DETERMINATION OF STEROID ESTROGENS IN WASTEWATER TREATMENT PLANT OF A CONTRACEPTIVES PRODUCING FACTORY

C. W. CUI, S. L. JI* and H. Y. REN
College of Environment and Energy Engineering, Beijing University of Technology, Beijing 100022, China
(*author for correspondence, e-mail: c.ch_w@126.com)

(Received 16 August 2005; accepted 14 November 2005)

Abstract. Steroid estrogens such as estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2) have been suspected to be the main contaminants, which can affect the endocrine system of animals. Many authors have investigated these chemicals in the domestic wastewater treatment plants (WTP). However, wastewater from industries producing steroid contraceptives has not got ample attention. From the environmental point of view, the four steroids are very significant because even very low concentrations (ng/L) can cause reproductive disturbances in human, livestock and wildlife. The main purpose of the present investigation was to develop an analytical method for the determination of the four steroid estrogens present in WTP of a pharmacy factory, mainly producing contraceptive medicine in Beijing, China. Analysis was performed by solid-phase extraction (SPE) system and liquid chromatography combined with tandem mass spectrometry (LC/MS/MS). The average recoveries from effluent samples ranged from 88% to 103% and the precision of the method ranged from 9% to 4%. Based on 0.5-L wastewater samples, the limit of quantification (LOQ) was determined at 0.7 ng/L for E1, 0.8 for E2, 0.9 ng/L for E3, and 0.5 ng/L for EE2 in influent, and 1.0 ng/L for E2 and EE2, and 2.0 ng/L for E1 and E3 in effluent. In the influent samples, average concentrations of 80, 85, 73 and 155 ng/L were determined for E1, E2, E3 and EE2, respectively, showing that they were removed in this WTP to the extent of 79, 73, 85 and 67%, respectively.

Keywords: E1, E2, E3, EE2, LC/MS/MS, LOQ, SPE, steroid estrogens, WTP

1. Introduction

Steroid estrogens have emerged as a new group of contaminants in the aquatic environment around the world (Desbrow et al., 1998; Jürgens et al., 2002; Peterson et al., 2001). Steroid estrogens are a group of biologically active compounds that are synthesized from cholesterol and have in common a cyclopentan-α-perhydrophenanthrene ring. Natural steroids such as E1, E2 and E3 are secreted by the adrenal cortex, testis, ovary and placenta in human and animal. EE2 is an important synthetic steroid and commonly used as a contraceptive or in estrogen-substitution hormonal therapies. These compounds enter aquatic ecosystems mainly through WTP effluents. Steroid estrogens are among the most potent endocrine disrupting chemicals (EDCs) causing effect in aquatic organisms even at trace-level concentrations (Johnson et al., 2001; Desbrow et al., 1998). For example, less than
1 ng/L of EE2 can stimulate male rainbow trout to produce vitellogenin, an egg yolk protein, which is normally associated only with the sexually mature females (Purdom et al., 1994), and 4 ng/L of EE2 can cause male fathead minnows not to develop normal secondary sexual characteristics (Länge et al., 2001). Many sewage treatment work effluents in the United Kingdom (Harries et al., 1997; Desbrow et al., 1998; Purdom et al., 1994; Harries et al., 1996; Aherne et al., 1989; Montagnani et al., 1996), Germany (Ternes et al., 1999a; Italy (Johnson et al., 2000), the Netherlands (Belfroid et al., 1999; Johnson et al., 2000), Sweden (Larsson et al., 1999), the United States (Snyder et al., 1999), Canada (Ternes et al., 1999a; Lee et al., 1998), and Israel (Shore et al., 1993) have been shown to contain one or more these estrogenic compounds in the low-ng/L range.

Analytical methods for determination of estrogen in wastewater such as capillary gas chromatography-mass spectrometry (GC/MS) techniques with or without derivatization have been widely used (Ternes et al., 2002; Peck et al., 2004; Williams et al., 2003), and liquid chromatography-mass spectrometry (LC/MS) and even LC/MS/MS have been performed with electrospray ionization (ESI) (Johnson et al., 2000; Baronti et al., 2000; Croley et al., 2000; Solé et al., 2000) and with atmospheric pressure chemical ionization (APCI) (Adler et al., 2001; Laganá et al., 2000). In contrast to GC/MS, LC/MS has the advantage of not requiring previous derivatization of the analytes, and its use for the analysis of water samples has grown considerably in recent years.

The work was structured to develop an analytical method for determining the four steroids (E1, E2, E3, EE2) in WTP of the contraceptives producing factory. The methodology used involved SPE and LC/MS/MS. To date, to the best of our knowledge, no LC/MS/MS methodology applied to the monitoring of steroid estrogens in WTP from pharmacy factory mainly producing contraceptive medicine, has been reported in the literature.

2. Materials and Methods

2.1. Chemicals

E1 (1,3,5(10)-estratriene-3-ol-17-one), E2 (1,3,5(10)-estratriene-3,17β-diol), E3 (1,3,5(10)-estratriene-3,16α,17β-triol) and EE2 (17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol) were from Sigma-Aldrich (Steinheim, Germany). The chemical structures of the steroids used in this study are shown in Figure 1. Stock solutions (1 mg/mL) of each standard, as well as mixtures were prepared in methanol and stored at 4°C. The 6-mL cartridges packed with 500 mg of ENVI-CARB, a graphitized nonporous carbon with a surface area of 100 m²/g (Supelco, Bellefonte, PA, USA), were used for SPE. Methanol, acetonitrile, and acetone were obtained from Fisher (Fair Lawn, NJ, USA). All solvents used were of HPLC grade or higher quality. The physiochemical properties of these chemicals are given in Table I.