Journal of Chromatography A, 1216 (2009) 7071–7080

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of endocrine-disrupting chemicals in the liquid and solid phases of activated sludge by solid phase extraction and gas chromatography–mass spectrometry

Yafeng Nie[a,b], Zhimin Qiang[a,*], Heqing Zhang[a], Craig Adams[c]

[a] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Chemical Defense Institute, Beijing 102205, China
[c] Department of Civil, Environmental, and Architectural Engineering, University of Kansas, Lawrence, KS 66045, USA

ARTICLE INFO

Article history:
Received 8 April 2009
Received in revised form 18 August 2009
Accepted 25 August 2009
Available online 27 August 2009

Keywords:
Endocrine-disrupting chemicals
Steroid estrogens
Phenolic compounds
Activated sludge
Solid phase extraction
Mass spectrometry

ABSTRACT

The highly complex matrix of activated sludge in sewage treatment plants (STPs) makes it difficult to detect endocrine-disrupting chemicals (EDCs) which are usually present at low concentration levels. To date, no literature has reported the concentrations of steroid estrogens in activated sludge in China and very limited data are available worldwide. In this work, a highly selective and sensitive analytical method was developed for simultaneous determination of two classes of EDCs, including estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2), 4-nonylphenol (NP) and bisphenol A (BPA), in the liquid and solid phases of activated sludge. The procedures for sample preparation, extracts derivatization, and gas chromatography–mass spectrometry (GC–MS) quantification were all optimized to effectively determine target EDCs while minimizing matrix interference. The developed method showed good calibration linearity, recovery, precision, and a low limit of quantification (LOQ) for all selected EDCs in both liquid and solid phases of activated sludge. It was successfully applied to determine the concentrations of EDCs in activated sludge samples from two STPs located in Beijing and Shanghai of China, respectively.

1. Introduction

In recent years, the presence of endocrine-disrupting chemicals (EDCs) in the environment has aroused a severe concern worldwide because they may alter the normal hormone functions as well as physiological status in wildlife and humans [1–3]. Steroid estrogens and endocrine-disrupting phenolic compounds are two major classes of EDCs which have attracted the most attention of environmental researchers. Steroid estrogens include estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2), which exhibit a high estrogenic capacity. Purdom et al. [4] found that even low concentrations (at ng/L level) of E2 or EE2 could induce vitellogenin in male fish. E2 is the most important estrogenic hormone, among the steroid estrogens, because it is associated with the reproductive system and maintenance of sexual characteristics of female. E1 and E3 are the metabolites of E2 and exert a lower biological activity than E2. EE2 is a synthetic estrogen used as a major ingredient in many oral contraceptives. The endocrine-disrupting phenolic compounds include bisphenol A (BPA) and 4-nonylphenol (NP) which have a less estrogenic capacity but are generally detected at a much higher concentration (μg/L level) than steroid estrogens (ng/L level) in the environment [5].

Past studies have shown that municipal sewage treatment plants (STPs) are a significant point source of EDCs released into the environment [6–8]. Most of the researches in respect of estrogens in STPs were conducted on wastewater side [9–11], while the activated sludge side has been largely overlooked. The difficulty in determining EDCs in the activated sludge is due to the facts that: (a) the serious matrix interference necessitates developing a highly efficient pretreatment procedure for sludge samples; and (b) the trace levels of EDCs (ng/g) generally present in sludge samples requires a highly sensitive and selective analytical method. Due to the limited data regarding the concentration levels of EDCs in activated sludge, it is not clear at present which process, biodegradation by sludge or sorption onto sludge, makes a major contribution to the removal of EDCs from wastewater in STPs [12]. Furthermore, the determination of EDCs partitioning between the solid and liquid phases in biological treatment units will undoubtedly help elucidate the fate and behavior of EDCs in STPs. As a result, there is an urgent need to develop a selective, sensitive and robust analytical method for simultaneous determination of EDCs in activated sludge.

To date, many analytical methods have been developed for detection of EDCs in environmental samples with varied matrices

* Corresponding author. Tel.: +86 10 62849632; fax: +86 10 62923541.
E-mail address: qiangz@rcees.ac.cn (Z. Qiang).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.08.064

based on either biological [13–15] or chromatographic methodology [16–18]. Biological techniques can be used to determine the endocrine-disrupting activity in total, whereas chromatographic techniques can identify unknown chemicals and quantify their concentrations individually. Gas chromatography (GC) or liquid chromatography (LC), in combination with mass spectrometry (MS) or preferably tandem MS (MS–MS), has been commonly used for the determination of EDCs. GC-based methods usually require derivatization of studied EDCs to improve the detection sensitivity. Ding et al. [19] evaluated the derivatization procedures with different silylating agents for detecting both natural and synthetic estrogenic chemicals by GC–MS. They found that *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilan (TMCS) showed the strongest trimethylsilylation power and provided the sufficient sensitivity and selectivity. Zhang et al. [20] also optimized the derivatization procedures for detection of EDCs in water by solid phase extraction (SPE) and GC–MS. LC-based methods do not require derivatization of studied EDCs, but are subjected to the interference from sample matrix that may significantly suppress the MS signals [21].

To determine trace levels of emerging organic contaminants in environmental samples, SPE is usually adopted to pre-concentrate and extract target organic analytes. Kuch and Ballschmiter [22] used high resolution GC–MS (operated in negative chemical ionization mode) coupled with SPE to detect phenolic compounds, natural and synthetic estrogens in surface and drinking waters and achieved a limit of detection (LOD) in the pg/L range. Liu et al. [23] employed SPE and GC–MS to simultaneously analyze steroid estrogens and endocrine-disrupting phenolic compounds in river and seawater samples. They examined the effects of different SPE cartridges, elution solvents and water properties on the extraction efficiency of target EDCs, and reported that the hydrophilic–lipophilic balance (Waters Oasis HLB) sorbent could achieve the best recovery among nine cartridges filled with different sorbents. These methods, however, were aimed to detect EDCs in water samples with relatively simple matrix. For an activated sludge sample, either its liquid phase or solid phase contains a high concentration of dissolved organic matter (DOM), thus new analytical methods should be developed with a primary aim to resolve the problem of severe matrix interference.

Particularly for analysis of the solid phase of activated sludge, highly efficient extraction and cleanup procedures are needed to remove interfering organic materials while retaining the most of target analytes. Gatidou et al. [24] detected NP, nonylphenol ethoxylates, triclosan and BPA in sewage sludge by GC–MS. Ternes et al. [25] used ultrasonic liquid extraction (ULE), preparative gel permeation chromatography (GPC) and silica gel cleanup as sample pretreatment method to detect four natural and synthetic estrogens in sludge and sediments by GC–MS–MS and achieved the limits of quantification (LOQ) at 2–4 ng/g. A rotary shaker was used to help extraction of the samples and the GPC and C18 SPE cartridge were used for sample cleanup by Gomes et al. [26]. However, the recoveries for E3 and EE2 obtained by either LC–MS or GC–MS were in a low range from about 17.8% to 43.6%. In addition, the incorporation of GPC complicated the sample cleanup procedure, thus limiting the method application.

The main objective of this study was to develop a sensitive and selective analytical method for simultaneous determination of endocrine-disrupting phenolic compounds and estrogens, including E1, E2, E3, EE2, BPA and NP, in both liquid and solid phases of activated sludge from municipal STPs. A highly efficient and relatively simple sample cleanup procedure was successfully developed to minimize the significant matrix interference. The quantification method for NP and the derivatization procedure for target analytes were also improved. The linearity of calibration curves, recovery efficiency and precision, and LOQ were all assessed for method validation. Thereafter, the developed method was applied to determine the concentration levels of studied EDCs in real activated sludge samples grabbed from the secondary sedimentation tanks in two STPs located in Beijing and Shanghai of China.

2. Experimental

2.1. Chemicals and standard solutions

E1 (99%) was purchased from Acros (New Jersey, USA), and E2 (97.0%), E3 (99.0%), BPA (99.9%) and NP (the technical isomer mixture of nine compounds with different branched side-chains) from Tokyo Chemical Industry (TCI, Tokyo, Japan). EE2 (98%) and the internal standards (IS), BPA-d_{16} (98%) and E2-d_2 (98%), were purchased from Sigma–Aldrich (St. Louis, USA). The chemical structures of studied EDCs are shown in Fig. 1. Methyl *t*-butyl ether (MTBE, HPLC grade) was purchased from Tedia (Fairfield, USA); methanol (HPLC grade) from Fisher Chemicals (Pittsburg, USA); methylene chloride (ultra resi-analyzed) from J.T. Baker (Deventer, The Netherlands); and hexane (HPLC grade) from J&K Chemical (Beijing, China). BSTFA with 1% of TMCS, used as the derivatization reagent, was supplied by Fluka (Buchs, Switzerland). Silica gel and neutral aluminum oxide, both of 100–200 mesh and chromatography grade, were purchased from Sinopharm Chemical Reagent Co. (SCRC, Beijing, China). Acetone, sodium sulfate anhydrous

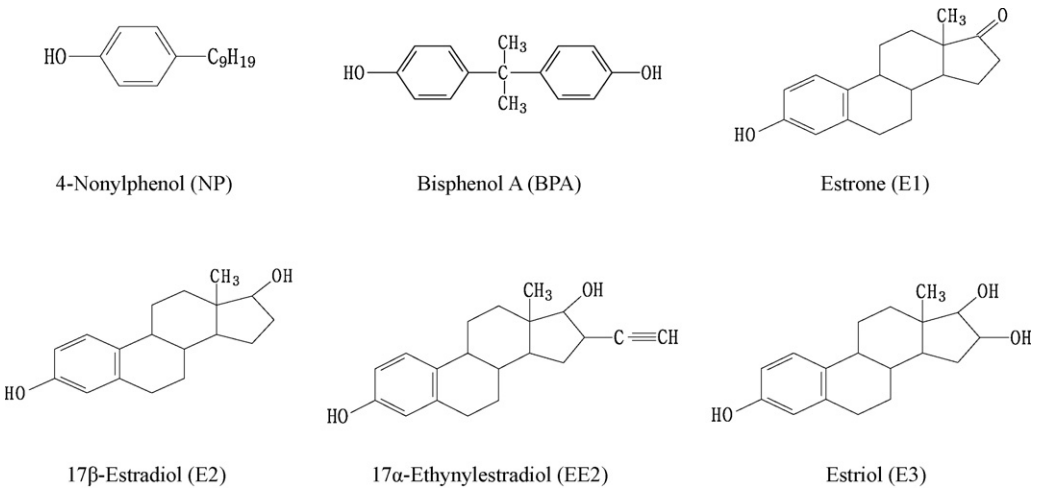

Fig. 1. Chemical structures of target EDCs.

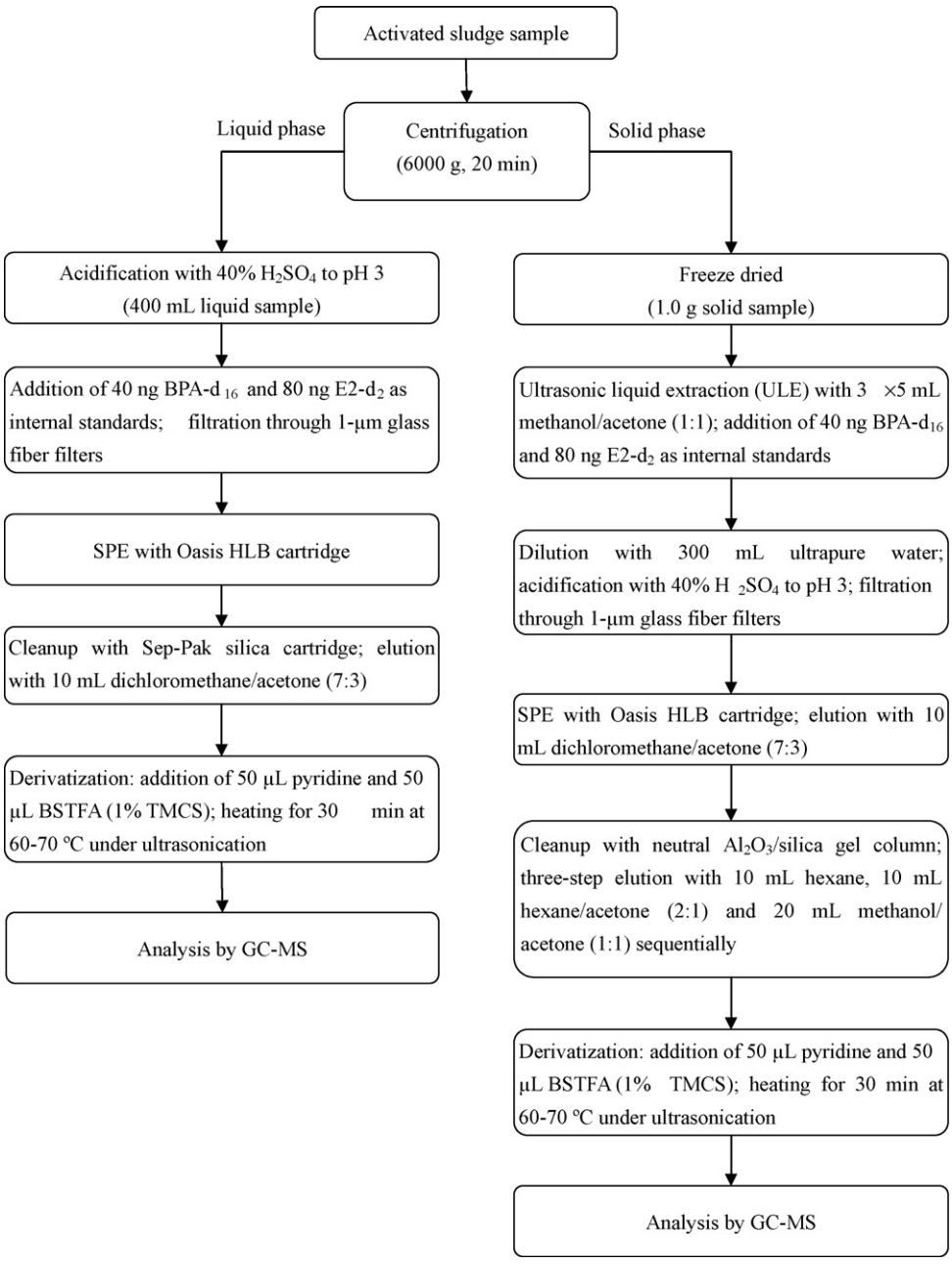

Fig. 2. Schematic diagram for analyzing target EDCs in the liquid and solid phases of activated sludge.

and other chemicals used were obtained from Beijing Chemical Reagents Company (Beijing, China) with at least analytical grade.

The stock solutions of individual EDCs were prepared by dissolving each compound in methanol at a concentration of 1000 mg/L. The mixed working solution containing E1, E2, E3 and EE2 was prepared at 1 mg/L by diluting the corresponding stock solutions with methanol. The working solutions of BPA and NP were prepared individually at a concentration of 1 and 10 mg/L, respectively. The working solutions of the internal standards (i.e., BPA-d_{16} and E2-d_2) were prepared individually in methanol at 1 mg/L each. All the stock and working solutions were stored at −18 °C prior to use.

2.2. Sample collection

Activated sludge samples were collected from the secondary sedimentation tanks of two STPs located in Beijing (STP-BJ, 500,000 m^3/d) and Shanghai (STP-SH, 50,000 m^3/d), respectively. STP-BJ employed a simple activated sludge process consisting of primary sedimentation, aeration tank and secondary sedimentation; while STP-SH employed an anoxic/oxic (A/O) process as biological treatment. To restrain the activity of microorganisms, 1% methanol (v/v) was immediately added to the sludge samples after sample collection. The samples were afterwards concentrated by natural sedimentation for volume reduction. The concentrated slurry was taken back to laboratory for experiments, while the supernatant was discarded on-site. The concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) of the concentrated slurry were analyzed according to the Standard Methods for the Examination of Water and Wastewater [27].

The treatment of activated sludge sample is illustrated in Fig. 2. The sample was first centrifuged at 6000 × *g* for 20 min by a centrifuge (Beckmann, J2-HS, USA) and divided into the liquid and solid phases. The pH of the liquid phase was immediately adjusted to 2.5–3.0 with 40% H_2SO_4 (v/v), while the solid phase was freeze-

dried. Thereafter, the prepared samples were stored at 4 °C in refrigerator and analyzed within 24 h.

2.3. Preparation of liquid phase sample

The liquid phase of activated sludge contains a high concentration of DOM which may severely interfere with the detection of trace-level EDCs. In this work, SPE was applied to extract the studied EDCs from sample and partially retain the interfering organic materials. In addition, a silica cartridge was used for further cleanup of the SPE eluate. The sample preparation procedures were modified on the basis of our previous work [28] as follows: (1) spike the internal standards of 40 ng of BPA-d_{16} (for phenolic compounds) and 80 ng of E2-d_2 (for steroids) into 400 mL of the liquid phase sample; (2) filter the sample through 1-μm glass fiber filters; (3) condition an Oasis HLB cartridge (500 mg/6 mL, Waters, Milliford, USA) with 5 mL of MTBE, 5 mL of methanol, and 5 mL of ultrapure water sequentially; (4) extract the sample with the HLB cartridge at a flow rate of 4–5 mL/min; (5) wash the cartridge with 5 mL of 10% methanol aqueous solution, 5 mL of ultrapure water, and 5 mL of 10% methanol aqueous solution containing 2% NH_4OH sequentially to partially remove the interfering organic materials; (6) dry the cartridge under vacuum for 40 min to remove moisture; (7) condition a Sep-Pak silica cartridge (500 mg/6 mL, Waters, Milliford, USA) with 5 mL of dichloromethane/acetone (7:3, v/v), and connect it to the bottom of the HLB cartridge; and (8) elute the studied EDCs into a conical-bottomed glass tube at a flow rate of 1–2 mL/min with 10 mL of dichloromethane/acetone (7:3, v/v). Thereafter, the eluate was dried under a gentle stream of N_2.

2.4. Preparation of solid phase sample

The freeze-dried solid phase sample (1.0 g) was placed into a 40-mL glass tube and treated with ULE for three times. For each ULE, 5 mL of methanol/acetone (1:1, v/v) was used and ultrasonication was continuously applied for 10 min. The slurry was centrifuged at 1600 × *g* for 8 min to collect the supernatant. The supernatants from the three ULEs were mixed together and the internal standards (i.e., 40 ng of BPA-d_{16} and 80 ng of E2-d_2) were spiked inside. The mixed supernatant was evaporated to 3–4 mL under a gentle stream of N_2, and diluted with 300 mL of ultrapure water. After the pH was adjusted to 2.5–3.0 with 40% H_2SO_4, the resulting solution was filtered through 1-μm glass fiber filters and further extracted by SPE with the Oasis HLB cartridge. The HLB cartridge was eluted with 10 mL of dichloromethane/acetone (7:3, v/v), and the eluate was dried to about 0.5 mL under a gentle stream of N_2.

Because the solid phase sample had a more complex matrix than the liquid phase sample, the silica gel cleanup process could not effectively remove the interfering matrix. A specially designed glass cleanup column was thus utilized to purify the extract post-SPE. The column cleanup procedures mainly consisted of three sequential elutions using hexane, hexane/acetone (2:1, v/v) and methanol/acetone (1:1, v/v), individually. These sequential eluents had an increasing polarity. Ideally, it was expected that the first-step and second-step elutions would remove non-polar and low-polarity interfering materials, respectively, and the third-step elution would completely elute the target EDCs.

The glass cleanup column was laboratory-made and had a length of 400 mm and an inner diameter (ID) of 10 mm. It was packed with four different layers from bottom to top: supporting glass wool (10 mm), silica gel (50 mm), neutral aluminum oxide (Al_2O_3, 50 mm), and sodium sulfate (Na_2SO_4, 15 mm). The glass wool was first inserted into the glass column to support the above layers and prevent solid particles from clogging the stopcock as well. A polytetrafluoroethylene (PTFE) stopcock was situated right below the glass wool to control the eluent. The silica gel and neutral Al_2O_3 were respectively heated at 130 and 400 °C for 16 h, cooled to room temperature, and deactivated with ultrapure water (3 mL water per 100 g silica gel/neutral Al_2O_3). The anhydrous Na_2SO_4 was heated at 400 °C for 4 h, cooled to room temperature, and placed on top of the neutral Al_2O_3 layer. To ease the packing, 15 mL of hexane was added into the cleanup column followed by the addition of silica gel and neutral Al_2O_3. The silica gel and neutral Al_2O_3 particles settled quite rapidly in hexane under tapping. The cleanup column was thereafter conditioned with 15 mL of methanol/acetone (1:1, v/v) and 5 mL of hexane prior to use. Based on our experimental results, the cleanup procedures were carried out as follows: (1) dilute the aforementioned 0.5-mL SPE extract with 1 mL of hexane, and transfer the resulting organic mixture onto the cleanup column with an additional 1 mL of hexane (for rinsing purpose); (2) just prior to exposure of the sodium sulfate layer to air, add 10 mL of hexane and 10 mL of hexane/acetone (2:1, v/v) sequentially to wash the cleanup column, and discard the eluates; and (3) elute the cleanup column with 20 mL of methanol/acetone (1:1, v/v) into a glass bottle to collect the target EDCs. The elution rate for all different steps was controlled at about 2 mL/min. It was observed that the SPE extract solution changed its color from brown to light yellow after column cleanup. The final eluate was evaporated to about 1 mL by a rotary evaporator at 10 kPa and 25 °C, and further dried with a gentle stream of N_2.

2.5. GC–MS analysis

The studied EDCs are polar organic compounds thus requiring derivatization prior to GC–MS analysis. To the dried residual originating from either the liquid or solid phase sample, 50 μL of pyridine (pre-dried with Zeolite 4A) and 50 μL of BSTFA (1% TMCS) were added. The mixed solution was heated in a 4-L ultrasonic-assisted water batch (KQ-100E, Kunshan, China) at 60–70 °C for 30 min. The ultrasonic had a power of 100 W and a frequency of 40 kHz. The solution was cooled to room temperature and dried under a gentle stream of N_2. The residual derivatives were dissolved in 100 μL of hexane for GC–MS analysis.

The GC–MS system (Agilent Technologies, USA) consisted of a gas chromatograph (Model 7890A), a quadrupole mass spectrometer (Model 5975C, VL MSD), an autosampler (Model 7683B), and an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). High purity helium gas (99.999%) was used as carrier gas at a constant flow rate of 1.5 mL/min. The GC oven temperature was programmed as follows: start from 100 °C and equilibrate for 1 min, ramp to 200 °C at 10 °C/min, ramp to 260 °C at 15 °C/min, further ramp to 300 °C at 3 °C/min and maintain for 2 min. The MS was operated in total ion chromatogram (TIC) mode for qualitative analysis from *m/z* 50 to 600 or selected ion monitoring (SIM) mode for quantitative analysis. The electron impact (EI) ionization was adopted. The temperatures for the inlet, MS transfer line, ion source, and quadrupole were set at 280, 280, 230 and 150 °C, respectively. For each analysis, 1 μL of sample was injected in the splitless mode. The quantification and confirmation ions for derivatived EDCs and internal standards are listed in Table 1.

2.6. Calibration curve and limit of quantification

For the liquid phase, a seven-point calibration curve was established. A desired amount of EDCs and a fixed amount of internal standards (i.e., 40 ng of BPA-d_{16} and 80 ng of E2-d_2) were spiked into 400 mL of ultrapure water at each concentration level. The prepared standard solutions were treated through the whole procedures for the liquid phase sample including acidification, filtration, addition of internal standards, SPE, cleanup and derivatization (the left part of Fig. 2), and the concentrations of EDCs were analyzed

Table 1
Quantification and confirmation ions and retention times for derivatized EDCs and internal standards.

Compounds	Molecular weight	Retention time (min)	Quantification ion(s)	Confirmation ion(s)
4-Nonylphenol (NP)	220	10.32–11.06	179, 193, 207, 221, 235, 292	–
Bisphenol A (BPA)	228	14.55	357	372
Bisphenol A-d_{16} (BPA-d_{16})	244	14.48	368	386
Estrone (E1)	270	17.66	342	257, 218
17β-Estradiol (E2)	272	18.09	416	285, 326
17β-Estradiol-d_2 (E2-d_2)	274	18.07	418	287
17α-Ethynylestradiol (EE2)	296	19.25	285	425, 232
Estriol (E3)	288	20.34	504	345, 311

by GC–MS.

For the solid phase, a five-point calibration curve was established. A desired amount of EDCs and a fixed amount of internal standards (i.e., 40 ng of BPA-d_{16} and 80 ng of E2-d_2) were spiked into 300 mL of ultrapure water at each concentration level. The prepared standard solutions were treated through the procedures post-ULE for the solid phase sample (the right part of Fig. 2), and the concentrations of EDCs were analyzed by GC–MS.

The calibration curve was established based on the peak area ratios of an individual EDC to its corresponding internal standard at various spiked concentration levels. According to the published literature [23,25], LOQ was defined as the analyte concentration corresponding to a signal to noise ratio of 10:1. The lowest point of the established calibration curve was slightly above the LOQ.

2.7. Determination of method recovery

The activated sludge collected from the secondary sedimentation tank of STP-BJ was separated into the liquid and solid phases by centrifugation (Fig. 2). To determine the method recovery for the liquid phase, the studied EDCs in methanol were spiked into 400 mL of the liquid phase sample at three different concentration levels. After the spiked sample was treated through the whole procedures for the liquid phase sample, the concentrations of EDCs were analyzed by GC–MS (Fig. 2). For each concentration level, triplicate samples were prepared and analyzed and the standard deviation was calculated. In addition, the inward concentrations of EDCs in the original liquid phase were also determined as sample blank. The method recovery was determined by subtracting the inward concentration from the total concentration of individual EDCs.

To determine the method recovery of the solid phase, the studied EDCs in methanol were spiked into 1.0 g of the freeze-dried solid phase sample at two concentration levels. The spiked sample was mixed vigorously by vortex for about 15 s to enable sufficient contact of EDCs with the solids. The sample was placed in a fume hood for 6 h to allow a complete evaporation of methanol. After the sample was treated through the whole procedures including ULE, addition of internal standards, dilution and filtration, SPE, cleanup, and derivatization, the concentrations of EDCs were analyzed by GC–MS (Fig. 2). Similarly, the inward concentrations of EDCs in the original solid phase were determined as sample blank and then subtracted from the total concentrations for determination of the method recovery. Besides, the recovery of the steps post-ULE was separately determined, through directly spiking the studied EDCs into the ULE extracts, to assess the contribution of ULE to the overall method recovery.

3. Results and discussion

3.1. GC–MS method

The SIM chromatograms of the derivatized EDCs and internal standards are shown in Fig. 3 for the standard solution and the liquid phase and solid phase of unspiked activated sludge from top

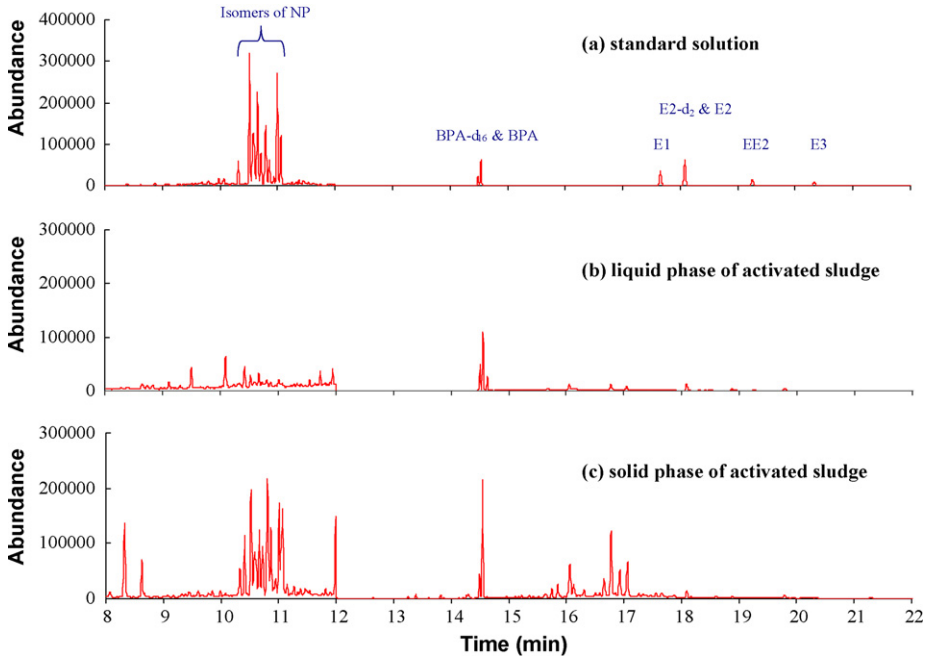

Fig. 3. SIM chromatograms of derivatized EDCs and internal standards in: (a) standard solution; (b) liquid phase of activated sludge; and (c) solid phase of activated sludge. The standard solution was directly analyzed under the conditions described in Section 2.5. The liquid and solid phase samples of activated sludge were analyzed according to the sample preparation procedures described in Fig. 2. The injected concentrations were 200 μg/L for BPA and BPA-d_{16}, and 800 μg/L for E1, E2, E2-d_2, EE2 and E3.

to bottom, respectively. Fig. 3(a) indicates that a good separation could be achieved for the studied EDCs under applied experimental conditions. The elution sequence of the EDC derivatives generally followed the order of their molecule weights (except BPA and E1). The cluster of nine NP isomers was first eluted from 10.32 to 11.06 min. BPA-d_{16} and BPA were closely eluted at 14.48 and 14.55 min, respectively, but a sufficient separation was observed. The estrogens were last eluted with a retention time ranging from 17.66 to 20.34 min. Although E2-d_2 (18.07 min) and E2 (18.09 min) were not separated, they could still be well quantified due to different quantification ions used. Furthermore, it is noted that the estrogens exhibited notably different response signals though they have similar chemical structures and a same concentration (i.e., 800 μg/L) was injected for each estrogen. The sensitivity of E1 and E2 was over two times that of EE2 and E3. As expected, the matrix effect increased from the liquid phase sample (Fig. 3(b)) to the solid phase sample (Fig. 3(c)). Overall, however, the matrix effect was relatively insignificant by comparing the chromatograms of the liquid and solid phase samples with that of standard solution. This demonstrates that our sample cleanup procedures were highly effective in removal of background interfering organic compounds. Results also show that most of the studied EDCs were present in the real activated sludge. The peaks of NP and BPA were distinguishable while those of E1, EE2 and E3 were undistinguishable depending on their specific concentrations present in the samples (Fig. 3(b) and (c)).

The molecular ions of studied estrogens, which were also the base peaks in the corresponding mass spectra, were selected as quantification ions except EE2 (Table 1). A base peak is the highest peak in a mass spectrum which is assigned a relative intensity value of 100. Using the base peak as the quantification ion can enhance both sensitivity and selectivity of the detection method because the interference from background organic materials is comparatively reduced. EE2 exhibited four main characteristic ions at *m*/*z* 440, 425, 285 and 232, among which the ion at *m*/*z* 425 was the base peak. According to Ternes et al. [9], however, sample matrix could produce interfering ions at *m*/*z* 425 and 440 within the same retention time. Therefore, the ion at *m*/*z* 285 was selected as quantification ion in this study. For BPA, the base-peak ion at *m*/*z* 357 was used for quantification to enhance the sensitivity.

With respect to NP, many past studies only used one or two quantification ion(s) to determine its concentration in environmental samples [23–24,29]. However, NP is frequently present in the activated sludge as a mixture of several technical isomers, particularly in the solid phase (Fig. 3). Its full-scan chromatogram clearly reveals that different NP isomers had considerably different characteristic ions and relative intensities. As a result, using one or two quantification ion(s) to determine the total concentration of NP isomers tends to reduce the detection accuracy. Hao et al. used five quantification ions to improve the detection of NP isomers in wastewater by GC–MS [30]. In this work, we selected six quantification ions from the mass spectra of derivatized NP isomers (i.e., *m*/*z* 179, 193, 207, 221, 235 and 292). Fig. 4 shows that the SIM chromatogram of NP is similar to its full-scan chromatogram, indicating that using six quantification ions can closely cover all the isomers. Theoretically, more quantification ions used in the SIM chromatogram result in a higher similarity to the full-scan chromatogram. However, an overuse of quantification ions may bring in more interfering matrix peaks, thus leading to a sacrifice of method selectivity.

3.2. Optimization of derivatization method

The trimethylsilyl derivatives of target compounds were reported to have characteristics of low thermal degradation and good solubility in common organic solvents [16]. Thereby, BSTFA containing 1% TMCS has been commonly used as a silylation reagent. The derivatization of studied EDCs followed the method developed by Zhang et al. [20] with minor modifications, which was detailed above. The estrogens contained both aliphatic and aromatic hydroxyl groups except E1 (Fig. 1). In general, aliphatic hydroxyl groups are more difficult to derivatize than aromatic ones [31]. Fig. 5(a) comparatively shows the effect of ultrasonication on the derivatization efficiency of studied EDCs. Results indicate that about 8% of E2 and E2-d_2, which were approximately calculated by the two peak areas ratio, were transformed to mono-*O*-TMS forms without the assistance of ultrasonication, and their aliphatic hydroxyl groups remained unsilylated. In contrast, all E2 and E2-d_2

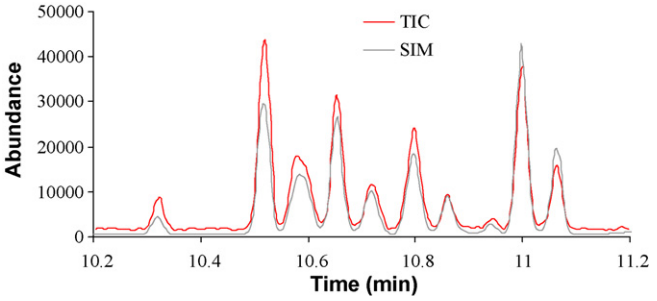

Fig. 4. Comparison of TIC and SIM chromatograms of the derivatized NP. The standard solution of NP was directly analyzed under the conditions described in Section 2.5. The injected concentration of NP was 2 mg/L.

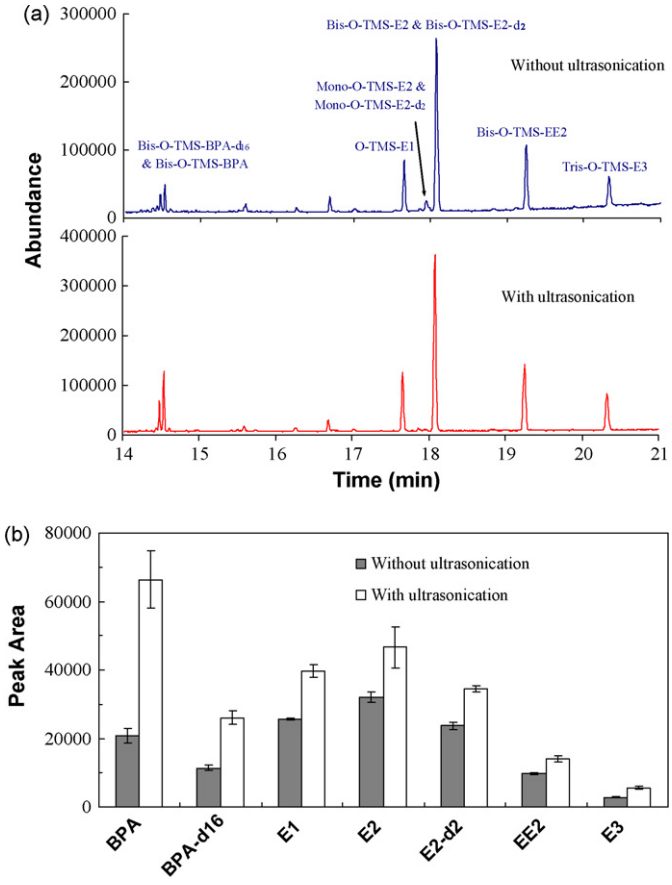

Fig. 5. Effect of ultrasonication on derivatization of studied EDCs and internal standards: (a) total ion chromatograms (TIC) of the derivatives; and (b) selected ion monitoring (SIM) peak areas of the derivatives ($n = 3$). The studied EDCs and internal standards were directly analyzed under the conditions described in Section 2.5. The injected concentrations were 200 μg/L for BPA and BPA-d_{16}, and 800 μg/L for E1, E2, E2-d_2, EE2 and E3.

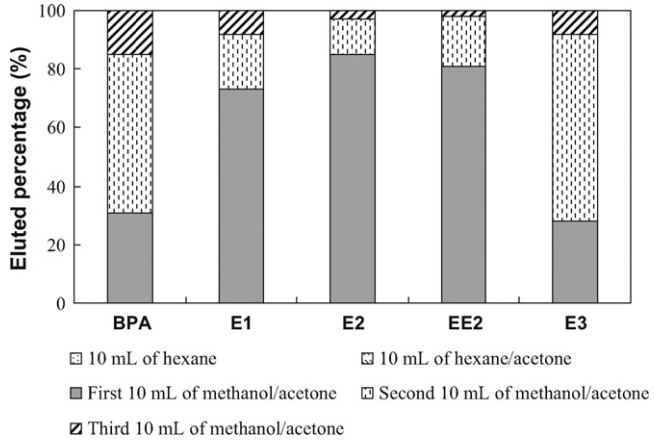

Fig. 6. Distribution of individual EDCs in sequential cleanup column eluates for solid phase sample preparation.

was transformed to bis-*O*-TMS forms with the assistance of ultrasonication, implying a complete silylation of both aliphatic and aromatic hydroxyl groups. Furthermore, Fig. 5(b) shows that ultrasonication could also significantly increase the peak area of the EDC derivatives by 44% (for EE2) to 219% (for BPA). It is seen that the derivatization of studied EDCs, if assisted with ultrasonication, could greatly improve the sensitivity of the detection method.

3.3. Optimization of elution steps in column cleanup for solid phase sample

Three sequential elutions using hexane (10 mL), hexane/acetone (2:1, v/v, 10 mL) and methanol/acetone (1:1, v/v, 30 mL) were applied to optimize the elution steps in column cleanup. Hexane and hexane/acetone were applied in batch mode in an attempt to effectively wash out non-polar and low-polarity interfering organic materials, while methanol/acetone was applied in an attempt to effectively elute the studied EDCs. To determine the optimal volume of methanol/acetone, it was divided into three aliquots and applied consecutively. It is noted that we had also tested two more aliquots of methanol/acetone (10 mL each), but the eluted EDCs were negligible compared to the first three aliquots. For simplicity purpose, the standard solution of studied EDCs prepared in hexane was directly used instead of the solution of SPE extracts. Experiments were conducted in triplicate.

Since the majority of studied EDCs could be eluted in the three sequential elutions, the total eluted amount of EDCs was approximated as 100%. The distribution pattern of individual EDCs in each elution step is illustrated in Fig. 6. Results indicate that only methanol/acetone could effectively elute the studied EDCs, while hexane and hexane/acetone could not elute any EDCs. For example, BPA was eluted by 31%, 54% and 15% using the three aliquots of methanol/acetone, respectively. It was found that most of BPA (54%) and E3 (64%) were eluted in the second aliquot, while most of E1 (73%), E2 (85%) and EE2 (81%) were eluted in the first aliquot. This can be correlated to their log K_{ow} values which are reported to be 2.81, 3.32, 3.43, 3.94 and 4.15 for E3, BPA, E1, E2 and EE2, respectively [8,32]. It is seen that in general the EDC with a higher log K_{ow} value (i.e., more hydrophobic) was more quickly eluted by methanol/acetone from the cleanup column because this column is similar to the normal phase chromatography. Since 85% of BPA and more than 90% of E1, E2, E3 and EE2 were eluted in the first two aliquots, 20 mL of methanol/acetone was selected as the optimal volume for the purpose of saving the eluent usage and the subsequent evaporation time meanwhile achieving a sufficient recovery for studied EDCs.

It should be pointed out that we did not particularly investigate the distribution pattern of NP in the three sequential elutions. NP has a chemical structure comparable to BPA (alkylphenols) but a much higher log K_{ow} value of 4.48 [5]. Thus, it is reasonably deduced that NP will be eluted by methanol/acetone more quickly than BPA. In addition, the elution steps in column cleanup optimized by the standard solution of EDCs could be well applied to the solution of SPE extracts, as demonstrated later by the recovery data of ECDs.

3.4. Recoveries

3.4.1. Recovery of the liquid phase sample

The recovery efficiency of studied EDCs was first examined in the liquid phase sample at three spiked concentration levels with or without the internal standards (i.e., BPA-d_{16} and E2-d_2), as shown in Table 2. Results indicate that in the presence of the internal standards, most of the recovery efficiencies were in the U.S. EPA recommended range of 70–120% (i.e., 87.2–117.8%). Only the recovery efficiencies of E3 (128.7%) and EE2 (129.1%) at the middle level slightly exceeded the recommended range, but these data were quite stable as reflected by their standard deviations (SD) of no more than 8.5%. The inward concentrations of individual EDCs had been corrected when calculating the recovery data. For the purpose of comparison, the recovery efficiency of studied EDCs was also examined in the absence of the internal standards. Table 2 shows that except BPA and E3, all the other EDCs had recovery efficiencies within the recommended range of 70–120% despite of their spiked concentration levels. The recovery efficiencies of BPA were 120.6% and 126.4% at the low and middle concentration levels, respectively, with SD values of no more than 9.2%. The recovery of BPA was acceptable because of its good stability, though slightly higher than 120%. The recovery efficiency of E3 remarkably decreased to 37.6–50.2% in the absence of E2-d_2, however. Gomes et al. [26] also reported a low recovery efficiency (57–58%) of E3 when analyzing estrogens in the river sediment samples. E3 has the highest polarity among the studied estrogens as reflected by its smallest log K_{ow} value (i.e., 2.81), which is attributed to three hydroxyl groups in its chemical structure. As a consequence, E3 is comparatively easier to bond to sample matrix and thus more difficult to recover. The

Table 2
Recovery of target EDCs in the liquid phase of activated sludge at three concentration levels (mean ± SD (%), $n = 3$).

Compounds	Low level		Middle level		High level	
	IS	No IS	IS	No IS	IS	No IS
NP	88.4 ± 2.6	105.7 ± 6.9	109.3 ± 3.0	99.3 ± 4.0	87.2 ± 0.4	110.1 ± 1.1
BPA	103.3 ± 8.8	120.6 ± 9.2	87.9 ± 0.3	126.4 ± 1.5	96.0 ± 2.2	105.3 ± 3.3
E1	94.8 ± 0.8	94.4 ± 1.8	111.5 ± 4.5	104.6 ± 0.2	90.3 ± 3.8	80.1 ± 10.1
E2	96.7 ± 2.3	95.5 ± 3.0	112.4 ± 2.2	104.7 ± 2.3	96.3 ± 1.1	83.7 ± 9.5
EE2	117.8 ± 3.5	113.3 ± 1.1	129.1 ± 7.7	117.3 ± 1.7	110.9 ± 1.6	94.1 ± 10.5
E3	114.9 ± 2.7	50.2 ± 1.3	128.7 ± 8.5	50.1 ± 1.0	103.3 ± 9.2	37.6 ± 6.4

Low level: 0.5 μg/L NP, 20 ng/L BPA, 40 ng/L each of E1, E2, EE2 and E3. Middle level: 2.0 μg/L NP, 40 ng/L BPA, 80 ng/L each of E1, E2, EE2 and E3. High level: 4.0 μg/L NP, 80 ng/L BPA, 140 ng/L each of E1, E2, EE2 and E3.

Table 3
Recovery for target EDCs spiked in the solid phase of activated sludge at two concentration levels (mean ± SD (%), $n = 3$).

Spiked level	NP	BPA	E1	E2	EE2	E3
Low						
$RE_{overall}$ [a]	104.9 ± 11.6	83.2 ± 6.4	80.4 ± 11.1	71.3 ± 0.9	91.0 ± 4.6	119.1 ± 9.0
$RE_{post\text{-}ULE}$ [b]	96.9 ± 3.2	125.7 ± 10.4	120.8 ± 13.3	104.2 ± 3.1	128.7 ± 8.6	109.5 ± 3.7
High						
$RE_{overall}$	107.1 ± 0.7	77.3 ± 1.4	72.8 ± 5.7	72.6 ± 0.1	106.0 ± 16.7	123.5 ± 0.9
$RE_{post\text{-}ULE}$	104.7 ± 2.4	101.8 ± 2.4	98.6 ± 4.8	100.3 ± 0.4	81.2 ± 5.5	81.9 ± 6.2

Low: 5 μg/g SS NP, 40 ng/g SS BPA, 40 ng/g SS each of E1, E2, EE2 and E3. High: 10 μg/g SS NP, 80 ng/g SS BPA, 80 ng/g SS each of E1, E2, EE2 and E3.
[a] Recovery efficiency of the overall method.
[b] Recovery efficiency of the steps post-ultrasonic liquid extraction.

two internal standards were added simultaneously with the studied EDCs, so they could compensate for the partial losses of target analytes in both extraction and cleanup procedures to minimize the matrix effect. It is seen from the above results that the use of E2-d_2 is necessary for significant improvement of the recovery efficiency of E3, while the use of BPA-d_{16} is not necessary for determination of BPA and NP in the liquid phase of activated sludge. However, considering that the solid phase of activated sludge has a more complex matrix, both internal standards were applied simultaneously in later experiments.

3.4.2. Recovery of the solid phase sample

The recovery efficiency of studied EDCs was further examined in the solid phase sample at two spiked concentration levels in the presence of internal standards, as shown in Table 3. Results indicate that the recovery efficiencies of the overall method ranged from 71.3% to 123.5% in the solid phase sample, showing a relatively greater fluctuation than those in the liquid phase sample (i.e., 86.6–129.1%). The solid phase method consisted of more extraction and cleanup procedures due to its complex matrix, which tended to impact the recovery efficiencies of studied EDCs more significantly. In particular, the recovery efficiencies of BPA, E1 and E2 in the solid phase sample were decreased to some extent in comparison to those in the liquid phase sample.

ULE was an important step in the solid phase method. Ternes et al. [25] ever reported that the recovery efficiency of the ULE step for EE2 in activated sludge was about 88% by using the ^{14}C-labled EE2. In this study, the recovery efficiencies of the overall method and the steps post-ULE were simultaneously determined (Table 3). Therefore, the recovery efficiencies of the ULE step for all studied EDCs could be readily calculated with the following equation:

$$RE_{ULE} = \frac{RE_{overall}}{RE_{post\_ULE}} \quad (1)$$

where RE_{ULE}, $RE_{overall}$ and RE_{post_ULE} represent the recovery efficiencies of the ULE step, the overall method and the steps post the ULE, respectively. The mean values of RE_{ULE} for BPA, E1 and E2 were determined to be 71.1, 70.2 and 70.4%, respectively, indicating an approximate 30% loss. This result reveals that the ULE step employed in the solid phase method mainly accounted for the decreased recovery efficiencies of the three compounds compared to those in the liquid phase sample as above mentioned. In contrast, the ULE step apparently exerted less impact on NP, E3 and EE2 whose mean values of RE_{ULE} were 105.3, 129.8 and 100.6%, respectively.

3.5. Method validation

The calibration curves of studied EDCs in the presence of internal standards were respectively established for the liquid phase and solid phase of activated sludge. The linear regression data are summarized in Table 4, of which the coefficients of determination (r^2) were all above 0.9950, indicating an excellent linearity of the calibration curves.

Because a part of studied EDCs usually had relatively high inward concentrations in the reference matrix, it was difficult to directly determine their LOQs. Alternatively, the LOQ of each selected EDC in ultrapure water was first determined at a spiked concentration level that could provide a signal to noise ratio of 10:1, and then the LOQ in the reference matrix was calculated on the basis of the LOQ in ultrapure water and the corresponding recovery efficiency [31]. Table 4 shows that for the steroid estrogens and BPA, the LOQs varied from 0.2 to 4.0 ng/L in the liquid phase and from 1.2 to 10.0 ng/g SS in the solid phase. In contrast, the LOQ of NP was 30.3 ng/L and 188.7 ng/g SS in the liquid and solid phases, respectively, which was one or two orders of magnitude higher than those of the steroid estrogens and BPA. To ensure the quantification accuracy for all isomers of NP, the smallest peak among the cluster of NP peaks was purposely selected to determine the signal/noise ratio and calculate the LOQ, which partially accounted for the high LOQ value for NP. Nevertheless, the LOQ for NP was still applicable because NP is usually present at a much higher concentration than other selected EDCs in the activated sludge of STPs.

The precision and accuracy of the developed method could be assessed by the recovery data shown in Tables 2 and 3. Results indicate that the majority of recovery efficiencies were within the range of 70–120% and all relative standard deviation (RSD) values were below 16% (as compared to the U.S. EPA recommended RSD value of no more than 20%). Therefore, both precision and accuracy are ensured for the developed method.

Table 4
Linear regression parameters of the calibration curves and LOQs of target EDCs for the developed method.

Compounds	Liquid phase					Solid phase				
	Linear range (ng/L)	Slope	y-Intercept	r^2	LOQ (ng/L)	Linear range (ng/g SS)	Slope	y-Intercept	r^2	LOQ (ng/g SS)
NP	100–10,000	0.0099	2.1199	0.9969	30.3	1000–20,000	0.0168	7.4723	0.9955	188.7
BPA	2.5–500	0.0114	0.0364	0.9995	0.2	4–150	0.0431	0.3213	0.9999	1.3
E1	2.5–500	0.0043	0.0299	0.9954	1.2	4–100	0.0198	0.0300	0.9967	1.5
E2	2.5–500	0.0040	0.0413	0.9960	0.8	4–100	0.0160	0.0281	0.9954	1.2
EE2	5–750	0.0013	0.0113	0.9997	4.0	10–100	0.0028	0.0012	0.9953	10.0
E3	5–750	0.0005	0.0078	0.9982	2.3	10–100	0.0033	−0.0011	0.9994	7.1

Table 5
Concentrations of target EDCs in the liquid and solid phases of activated sludge collected from two STPs.

STPs	EDCs (mean ± SD, n = 3)					
	NP	BPA	E1	E2	EE2	E3
STP-BJ						
Liquid phase (ng/L)	199.8 ± 23.2	135.3 ± 10.9	N.D.	N.D.	6.3 ± 1.1	17.6 ± 1.7
Solid phase (ng/g SS)	2308.7 ± 27.5	127.3 ± 13.7	11.2 ± 0.5	N.D.	N.D.	10.5 ± 1.1
STP-SH						
Liquid phase (ng/L)	229.6 ± 40.1	134.0 ± 23.3	4.0 ± 0.7	N.D.	6.7 ± 1.1	26.0 ± 2.1
Solid phase (ng/g SS)	2323.6 ± 222.1	101.3 ± 6.4	22.1 ± 4.1	N.D.	N.D.	9.7 ± 0.5

N.D.: not detected.

3.6. Method application

The developed method was applied to determine the concentrations of studied EDCs in the liquid and solid phases of activated sludge samples collected from the secondary sedimentation tanks of STP-BJ and STP-SH in June 2008. The TSS and VSS concentrations were 12,081 and 7193 mg/L for the STP-SH sludge and 14,270 and 8227 mg/L for the STP-SH sludge, respectively. Triplicate analyses were performed for each sample. The mean concentration of individual EDCs was calculated together with its standard deviation, as summarized in Table 5. Results indicate that the steroid estrogens were present with a low concentration which ranged from <LOQ to 26.0 ng/L in the liquid phase and from <LOQ to 22.1 ng/g SS in the solid phase of activated sludge, respectively. In comparison, BPA and particularly NP were detected with a much higher concentration. In the liquid phase, about 135 ng/L BPA and 200–230 ng/L NP were detected; while in the solid phase, about 101–127 ng/g SS BPA and 2309–2324 ng/g SS NP were detected. The concentration levels of studied EDCs in the activated sludge from STP-BJ and STP-SH were very similar probably due to the similar concentrations of TSS and VSS.

To date, no data about the concentration levels of steroid estrogens in the activated sludge of STPs have been reported in China and limited data are available worldwide. Andersen et al. [33] investigated the fate of steroid estrogens in a STP whose treatment processes mainly included denitrification, nitrification and chemical phosphate precipitation. They reported that the concentrations of E1, E2 and E3 in the solid phase of activated sludge ranged from 3 to 7 ng/g SS, which is quite comparable to our results. In this study, all the selected estrogens except E2 were detected. Some laboratory-scale experiments on the degradation of externally spiked estrogens in activated sludge showed that E2 could be quickly degraded with a removal efficiency up to 90% within 30 min [34,35]. Therefore, the concentration of E2 in the activated sludge of STPs is usually low or even undetectable. It was reported that the biodegradation of E2 could produce E1 and E3 [19]. The two metabolites, E1 and E3, were more resistant to biodegradation than E2, thus being frequently detected in STPs. EE2 was detected in the liquid phase of activated sludge with a low concentration, but not detected in the solid phase. It is probably due to the highest LOQ value of EE2 in the solid phase among the steroid estrogens (i.e., 10 ng/g SS, Table 4). Qiao et al. [36] analyzed the concentrations of NP and BPA in the solid phase of activated sludge collected from three STPs in Beijing. They reported that NP was detected with a concentration ranging from 0.62 to 12.42 μg/g SS, but BPA was not detected at all. It seems that their sample treatment procedure, which only utilized SPE, was not effective in removal of matrix interference, thus sacrificing the sensitivity for BPA detection.

Due to the lipophilic nature of studied EDCs, these compounds tend to adsorb on the solid phase of activated sludge. The adsorbability of studied EDCs appears to correlate with their $\log K_{ow}$ values to some extent. For example, the partitioning coefficients of NP, BPA and E3 between the liquid and solid phases, which were all detected in two activated sludge samples (Table 5), increased as their $\log K_{ow}$ values decreased. Among the selected EDCs, NP was most lipophilic, thus having the highest concentration in the solid phase. It is seen that the activated sludge in STPs may adsorb a significant amount of EDCs which have relatively high $\log K_{ow}$ values, thus appropriate treatment is required for waste sludge prior to its discharge into the environment.

4. Conclusions

This work developed a selective, sensitive and robust analytical method for simultaneous determination of six EDCs including E1, E2, E3, EE2, BPA and NP in the liquid and solid phases of activated sludge from STPs. Relatively simple and highly efficient procedures were established for sample preparation. For the liquid phase sample, an Oasis HLB and a Sep-Pak silica cartridge were used for sample extraction and matrix cleanup. For the solid phase sample, ULE, SPE and matrix cleanup with a laboratory-made glass column that was packed with sodium sulfate, neutral aluminum oxide, silica gel, and glass wool at different layers, were used for sample preparation. Results indicate that a three-step sequential elution procedure, which used 10 mL hexane, 10 mL hexane/acetone (2:1, v/v), and 20 mL methanol/acetone (1:1, v/v) could efficiently remove matrix interference meanwhile adequately recovering the studied EDCs in the solid phase sample. The refined extracts were derivatized with BSTFA (1% TMCS) with the assistance of ultrasonication and analyzed by GC–MS. The application of ultrasonic to derivatization, the adoption of six quantification ions for NP quantification, and the use of deuterated internal standards (i.e., E2-d_2 and BPA-d_{16}) could significantly increase the recovery and sensitivity of the developed method. In the presence of internal standards, the majority of recovery data were in the U.S. EPA recommended range of 70–120% for all selected EDCs in the solid and liquid phases of activated sludge. Only a few of recovery data were above 120%, but all below 130% and with low standard deviations.

This method was successfully applied to determine the concentration levels of target EDCs in the activated sludge collected from two STPs in Beijing and Shanghai, respectively. Results indicate that except E2, all the other five EDCs were detected. The concentrations of phenolic compounds (i.e., BPA and NP) were much higher than those of steroid estrogens in both liquid and solid phases of activated sludge.

This work has thus raised a severe concern regarding the disposal of waste sludge which may accumulate a significant amount of EDCs. In addition, because the activated sludge has a highly complex matrix, the sample preparation procedures for the liquid and solid phases may be readily extended to other similar matrices, such as animal feeding wastewater and manure, and digested anaerobic sludge with minor modifications.

Acknowledgements

The authors greatly appreciate the financial support from the National Natural Science Foundation of China for Young Scholars (Project #20707032) and for Creative Research Groups (Project #50621804) as well as the funds from the Ministry of Science and Technology of China to support the State Key Laboratory of Environmental Aquatic Chemistry (Project #S133). The valuable comments from Dr. Yiqi Zhou on EDCs analysis are acknowledged.

References

[1] O. Tsutsumi, J. Steroid Biochem. 93 (2005) 325.
[2] L.J. Mills, C. Chichester, Sci. Total Environ. 343 (2005) 1.
[3] J.E. Harries, D.A. Sheahan, S. Jobling, P. Matthiessen, P. Neall, J.P. Sumpter, T. Tylor, N. Zaman, Environ. Toxicol. Chem. 16 (1997) 534.
[4] C. Purdom, P. Hardiman, V. Bye, N. Eno, C. Tyler, J. Sumpter, Chem. Ecol. 8 (1994) 275.
[5] G. Ying, B. Williams, R. Kookana, Environ. Int. 28 (2002) 215.
[6] A.C. Johnson, J. Sumpter, Environ. Sci. Technol. 35 (2001) 4697.
[7] G.D. Ascenzo, A.D. Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, R. Samperi, Sci. Total Environ. 302 (2003) 199.
[8] G. Ying, R.S. Kookana, Y. Ru, Environ. Int. 28 (2002) 545.
[9] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.-D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81.
[10] R.L. Gomes, M.D. Scrimshaw, J.N. Lester, Trac-Trend Anal. Chem. 22 (2003) 697.
[11] A.K. Sarmah, G.L. Northcott, F.D.L. Leusch, L.A. Tremblay, Sci. Total Environ. 355 (2006) 135.
[12] M. Auriol, Y. Filali-Meknassi, R.D. Tyagi, C.D. Adams, R.Y. Surampalli, Process Biochem. 41 (2006) 525.
[13] R. Bogers, S.D. Vries-Buitenweg, I. Geuijen, B.V.D. Waart, R. Kuiper, S.V.D. Linden, L. Puijker, A. Murk, B.V.D. Burg, J. Legler, Environ. Int. 33 (2007) 292.
[14] J. Nelson, F. Bishay, A.V. Roodselaar, M. Ikonomou, F.C.P. Law, Sci. Total Environ. 374 (2007) 80.
[15] T. Schultis, J.W. Metzger, Chemosphere 57 (2004) 1649.
[16] M.D. Hernando, M. Mezcua, M.J. Gomez, O. Malato, A. Aguera, A.R. Fernandez-Alba, J. Chromatogr. A 1047 (2004) 129.
[17] P. Labadie, E.M. Hill, J. Chromatogr. A 1141 (2007) 174.
[18] A. Laganà, A. Bacaloni, I.D. Leva, A. Faberi, G. Fago, A. Marino, Anal. Chim. Acta 501 (2004) 79.
[19] W. Ding, C. Chiang, Rapid Commun. Mass Spectrom. 17 (2003) 56.
[20] Z.L. Zhang, A. Hibberd, J.L. Zhou, Anal. Chim. Acta 577 (2006) 52.
[21] T. Reemtsma, J. Chromatogr. A 1000 (2003) 477.
[22] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[23] R. Liu, J.L. Zhou, A. Wilding, J. Chromatogr. A 1022 (2004) 179.
[24] G. Gatidou, N.S. Thomaidis, A.S. Stasinakis, T.D. Lekkas, J. Chromatogr. A 1138 (2007) 32.
[25] T.A. Ternes, H. Andersen, D. Gilberg, M. Bonerz, Anal. Chem. 74 (2002) 3498.
[26] R.L. Gomes, E. Avcioglu, M.D. Scrimshaw, J.N. Lester, Trac-Trend Anal. Chem. 23 (2004) 737.
[27] American Public Health Association (APHA), Standard Methods for the Examination of Water & Wastewater, the 21st Edition, American Public Health Association, Washington, DC, USA, 2005.
[28] H. Zhang, H. Yamada, H. Tsuno, Environ. Sci. Technol. 42 (2008) 3375.
[29] D. Li, J. Park, J.R. Oh, Anal. Chem. 73 (2001) 3089.
[30] R. Hao, P. Liang, M. Zhao, J. Wang, Y. Zhou, Environ. Sci. 27 (2006) 2222 (in Chinese).
[31] J.B. Quintana, J. Carpinteiro, I. Rodriguez, R.A. Lorenzo, A.M. Carro, R. Cela, J. Chromatogr. A 1024 (2004) 177.
[32] G. Ying, R.S. Kookana, P. Dillon, Water Res. 37 (2003) 3785.
[33] H. Andersen, H. Siegrist, B. Halling-Sorensen, T.A. Ternes, Environ. Sci. Technol. 37 (2003) 4021.
[34] F. Li, R. Desmiarti, A. Yuasa, A. Horio, Bioresour. Technol. 99 (2008) 2964.
[35] T. Hashimoto, T. Murakami, Water Res. 43 (2009) 573.
[36] Y. Qiao, J. Zhang, Y. Zhang, M. Chen, M. Yang, D. Xu, Environ. Chem. 26 (2007) 671 (in Chinese).