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Sensitive analysis of steroid estrogens and bisphenol a in small volumes of water using isotope-dilution ultra-performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

An isotope-dilution ultra-performance liquid chromatography–electrospray tandem mass spectrometry method combined with dansylation was established to sensitively quantify four steroid estrogens (estrone, 17α-estradiol, 17β-estradiol and 17α-ethynylestradiol) and bisphenol A in sewage influent and effluent. A simple hexane extraction was performed from a small volume (10 mL), followed by dansyl chloride derivatization and purification with a silica cartridge. The method effectively reduced the matrix effects in sample extract and permitted the selective and sensitive determination of target compounds from complicated matrices. The detection limits of the method for steroid estrogens were 0.20 −0.90 ng L^{-1} in influent and 0.10–0.20 ng L^{-1} in effluent samples. For bisphenol A, the limits detection of the method were 20 and 0.80 for influent and effluent samples, respectively. Recoveries of 85%–96% were observed in all matrices. The method was applied to analyze residual estrogens and bisphenol A in sewage influent and effluent samples from Beijing, China. The concentrations of bisphenol A (636 −1200 ng L^{-1}) were up to 250 times higher than those of steroid estrogens. Estrone was the dominant estrogen in influent and effluent samples, while similar concentrations of 17α-estradiol and 17β-estradiol were detected in all samples.

1. Introduction

The presence of endocrine-disrupting chemicals in aquatic environments has attracted considerable attention as they may interfere with the reproduction and development of aquatic organisms (Allen et al., 1999; Matozzo et al., 2008; Snyder, 2008; Ashrap et al., 2017). Phenolic compounds of concern include bisphenol A (BPA) and steroid estrogens, such as the naturally occurring female hormone 17β-estradiol (βE2) and estrone (E1), as well as the synthetic contraceptive additive, 17α-ethynylestradiol (EE2). Much attention has focused on BPA as an endocrine disruptor because of its worldwide production, chronic toxicity, and extensive use in industrial, agricultural, and household applications (Manfred et al., 2004; Bautista-Toledo et al., 2005; Rubin, 2011). Steroid estrogens showed the highest potency of estrogenic activities in aquatic environments. In a recent report, steroid estrogens produced chronic-reproductive effects on fish at concentrations as low as 0.1 ng L^{-1} (Caldwell et al., 2012). Although the sewage treatment process removed most steroid estrogens and BPA, sub-ng L^{-1} concentrations were still detected in the effluent and receiving waters (Snyder et al., 1999; Ternes et al., 1999; Baronti et al., 2000; Johnson and Williams, 2004; Chang et al., 2009, 2011; Backe, 2015; Liu et al., 2016). Therefore, ultrasensitive analytical methods are required to quantify and assess the risk of such compounds in the environment.

Many analytical methods have been reported for analyzing steroid estrogens or BPA in environmental waters. Traditional analyses involved gas chromatography-mass spectrometry (GC-MS) combined with solid phase extraction (SPE). However, those methods require time-consuming derivatization to reduce analyte polarity and thermal instability, and exhibit high method detection

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limits (typically >1 ng L^{-1} for estrogens (Lee and Peart, 1998; Kuch and Ballschmiter, 2000; Gunatilake et al., 2014) and >100 ng L^{-1} for BPA (Gatidou et al., 2007)). Alternative methods based on SPE-liquid chromatography–electrospray tandem mass spectrometry (LC-ESI-MS/MS) have been developed for the direct analysis of compounds in sample extracts. Such methods are more sensitive than GC-MS methods for the analysis of surface waters (estrogen: <0.1–10 ng L^{-1} (Croley et al., 2000; Ingrand et al., 2003; Chang et al., 2009); BPA: >20 ng L^{-1} (Martín et al., 2014)). However, the high matrix effects in sewage influent samples can cause marked losses in the sensitivity of LC-ESI-MS/MS analyses (Gangl et al., 2001). SPE methods reduce the matrix effects only moderately because the matrix components with similar polarities co-eluted with analytes, causing signal interferences during LC-ESI-MS/MS analyses. Furthermore, the direct LC-MS/MS analytical methods required large sample volumes (200 mL–4 L), and involved complicated extraction and purification steps to reduce matrix interferences. For example, although three solid phase cartridges were used for purification, the coeluting matrix from the large volumes of wastewater still reduced the sensitivity by >80% in negative mode (Chang et al., 2009). Such requirements render analytical procedures time-consuming and expensive, which has, in part, limited extensive surveys on the occurrence and abundance of steroid estrogens and BPA in aquatic environments.

Recently, chemical derivatization was used to increase the ionization efficiency, and enhance the signal intensity of LC-ESI-MS/MS analyses. The derivatizing agents included dansyl chloride (Anari et al., 2002; Chang et al., 2010), bromide pentafluorobenzoyl (Courant et al., 2007), and 2-fluoro-1-methylpyridinium p-toluenesulfonate (Lin et al., 2007), among others. Dansyl derivatives were reported to produce the highest signal (one or two orders of magnitude greater than underivatizaed estrogens); however, similar to parent estrogens, dansyl derivatives were susceptible to matrix effects when the derivatized solutions were directly injected into the detection system. Lin et al. (2007) reported a large degree of signal suppression of 85.8%–94.15% and 94.5%–99% for dansylated estrogens (E1, E2, E3, EE2) in river water and in sewage effluents, respectively.

In this study, we aimed to develop a simple and reliable UPLC-MS/MS method, combined with dansylation for the accurate and sensitive determination of steroid estrogens and BPA in sewage water. Due to the high sensitivities of dansyl derivatization coupled to UPLC-MS/MS analyses, 10 mL water samples and liquid–liquid extractions (LLE) method were used. Hexane extractions and silica cartridge purifications of the dansylated compounds resulted in the removal of co-eluted interferences and reduced the matrix effects. The isotope-dilution method was also used to compensate matrix effects. The developed method was applied to the analysis of steroid estrogens and BPA in sewage influent and effluent water samples (10 mL). Notably, the simple sample preparation process and high assay sensitivity allowed for extensive surveys of compounds from complex environment matrices.

2. Experimental

2.1. Chemicals and reagents

Four steroid estrogens (E1, αE2, βE2, EE2) and BPA were purchased from Sigma-Aldrich (St Louis, MO, USA) (Table 1). Deuterated standards, including estradiol-2,4,16,16-d_4 (d_4-E1), 17β-estradiol-2,4,16,16-d_4 (d_4-βE2), 17α-ethinylestradiol-2,4,16,16-d_4 (d_4-EE2) and bisphenol-A-2,2′,6,6′-d_4 (d_4-BPA) were purchased from C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada). HPLC-grade ethyl acetate (EtOAc), hexane, acetone, and acetonitrile were obtained from Fisher Chemical Co. (Beijing, China). Formic acid, dansyl chloride (>99% purity) and sodium carbonate (>99.999% purity) were obtained from Sigma-Aldrich. HPLC-grade water was prepared using Milli-Q RC apparatus (Millipore, Billerica, MA).

2.2. Sample collection and preparation

Influent and effluent samples were collected in 1 L amber glass bottles, which were previously washed with methanol and HPLC-grade water, from four sewage treatment plants (STPs) of Beijing, China in April and May 2015. The four STPs receive mainly domestic wastewater, and are operated with primary and secondary treatments. After the samples were collected, they were brought back to the lab and extracted within 6 h from the time of collection.

10 mL of sewage influent or effluent samples was spiked with 5 μg L^{-1} of surrogate standard mixture (50 μL) and extracted with 5 × 3 mL of hexane. The hexane extract was evaporated to dryness and redissolved in 100 μL of aqueous sodium bicarbonate (100 mmol L^{-1}, pH adjusted to 10.5 with sodium hydroxide) and 100 μL of dansyl chloride (1 mg mL^{-1}in acetone). Each sample was vortex-mixed for 1 min and incubated at 60 °C for 5 min. Next, 1 mL of HPLC-grade water and 2 × 3 mL of hexane were added to the derivatization solutions. The organic layer was removed and passed through a silica cartridge (3 mL, 500 mg, Waters, Milford, MA, USA), which was pre-conditioned with 4 mL of hexane. After application of the extract, the cartridge was rinsed with 3 mL of hexane/EtOAC (1:9, v/v) and eluted with 4 mL of hexane/EtOAC (1:1, v/v). The eluate was evaporated to dryness and reconstituted with 100 μL of acetonitrile for UPLC-MS/MS analysis.

2.3. UPLC-ESI-MS/MS analysis

The LC apparatus was an Acquity UPLC (Waters). All analytes were separated using a Waters Acquity UPLC HSS T3 column (50 mm × 2.1 mm, 1.8 μm particle size). The column was maintained at 40 °C with a mobile phase flow rate of 0.25 mL/min, and the injection volume was 5 μL. Acetonitrile (A) and water containing 0.1% formic acid were used as mobile phases. The mobile phase gradient condition was initiated with 60% A, followed by a linear increase to 95% A in 6 min, and held for 2 min to allow re-equilibration. The injection volume was 5 μL.

Mass spectrometry was performed using a Quattro Premier XE tandem quadrupole mass spectrometer (Waters) with an ESI interface in positive ionization mode. The capillary voltage was set at 3.2 kV. The flow rates of the desolvation gas and cone gas were set to 600 and 10 L h^{-1}, respectively. The source temperature and desolvation gas temperature were held at 150 and 500 °C, respectively. Quantitative analysis was performed in multi-selected reaction monitoring (MRM) mode, and the two most abundant MRM transitions were monitored. The relative ratios between these transitions are listed in Table 1. Dwell time for each MRM transition was selected at 50-200 ms under time-segmented conditions based on the chromatographic separation of the target compounds (Table 1). The instrumental detection limits (IDLs) were defined as the amount of analyte that produced a signal-to-noise (S/N) of 3 (peak to peak) in the standard solution. The method detection limits (MDLs) were estimated based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyzing field samples and on a minimal value of S/N of 3.

3. Results and discussion

3.1. UPLC-ESI-MS/MS analysis

Due to the extremely low concentrations of steroid estrogens