A novel hybrid mesostructured silica for the solid-phase extraction of estrogenic hormones from waters

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Mesoporous silica functionalized with octadecyl groups (denoted as SBA-15-C_{18}) was prepared and characterized. The adsorption capacity of the prepared SBA-15-C_{18} for a mixture of twelve endocrine disrupting compounds (synthetic and natural estrogenic hormones) in aqueous media was evaluated by off-line solid-phase extraction (SPE) and compared with a commercial phase for SPE (ExtraBond^{R} C_{18}). Analytes were determined by high performance liquid chromatography with UV-Vis detection. Under optimized conditions (100 mg of SBA-15-C_{18} sorbent; conditioning: 2 mL methanol and 2 mL Milli-Q water; loading: 150 mL water sample; washing: 5 mL Milli-Q water; elution: 2 mL methanol; flow rate 0.5 mL min^{-1}) the preconcentration factor achieved was 10^3. The analytical characteristics of this methodology were evaluated, showing good precision, accuracy and linerarity, with method quantification limits (MQL) between 0.16 and 3-00 µg L^{-1}. The SBA-15-C_{18} material enabled the extraction of the twelve estrogenic hormones in tap water (pH 6.6), mineral water (pH 5.0), ground water (pH 7.3), river water (pH 5.0) and effluent wastewater (pH 5.0) with recoveries between 71 and 112% at the lower level studied (MQL).

Keywords: Solid-phase extraction . Mesoporous silica . endocrine disrupting compounds . estrogens . water

1. Introduction

The presence of emergent contaminants in the environment is one of the main issues concerning organizations committed to public and environmental health, such as the World Health Organization (WHO) and the European Commission. A wide variety of organics chemicals have been identified in aquatic systems, such as pharmaceuticals, products of personal care or endocrine disrupting compounds (EDCs).

We can consider as EDCs any natural or synthetic chemical compound that interferes with normal endocrine function. These EDCs are thought to affect the binding, synthesis, or decomposition of essential hormones. The effect produced in a number of species of wildlife (fishes, amphibians, birds and mammals) may be cumulative and irreversible[1]. Thus, as environmental and social concerns about water quality are increasing, consequently the study of the environmental impact of EDCs will become more prevalent in the next years[1-3].

Due to the growing populations and increased discharges from wastewater treatment plants (WWTPs), the presence of EDCs in waters could be a subject of concerns, as conventional treatment methods have proven to be inadequate to sufficiently eliminate them.[4] EDCs have been targeted and detected in wastewaters (influents and effluents of WWTPs), natural waters and drinking waters.[5-11]

Some natural and synthetic estrogenic compounds are EDCs, introduced to the environment by anthropogenic inputs, since they are used in medicine as contraceptives in some hormonal therapies and in veterinary, or because they are naturally generated by the human body (e.g. steroidal sex hormones). These compounds are very powerful and can produce deep effects at very low concentration.

For example, it has been demostrated that estrone, 17α- and 17β-estradiol, ethinylestradiol and estriol, which can be found in surface waters, are some of the major contributors to estrogenic activity with physiological effects on organisms.[3] In a recent review of LaFleur and Schug[12] some analytical methods developed for the quantification of selected EDCs from aqueous systems are detailed. In most cases, these methods consist of an extraction and pre-concentration step followed by the determination of the analytes using gas or liquid chromatography.

In spite of the rapid development of various technologies in analytical chemistry, sample preparation is still a crucial step to achieve higher sensitivity and/or better selectivity for the analysis of various analytes, especially for trace level analytes. In that sense, investigation and application of new materials has become a very interesting research area in field of analytical chemistry.[13,14] For example, a great range of new mesoporous materials have been used in sample preparation, such as: extraction of metal ions, adsorption of organic compounds, selective size enrichment of peptides and proteins, etc. [13]. These materials are gaining interest in sample preparation because of their desirable characteristics: (a) highly ordered and size-controlled mesoporous structures, (b) extremely high surface areas and large pore volumes, (c) very good thermal and chemical stability and (d) high flexibility in functionalization to enable the introduction of hydrophilic, hydrophobic, polar as well as charged functional moieties on surface.[13]

The adsorption of trace contaminants onto solid stationary phases (solid-phase extraction, SPE) has proved to be an effective and valuable technique due to its flexibility, environmental friendly, and simplicity. Indeed, there is no doubt that SPE is currently the most popular sample-preparation technique in areas such as environmental and biological chemistry and food analysis.[15] The most remarkable increase in the use

of SPE has occurred in the last few years, with multiple improvements in terms of supporting formats, and the introduction of new phases. In this respect, although numerous types of materials have been used as stationary phases in SPE (*e.g.* activated carbon, amorphous silica, clays, zeolites, organic chelating resins, ion-imprinting polymers, etc.), many of these materials suffer from inherent problems such as low capacity, low selectivity, long equilibrium time, and mechanical and/or thermal instability, etc. In this context, and to avoid these limitations, the goal of some research groups in this field is to develop novel sorbent materials for SPE of different contaminants.[16] For example, recently, various promising sorbents prepared by functionalization of mesoporous silicas have been studied for the SPE of toxic metals from waters.[17]

Amorphous silica chemically bonded with various groups has been the most conventional material for SPE of organic contaminants.[18] However, in a previous paper, our research group has demonstrated the good extraction capacity and elution efficiency of functionalized mesoporous silica for SPE of 17β-estradiol from aqueous media.[19] With this in mind, the objective of the present paper was to prepare a new hybrid mesoporous silica (SBA-15 type), functionalized by one-pot procedure with octadecyl (C_{18}) groups, and to study their applicability as sorbent for SPE of a mixture of twelve estrogenic compounds in waters: estrone (E1), 17β-estradiol (17β-E2), estriol (E3), progesterone (P), hexestrol (HEX), diethylstilbestrol (DES), 4-androstene-3,17-dione (AND), ethinylestradiol (EE2), 17α-methyltestosterone (17α-MT), nandrolone (NAN), prednisolone (PRED) and testosterone (T) (see supporting information Fig.A1) by HPLC-DAD. To the best of our knowledge, this is the first time that hybrid mesoporous silica has been used for SPE of a mixture of steroids hormones from five different waters (tap water, mineral water, groundwater, river water and effluent wastewater).

Besides, with the aim of evaluating the performance of this new stationary phase, results were compared with that obtained on commercial C_{18} stationary phase (ExtraBond^R C_{18} cartridge).

2. Experimental

2.1. Reagents and materials

Tetraethylorthosilicate (TEOS) 98% (M = 208.33 g mol^{-1}, d = 0.934 g mL^{-1}), poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (EO20PO70EO20, Pluronic 123, M_{av} = 5800 g mol^{-1}, d = 1.019 g mL^{-1}), cetyltrimethylammonium bromide (CTAB) 98%, (M= 364,46 g mol^{-1}), octadecylsilane (OTES) 97% (M = 284.61 g mol^{-1}, d = 0.795 g mL^{-1}), E1, 17β-E2, E3, P, HEX and DES were purchased from Sigma-Aldrich (St. Louis, MO, USA). AND, EE2, 17α-MT, NAN, PRED and T were purchased from Fluka (Busch, Switzerland). ExtraBond^R bulk C_{18} sorbent (irregular morphology, 500 m^2 g^{-1}, 60 Å pore diameter, 17% carbon loading) was obtained from Scharlab (Barcelona, Spain). Ethanol absolute was purchased from SDS (Peypin, France). Hydrochloric acid 35% (M = 36.45 g mol^{-1}, d = 1.19 g mL^{-1}) was purchased for Panreac (Castellar del Vallès, Barcelona, España). HPLC-grade solvents acetonitrile (ACN) and methanol (MeOH) were purchased from Sigma-Aldrich. Water (typically 18.2 MΩ·cm at 25 °C) was obtained from a Milli-Q water system (Millipore Iberica, Madrid, Spain).

2.2. Standard solutions

Stock standard solutions of 4000 mg L^{-1} were prepared by diluting in MeOH adequate amounts of each compound and stored at – 20ºC. Working solutions were prepared at various concentrations by appropriate dilution of the stock solution in MeOH.

2.3. Water samples

Five types of water (tap water, mineral water, river water, ground water and effluent wastewater samples) were analysed in order to demonstrate the applicability of the material. Tap water was collected in our laboratory from the Canal de Isabel II water treatment plant that supplies water for human consumption to Madrid City. Bottled mineral water (Manantial Fuenteblanca, Sierra de Segura) was bought in a local market. Ground water was collected in Escalona (Toledo) and stored at -20 º C until extraction. River water was collected in Alberche River (Toledo), filtered through a glass fiber filter to eliminate particulate material and stored at -20 º C until extraction. Effluent wastewater was taken from the WWTP of the Rey Juan Carlos University, filtered through a glass fiber filter to eliminate particulate material and stored at -20 º C until extraction.

2.4. Synthesis of SBA-15-C_{18}

Octadecyl-functionalized SBA-15 (denoted SBA-15-C_{18}) was prepared according to the methodology described in our previous work.[19] However, in the current study the amount of OTES was reduced, in order to obtain a lower functionalization degree, with a carbon loading similar to the commercial amorphous silica ExtraBond^{R} C_{18} (17% C) that was used for comparative purposes. SBA-15-C_{18} was prepared as follows: 12 g of poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) was

dissolved in 361 g of water and 375 g of 2.0 M HCl solution with stirring at room temperature. After 22 mL of TEOS was added to that homogeneous solution with stirring at room temperature. The resulting mixture was stirred at 40 °C for 3 h for prehydrolysis, and then 4.15 g of OTES was slowly added into the solution. The resulting mixture was stirred at 40 °C for 20 h and then transferred into a polypropylene bottle and reacted under static condition at 50 ºC for 2 h and 90 °C for 24 h. The solid product was recovered by filtration, washed with water, and dried at room temperature overnight. The template was removed from the synthesized material by refluxing in ethanol:H_2O (95:5, v/v) for 24 h. Finally, the material was dried at 50 °C for 24 h.

2.5. Characterization of SBA-15-C_{18}

X-ray diffraction (XRD) patterns of the silicas were obtained on a Philips Diffractometer model PW3040/00 X'Pert MPD/MRD at 45 KV and 40 mA, using Cu-Kα radiation (λ= 1.5418 Å). Scanning electron micrographs and morphological analysis was carried out on a XL30 ESEM Philips with an energy dispersive spectrometry system. Conventional transmission electron microscopy was carried out on a TECNAI 20 Philips, operating at 200 kV. N_2 gas adsorption-desorption isotherms were obtained using a Micromeritics ASAP 2020 analyzer, and pore size distributions were calculated using the Barret-Joyner-Halenda (BJH) model on the adsorption branch. Proton-decoupled ^{29}Si MAS-NMR spectra were recorded on a Varian-Infinity Plus 400 MHz Spectrometer operating at 79.44 MHz proton frequency. Cross Polarization ^{13}C MAS-NMR spectra were recorded on a Varian-Infinity Plus 400 MHz Spectrometer operating at 100.52 MHz proton frequency. Elemental analysis (%C) was performed with a LECO CHNS-932 analyzer (Universidad Complutense de Madrid, Spain). Thermogravimetric

analyses were carried out using a Setsys 18 A (Setaram) analyzer (from 25 to 800 ºC at 10 ºC per min).

2.6. SPE experiments

To prepare the SPE cartridges, 100 mg of SBA-15-C_{18} were packed into a 6 mL syringe type cartridge (65 mm length, 11 mm diameter) plugged with porous PTFE disks at both ends. To prevent the material lost during sample loading, a 0.45 μm pore size nylon filter membrane was also inserted at the bottom of the mesoporous silica bed. Extraction was performed using a Supelco Visiprep™ DL solid phase extraction vacuum manifold 12 port model (Sigma Aldrich) connected to a vacuum pump at 7.6 psi. Conditioning of the cartridges was accomplished by passing 1 x 2 mL MeOH and 1 x 2 mL Milli-Q water at a flow rate of 1 mL min^{-1}. Then, cartridges were loaded with 150 mL of water (adjusted to pH 5 in the case of mineral water). After extraction of the sample, each cartridge was washed with 1 x 5 mL Milli-Q water to remove interferences. Elution was performed by passing 1 x 2 mL MeOH at a flow rate of 0.5 mL min^{-1} (see supporting information fig. A2). At any point of the process, the bed was not allowed to dry, from conditioning with organic solvent, washing with water, through sample loading, in order to achieve reproducible recoveries. Finally, the corresponding extracts were evaporated and reconstituted with 150 μl of MeOH for subsequent analysis.

2.7. Chromatographic analysis

HPLC analyses were performed on a Varian ProStar chromatographic system (Varian Ibérica, Madrid, Spain). The system consisted of a 230 ProStar ternary pump, a ProStar 410 autosampler with a six-port injection valve equipped with a 20 μL injection loop

(Rheodyne), a photodiode array detector DAD 335 ProStar UV-vis detector and a PC-based data acquisition system Varian Star Workstation.

Separation was achieved on an Ascentis C_{18} column (250 x 4.6 mm, 5µm). The mobile phase composition was water (mobile phase A) and ACN (mobile phase B). It was employed a mobile phase gradient in order to achieve the complete resolution of all the studied steroids hormones, that consisted of: t = 0 min 35 % B, t =5 min 40 % B (5 min), t =10.5 min 45 % B (1 min) and t = 16 min 100% B (4 min). The flow rate was 1.0 mL min^{-1}. The detection was recorded following a dual wavelength method at 200 nm for E1, 17β-E2, E3, EE2 and HEX and 242 nm for PRED, NAN, T, 17α-MT, AND, DES and P in order to obtain the maximum sensitivity for all the compounds.

3. Results and discussion

3.1. Adsorbent characterization

XRD pattern of the SBA-15-C_{18} displayed a well-resolved pattern at low 2θ values with a very sharp (100) diffraction peak at 0.89 and a weak diffraction peak (110) at 1.68. d_{100}-spacing value and unit cell parameter (a_0) were: 99 and 115 Å. This pattern was similar to the pure SBA-15,[19] indicating that the prepared functionalized silica contains well-ordered hexagonal arrays of one-dimensional channel structure. However, the peak intensity decreases and the higher order (110) and (200) diffractions become less or not resolved in the functionalized material, showing that the mesopore ordering decreases with the presence of organic groups in the structure. These phenomenon can be explained by that the OTES would perturb the self-assembly of surfactant micelles and the silica precursor.

The N_2 adsorption-desorption isotherms for mesoporous silica are shown in Fig.1a. For this material the isotherm is type IV according to the I.U.P.A.C. classification and has an H1 hysteresis loop that is representative of materials with pores of constant cross-section. The synthesized material possessed very high S_{BET} (796 $m^2 g^{-1}$), a pore volume of 0.88 $cm^3 g^{-1}$ and a BJH pore diameter of 76 Å, typical of surfactant-assembled mesostructures (see supporting information Table A1). The higher wall thickness for the material (39 Å) in comparison with the pure SBA-15 (16 Å) confirmed the presence of ligand inside of silica pores.[19] The narrow pore size distribution found for this material (Fig. 1b) provides evidence for its uniform framework mesoporosity.

Scanning electron microscopy (SEM) images showed that SBA-15-C_{18} has cylindrical shape, with an average particle size of 1.4 μm (length) and 750 nm (wide) (Fig. 1c). Transmission electron microscopy (TEM) images demonstrated a clear arrangement of hexagonal pores with uniform size for this material (Fig. 1d). These results confirmed that the functionalized mesoporous silica synthesized contain well ordered, one-dimensional pore structure, similar to that of the pure SBA-15.[19]

The successful incorporation of functional groups was confirmed by ^{29}Si MAS-NMR spectroscopy. The ^{29}Si MAS-NMR spectra in the solid state for SBA-15-C_{18} showed three main peaks at -112, -105 and -95(*sh*) ppm and these were assigned to Q^4, Q^3 and Q^2 silanol sites, respectively. The dominant peak in the SBA-15-C_{18} spectrum was due to Q^3 silanol sites ($(SiO)_3$***Si***OH). Since the ratio Q^4/Q^3 was lower than 1, the number of silanol groups in the surface of this material was high (see supporting information Fig. A3). In addition, the peak that appeared at -37 ppm, assigned to D^H siloxane units, confirmed that the C_{18} organic moieties were incorporated as a part of the silica wall structure.[21] Comparing this spectrum with the obtained in our previous work,

the lower intensity of the D^H peak and the higher intensity of the Q^3 peak in this material can be attributed to the lower amount of OTES used for its preparation.[19]

Important features related to the immobilization of pendant groups onto the silica structure can be obtained from ^{13}C MAS-NMR spectra. The spectrum clearly display peaks at 28, 19 and 14 ppm, corresponding to the carbon atoms on the C_{18} group ($-(CH_2)_{16}-$, $-CH_3$ and $Si–CH_2-$, respectively). It further confirms that this material was indeed functionalized with C_{18} groups and the organic moiety was not decomposed during the preparation procedure.

The amount of attached C_{18} molecules onto the mesoporous silica surface (L_o = 0.69 mmol g^{-1}) was estimated from the percentage of carbon in the functionalized mesoporus silica, calculated by elemental analysis (17% C). Finally, thermogravimetric analysis (TGA) of the modified mesoporous silica allowed the establishing of information on thermal stability of this material. The TGA curve of the SBA-15-C_{18} prepared (see supporting information Fig. A4) show that degradation process occurs between 200-600 ºC and the weight loss is about 17 %, due to the breakage of pendant groups anchored on the silica surface (exothermic degradation process). The mass loss observed in the SBA-15-C_{18} is in agreement with the amount of C_{18} groups covalently bound to the support, calculated by elemental analysis. The thermal stability of these samples is in agreement with previous results given in the literature for other functionalized mesoporous silicas.[19]

3.2. Optimization of the chromatographic conditions

For the development of the chromatographic methodology was taken, as starting point, a previous paper of our research group[20] on the separation of E1, E2, E3, EE2, T, DES and P. This methodology consisted of the employ of a mobile phase with water (mobile

phase A) and ACN (mobile phase B) and a elution gradient starting at 35 % B that was linearly increased to 45 % B in 5 min, this composition was maintained for 8 min, increased to 100 % B in 1 min and finally it was maintained this proportion till 20 min with a flow rate of 1 mL min^{-1}. This method enabled the partial separation of twelve EDCs in 18 min. Thus, in order to increase the resolution of NAN and 17β-E2 that eluting at 9 min and 17α-MT, AND and DES that eluting around 13 min, several experiments were carried out in the current work to develop a proper gradient to separate all the analytes in the lowest time. In order to achieve the baseline separation of analytes that eluting in the middle part of the chromatogram it was slowed down the intermediate part of the elution gradient. After optimization, the gradient that achieved the complete resolution of all analytes consisted of: t = 0 min 35 % B, t =5 min 40 % B (5 min), t =10.5 min 45 % B (1 min) and t = 16 min 100% B (4 min). The flow rate was 1.0 mL min^{-1}. Under these conditions the baseline separation of all the compounds was achieved in less than 19 min with Rs ≥ 1.5 except for 17α-MT and AND (Rs ≥ 1.3). The retention time (t_r) for the compounds were: 3.97, 4.56, 9.75, 10.21, 11.94, 13.26, 14.08, 14.44, 14.80, 15.64, 16.14 and 18.44 min for E3, PRED, NAN, 17β-E2, T, EE2, E1, 17α-MT, AND, DES, HEX and P, respectively. Finally, in order to obtain the maximum sensitivity for all the compounds, the detection was recorded following a dual wavelength method at 200 nm for E1, 17β-E2, E3, EE2 and HEX and at 242 nm for PRED, NAN, T, 17α-MT, AND, DES and P. Fig. 2 show the separation obtained for twelve EDCs in the optimized elution gradient recorded at two wavelengths.

3.3. Optimization of the SPE procedure

Two different sorbents packed in disposable syringe type cartridges were evaluated: SBA-15-C_{18} and ExtraBond^R C_{18}. Conditioning of the cartridges was accomplished by

passing 3 x 2 mL of MeOH and 3 x 5 mL of Milli-Q water at a flow rate of 1 mL min^{-1} according to our previous study.[19] Then 50 mL of Milli-Q water spiked to a final concentration of 450 μg L^{-1} of each EDCs was loaded into the cartridges. After extraction of the spiked water sample, each cartridge was washed with 3 x 5 mL of Milli-Q water. Finally, elution was performed by passing 3 x 2 mL of MeOH.[19] Four different samples were prepared for the evaluation of the recoveries, three of them were Milli-Q water samples spiked with the twelve EDCs at a known concentration, and another one was a simulated sample, prepared in the same way but spiked with the analytes at the end of the SPE process. The recoveries (%) were calculated by comparison of the areas of the samples with the areas of the simulated sample.

The adsorption of the twelve EDCs onto SBA-15-C_{18} and ExtraBond^R C_{18} is showed in Fig. 3. As it can be seen, SBA-15-C_{18} sorbent proved good extraction capacity and elution efficiency for the target ECDs, so a cartridge with 100 mg of this sorbent retained between 89 – 111 % of the analytes from 50 mL of 450 μg L^{-1} in Milli-Q water. The repeatability of the procedure was good, with relative standard deviation (RSD, %) between 1 – 4 % (n = 3). On the other hand, under similar conditions, ExtraBond^R C_{18} was not capable of extracting most of these compounds satisfactorily, with recoveries lower than 60 % for five of the twelve target EDCs (recoveries between 7 and 90 %).

A good knowledge of the interactions between EDCs and silica sorbents is important for setting up efficient multiresidue extraction schemes. In supporting information (see supporting information Fig. A1) are showed the structures of the analytes investigated. ExtraBond^R C_{18} is a reversed-phase packing material commonly used in SPE when aqueous samples are involved. This material is “en-capped” so the silanol groups onto the silica surface are derivatized with trimethylchlorosilane reagent

that makes the overall surface of the silica somewhat more hydrophobic. On the other hand, the mesoporous silica synthesized provided mixed retention mechanisms for the analytes, although the type and relative importance of each one depend on the type and amount of functional organics groups on the silica surface. In SBA-15-C_{18} material, the analytes will experiment a reversed-phase sorption (by hydrophobic interactions) to the C_{18} groups and in some cases interaction with the silanol groups as a function of pH (mixed-mode application).[22] It is well know that the presence of hydrophobic C_{18} groups onto the silica surface generates advantages to the adsorption of hydrophobic organic compounds, such as the ones studied in this work, and that the capacity of the sorbent to do so improves as the percentage of C_{18} loading increases.[20] Since the carbon loading of SBA-15-C_{18} and ExtraBond^R C_{18} sorbents was similar (17 % C), the better results achieved with the first sorbent can be attributed not only to its higher loading by the C_{18} groups (L_{0C18} = 0.69 mmol g^{-1}) but also to its uniform surface coverage and better accessibility to these groups. This fact has a pronounced effect on the hydrophobic interactions between the analytes and the sorbent, allowing very good recoveries with SBA-15-C_{18} material. In addition, the higher number of residual non-modified silanol groups in the SBA-15-C_{18} surface ($Q^4/Q^3 < 1$), in comparison with the "end-capped" ExtraBond^R C_{18}, could be the reason for polar secondary interactions (hydrogen bonding interactions) in this material with the more polar compounds, especially with E3, PRED, NAN and 17β-E2.

Several experiments were run to assess the optimal conditions for the SPE procedure with the SBA-15-C_{18} material. To optimize the washing step, after loading of the spiked Milli-Q water sample, each cartridge was washed with 3, 2 and 1 x 5 mL of Milli-Q water to remove interferences. Elution was performed by passing 3 x 2 mL of MeOH. Results obtained indicated that better recoveries were obtained reducing the

volume of water in the washing step. Another important step in the SPE is the efficient elution of the retained analytes. For this reason, we investigated different volumes of MeOH (3, 2 and 1 x 2 mL) for eluting the EDCs from the cartridges. Results indicated not significant differences between the different volumes tested to desorb the analytes, hence 1 x 2 mL of MeOH was selected as eluent for the subsequent experiments to reduce time and reagent consumption. Finally, the conditioning step was also optimized, so the MeOH and water amounts used for this purpose were reduced to 2 mL in both cases. As it can be seen in supporting information (see supporting information Table A2) under optimized conditions good recoveries, near 100 % in all cases, were obtained with very good RSD (1 – 8 %). These results confirmed the excellent adsorption capacity of this silica, so the SBA-15-C_{18} material might be appropriate for simultaneous extraction of a wide variety of moderately polar to non-polar EDCs in waters for monitoring purposes.

In the literature we can find that different commercial C_{18}-modified amorphous silicas have been used to preconcentrate estrogenic compounds as endocrine disrupters in waters. For example, López-de Alda et al.[22] evaluated octadecyl-bonded silica cartridge (RP-C_{18} from Baker) for on-line SPE of steroid sex hormones and related synthetic compounds. Recoveries percentages obtained from the analysis of 50 mL of spiked Milli-Q water extracted with this cartridge were between 70 – 99 % for DES, E3, EE2, P, E1 and E2. Chen et al.[23] evaluated a reversed phase PolarPlus C_{18} (not end-caped) adsorbent for off-line SPE of estrogenic steroids in waters. Mean recoveries of spiked Milli-Q water were 65-79 % for E3, EE2, E1 and E2 (RSD = 2 – 20 %). More recently, Kuster et al.[24] tested LiChrolut RP-18 cartridges (500 mg) to extract a mixture of estrogenic compounds in waters. From the replicate analysis of spiked Milli-Q water, adjusted to pH 5 prior SPE, the recoveries calculated were between 65 and 92 % for

DES, E2, EE2, E3 and E1 (RSD = 6 %). It is interesting to mention that the recovery percentage obtained in these studies for DES (65 and 70 %) was somewhat lower than the obtained for the other analytes studied,[24] that was attributed to a phenomenon in which some kind of equilibrium process between two different isomeric forms of the compounds would take place.[22] According to the results obtained in the current work, higher recoveries and lower RSDs were obtained for the same analytes with the cartridges packed with the SBA-15-C_{18}, taking into account that the cartridges used in our study had only 100 mg of the mesoporous sorbent. In addition, under optimized conditions a recovery of 100 % (RSD = 8 %) was observed for DES.

The effect of sample volume (from 50 to 500 mL) on the recovery was also studied with Milli-Q water spiked with a mixture of the analytes (to a final concentration from 450 to 45 μg L^{-1}). As it can be seen in supporting information (see supporting information Fig. A5), EDCs were quantitatively retained from 150 mL of spiked water with cartridges packed with SBA-15-C_{18}. The increase of the water volume to 250 mL produced an important reduction in the recovery of E3, PRED and DES. Thus, the preconcentration factor that could be achieved with this material was 10^3.

3.4. SPE of water samples

Table 1 shows recoveries (%) for the twelve EDCs in ground water (pH 7.3) and tap water (pH 6.6) using SBA-15-C_{18} and ExtraBond^R C_{18} as packing materials for SPE. As it can be seen, SBA-15-C_{18} sorbent proved good extraction capacity and elution efficiency, so recoveries > 90% were achieved for the target ECDs, with exception of E3, PRED and DES. The repeatability of the procedure was good, with RSD between 1 – 9 % (n = 3). On the other hand, under similar conditions, ExtraBond^R C_{18} was not

capable of extracting most of these compounds satisfactorily, with recoveries lower than 85 % for eleven of the twelve target EDCs. In addition very bad repeatability of the procedure with this material was observed in most cases.

Since the pH of natural water samples may vary considerable and this can affect the extraction efficiency, an experiment to assess the extent of this effect was performed. Tap water, mineral water, groundwater, river water and effluent wastewater (spiked to 150 μg L^{-1} of each target analyte) were adjusted to pH 5.0 with HCl prior to extraction. For comparative purpose, the assays were also carried out with the same waters at their original pH. As it can be seen in Fig. 4, in general, higher extraction efficiency for the twelve EDCs was observed at pH 5.0 in mineral water, effluent wastewater and river water. On the other hand, quantitative recoveries and relatively small standard deviations were obtained for all compounds, with exception of E3, PRED and DES, in tap water and ground water at their original pH (6.6 and 7.3, respectively). This retention behaviour means that these samples can be submitted to SPE pretreatment without adjusting the pH.

3.5. Reusability of the SPE cartridges

Reusability is one of the key factors to assess the effectiveness of a sorbent. For this reason, three series of sorption/desorption experiments were carried out to evaluate the reusability of the cartridges packed with the SBA-15-C_{18} material. After the sorption step (passing 150 mL of spiked Milli Q water with 150 μg L^{-1} of E1, 17β-E2, EE2, E3, P, T, 17α-MT, AND, NAN, PRED, HEX and DES) the sorbent was washed with Milli-Q water to remove interferences. Elution was performed by passing 2 mL of MeOH to desorb the EDCs retained in the cartridge. After each desorption step, the sorbent was conditioned with 2 mL MeOH and 2 mL Milli-Q water for a new reusability

experiment. This sorption/desorption procedure was repeated four times. The experimental results indicated that the SBA-15-C_{18} silica is stable in this operation process, enabling three loading and elution cycles without significant decrease in the recoveries of the studied target analytes (see supporting information Fig. A6).

3.6 Performance of the method

The linearity of the method was evaluated using standard mixtures of the twelve target EDCs in MeOH at seven concentration levels, covering a range between the method quantification limit (MQL) and 150 μg L^{-1} for each analyte, taking into consideration a preconcentration factor of 10^3. The slope and intercept values of the calibration curves were determined using regression analyses. Linear relationship was found between peak areas and the concentration of the analyte in all cases, with determination coefficients (R^2) ≥ 0.99 (Table 2). RSD (%) values for the slope of the calibration curves obtained in three different days were between 3 and 11%. These results showed that linearity of the method was good for the analytes studied.

The instrumental detection limit (IDL) and instrumental quantification limit (IQL) were estimated as the concentration level corresponding to a signal-to-noise of 3 and 10, respectively, from injection of a standard solutions successively diluted. Sensitivity of the method was estimated by application of the preconcentration factor of 10^3 to the IDL and IQL calculated. The method detection limit (MDL) and method quantification limit (MQL) obtained was confirmed by injection of a spiked water extracted following the final SPE procedure (obtaining a signal-to-noise ratio of 3 and 10, respectively). The MDL and MQL obtained for each analyte are shown in Table 2.

Instrumental precision of the method was studied in terms of repeatability and intermediate precision at two levels concentration (MQL and 150 μg L^{-1}). Results were

obtained in terms of RSD (%) for peak areas (A). As shown in Table 2, the instrumental repeatability, determined for six consecutive injections of each standard mixture (n = 6), was acceptable at both concentration levels, with RSD < 9 %. Intermediate precision was determined for three consecutive injections of each standard mixture, carried out on three different days (n = 9, k = 3). RSD obtained for intermediate precision was between 2 and 16 % (Table 3).

The accuracy of the method was assessed using three independent aliquots of 150 mL of water samples, freshly spiked with the appropriate amount of standard mixtures of the twelve target EDCs, in order to obtain a final concentration of MQL and 150 μg L^{-1}. Non spiked samples (blanks) were also processed and demonstrated that the concentration of the analytes in the non spiked samples was below the MQL. As presented in Table 3**,** mean recovery values obtained were between 71 and 112 % with a RSD ≤ 10% for the lowest concentration and between 50 and 105 % with a RSD ≤ 11% for the highest concentration.

4. Conclusions

In the current work, SBA-15-C_{18} was synthesized, characterized and investigated as new sorbent in SPE for the extraction of twelve estrogenic hormones from waters using HPLC with DAD detection. Although small quantities (100 mg) of the sorbent were used, this new material exhibited excellent extraction capability for the compounds studied with a preconcentration factor of 10^3. In addition, the target analytes were successfully determined with satisfactory precision and good recovery in tap water, mineral water, groundwater, river water and effluent wastewater.

Abbreviations

AND	4-androstene-3,17-dione
ACN	Acetonitrile
BJH	Barret-Joyner-Halenda
DES	Diethylstilbestrol
EDCs	Endocrine disrupting compounds
17β-E2	17β-estradiol
E3	Estriol
E1	Estrone
EE2	Ethinylestradiol
HEX	Hexestrol
IDL	Instrumental detection limit
IQL	Instrumental quantification limit
MeOH	Methanol
MDL	Method detection limit
MQL	Method quantification limits
17α-MT	17α-methyltestosterone
NAN	Nandrolone
OTES	Octadecylsilane
PRED	Prednisolone
P	Progesterone
RSD	Relative standard deviation
SBA-15-C_{18}	Santa Barbara Amorfous silica functionalized with octadecyl groups
SEM	Scanning electron microscopy
SPE	Solid-hase extraction
T	Testosterone
TEOS	Tetraethylorthosilicate

TGA	Thermogravimetric analysis
TEM	Transmission electron microscopy
WWTPs	Wastewater treatment plants
WHO	World Health Organization
XRD	X-ray diffraction

Acknowledgements

Authors thank financial support from the Comunidad Autónoma of Madrid and European funding from FEDER program (project S2013/ABI-3028, AVANSECAL).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

References

1 J.Q. Jiang, Z Zhou and V.K. Sharma, *Microchem. J*., 2013, **110,** 292-300.

2 T. Deblonde, C. Cossu-Leguille and P. Hartemann, *Int. J. Hyg. Environ. Health*, 2011, **214**, 442-448.

3 K.E. Murray, S.M. Thomas and A.A. Bodour, *Environ. Pollut.*, 2010, **158,** 3462-347.

4 P. B. Fayad, M. Prévost and S. Sauvé, *Talanta*, 2013, **115**, 349-360.

5 N. A. Al-Odaini, M. P. Zakaria, M. I. Yaziz and S. Surif, *J. Chrom. A*, 2010, **1217,** 6791-6806.

6 R. Liu, J.L. Zhou and A. Wilding, *J. Chrom. A*, 2004, **1022**, 179-189.

7 M.H. Dévier, K. Le Menach, L. Viglino, L. Di Gioia, P. Lachassagne and H. Budzinski, *Sci. Total Environm*., 2013, **443,** 621-632.

8 B.J. Vanderford, R. A. Pearson, D.J. Rexing and S.A. Snyder, *Anal. Chem*., 2003, **75**, 6265-6274.

9 Y. Yoon, J. Ryu, J. Oh, B. G. Choi and S. A. Snyder, *Sci. Total Environm*., 2010, **408**, 636-643.

10 M. Gorga, M. Petrovic and D. Barceló, *J. Chrom A*, 2013, **1295,** 57-66.

11 R. Guedes-Alonso, S. Montesdeoca-Esponda, Z. Sosa-Ferrera and J. J. Santana-Rodríguez, *Trends Environ. Anal.Chem*., 2014, **3–4**, 14–27.

12 A.D. LaFleur and K.A. Schug, *Anal. Chim. Acta*., 2011, **696,** 6-26.

13 L. Zhao, H. Qin, R. Wu and H. Zou, *J. Chromatogr. A*, 2012, **1228,** 193-204.

14 J. Tian, J. Xu, F. Zhu, T. Lu, C. Su and G. Ouyang, J. *Chromatogr. A*, 2013, **1300** 2-16.

15 E.M. Thurman and M.S. Mills, Solid-Phase Extraction. Principles and practice, John Willey & Sons, New York, 1998.

16 A. Ballesteros-Gómez and S. Rubio, *Anal. Chem*., 2011, **83,** 4579-4613.

17 I. Sierra and D. Pérez-Quintanilla, *Chem. Soc. Rev*., 2013, **42**, 3792-3807.

18 M.C. Hennion, *J. Chromatogr. A*, 1999, **856**, 3-54.

19 J. Gañán, D. Pérez-Quintanilla, S. Morante-Zarcero and I. Sierra, *J. Hazard. Mater*., 2013, **260,** 609-617.

20 V. Pérez-Fernández, S. Morante-Zarcero, D. Pérez-Quintanilla, M.A. García, M.L. Marina and I. Sierra, *Electrophoresis*, 2014, **35**,1666-1676.

21 D.J.T. Hill, C.M.L. Preston and A.K. Whittaker, *Polymer*, 2002, **43,** 1051-1059.

22 M.J. López de Alda and D. Barceló, *J. Chromatogr. A*, 2001, **911,** 203-210.

23 C.Y. Chen, T.Y. Wen, G.S. Wang, H.W. Cheng, Y.H. Lin and G.W. Lien, *Sci. Total. Environ*., 2007, **378,** 352-365.

24 M. Kuster, D.A. Azevedo, M.J. López de Alda, F.R. Aquino Neto and D. Barceló, *Environment. Inter*., 2009, **35,** 997-100.

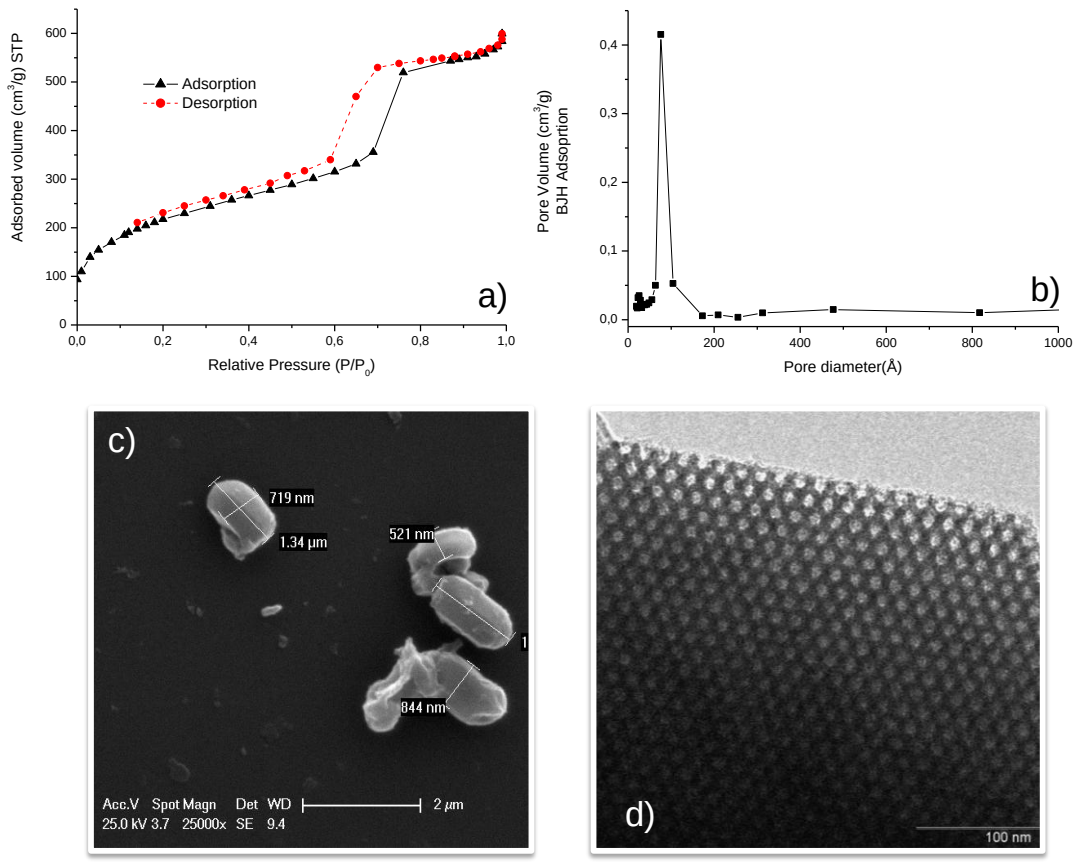

Fig. 1 (a) N2 adsorption-desorption isotherms, (b) pore size distribution, (c) SEM image and (d) TEM image of SBA-15-C_{18}.

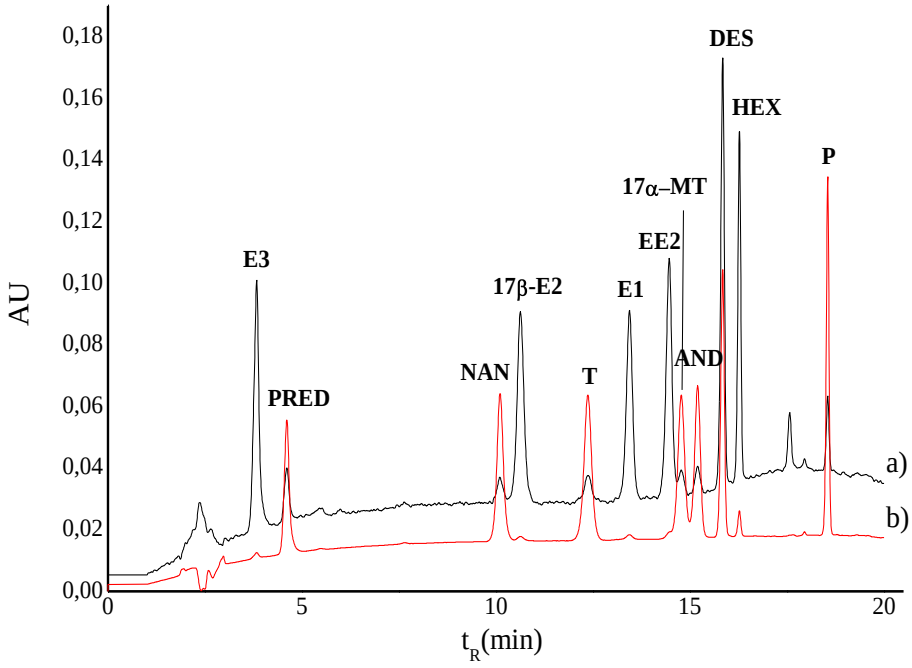

Fig. 2 Chromatographic separation obtained for twelve endocrine disrupting compounds with the optimized gradient elution: t = 0 min 35 % B – 65 % A, t =5 min 40 % B– 60 % A (5 min), t =10.5 min 45 % B – 55 % A (1 min) and t = 16 min 100 % B (4 min) (water as mobile phase A and acetonitrile as mobile phase B). The flow rate was 1.0 mL/min and the detection was recorded following a dual wavelength method at (a) 200 nm for E1, 17β-E2, E3, EE2 and HEX and (b) 242 nm for PRED, NAN, T, 17α-MT, AND, DES and P.

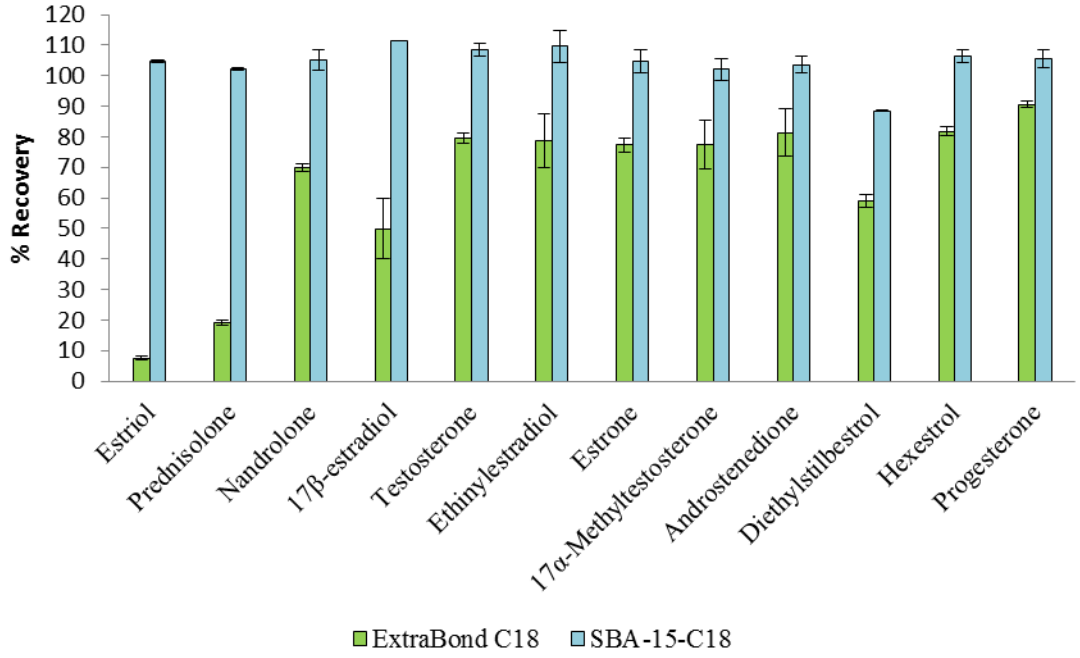

Fig. 3 Comparison of the recovery percentages obtained from the analysis (n = 3) of 50 mL sample volume of spiked Milli-Q water at 450 µg/L extracted with SPE cartridges packed with commercial silica (ExtraBond^{R} C_{18}) and mesoporous silica (SBA-15-C_{18}).

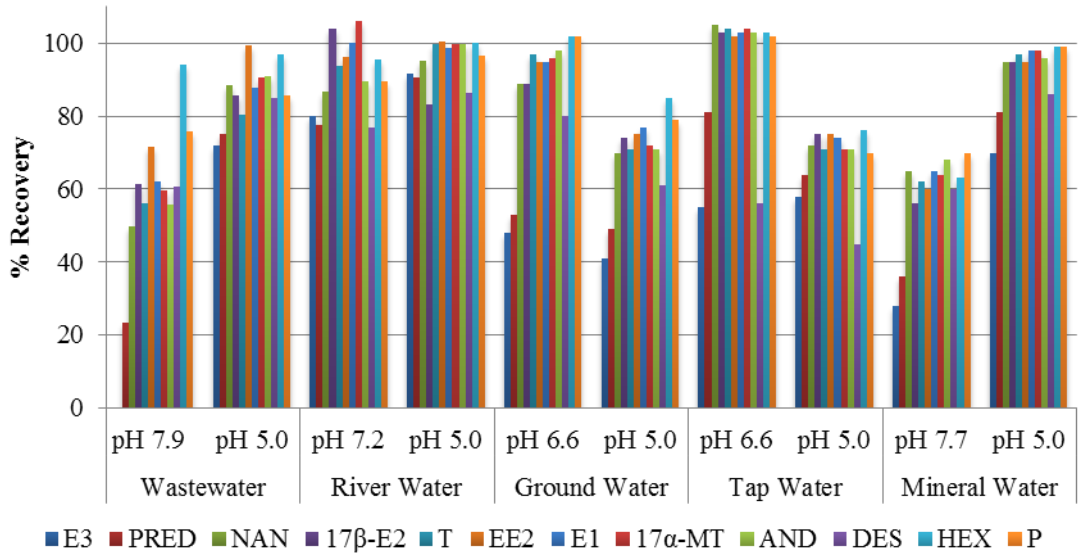

Fig. 4 Effect of the water pH on the extraction efficiency of estrogenic hormones in different samples with SPE cartridges packed with mesoporous silica SBA-15-C_{18}. Spiked concentration level: 150 μg L^{-1}. Estriol (E3), prednisolone (PRED), nandrolone (NAN), 17β-estradiol (17β-E2), testosterone (T), ethinylestradiol (EE2), estrone (E1), 17α-methyltestosterone (17α-MT), 4-androstene-3,17-dione (AND), diethylstilbestrol (DES), hexestrol (HEX) and progesterone (P).

Table 1 Comparison of the recovery percentages obtained from the analysis (n = 3) of 150 mL of spiked ground water and tap waterextracted with SPE cartridges packed with 100 mg of SBA-15-C_{18} and ExtraBond^{R} $C_{18.}$ [a] Spiked level = 150 µg L^{-1}

	Recovery (%) ± S.D.			
	Ground water (pH 7.3) [a]		**Tap water (pH 6.6) [a]**	
Analytes	**SBA-15-C_{18}**	**ExtraBond^{R} C_{18}**	**SBA-15-C_{18}**	**ExtraBond^{R} C_{18}**
Estriol	48 ± 3	3 ± 1	55 ± 5	5 ± 2
Prednisolone	53 ± 2	2 ± 3	81 ± 5	3 ± 5
Nandrolone	89 ± 6	33 ± 10	105 ± 6	59 ± 22
17β-Estradiol	89 ± 4	29 ± 7	103 ± 4	49 ± 18
Testosterone	97 ± 5	51 ± 15	104 ± 7	73 ± 23
Ethinylestradiol	95 ± 4	40 ± 10	102 ± 4	61 ± 19
Estrone	95 ± 3	54 ± 16	103 ± 4	69 ± 14
17α-Methyltestosterone	96 ± 4	60 ± 17	104 ± 7	82 ± 21
4-androstene-3.17-dione	98 ± 3	62 ± 16	103 ± 6	85 ± 20
Diethylstilbestrol	80 ± 4	26 ± 9	56 ± 4	46 ± 13
Hexestrol	102 ± 1	57 ± 12	103 ± 3	71 ± 12
Progesterone	102 ± 2	89 ± 19	102 ± 6	91 ± 15

Table 2 Analytical characteristics of the developed method. Estriol (E3), prednisolone (PRED), nandrolone (NAN), 17β-estradiol (17β-E2), testosterone (T), ethinylestradiol (EE2), estrone (E1), 17α-methyltestosterone (17α-MT), 4-androstene-3,17-dione (AND), diethylstilbestrol (DES), hexestrol (HEX) and progesterone (P). [a] Spiked concentration level: low level = MQL; high level = 150 µg L^{-1}

Analytical characteristics	**E3**		**PRED**		**NAN**		**17β-E2**		**T**		**EE2**		**E1**		**17α-MT**		**AND**		**DES**		**HEX**		**P**	
Precision																								
Concentration level[a] (µg L^{-1})	1.30	150	0.20	150	0.16	150	3.00	150	0.50	150	2.70	150	3.00	150	0.16	150	0.30	150	0.10	150	0.70	150	0.20	150
Instrumental repeatability (*n* = 6)																								
Area, RSD (%)	2.3	8.5	4	12	6.6	6.2	6.7	4.9	3.1	7.1	4.6	4.7	5.4	7.1	2.6	7.3	6.6	5.0	7.7	3.2	5.2	2.8	4.5	2.9
t_R, RSD (%)	1.2	1.1	1.2	1.2	1.2	1.4	1.4	1.7	1.8	1.6	1.4	1.7	1.1	1.2	1.1	1.1	1.1	1.2	0.6	0.7	0.3	0.5	0.2	0.3
Intermediate precision (*n* = 6)																								
Area, RSD (%)	11.9	4.5	7.2	5.3	2.7	8.0	8.8	6.2	5.6	7.1	6.2	4.6	5.9	13.9	2.3	7.6	16	11.1	14.1	5.3	7.4	7.5	6.4	9
t_R, RSD (%)	1.5	1.4	1.6	1.4	1.4	1.4	1.6	1.5	2.2	1.5	1.6	1.5	1.2	1.0	1.3	1.0	1.3	1.0	0.6	0.6	0.4	0.4	0.1	0.2
Linearity																								
Linear range (µg L^{-1})	1.30 - 150		0.20 - 150		0.16 - 150		3.00 - 150		0.50 - 150		2.70 - 150		3.00 - 150		0.16 - 150		0.30 - 150		0.10 - 150		0.70 - 150		0.20 - 150	
Linear equation (bx + a)	23.229x + 173.35		36.010x + 16.19		59.599x + 10.235		57.103x + 231.11		53.319x + 279.08		77.115x - 182.51		79.455x + 18.848		44.511x + 147.48		55.746x - 45.155		30.805x + 141.66		72.181x - 300.76		43.446x + 181.56	
R^2	0.998		0.989		0.998		0.995		0.999		0.999		0.999		0.995		0.999		0.997		0.986		0.995	
MDL (µg L^{-1})	0.40		0.07		0.05		0.90		0.17		0.80		0.90		0.05		0.10		0.03		0.20		0.06	
MQL (µg L^{-1})	1.30		0.20		0.16		3.00		0.50		2.70		3.00		0.16		0.30		0.10		0.70		0.20	

Table 3. Accuracy of the developed method. Estriol (E3), prednisolone (PRED), nandrolone (NAN), 17β-estradiol (17β-E2), testosterone (T), ethinylestradiol (EE2), estrone (E1), 17α-methyltestosterone (17α-MT), 4-androstene-3,17-dione (AND), diethylstilbestrol (DES), hexestrol (HEX) and progesterone (P). [a] Spiked concentration level: low level = MQL; high level = 150 μg L^{-1}

		Recovery (%) ± S.D.											
Sample	**Concentration level[a]**	**E3**	**PRED**	**NAN**	**17β-E2**	**T**	**EE2**	**E1**	**17α-MT**	**AND**	**DES**	**HEX**	**P**
Tap water	Low	80 ± 8	88 ±15	98±16	98±14	96±13	96±9	99±12	100±19	98±15	77±5	111±9	87±11
(pH 6.6)	High	55±5	81±5	105±6	103±4	104±7	102±4	103±4	104±7	103±6	56±4	103±3	99±6
Mineral water	Low	108±4	101±9	108±10	90±4	70±6	89±10	110±13	87±6	107±10	78±8	108±9	110±8
(pH 5.0)	High	70±2	81±8	95±6	95±7	97±4	95±5	98±7	98±3	96±4	86±4	99±3	99±2
Ground water	Low	82±8	100±8	108±9	112±8	111±5	101±19	100±10	102±10	112±9	80±8	99±16	98±11
(pH 7.3)	High	48±3	53±2	89±6	89±4	97±5	95±4	95±3	96±4	97±3	80±4	102±1	102±2
River water	Low	72±5	78±6	85±9	85±10	87±6	91±4	87±8	88±6	89±9	89±8	89±6	93±8
(pH 5.0)	High l	92±6	90±10	95±8	83±8	100±7	100±4	99±5	100±8	100±8	86±4	100±1	97±6
Wastewater	Low	71±1	77±6	81±5	83±7	81±5	79±4	84±4	79±6	82±5	71±4	92±5	86±2
(pH 5.0)	High	77±9	80±12	88±11	86±10	80±9	99±7	88±10	91±8	91±9	85±16	97±1	86±5