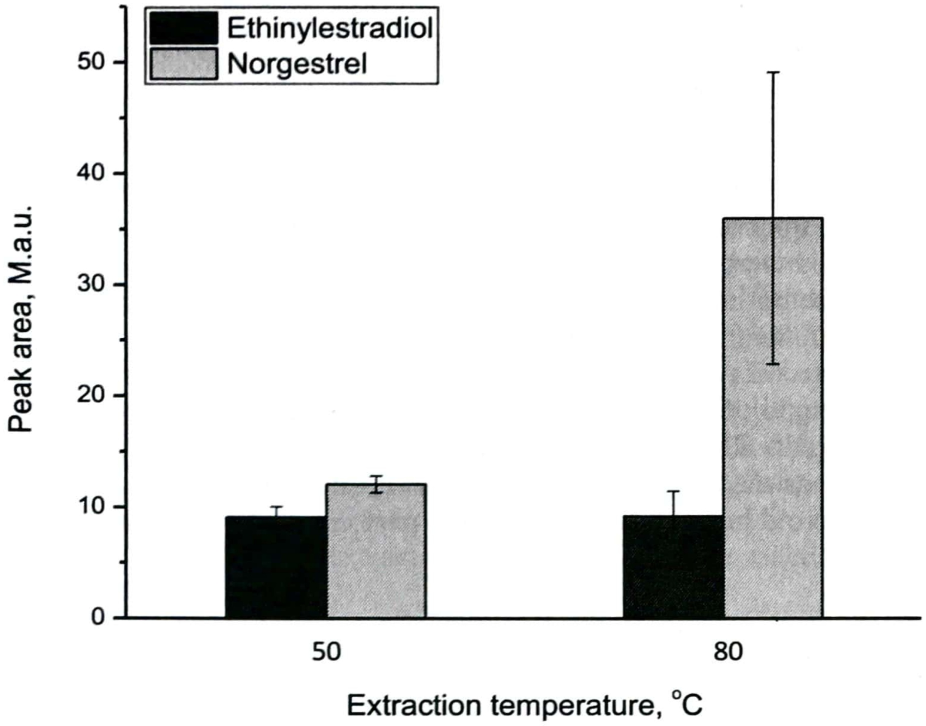

Figure 1. Effect of extraction temperature on peak area of ethinylestradiol and norgestrel

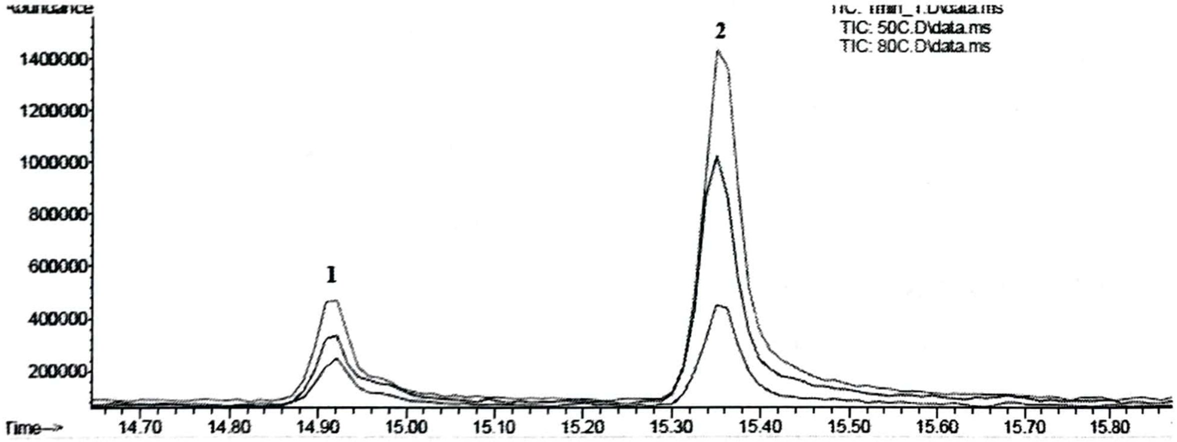

Figure 2. Chromatogram of the experiment of optimization extraction temperature (1 – ethinylestradiol, 2 – norgestrel)

Effect of extraction time

According to obtained results (Figure 3), analytes responses increase with increasing of extraction time. Using extraction time 1 minute, norgestrel extracted less efficiently than extracting during 3 minutes (Figures 3 and 4). Extraction time of 1 minute is not enough to fully saturate the hormones on fiber and obtain reliable results. Increasing of ethinylestradiol response with increase of extraction time from 3 to 5 min is not significant (50 %). Decreasing of norgestrel response with increasing of extraction time from 3 to 5 min may be explained by competition of analytes for sorption sites. Extraction time 3 min was selected as optimal for simultaneous extraction of ethinylestradiol and norgestrel from water samples in order to avoid losses of norgestrel during extraction process.

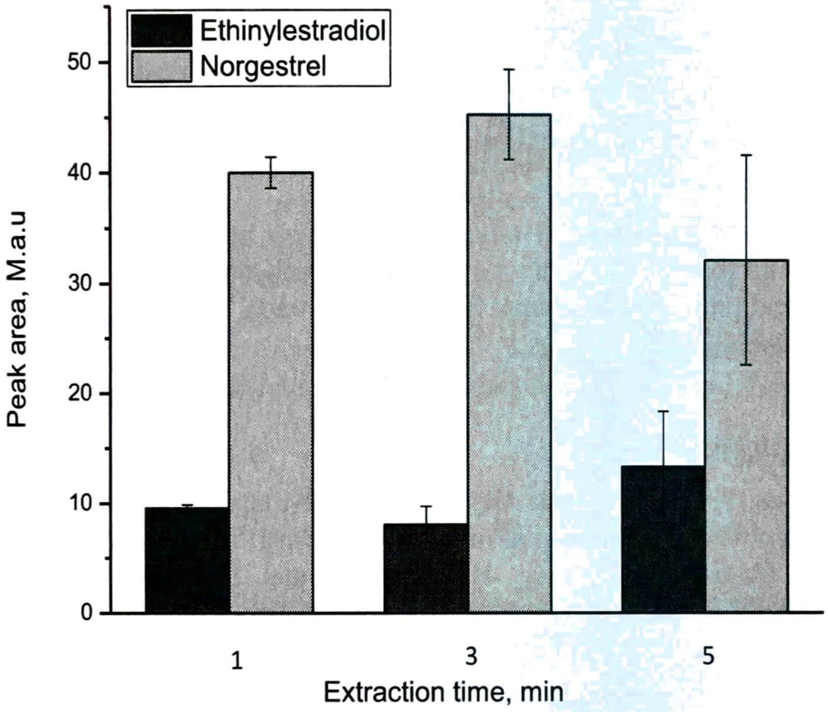

Figure 3. Effect of extraction time on responses of ethinylestradiol and norgestrel

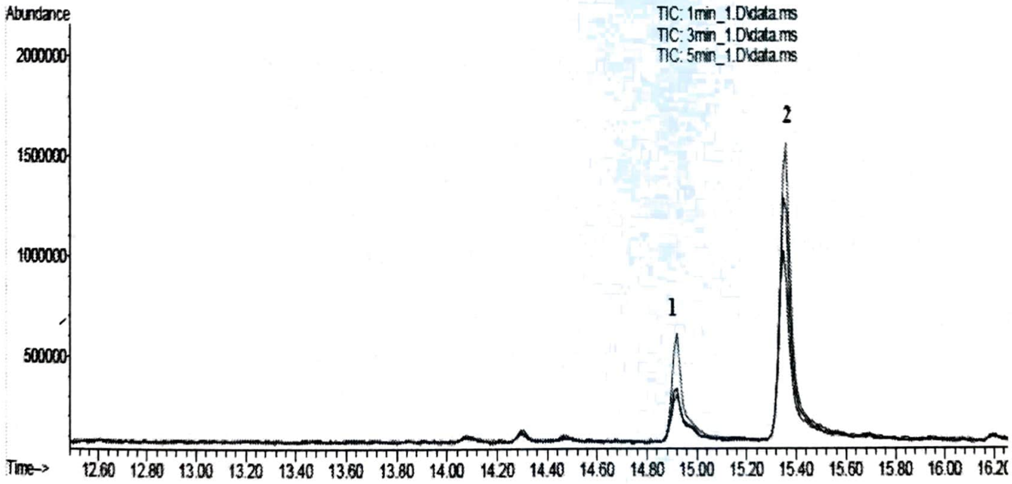

Figure 4. Chromatogram of the experiment of optimization extraction time
(1 – ethinylestradiol, 2 – norgestrel)

CONCLUSION

Results of this research work demonstrate that the SPME-GC-MS method can be used as a fast and simple method of water analysis for determination of ethinylestradiol and norgestrel. In comparison with other analytical method based on using expensive organic solvents, the proposed SPME method is cheaper (one fiber can be used appr. 100 times for water analyses) and does not require a lot of time for sample preparation process. Investigated optimal parameters of SPME on-fiber mode method for ethinylestradiol and norgestrel determination in water samples are: extraction time 3 min and extraction temperature 80°C. Fiber coating based on 100 µm PDMS provides an extraction of sufficient amount of analytes from the one of the most difficult matrix – water sample. Thus, optimized SPME parameters can be used in future for efficient extraction and determination of ethinylestradiol and norgestrel from samples of drinking and pre-filtered natural water samples.

Subsequently, the proposed method might be improved by optimization of others parameters of solid-phase microextraction method, such as salt effect, pH effect, desorption time and pre-incubation time. Sample preparation technique prior to SPME also can be improved.

ACKNOWLEDGEMENTS

This research was conducted under the project "Development of methodological framework for identification of endocrine disruptors in water resources of the Republic of Kazakhstan" funded by Ministry of Education and Science of Republic of Kazakhstan.

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