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Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices — A review☆

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ABSTRACT

The estrogens 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2) are reported as highly endocrine-disrupting agents, being recently included in an EU watch list regarding emerging aquatic pollutants. Therefore, the monitoring of these chemicals in the different environmental compartments assumes great importance. Moreover, due to the possible adverse effects on living beings, their occurrence on animal tissues and fluids must also be addressed. In recent years, a significant number of studies have described and proposed different analytical methodologies to detect and/or quantify E2 and EE2 mostly in environmental aqueous samples, including sludge and sediments and also in biological matrices such as plasma and tissues. Taking into account the complexity of real matrices and that both estrogens are generally present at trace levels, the development of accurate and reliable techniques for their determination can be quite a challenge. The present review aims at describing the main characteristics of the analytical methods recently used for E2 and EE2 determination in environmental and biological samples. The steps for sample preparation such as analytes extraction, preconcentration and clean-up are discussed and the instrumental based analytical techniques are compared. Furthermore, the application of biological tools to determine the total estrogenicity of environmental samples, as well as their potential combination with instrumental analyses, is highlighted.

Abbreviations: AAE, aqueous alkali extraction; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; ASE, accelerated solvent extraction; BPA, bisphenol A; BSTFA, *N,O*-bis(trimethylsilyl)-trifluoroacetamide; cEEQ, estradiol equivalents calculated from concentration data; CI, chemical ionization; CLEIA, chemiluminescence enzyme immunoassay; CPRG, chlorophenol red-β-D-galactopyranoside; CV, cyclic voltammetry; DAD, diode array detector; DLLME, liquid–liquid microextraction; DLLME-SFO, dispersive liquid–liquid microextraction based on the solidification of a floating organic drop; DLLSME, dynamic liquid–liquid–solid microextraction; DNS-Cl, dansyl chloride; E1, estrone; E2, 17-β-estradiol; EE2, 17-α-ethinylestradiol; EDCs, endocrine-disrupting chemicals; EEQ, estradiol equivalents; EI, electron ionization; ELISA, enzyme-linked immunosorbent assay; ER-α, recombinant human estrogen related receptor alpha; ERE, estrogen-responsive sequences; ESI, electrospray ionization; FD, fluorescence detector; FPSE, fabric phase sorptive extraction; GC–MS, gas chromatography coupled to mass spectrometry; GC–MS/MS, gas chromatography coupled to tandem mass spectrometry; GPC, gel permeation chromatography; hER, human estrogen receptor; HF-LPME, hollow-fiber liquid-phase microextraction; HPLC, high performance liquid chromatography; HLB, hydrophilic–lipophilic balance; IS, internal standard; K_{ow}, octanol/water partition coefficient; LC–MS, liquid chromatography coupled to mass spectrometry; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; LLE, liquid–liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MAE, microwave accelerated extraction; MASE, membrane-assisted solvent extraction; MEKC, micellar electrokinetic chromatography; MIPs, molecularly imprinted polymers; MPs, magnetic particles; MRM, multiple reaction monitoring; MSPD, matrix solid-phase dispersion; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; MTBE, methyl tert-butyl ether; MWCNTs/GCE, glassy carbon electrodes modified with multi-walled carbon nanotubes; NiTPPS, Ni(II)tetrakis(4-sulfonatophenyl) porphyrin; POCIS, polar organic chemical integrative samplers; PFBBr, pentafluorobenzyl bromide; PFBCl, pentafluorobenzoyl chloride; RAM, restricted access material; SIM, selected ion monitoring; SPE, solid-phase extraction; SPME, solid-phase microextraction; SRM, selected reaction monitoring; S_w, water solubility; SWV, square wave voltammetry; TBDMSCl, *tert*-butyldimethylsilyl chloride; TMCS, trimethylchlorosilane; TMSI, trimethylsilylimidazole; TWA, time-weighted average; UPLC, ultra-high performance liquid chromatography; WWTP, wastewater treatment plant; YES, Yeast Estrogen Screen.

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1. Introduction

Endocrine-disrupting chemicals (EDCs) constitute a group of organic pollutants with increasing importance due to their impact in the environment and human health. EDCs are generally defined as chemicals that may interfere with the function of the endocrine system in wildlife and humans by blocking or mimicking the normal effect of hormones, affecting their synthesis or metabolism, and altering hormone receptor levels [1]. The compounds exhibiting endocrine disrupting properties include a wide range of chemical groups, among which are steroid estrogens, both of natural and synthetic origin. This group of compounds, and particularly the natural hormone 17-β-estradiol (E2) and the synthetic estrogen 17-α-ethinylestradiol (EE2) (Fig. 1), is described as the EDCs with higher disrupting potency [2,3].

Estradiol (E2) is the major estrogen in vertebrates, being associated with the female reproductive system and maintenance of sexual characteristics [4]. Ethinylestradiol (EE2) is a synthetic hormone derived of the natural estrogen E2 which is mainly used as a component of oral contraceptives. Other applications of EE2 in human medicine include estrogen replacement therapy and suspension of breastfeeding [5]. Both estrogens (E2 and EE2) are largely excreted by human and animals in urine and feces as active free forms or inactive glucuronide and sulfate conjugates. The estrogen excretion varies as a function of gender, physiological and developmental state. The highest contributors are pregnant and menstrual women, excreting, respectively, 308 and 4.66 μg per day of E2 in urine and 202 and 0.2 μg per day of E2 in feces [6]. Except during these periods, woman and man present a similar E2 excretion, between 1.5–7 μg per day [7,8]. Assuming that approximately 17% of the total female population take the contraceptive pill regularly in western countries, it is estimated that 4.5 and 6 μg per day of EE2 are excreted per head in urine and feces, respectively [6,7]. These data were used to predict a total estrogen discharge of 4.4 kg per year per million inhabitants [8]. These estrogens eventually end up in the environment through discharge of wastewater treatment plant (WWTP) effluents and disposal of animal waste [2,8]. In fact, numerous studies have reported the presence of E2 and EE2 in waste and surface waters of various countries, in concentrations ranging from low ng L^{-1} to μg L^{-1} levels [7,9–14]. The two estrogens have also been detected in sludge and sediment samples [11,15–17], showing their potential to persist in the environment [18].

Solubility in water (S_w) and the octanol/water partition coefficient (K_{ow}) are considered crucial to assess the fate of chemicals in the environment [2]. In general, chemicals with K_{ow} values <10 are considered relatively hydrophilic, tending to have high S_w and low adsorption and bioconcentration factors. On the opposite, compounds with K_{ow} values >10^4 are considered very hydrophobic and have high sorption potential [2,19], such as E2 and EE2 with values log K_{ow} c.a. 4. Both E2 and EE2 also present low water solubility values (13 and 4.8 mg L^{-1}, respectively), reinforcing their hydrophobic nature and sorption potential.

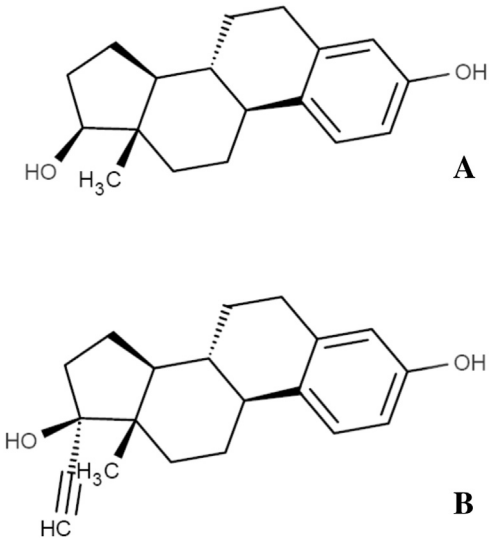

Fig. 1. Chemical structures of the estrogenic compounds 17-β-estradiol (E2) (A) and 17-α-ethinylestradiol (EE2) (B).

In a world with increasing population and resultant higher WWTP discharges, the presence of estrogens in the environment is an emerging problem due to the possible negative effects on ecosystems and living beings, even at low ng L^{-1} levels. This is particularly important in the case of E2 and EE2 as these estrogens have been identified as the major sources of estrogenic activity [2,4,8,13]. Indeed, the presence of E2 and EE2 in the environment has been associated with fish feminization (synthesis and secretion of vitellogenin), reproduction and behavior modifications, fertility reduction, increase of breast and testicular cancer in humans and promotion of abnormal reproductive processes [2,10,20,21]. Moreover, these chemicals have the potential to bioaccumulate and enter the food chain [22,23]. For all these reasons, the European Union has recently added E2 and EE2 to a new "watch list" of emerging aquatic pollutants included in the Water Framework Directive [24].

Having in mind the risks that steroid estrogens pose to humans and wildlife, the monitorization of these chemicals in the environment is of crucial importance and claim for development of valid and robust analytical methods. Therefore, the main purpose of the present paper is to provide a state of the art review of the current techniques applied to detect and quantify the estrogens E2 and EE2 in environmental and also in biological matrices. Due to the complexity of these matrices and the trace levels of estrogens normally detected, multi-step sample preparation is often needed to enrich analytes and reduce interferences [25]. Thus, this review targets recent methods describing the steps of sample extraction, clean-up and analytical determination, highlighting the most used techniques in each step for both types of matrix. It is based on information retrieved in a literature search performed on the *ISI Web of Knowledge* search engine for papers containing the words "estradiol" or "ethinylestradiol" and published in the last six years (2009–2014). A total of 114 papers were considered for this review.

2. Determination of E2 and EE2 in biological samples

In what concerns biological matrices, E2 and EE2 have been mainly determined in plasma and tissues of humans and other mammals, fish, invertebrates, and milk samples as summarized in Table 1 and a schematic representation about sample treatment strategies is presented in Fig. 2. The quantification of the estrogens E2 and EE2 in tissues and biofluids such as plasma or serum is essential to understand human biology and health [26,27], to determine the possible interaction of oral contraceptives (EE2) with co-administered drugs [28,29] or even to assess the possible impact of animal exposure to endocrine disruptive agents. Furthermore, one study has reported the development and validation of an analytical method for the simultaneous quantification of testosterone and E2 in a human cell line (H295R), in accordance with the requirements of the current EPA Steroidogenesis guideline [30]. Endogenous compounds such as E2 derivatives can potentially be used as growth promoters. The direct analysis of these chemicals in bovine hair has been proposed by Bichon et al. [31] as an efficient strategy for the detection of "natural" steroid abuse in cattle. Similarly, the determination of estrogens in milk and dairy products appears as a useful tool to monitor the introduction of natural and synthetic estrogens in the milk cycle by human action [32]. In order to assess the environmental fate of estrogens, several studies have focused on the analysis of fish tissues and plasma samples [33–35]. The main purpose of those studies has been to determine the uptake, elimination and bioaccumulation of estrogens, namely EE2, in fish or other aquatic organisms from natural ecosystems exposed to these compounds. The potential for transfer