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Determination of estrogenic compounds in wastewater using liquid chromatography–tandem mass spectrometry with electrospray and atmospheric pressure photoionization following desalting extraction

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ABSTRACT

Two complementary LC–MS ionization methods, electrospray (ESI) and atmospheric pressure photoionization (APPI), have been optimized to determine three natural estrogenic compounds (estrone, 17β-estradiol and estriol) and two synthetic estrogenic compounds (17α-ethynylestradiol and diethylstilbestrol) in the influent and effluent of wastewater treatment plants (WWTPs). The wastewater samples were first subjected to solid-phase extraction coupled with desalting extraction to remove matrix interference. The analytes were then detected using liquid chromatography–tandem mass spectrometry (LC–MS–MS) with ESI and dopant-assisted (DA) APPI to evaluate the ion suppression effect and to complement the detection and quantification of estrogenic compounds in complex wastewater samples. The average ion suppression factors for the extracts of the WWTP influent analyzed using ESI and APPI were 52 ± 5% and 27 ± 7%, respectively. The sensitivity and ionization efficiency of the LC–ESI–MS–MS system decreased dramatically when a complex matrix was present in the WWTP influent sample. Estrogenic compounds could be detected in the WWTP influent and effluent samples at concentrations below the parts-per-billion level. The lower detection limits obtained when using ESI and the higher matrix tolerance of the APPI method allowed the complete quantification of estrogenic compounds in very complex samples in a complementary manner.

1. Introduction

Endocrine-disrupting chemicals (EDCs) have become a public health concern in modern times because of their detrimental effects on the human endocrine system (Colborn et al., 1993; Roda et al., 2006; Richardson, 2006). These EDCs have the ability to alter or disrupt normal hormonal functions through their mimicking of the behavior of the sex steroid hormones estrogen and androgen. Estrogens, which are the most potent EDCs, are of particular interest because of their high estrogenic potency and endocrine-disrupting properties. Natural steroid estrogens, which exert estrogenic effects in fish at low ng L^{-1} levels (Purdom et al., 1994), are increasingly becoming a concern worldwide because of their potential risk to humans and wildlife (Beck et al., 2006; Grung et al., 2007; Beck et al., 2008). The natural estrogens, 17β-estradiol (E2) and its main metabolites, estrone (E1) and estriol (E3), exhibit high degrees of estrogenic activity in aquatic environments. Moreover, the synthetic estrogenic compounds, 17α-ethynylestradiol (EE2) and diethylstilbestrol (DES), also have the ability to interfere with the functions of hormone systems. Although natural and synthetic estrogenic compounds can be degraded biologically, they cannot be removed completely in wastewater treatment plants (WWTPs), thus they are often discharged into surface waters (Bila et al., 2007; Esperanza et al., 2007; Lee et al., 2008; Auriol et al., 2008). These estrogenic compounds are usually detected in WWTP effluents and receiving surface waters at concentrations on the ng L^{-1} levels (de Mes et al., 2005; Campbell et al., 2006; Cui et al., 2006; Fernandez et al., 2007; Hutchins et al., 2007; Nelson et al., 2007; Salvador et al., 2007; Tan et al., 2007). To evaluate the removal efficiency of specific estrogenic compounds from WWTPs, and due to their low concentrations present in complex matrices, it is necessary to develop highly sensitive and selective methods to determine these estrogenic compounds at trace levels.

Liquid chromatography–tandem mass spectrometry (LC–MS–MS)-based analyses of estrogenic compounds are most commonly undertaken using an electrospray interface operated in the negative ionization mode (Rodriguez-Mozaz et al., 2004a,b; Reddy et al., 2005; Beck et al., 2006; Salvador et al., 2007). Electrospray ionization (ESI) often suffers, however, from the setback of ion suppression (Buhrman et al., 1996; Annesley, 2003; Jessome and Volmer, 2006; Lin et al., 2007) or signal enhancement (Rodil et al., 2005) when using complex matrices. Ion suppression is one of the most adverse problems affecting LC–MS techniques during quantification, regardless of the sensitivity or selectivity of the mass analyzer used. Although, tandem mass spectrometry techniques

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can be used to remove large amounts of noise and, hence, increase signal-to-noise ratios, the signals of the target ions of analytes usually remain suppressed during the ionization process. Moreover, co-eluting matrix components can also reduce the ion intensities of the signals of interest. Because there is only limited information available regarding the origin and mechanism of ion suppression, it is a difficult problem to solve in many cases. Several strategies have been developed not only to evaluate its presence but also to account for its effects and to eliminate the risk of ion suppression. Standard addition method was the ideal approach to compensate matrix effect during quantification (Koester et al., 2000; Magnuson et al., 2000), but it would be too time-consuming to analyze every sample. Recently, a novel atmospheric pressure photoionization (APPI) technique has been applied to the analysis of estrogenic compounds in biological matrices and river samples (Leinonen et al., 2002; Yamamoto et al., 2006). APPI is less susceptible to salt buffer effects and ion suppression caused by complex matrix than are atmospheric pressure chemical ionization (APCI) and ESI. The environmental application of ESI and APPI to determine estrogenic compounds in urban and tidal rivers has been reported (Yamamoto et al., 2006), however, in the analysis of more complex of WWTP influent and effluent samples has not been reported. One of our goals in this study was to evaluate the applicability of LC–MS–MS with three different atmospheric pressure ionization (API) interfaces to determine estrogenic compounds at trace levels in complex environmental samples from Taiwan. Complex WWTP influent and effluent samples were prepared through desalting extraction to remove a portion of the ionic and hydrophilic interference, and then optimized LC separation to minimize any remaining interferences. The use of ESI, APCI and APPI techniques to evaluate their ionization efficiencies and ion suppression factors for the solid-phase extraction (SPE) and desalting extracts of samples obtained from WWTP influents and effluents were examined. Herein, we also demonstrated the effectiveness of the proposed method for the determination of selected estrogenic compounds in complex WWTP wastewater samples at trace levels.

2. Experimental

2.1. Reagents and chemicals

The standard estrogenic compounds: estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2), diethylstilbestrol (DES) and 17β-estradiol-17-acetate (E2-acetate, as an internal standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and were used as received. Deionized water was further purified using a Millipore water purification device (Millipore, Bedford, MA, USA).

2.2. Sample collection

Tap water and groundwater samples were collected on the campus of National Central University (Chung-Li, Taiwan). River water samples were collected from Pu-Tzu River, the major river of the Chainan Plain in southwestern Taiwan. WWTP influents and effluents were collected from the An-Ping community in Tainan city, Taiwan. This WWTP performs mechanical clarification, biological treatment, and flocculation filtration (population equivalent: 380 000). All samples were pretreated as described in our previous study (Chen et al., 2008).

2.3. Sample pretreatment

The procedure used to extract the estrogenic compounds from the water samples was described previously (Ding and Chiang, 2003), and used here with minor modifications. Water samples were preconcentrated using Oasis HLB cartridges (3 mL, 60 mg, surface area 810 $m^2 g^{-1}$; Waters, Milford, MA, USA). Before extraction, each HLB cartridge was conditioned with 3 mL of MTBE, and then rinsed with 3 mL of methanol and 3 mL of deionized water on an SPE manifold (VacMaster, IT Sorbent Technology, Cambridge, UK). The acidified water samples 50 mL (pH 3.0) mixed with 50 mL of deionized water were passed through the HLB cartridge at a flow rate of ca. 3–5 mL min^{-1}. When the extraction was complete, the cartridge was air-dried under vacuum for 10 min. The selected estrogenic chemical residues were then eluted from the cartridge using 3 mL of MTBE, and collected in a 5-mL mini-vial. The extracts were concentrated to ca. 1 mL. A desalting procedure with liquid–liquid extraction was executed to reduce the interference of a portion of the ionic matrix (Ingrand et al., 2003). To salting-out the extract, 1 mL of 5% sodium chloride was added and then the mixture was shaken for 10 min on a Vortex-2 Genie (Scientific Industries, USA). After separation of the phases, the organic layer (MTBE) was withdrawn using a syringe and then dried by passing through a column of anhydrous sodium sulfate. Finally, prior to LC–MS–MS, the extract was evaporated to dryness under a stream of nitrogen gas and redissolved with 200 μL of methanol containing E2-acetate (200 μg L^{-1}).

2.4. LC–MS–MS analysis

LC separation and mass spectrometric detection of estrogenic compounds were performed on an Agilent 1100 series LC-MSD Trap SL system with ESI, APCI and APPI ionization interfaces (Palo Alto, CA, USA). The injection volume was 20 μL, for ESI and APPI, gradient elution was programmed at a flow rate of 0.1 mL min^{-1}; for the APCI interface, the linear gradient was performed at a flow rate of 0.3 mL min^{-1} (see online Supplementary material). A photoionization lamp generating 10-eV photons was located within the APPI interface. One-mL and 10-mL SGE syringes (Ringwood, Australia) were used to evaluate the ionization parameters and to infuse the dopant for the APPI, respectively. Mass spectra were collected in the scan range m/z 100–450. The optimal operating parameters for each ion source were applied (see online Supplementary material). For tandem mass spectrometric detection, collision-induced dissociation (CID) in product ion scan was performed using helium as the background gas and collision gas at a pressure of 6×10^{-6} Torr.

3. Results and discussion

3.1. Optimization of LC–MS–MS parameters

In this study we used tandem mass spectrometric technique (MS–MS) for identification and quantification purposes because of its high specificity. The behavior of five selected estrogenic compounds in both positive and negative ionization modes using ESI, APCI and APPI interfaces was also evaluated. Irrespective of the API interface chosen, these five estrogenic compounds were all ionized efficiently in the negative ionization mode to form their deprotonated ions $[M–H]^-$, consistent with previous reports (Rodriguez-Mozaz et al., 2004a; Labadie and Hill, 2007). Therefore, we performed all subsequent analyses in the negative ionization mode using the LC–MS–MS method.

The detection parameters in the MS system for the three interfaces were optimized preliminarily by evaluating the signal intensities and fragmentations in a series of continuous-infusion experiments. Quantification of all target compounds was performed through product ion scan recording of two to five transitions simultaneously. Under the optimized MS–MS conditions, the precursor ions were stored in the ion trap by adjusting the isolation segment window and then fragmented with an appropriate fragmentation amplitude to maximize the sensitivity and maintain