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## Solid-phase extraction of estrogen hormones onto chemically modified carbon cryogel

DANIJELA B. PROKIĆ<sup>1\*</sup>, MARIJA M. VUKČEVIĆ<sup>2</sup>, MARINA M. MALETIĆ<sup>1</sup>, ANA M. KALIJADIS<sup>3</sup>, JOVANKA N. PEJIĆ<sup>4</sup>, BILJANA M. BABIĆ<sup>5</sup> AND TATJANA M. ĐURKIĆ<sup>2</sup>

<sup>1</sup>Innovation Center of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia, <sup>2</sup>Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia, <sup>3</sup>Department of Materials „Vinča” Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11000 Belgrade, Serbia, <sup>4</sup>Institute for Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia, and <sup>5</sup>Institute of Physics - National Institute of the Republic of Serbia, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia.

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**Abstract:** This study introduces a novel solid-phase extraction (SPE) method utilizing pristine and chemically treated carbon cryogel (CC) as an adsorbent for the isolation and enrichment of estrogen hormones (estrone, 17 $\beta$ -estradiol, and 17 $\alpha$ -ethinylestradiol) from water samples. High recovery values (82-95 %) were obtained after optimizing the SPE technique, which included adsorbent mass and chemical treatment, sample volume and pH, and elution solvent type and volume. The developed analytical method, based on SPE coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS), proves to be selective, efficient, and cost-effective for the determination of selected estrogens. The utilization of self-made cartridges with chemically modified CC produced results comparable to those obtained with commercial cartridges while employing significantly less material. Furthermore, the selectivity of the employed materials contributed to minor matrix effects. The optimized method was successfully applied to analyze estrogen hormones in groundwater, surface water, and wastewater samples, with the results highlighting the importance of monitoring these contaminants in the aquatic environment.

**Keywords:** estrogens, SPE, surface water, groundwater, waste water, liquid chromatography-tandem mass spectrometry.

### INTRODUCTION

Estrogen hormones are acknowledged as endocrine-disrupting compounds (EDCs) capable of disrupting the endocrine systems of both humans and animals, leading to adverse health effects.<sup>1</sup> Wastewater is the main route by which these

\* Corresponding author. E-mail: [dprokic@tmf.bg.ac.rs](mailto:dprokic@tmf.bg.ac.rs)  
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hormones get into the environment. Existing wastewater treatment plants (WWTPs) struggle to entirely eliminate hormones, contributing to their pervasive presence in environmental water.<sup>2</sup> The presence of these compounds in the aquatic environment can affect fish sexual development and reproduction.<sup>3,4</sup> Long-term estrogen exposure also has negative effects, such as bioaccumulation in aquatic species, which can eventually reach people through the food chain.<sup>3</sup> Therefore, the removal of these substances from wastewater and their monitoring in the aquatic environment is becoming increasingly important.

Estrogens are generally present in the aquatic environment at very low concentrations ( $\text{ng dm}^{-3}$ ), so their detection requires an efficient isolation and preconcentration method before analysis. This step is of crucial importance for the outcome of further analysis, especially when dealing with complex matrix samples where the components of interest are present at trace concentrations. Solid-phase extraction (SPE) is commonly used to enrich ambient water samples prior to analysis.<sup>4-8</sup> The choice of the adsorbent is essential in the application of the SPE technique since it affects parameters such as affinity, selectivity, and extraction capacity.<sup>9</sup>

Due to their well-developed specific surface area, wide porosity range, and consequently high adsorption capacity, numerous carbon-based materials have been used as efficient adsorbents for the removal or extraction of different environmental pollutants from water.<sup>10-12</sup> Additionally, the surface of carbon materials can be easily tailored or modified by various treatments in order to improve the adsorption features of the examined materials for specific water pollutants.<sup>13-15</sup> Due to their easily controllable mesoporosity, carbon cryogel (CC) has become an attractive material for adsorption purposes.<sup>10</sup> By selecting the right precursor material and managing the synthesis settings, it is possible to customize the pore structure of CC.<sup>16</sup> The predominantly mesoporous structure of CC provides a fast transfer of adsorbate through the pore network, so this material has been used as an adsorbent for different organic and inorganic solutes from the liquid phase.<sup>10,14,16</sup>

Previously, it was shown that modified and unmodified CC have high efficiency in the removal of estrone (E1),  $17\beta$ -estradiol (E2), and  $17\alpha$ -ethinylestradiol (EE2) from water, showing higher Langmuir adsorption capacities for all three hormones in comparison with carbonized and activated hydrothermal carbons, multi-walled carbon nanotubes, and activated carbon cloths.<sup>14,15</sup> The results also demonstrated that the matrix component of surface water, groundwater, and wastewater samples did not significantly affect the adsorption capacity of CC towards E1, E2 and EE2.<sup>14</sup>

The objectives of this study were to assess the possibility of using self-made cartridges packed with pristine and chemically modified CC as SPE adsorbents and to develop a new, reliable, efficient, and cost-effective method for the

determination of E1, E2, and EE2 from environmental water samples. Also, hormone recoveries obtained using the most efficient CC adsorbent were compared with those obtained by commercially available cartridges. To the best of our knowledge, CC material has not been used as an adsorbent for hormone extraction from water so far. The instrumental method used for hormone detection was liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization. The optimized and validated method was applied to the analysis of real water samples.

## EXPERIMENTAL

### *Material preparation*

The CC was manufactured at the Vinča Institute of Nuclear Sciences, and a detailed synthesis procedure is described in the literature.<sup>10</sup> Briefly, a solution of resorcinol (R) and formaldehyde (F) with sodium carbonate as a basic catalyst was poured into a glass tube, sealed, and gelled for 7 days. Wet RF gel was washed in *t*-butanol, pre-frozen (-30 °C), and freeze-dried for 24 hours under a vacuum (0.4 mbar). The obtained RF cryogel was finally carbonized to 800 °C under a nitrogen atmosphere with a heating rate of 5 °C min<sup>-1</sup> and cooled to room temperature. The resulting material was squashed into powder and stored closed in a PVC box.

Chemical modification of CC was carried out by heating a suspension of the material in HNO<sub>3</sub> or KOH water solution, and modified materials were labeled as CC/HNO<sub>3</sub> and CC/KOH, respectively. Applied treatment conditions during the chemical modification process are described in a previous paper.<sup>14</sup> Chemical modification with the mentioned agents leads to the formation and/or alternation of oxygen functional groups<sup>17,18</sup>. An increased amount of oxygen groups may enhance the adsorption efficiency of tested estrogens since these hormones possess hydroxyl groups, which may form hydrogen bonds on the adsorption surface.<sup>15,19</sup>

### *Solid-phase extraction*

In order to obtain high recoveries of the SPE method, the following parameters were optimized: the mass of the adsorbent, the volume and initial pH value of the water samples, and the type and volume of the organic solvent for hormone elution. In addition, the possibility of improving the method through CC modification was investigated.

Initially, spiked water samples were prepared by spiking deionized water with a mixed hormone stock solution (5 mg dm<sup>-3</sup> of each hormone in methanol) to a concentration of 2.5 µg dm<sup>-3</sup> per hormone. The SPE cartridges (3 cm<sup>3</sup> volume) were made by packaging a selected amount of CC between two Teflon frits. The cartridges were conditioned by passing 5 cm<sup>3</sup> of a chosen organic solvent followed by 5 cm<sup>3</sup> of pH-adjusted deionized water. After conditioning, spiked water samples of the required volume and pH value were passed through the cartridges. Then, the cartridges were dried under vacuum for 10 min and eluted with a chosen organic solvent until the optimal eluent volume was achieved. The eluents were collected in glass tubes, evaporated to dryness under N<sub>2</sub>, and reconstituted in 1 cm<sup>3</sup> of the mobile phase. After reconstitution, all samples were vortexed and filtered through the polyvinylidene difluoride (PVDF) 0.45 µm filters into glass vials.

The optimal parameters of the extraction procedure were selected based on the highest recovery values obtained. The optimization process involved varying the adsorbent mass, using 20 mg, 50 mg, and 100 mg of material. Subsequently, the optimal volume of water samples was selected based on recoveries obtained by performing the extraction from 25 cm<sup>3</sup>, 50 cm<sup>3</sup>, 100

cm<sup>3</sup>, and 200 cm<sup>3</sup>. To find an optimal pH value of water samples, extraction of selected hormones was carried out by using 20 mg of adsorbent and 200 cm<sup>3</sup> of water sample with an initial pH adjusted to 5, 6, 7, 8, 9, 10, and 11. An optimal organic elution solvent was selected according to SPE recoveries gained using methanol (MeOH), acetonitrile (ACN), ethyl acetate (EtOAc), a 1:1 (vol) mixture of dichloromethane and methanol (DCM/MeOH), and a 1:1 (vol) mixture of ethyl acetate and methanol (EtOAc/MeOH). Further optimization steps involved determining the appropriate eluent volume, with elution performed using 5, 10, and 15 cm<sup>3</sup> of the optimal organic solvent. In the final optimization step, three types of materials (CC, CC/HNO<sub>3</sub>, and CC/KOH) were evaluated as potential adsorbents, maintaining the parameters optimized in previous steps. Additionally, recovery values gained using cartridges packed with the most efficient CC material were compared with the recoveries gained using commercially available cartridges: Supelclean Envi Carb, Supelclean Envi-18, Supelclean LC-SCX, Supelclean LC-18 (Sigma–Aldrich), and Oasis HLB (Waters, USA).

#### *LC–MS/MS analysis*

The concentrations of the hormones in the final extracts were measured by liquid chromatography coupled with tandem mass spectrometry. The separation of tested hormones was performed using a Surveyor LC system (Thermo Fisher Scientific, USA). The analytical column used for reverse-phase separation was an Agilent Zorbax Eclipse XDB-C18 column (75 mm × 4.6 mm × 3.5 μm). According to literary sources, a mixture of water, MeOH, and/or ACN is often utilized during LC-MS analysis of E1, E2, and EE2, with NH<sub>4</sub>OH as a mobile phase modifier in negative mode and formic acid in positive mode.<sup>7,20,21</sup> During the optimization of the LC-MS method, we obtained the most stable and intense signals in positive mode, with the optimal mobile phase composition of 25% formic acid (0.1% water solution) and 75% methanol. The method was isocratic, with a constant flow rate of 0.3 cm<sup>3</sup> min<sup>-1</sup>.

For detection and quantification of the hormones, LCQ Advantage (Thermo Fisher Scientific, USA) mass spectrometer with an electrospray ion source and quadrupole ion trap mass analyzer was used. The measurements were conducted in positive ionization mode, with optimal source parameters set at: source voltage, 4.5 kV; sheath gas, 23 au; auxiliary gas, 5 au; and capillary temperature, 350 °C. The selected reaction monitoring mode (SRM) was used for quantification purposes. Table S-I of the Supplementary material lists the selected precursor ion, the optimal collision energy, the most abundant fragment ion, and its isolation width for each hormone, whereas Fig. S-2 shows an example of a SRM chromatogram.

#### *Real water samples analysis*

The applicability of the optimized SPE method was tested by analyzing selected hormones in groundwater, surface water, and wastewater samples. Two groundwater, four surface water, and four wastewater samples were collected. Groundwater samples GW1 and GW2 were collected from observation wells close to the river Danube in the vicinity of Kovin. Surface water samples were collected from the rivers Danube (locations Novi Sad and Kladovo, labeled SW1 and SW2 respectively), the Velika Morava (location 1 km from the confluence with the Danube, labeled SW3), and the Pek (location 8 km from the confluence with the Danube, labeled SW4). Two wastewater samples were taken from Belgrade (WW1, sampled at discharge into the Danube near the confluence of the Sava into the Danube, and WW2, sampled at discharge near Belgrade Fair), while two samples were from the entrance and exit of the wastewater treatment plant (WWTP) Arandelovac (WW3 and WW4). Water samples were stored in 1 dm<sup>3</sup> plastic bottles and stored in a freezer. Prior to the SPE procedure, the water samples were filtered through 1-3 μm glass fiber filters (Whatman GmbH, Dassel, Germany).

## RESULTS AND DISCUSSION

*Optimization of solid-phase extraction*

The initial step in optimizing the SPE method for hormone analysis from water samples was to determine an adequate adsorbent mass. The influence of the adsorbent mass on the extraction efficiency is demonstrated in Fig. 1a. Results showed that recoveries of all three investigated hormones increased as the mass of CC decreased. It can be assumed that the increase in adsorbent mass leads to a decrease in the homogeneity of close-packed material, preventing satisfactory contact between the adsorbent particles and solution. The highest recovery values, ranging from 65 to 70 %, were obtained for the adsorbent mass of 20 mg. Therefore, 20 mg of adsorbent was chosen for further experiments.

Choosing the appropriate volume of the water sample is an important step in the SPE optimization process. Low sample loading volumes are advantageous when considering potential matrix effects and extraction times, yet extraction efficacy and pre-concentration factors generally increase as sample volume is increased<sup>22</sup>. Fig. 1b illustrates the SPE method's recoveries for all tested hormones. Recoveries increase with sample volume, yet volumes above 200 cm<sup>3</sup> weren't tested due to analysis time constraints. Thus, 200 cm<sup>3</sup> was chosen as optimal. The effect of the initial pH value of the water sample was also investigated by varying the initial pH values in the range from 5 to 11. Recoveries obtained for all selected hormones (Fig. 1c) in the tested pH range were high and acceptable (72–85 %). As pH did not have a significant influence on extraction efficiency, the neutral pH value was selected for further experiments. Additionally, at a neutral pH value, the highest recoveries for EE2 were achieved.

Fig. 1d shows the effects of different eluents on extraction efficiency. The experiment was done under the following conditions: material mass was 20 mg, sample volume was 200 cm<sup>3</sup> and initial pH value was 7. The elution conditions were chosen based on the optimization of the parameters in the previous steps. The tested elution solvents were MeOH, ACN, EtOAc, DCM/MeOH, and EtOAc/MeOH. All tested solvents used individually yielded low recoveries, with ACN providing the lowest recovery. When using DCM/MeOH and EtOAc/MeOH mixtures, high and acceptable recoveries for all tested hormones were obtained. Even though the DCM/MeOH mixture produced slightly higher recoveries than the EtOAc/MeOH mixture, the latter was chosen as a suitable elution solvent due to its lower toxicity when compared to DCM.<sup>23,24</sup>

In the next step, the eluent volume was optimized. As shown in Fig. 1e, the recoveries of the tested hormones consistently improved with the increase in eluent volume. A 15 cm<sup>3</sup> eluent volume proved to be adequate, providing satisfactory recoveries for all observed hormones (74–85 %). Therefore, this volume was selected as the optimal choice for subsequent analyses.

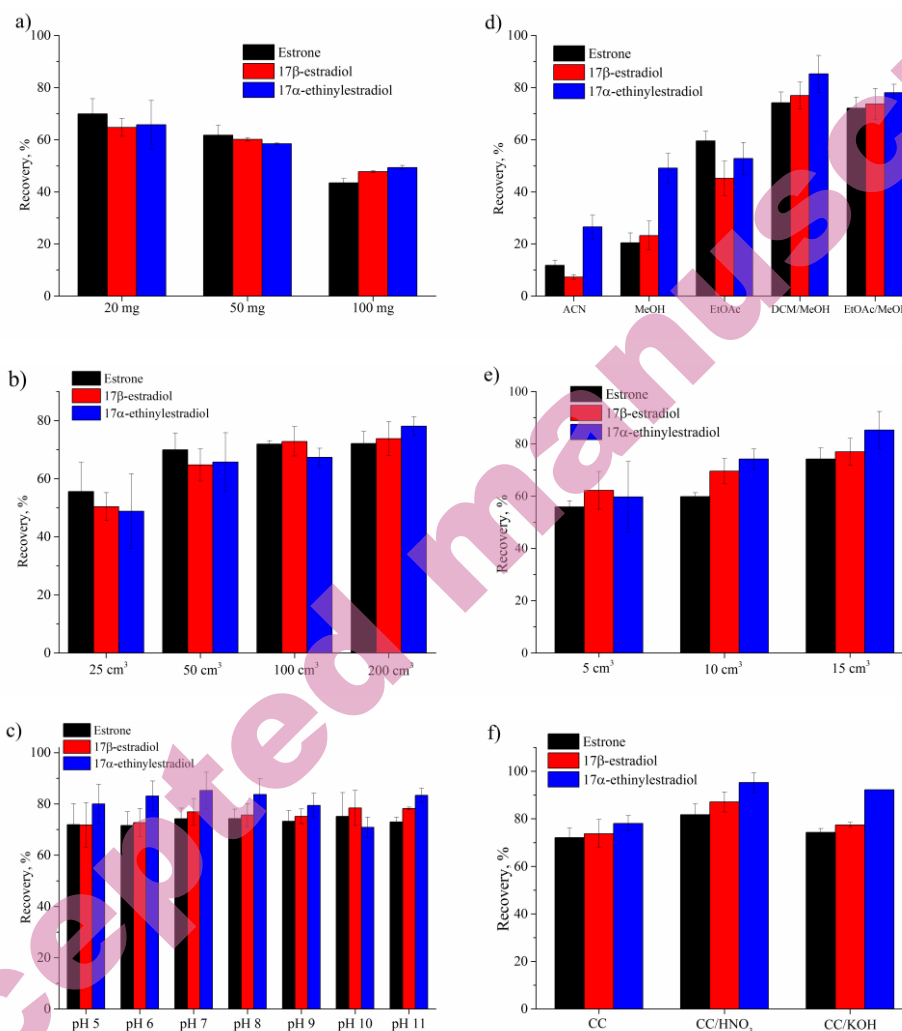


Fig. 1. Recoveries of selected hormones obtained using different a) adsorbent masses, b) sample volumes, c) initial pH values of the sample, d) elution solvents, e) eluent volumes, and f) unmodified and chemically modified CCs

The final step in SPE optimization was the selection of the material modification method that would provide the highest efficiency of CC for the extraction of tested hormones. The obtained results (Fig. 1f) show that after the chemical modification of CC, modest variations in recovery values were found. However, utilizing CC/HNO<sub>3</sub> as an adsorbent produced better results for all three hormones with high recoveries (82–95 %), hence CC/HNO<sub>3</sub> was chosen for real water sample analysis. In our previous study, we found that modifying the material



with  $\text{HNO}_3$  resulted in an increase of carboxyl functional groups.<sup>14</sup> In the present study, it is observed that functionalization leads to a slight increase in the recovery values of the tested hormones, possibly due to the prevalence of hydrogen bonds in the mechanism of adsorption, which is a consequence of the increase in oxygen surface groups.<sup>25</sup> The final optimized analysis procedure was as follows: the 3 cm<sup>3</sup> cartridge, packed with 20 mg CC/ $\text{HNO}_3$ , was conditioned with 5 cm<sup>3</sup> of EtOAc/MeOH (1:1) mixture followed by 5 cm<sup>3</sup> of deionized water; 200 cm<sup>3</sup> of the water sample, with the initial pH adjusted to 7, was passed through the preconditioned cartridge; the cartridge was dried under vacuum for 10 min, and analytes were eluted with EtOAc/MeOH mixture (1:1) until 15 cm<sup>3</sup> of extract was collected in a glass test tube; the extract was evaporated to dryness, reconstituted with 1 cm<sup>3</sup> of mobile phase, and the final extract was filtered through a PVDF filter (0.45  $\mu\text{m}$ ) into the glass vial and analyzed.

#### *Method validation*

In order to determine the applicability of the developed analytical method for the extraction of observed hormones from real water samples, the linearity, repeatability, matrix effect, limit of detection (LOD), and limit of quantification (LOQ) were estimated. Details related to method validation are given in Supplementary material to this paper. The calibration curves based on six calibration levels ranging from 5 to 500  $\mu\text{g dm}^{-3}$  showed good linearity, with determination coefficients ( $R^2$ ) of 0.9981 for E1, 0.9909 for E2, and 0.9971 for EE2. The calibration curves are presented in Fig. S-3. in Supplementary section. The relative standard deviation (RSD) was in the range of 3.2 – 5.9 %, indicating good repeatability. For all observed hormones, the calculated values of LOD and LOQ were in the range of 2.63-6.36  $\text{ng dm}^{-3}$  and 8.77–21.19  $\text{ng dm}^{-3}$ , respectively. The recoveries, RSD, LOD, LOQ, and  $R^2$  values are given in Table S-II of the Supplementary material. The LOD values of our method are lower than the corresponding values obtained in some studies<sup>20-26,27</sup> and comparable to some recently published studies.<sup>1,7</sup> Glineur et al.<sup>28</sup> developed an analytical method in which lower LOD and LOQ were achieved, but that method requires more time because an additional sample purification technique is necessary.

The existence of certain co-extracted substances, including natural organic matter and other contaminants, in environmental samples has the potential to impact the signal intensity of LC-MS/MS through either suppression or enhancement of the signal. Hence, assessing the matrix effect becomes crucial for a comprehensive understanding of the analytical results. The results presented in Table S-II of the Supplementary material show that the matrix effect has no significant influence on the determination in this case. In all studied matrices, the deviation of the results was less than 20 %, indicating the strong selectivity of CC/ $\text{HNO}_3$  for the investigated hormones. However, in order to improve accuracy, the standard addition method was used to analyze real water samples.

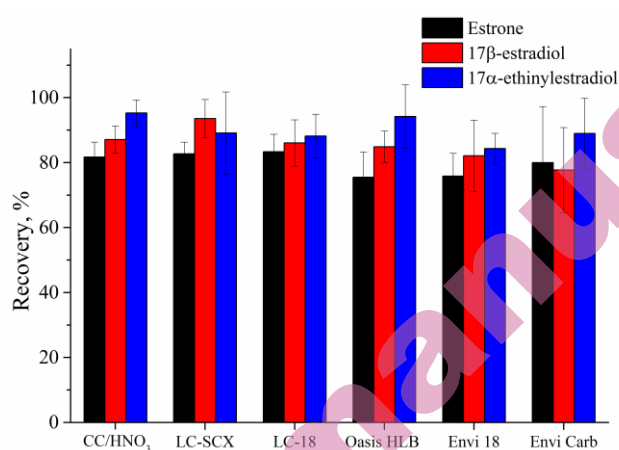


Fig. 2. Recoveries of selected hormones obtained using commercial cartridges and CC/HNO<sub>3</sub>

By comparing recoveries gained using commercially obtained cartridges and cartridges packed with CC/HNO<sub>3</sub> (Fig. 2), it was demonstrated that CC/HNO<sub>3</sub> could be successfully used as a solid-phase adsorbent for the analysis of tested hormones in water samples. According to the results presented in Fig. 2, it is evident that recoveries obtained using cartridges packed with CC/HNO<sub>3</sub> were comparable to the recoveries gained by commercial cartridges. The performance of the proposed method also was on par with a few previously documented methods<sup>7,29</sup>, which utilized expensive commercial cartridges. Notably, our cartridges utilized a significantly smaller amount of material, 20 mg as opposed to 200-500 mg in the referenced studies, underscoring the cost-effectiveness of this approach.

#### *Real water samples*

In Table I, detected concentrations of examined hormones in groundwater, surface water, and wastewater samples are presented. At least one hormone was found in eight, out of the ten samples that were tested, indicating a considerable prevalence of these compounds in the aquatic environment.

In GW samples, only trace amounts of the studied hormones were detected, below LOQ levels, which is in accordance with results obtained in some previous studies.<sup>30,31</sup> Higher concentrations were detected in GW samples in Poland ( $\leq 43$  ng dm<sup>-3</sup> for estrone and  $\leq 48$  ng dm<sup>-3</sup> for 17β-estradiol).<sup>32</sup> None of the tested hormones were found in the SW2 sample collected from the Danube, Kladovo, while in the remaining SW samples, hormone concentrations ranged from 8.8 to 36.2 ng dm<sup>-3</sup>, which was comparable with hormone concentrations obtained in

previous studies in Italy,<sup>33</sup> Poland,<sup>34</sup> China,<sup>29</sup> Indonesia,<sup>35</sup> and Malaysia (river Pahang).<sup>36</sup>

TABLE I. Hormone concentrations detected in ground, surface, and wastewater samples

| Sample               | Location                   | Concentration, ng dm <sup>-3</sup> |       |       |
|----------------------|----------------------------|------------------------------------|-------|-------|
|                      |                            | E1                                 | E2    | EE2   |
| <u>Groundwater</u>   |                            |                                    |       |       |
| GW1                  | Kovin 1                    | < LOQ <sup>a</sup>                 | < LOQ | < LOQ |
| GW2                  | Kovin 2                    | — <sup>b</sup>                     | < LOQ | —     |
| <u>Surface water</u> |                            |                                    |       |       |
| SW1                  | Danube, Novi Sad           | 36.2                               | 10.1  | —     |
| SW2                  | Danube, Kladovo            | —                                  | —     | —     |
| SW3                  | Velika Morava              | —                                  | 8.8   | —     |
| SW4                  | Pek                        | 22.8                               | 10.2  | 9.6   |
| <u>Wastewater</u>    |                            |                                    |       |       |
| WW1                  | Belgrade, Confluence       | 99.3                               | —     | —     |
| WW2                  | Belgrade, Belgrade Fair    | 71.3                               | —     | —     |
| WW3                  | WWTP Arandelovac, influent | 163.5                              | 101.8 | 91.0  |
| WW4                  | WWTP Arandelovac, effluent | —                                  | —     | —     |

<sup>a</sup> (< LOQ) detected, but below LOQ.

<sup>b</sup> (—) not detected.

Higher concentrations, up to 820 ng dm<sup>-3</sup> were recorded in the Bacanga River in Brazil.<sup>1</sup> This river is positioned in an area affected by urbanization, burning, deforestation, water contamination, and siltation, which may explain the high concentration of the selected compounds detected in the water samples of that river.<sup>1</sup> Zhang et al. detected observed hormones in river water in Switzerland at concentrations up to 3.7 ng dm<sup>-3</sup>,<sup>37</sup> which was lower than the results obtained in the present study. Rocha et al. determined estrone, 17 $\beta$ -estradiol, and 17 $\alpha$ -ethinylestradiol in a river estuary in Portugal at concentrations  $\leq 16$  ng dm<sup>-3</sup>,  $\leq 18$  ng dm<sup>-3</sup>, and  $\leq 11$  ng dm<sup>-3</sup>, respectively.<sup>38</sup> As anticipated, wastewater samples contained the highest quantities of the hormones. Concentrations of E1, E2, and EE2 in the present work were up to 163.5 ng dm<sup>-3</sup>, 101.8 ng dm<sup>-3</sup>, and 91.0 ng dm<sup>-3</sup>, respectively. The relatively high concentrations of E1 can be explained by the conversion of E2 and EE2 into E1 before it can be transformed further.<sup>39</sup> The highest concentrations of hormones were detected in sample WW3, from the inlet of WWTP Arandelovac, while there was no detectible amount left in the sample from the exit of the same WWTP. Concentrations of E1 in sample WW3 were comparable with obtained E1 concentrations of wastewater influents in France and Slovenia.<sup>40,41</sup> The concentration of E2 in the WW3 sample was also comparable with the E2 concentration of influent wastewater in Malaysia,<sup>42</sup> while higher levels of E1 and EE2 were recorded in wastewater influents in Brazil.<sup>39</sup>

Taking into account the obtained results, it can be concluded that the developed method was efficiently applied for the selective determination of trace estrogens in complex environmental water samples.

#### CONCLUSION

In the present study, pristine and chemically modified CC was used as a new solid-phase extraction adsorbent for the determination of estrogen hormones in environmental water samples. An efficient SPE method was developed by optimizing the adsorbent mass (20 mg), volume (200 cm<sup>3</sup>), and initial pH value (pH 7) of the water sample, the type and volume of elution solvent (15 cm<sup>3</sup> of EtOAc/MeOH 1:1 mixture), and by selecting HNO<sub>3</sub> treated CC as an adsorbent. The optimized SPE method provided high recovery values for all tested hormones (82–95 %), comparable with the recoveries obtained using commercially available cartridges. Notably, our method used significantly less material than is customary, demonstrating the cost-effectiveness of this approach. The developed SPE/LC/MS-MS method was successfully applied to the analysis of ground, surface, and wastewater samples, whereby the matrix effect of examined water samples did not have a significant impact on method accuracy. The highest concentrations of tested hormones were found in wastewater samples. However, the fact that the tested hormones were detected and quantified in most of the tested samples indicates the significant presence of these pollutants in the aquatic environment, which requires further monitoring.

#### SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/12844>, or from the corresponding author on request.

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#### ИЗВОД

##### ЕКСТРАКЦИЈА ЕСТРОГЕНИХ ХОРМОНА НА ХЕМИЈСКИ МОДИФИКОВАНОМ УГЉЕНИЧНОМ КРИОГЕЛУ

ДАНИЈЕЛА Б. ПРОКИЋ<sup>1</sup>, МАРИЈА М. ВУКЧЕВИЋ<sup>2</sup>, МАРИНА М. МАЛЕТИЋ<sup>1</sup>, АНА КАЛИЈАДИС<sup>3</sup>, ЈОВАНКА Н. ПЕЈИЋ<sup>4</sup>, БИЉАНА М. БАБИЋ<sup>5</sup> И ТАТЈАНА БУРКИЋ<sup>2</sup>

<sup>1</sup>Иновациони Центар Технолошко–металуричког факултета Карнегијева 4, 11000 Београд, Србија,

<sup>2</sup>Технолошко–металурички факултет, Универзитет у Београду, Карнегијева 4, 11000 Београд, Србија,

<sup>3</sup>Лабораторија за материјале, Институт за нуклеарне науке Винча–Институт, од националног значаја Републике Србије, Универзитет у Београду, Мике Пешировића Аласа 12-14, 11000 Београд,

<sup>4</sup>Институт за хемију, технологију и металургију, Универзитет у Београду, Његошева 12, 11000 Београд, Србија и <sup>5</sup>Институт за физику–Институт од националног значаја, Универзитет у Београду, Предревица 118, Београд, Србија.

У овом раду је представљена нова метода екстракције на чврстој фази, коришћењем немодификованог и хемијски модификованог угљеничног криогела (енгл. carbon cryogel, CC) као адсорбента за изоловање и предконцентрисање естрогенних хормона (естрона, 17 $\beta$ -естрадиола и 17 $\alpha$ -етинилестрадиола) из узорака воде. Након оптимизације методе, која је обухватила оптимизацију масе и хемијског третмана адсорбента, запремине и рН-вредности узорка и типа и запремине растварача за елуирање, добијене су високе вредности приноса (82-95 %). Развијена аналитичка метода, базирана на SPE екстракцији и течном хроматографији–тандем масеној спектрометрији, показала се селективном, ефикасном и економичном за одређивање одабраних естрогенних хормона. Коришћењем кертрица са модификованим CC постигнути су резултати који су били упоредиви са резултатима добијеним при употреби комерцијалних кертрица, уз коришћење знатно мање масе материјала. Поред тога, селективност одабраног материјала је допринела малом ефекту матрице. Оптимизована метода је успешно примењена за анализу естрогенних хормона у подземним, површинским и отпадним водама, при чему резултати указују на важност праћења ових загађујућих материја у воденој средини.

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