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Efficient analysis of selected estrogens using fabric phase sorptive extraction and high performance liquid chromatography-fluorescence detection

Rajesh Kumar[a], Gaurav[a,c], Heena[a], Ashok Kumar Malik[a,*], Abuzar Kabir[b,**], Kenneth G. Furton[b]

[a] Department of Chemistry, Punjabi University, Patiala-147002, Punjab, India
[b] International Forensic Research Institute, Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA
[c] Punjabi University College of Engineering & Management, Rampura Phul-151103, Punjab, India

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ABSTRACT

A simple, fast and sensitive analytical method using fabric phase sorptive extraction (FPSE) followed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) has been developed for efficient quantification of biologically important molecules *e.g.*, 17α-ethynylestradiol (EE2), β-estradiol (E2) and bisphenol A (BPA). FPSE is a new sorptive extraction technique that integrates the advantages of permeable sol–gel derived hybrid organic–inorganic sorbents with flexible and permeable fabric substrates, resulting in a highly efficient and sensitive extraction media that can be introduced directly into any kind of fluidic matrix. Various factors affecting the performance of FPSE technique were optimized. The chromatographic separation was carried using mobile phase acetonitrile/methanol/water (30:15:55; v/v) at a flow rate 1.0 mL/min on C_{18} column with fluorescence detection (λ_{ex} = 280 nm and λ_{em} = 310 nm). The calibration curves of the target analytes were prepared with good correlation coefficient values (R^2 > 0.992). Limit of detection (LOD) values range from 20 to 42 pg/mL. The developed method was applied successfully for the analysis of estrogen molecules in urine and various kinds of aqueous samples.

1. Introduction

Estrogens, primary female sex hormones, are a group of compounds crucial in menstrual and estrous reproductive cycles. This group of compounds is mainly used as drugs in the treatment of hormonal imbalances, breast cancer, and impotence. They can be divided into endogenous estrogens, natural and artificial estrogens, or synthetic estrogens [1]. The endogenous estrogens enter into the environment primarily through the sewage discharge and animal waste disposal [2,3]. These estrogens can be excreted in different amounts depending upon gender, age, state of health, diet or pregnancy. The artificial estrogens are often used as contraceptives and agents for animal growth, which are discharged into the environment with pharmaceutical and aquaculture wastewater. They enter into the human body through the food chain and may instigate harmful effects such as reproductive disorder, birth defects and cancer risks even at very low concentrations [4].

17α-ethynylestradiol (EE2) is an orally bioactive estrogen used in many formulations of contraceptive pills. 17β-estradiol (E2) is secreted by mature follicles of ovaries which promotes and adjusts the feminine reproductive organ and controls the growth of secondary sexual characteristics. It may also be used in replacement therapy for female hypogonadism or primary ovarian failure. Bisphenol A (BPA) is widely used in industry as a manufacturing intermediate in synthesis of epoxy, polysulfone and certain polyester resins [5,6]. It is mainly used as monomer in polycarbonate manufacturing. It is released from polycarbonate flasks during autoclaving and displays estrogenic activity. These estrogens are found in diverse environmental compartments around the world including ground water, river water, sewage water, hospital wastewater and biological samples [1,7–13,17,19].

Quantification of estrogens in environmental and biological samples presents unique challenges due to their ultra-trace level concentrations in these complex matrices and need of a series of

* Corresponding author. Tel.: +91 175 3046598; fax: +91 175 2283073.
** Corresponding author. Tel.: +1 305 348 2396; fax: +1 305 348 4172.
E-mail addresses: malik_chem2002@yahoo.co.uk (A.K. Malik), akabir@fiu.edu, abuzarkabir@gmail.com (A. Kabir).

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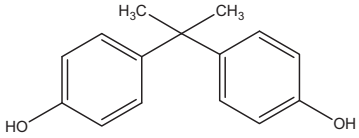
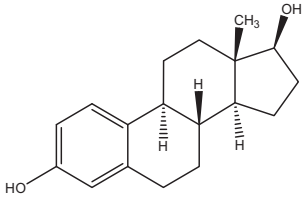
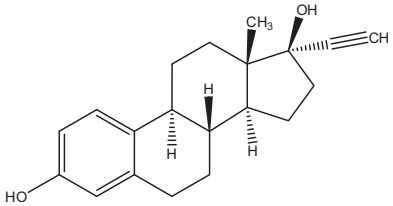
Table 1
Basic characteristics of selected estrogens.

Estrogen	Abbreviation	Molecular structure	Therapeutic class	Log K_{ow}
Bisphenol A	BPA		Plasticizer	3.64
17β-Estradiol	E2		Natural estrogen	4.01
17α-Ethynylestradiol	EE2		Ovulation inhibitor	4.11

rigorous sample preparation steps prior to injection into the analytical system. Table 1 presents the chemical structures and other pertinent properties of the selected estrogens.

In recent years, several chromatographic methods have been reported for the determination of EE2, E2 and BPA. Most of the existing chromatographic methods for the analysis of these estrogens are based on gas chromatography-mass spectrometry (GC-MS) [3,4,11–14,20,28] and liquid chromatography-mass spectrometry (LC-MS) [4,7–9,15–18,24]. However, the use of these techniques encounters several drawbacks, such as highly expensive instrumentation and huge maintenance cost. Before GC analysis, generally a derivatization process is needed to increase the volatility of estrogens as most of them are high boiling compounds, which is a tedious process and causes sample loss [3,11–14,17,20,28]. In comparison to LC-MS, high performance liquid chromatography (HPLC) coupled with a UV or fluorescence detector is simpler, inexpensive and a faster technique. However, a fluorescence detector is more sensitive than a UV detector for selected estrogen molecules.

Among the sample preparation techniques that are commonly used include solid phase extraction (SPE) [3,7–12,15–19], Solid-phase microextraction (SPME) [20–22], liquid–liquid microextraction (LLME) [23] and stir bar sorptive extraction (SBSE) [28–31]. These sample preparation techniques have many shortcomings. The drawbacks of SPE include the requirement of a filtered and particulate free sample to prevent clogging of the sorbent bed, involvement of multiple time consuming steps, consumption of high volume of organic solvents in conditioning as well as elution step, the requirement of solvent evaporation and sample reconstitution steps [7,8,10]. Shortcomings of the SPME technique include small sorbent loading, low sample capacity, bending and damage of the sorbent coating during operation, which leads to loss of sensitivity [33]. Other drawbacks of SPME include limited number of commercially available stationary phases, high cost, instability and swelling of the fiber in organic solvents, fiber breakage during its passage through ferrule, which greatly restrict its use in hyphenation with HPLC [25]. LLME requires an extraction solvent capable of selectively extracting the target analytes, low solubility in aqueous phase, and be compatible with the analytical instrument [26]. Major limitations of SBSE include the possibility of physical damage to the extraction phase when stirring at high speed, limited number of available extraction sorbents, and its incompatibility with viscous biological or environmental samples containing particulates, debris and proteins [27]. Therefore, in present study, pretreatment of the estrogens was achieved by using a new novel sample preparation technique, fabric phase sorptive extraction (FPSE). FPSE, recently developed by Kabir and Furton [32], has successfully integrated the advantages of sol–gel derived microextraction sorbents [33] and rich surface chemistry of cellulose fabric, resulting in a highly sensitive, efficient and solvent minimized sample preparation media. This technique has innovatively addressed two major shortcomings of all sorptive extraction techniques *viz.*, low sample capacity and longer sample preparation time. The combination of an intrinsically porous cellulose surface as the substrate and the vigor of sol–gel derived hybrid organic–inorganic material system, with tunable selectivity and adjustable porosity, has allowed for the utilization of high amounts of extraction sorbents dispersed as an ultra-thin sorbent film into the cellulose substrate matrix, leading to a phenomenal increase in analyte retention capacity with very fast extraction equilibrium. Compared to the sorbent loading in a typical SPME fiber, FPSE media contains approximately 400 times higher sorbent loading. However, unlike stir bar sorptive extraction, the extraction sorbent is distributed uniformly on the surface of nanometer size cellulose micro fibrils of FPSE. Due to the ultra-thin coating of the sol–gel poly-THF sorbent on the substrate matrix, the inherent porosity of cellulose fabric remains well preserved. As a consequence, aqueous solutions containing the target analytes can easily access the active sites of sol–gel poly-THF sorbent for effective analyte–sorbent interaction, leading to highly efficient extraction. Due to the uniform dispersion of the ultra-thin film of extraction sorbent throughout the substrate matrix, the FPSE media is capable of reaching extraction equilibrium for target analytes in a very short period of time. In the same way, back extractions of the accumulated analytes from the FPSE media in organic solvents is even faster due to the low viscosity of the organic solvents used, typically in the range of 2–10 min, without the potential carryover risk. Due to the strong chemical bonding between the extraction sorbent and the host substrate, FPSE media can be exposed to any organic solvent of choice as well as to harsh chemical environments (highly acidic and basic). There are several advantageous features of this technique which make it more