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ORIGINAL

Analysis of Six Phenolic Endocrine Disrupting Chemicals in Surface Water and Sediment

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Abstract An efficient and reliable method based on gas chromatography–mass spectrometry (GC–MS) was developed for the extraction and analysis of six phenolic endocrine disrupting chemicals (EDCs), such as 4-nonylphenol (4-NP), nonylphenol-mono-ethoxylate (NP1EO), nonylphenol-di-ethoxylate (NP2EO), 4-*tert*-octylphenol (4-*t*-OP), bisphenol A (BPA) and 4-cumylphenol (4-CP) in surface water and sediment. The method was developed by using microwave-assisted extraction (MAE), solid phase extraction (SPE) and derivatization procedure. The MAE procedures were performed by optimizing three key process factors, consisted of extraction solvent, extraction temperature and holding time, affecting the extraction efficiency from sediment samples. For SPE, various parameters that may affect the recovery efficiency of water samples, such as SPE phase cartridge, elution solvent, as well as pH of water samples, were investigated. A series of derivatization conditions, such as derivatization reagent, reaction temperature and reaction time, were improved. The method achieved good repeatability and reproducibility with relative standard deviations <13% for all target EDCs in the both samples. Satisfactory recoveries for spiked water and sediment samples ranged from 85 to 101% and 74 to 105%, respectively. The limits of quantification varied from 0.20 (4-*t*-OP) to 11.50 ng L^{-1} (NP2EO) and from 0.31 (4-*t*-OP) to 9.50 ng g^{-1} dry weight (dw) (NP2EO) for water samples and sediment samples, respectively. The established method was successfully applied to the analysis of target EDCs in surface water and sediment samples collected from Caohai site of Dianchi Lake, China. The results showed that NP1EO, NP2EO and BPA were the three dominant phenolic EDCs in the site, reaching 114, 97 and 149 ng L^{-1} in surface water, while 444, 186 and 178 ng g^{-1} dw in surface sediment, respectively.

Keywords Gas chromatography-mass spectrometry · Solid phase extraction · Microwave-assisted extraction · Phenolic endocrine disrupting chemicals · Surface water · Surface sediment

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Introduction

Endocrine disrupting chemicals (EDCs) as exogenous substances can block or mimic the normal function of hormones, including their synthesis, secretion, transportation, integration and metabolism, leading to loss of hormonal balance [1–3]. Recently, the occurrence and fate of phenolic EDCs in matrixes of surface water [4–7], sediment [3, 4, 8], atmosphere [9], vegetables [10] and fish [11] have generated considerable concern due to their potential adverse effects on humans and other organisms. 4-nonylphenol (4-NP), nonylphenol-mono-ethoxylate (NP1EO), nonylphenol-di-ethoxylate (NP2EO), 4-*tert*-octylphenol (4-*t*-OP), bisphenol A (BPA) and 4-cumylphenol (4-CP) are the major phenolic EDCs contributors because of their widespread application in industrial, agricultural and household applications. 4-NP, NP1EO, NP2EO and 4-*t*-OP are more toxic, lipophilic, estrogenic and persistent than the long chain ethoxylates [12, 13]. The estrogenicity of BPA is about 500 times stronger than 4-*t*-OP [14]. 4-CP lacks one of two phenol-hydroxyl groups of BPA, but it has been noted that 4-CP has an intense estrogenic activity [15, 16].

As is well known, most of the methods have focused on the development of analytical methods for phenolic EDCs in water samples [17–19], but limited studies are devoted to the development of sediment samples due to their complex physiochemical processes and low LODs [1, 20]. So far, none of published studies reported the simultaneous analysis of 4-NP, NP1EO, NP2EO, 4-*t*-OP, BPA and 4-CP in surface water and sediment samples with a simple, fast, cheap and precise integrated analytical method.

Although liquid–liquid extraction (LLE) [3, 4] and solid-phase microextraction (SPME) [21] have also been reported for water samples, the most common pre-treatment method was solid-phase extraction (SPE) [17–19]. Sediment acts as a potential secondary source of phenolic EDCs in water systems. Therefore, EDC adsorption process on sediment can be significant in terms of the ecotoxicological impacts [2]. There were several extraction methods for phenolic EDCs from sediment samples such as sonication [18], pressurized liquid extraction (PLE) [22], soxhlet extraction (SE) [4], accelerated solvent extraction (ASE) [23] and microwave-assisted extraction (MAE). Recently, MAE has been widely used for the extraction of organic pollutants from sediments [2, 17, 20]. The advantages of this technique include small volumes of solvents, considerable saving in processing time and multiple sample extractions [1, 24].

Gas chromatography–mass spectrometry (GC–MS), gas chromatography–tandem mass spectrometry (GC–MS/MS), liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been shown to be efficient for the analysis of phenolic compounds [25–31]. GC–MS with lower cost can be very satisfactory for the analysis in derivatized forms, while LC-MS with the higher cost enables the analysis without derivatization. Thus, derivatization technology is critical for the determination of phenolic EDCs using GC–MS. The purpose of derivatization is to convert the target compounds into other products with greater volatility, thermal stability, superior chromatographic properties and ideally increased response [19].

The objective of this work was to develop and validate an efficient and reliable method based on MAE-SPE-derivatization-GC-MS for the analysis of six phenolic EDCs such as 4-NP, NP1EO, NP2EO, 4-*t*-OP, BPA and 4-CP in surface water and sediment. Various technical conditions were investigated in order to achieve the highest recoveries for the analysis of phenolic EDCs in water and sediment. In the paper, three key parameters of MAE procedure, including extraction solvent, extraction temperature and holding time, were carefully studied and developed. The important parameters of SPE, such as SPE cartridge, elution solvent, as well as pH of water samples, were also investigated. A series of derivatization conditions, such as derivatization reagent, reaction temperature and reaction time, were improved. And finally, the developed analytical method was applied to the analysis of phenolic EDCs in surface water and sediment samples collected from the Caohai site of Dianchi Lake, China.

Experimental

Chemicals and Standards

All the organic solvent, including dichloromethane (DCM), ethyl acetate (EA), acetone, methanol (MeOH) and hexane, were HPLC grade (Merck, Darmstadt, Germany).

Pyridine, copper granules, surrogate standard (bisphenol A-d_{16}, 98%), internal standard (5α-androstane), derivatization reagents (*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and phenyltrimethylammonium hydroxide (PTA-OH)) and phenolic EDCs consisted of 4-nonylphenol (4-NP, 99%), 4-*tert*-octylphenol (4-*t*-OP, 97%), 4-cumylphenol (4-CP, 99%) and bisphenol A (BPA, ≥99%), were supplied by Sigma-Aldrich (St. Louis MO, USA). Nonylphenol-mono-ethoxylate (NP1EO, 99%) and nonylphenol-di-ethoxylate (NP2EO, 99%) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). SPE cartridges of Sep-Pak C-18 (6 mL, 500 mg) and Oasis® HLB (6 mL, 500 mg) cartridges were obtained from Waters (Milford, MA, USA), whereas LC-18 cartridges (3 mL, 500 mg) were purchased from Supelco (Bellefonte, PA, USA).

Stock solution of 5α-androstane was prepared in hexane at 1,000 mg L^{-1}. All stock solutions (1,000 mg L^{-1}) were prepared in methanol and stored in a freezer at −20 °C. HPLC grade water was prepared in the laboratory using a Milli-Q ultrapure system (Millipore, Molsheim, France).

Sample Collection and Preservation

All glassware was cleaned by SC 1160 automatic bottle-washer (SalvisLab, Switzerland), and then pyrolyzed at 450 °C for 4 h prior to use. Glass microfiber filters (GF/F, 0.45 μm nominal, Millipore, MA, USA) were also pyrolyzed at 450 °C for 4 h prior to use.

Milli-Q grade water and sediment spiked with 200 ng L^{-1} and 25 ng g^{-1} dw of phenols, respectively, were used for the method development and validation experiments. Concentration of injected standard solution was 1 ng μL^{-1}. Sediment samples for spiking procedures were collected from the Aquaculture Base of Dianchi Lake as a representative for low polluted matrix. Surface sediment samples were collected from the superficial 10 cm of sediment using a stainless-steel Van Veen grab, bottled and then stored at about −45 °C until analysis. Sediment samples were freeze-dried (Eyela FDU-1200, Japan) for 4 days, and

then homogenized with a stainless steel spoon, placed into pre-cleaned brown glass bottles, and finally stored at 4 °C on board.

Environmental samples collected from Caohai site of Dianchi Lake were analyzed in order to validate the developed method. Water sample was collected in 4 L pre-cleaned amber glass bottles. Methanol (1:99, *v/v*) was immediately added to water samples, and then the bottle was capped and agitated by hand, in order to inhibit biological activity. Water samples were transported in boxes packed with ice and stored at 4 °C in a refrigerator upon arrival at the laboratory. Water samples were subsequently filtered through a pre-combusted GF/F filter to remove particle contaminants and analyzed within 24 hours. Surface sediment samples were collected and pre-treated as well as the sediment samples collected from the Shizao Aquaculture Base.

Microwave-Assisted Extraction

Microwave-assisted extraction experiments were performed by ETHOS 1 advanced microwave extraction system (Milestone, Italy) equipped with a 12-sample tray and temperature controlling system. A portion of the collected sediment samples was analyzed prior to being spiked, to determine possible background concentrations of target compounds in the matrix. For the method development and validation experiments, 4.0 g dried samples were transferred into Teflon lined extraction vessels and spiked with 100 ng of phenolic EDCs. 100 ng of BPA-d_{16} were added in order to correct the possible losses in the procedure. The samples were carefully mixed with a spatula and then left for 48 h to allow sorption processes to occur, as in nature. Copper granules were treated with 30% HNO_3 for ca. 30 s, and then cleaned with Milli-Q grade water, acetone and hexane, and finally dried with a stream of nitrogen [32]. The activated copper granules were added to remove sulfur in the samples. When the irradiation period was completed, the vessels were removed from the microwave cavity and cooled to room temperature before opening. The extracts were carefully filtered through absorbent cotton into the flat-bottomed flasks (250 mL). The sediment samples were washed with 15 mL of the same solvent or solvent mixture for three times. The extracts were concentrated to near dryness leaving a small amount of residue by a rotary evaporator (Buchi Rotavapor RII, Switzerland), and then diluted to a final volume of 250 mL using Milli-Q grade water, subjected to SPE clean-up procedure.

Solid Phase Extraction

100 ng of 4-NP, NP1EO, NP2EO, 4-*t*-OP, BPA and 4-CP was spiked in 500 mL of Milli-Q grade water for recovery tests. The pH value of water sample was adjusted using hydrochloric acid or sodium hydroxide. Three different SPE cartridges (Sep-Pak C-18, LC-18 and Oasis® HLB cartridges) were placed on a vacuum manifold (12, Supelco, USA) and vacuum pressure was adjusted to achieve the required flow rate. All the cartridges were conditioned with 5 mL of ethyl acetate to remove residual bonding agents, followed by 5 mL of methanol to ensure that the sorbents were soaked in methanol. After 5 min of soaking in methanol, 3×5 mL Milli-Q grade water was passed through the cartridges at a rate of 1–2 mL min^{-1}. Then, water samples spiked with surrogates (100 ng L^{-1}) were extracted at a flow rate less than 5 mL min^{-1}. For real samples, surface water was filtered through glass microfiber filters in order to remove fine particles and thus prevent cartridges from becoming blocked. After that, the cartridges were washed with 2×5 mL Milli-Q grade water:MeOH (9:1, *v/v*) and then dried under vacuum for 40 min. The analytes were eluted into 15 mL vials from the sorbents with 10 mL of solvents (e.g. DCM) at a flow rate of 0.5 mL min^{-1}. The eluates were blown down to dryness under a gentle flow of nitrogen and then derivatized.

Derivatization

The high polarity of some compounds gave rise to bad chromatographic peaks, and derivatization was done to reduce the polarity of these compounds. The standards were transferred into 2 mL reaction vials, and the solvent was further evaporated to dryness under a gentle nitrogen stream. The dry residues were derivatized by adding 50 μL of derivatization reagents (e.g. BSTFA). The vials were closed and completely mixed for 1 min using a vortex system, and then heated at different reaction temperatures (room temperature, 40, 50, 60, 70, 80, 90 °C) for different reaction times (10, 20, 30, 40, 50, 60, 90, 120 min). After the derivatization reaction, the derivatives were cooled to room temperature and then 50 μL internal standard (2 ng μL^{-1}) was added. 1 μL of the mixtures was injected for GC–MS analysis.

GC–MS Analysis

Gas chromatography-mass spectrometry analysis was performed with a Trace GC (Thermo Fisher Scientific, USA), equipped with a Trace DSQ quardrupole mass spectrometer and an autosampler Triplus AS. The separation of the compounds was achieved by using a DB-5MS capillary column (30 m length) with a 0.25 mm inner diameter and 0.25 μm film thicknesses (Agilent, USA). The carrier gas was helium (99.999%) and maintained at a constant flow rate of 1 mL min^{-1}. A sample volume of 1 μL was

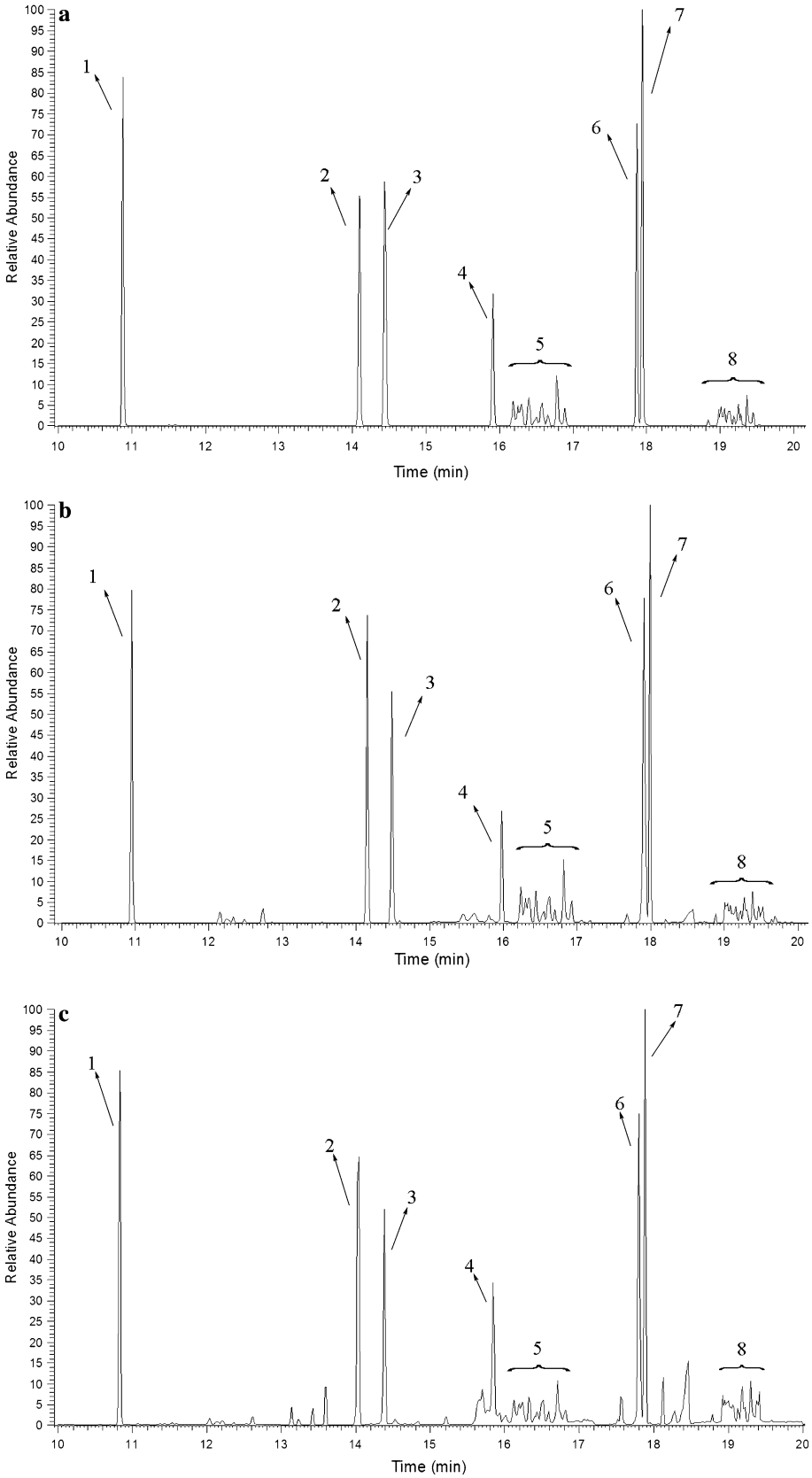

Fig. 1 SIM chromatogram of target compounds. **a** Standard solution (1 ng μL^{-1}); **b** spiked water sample (200 ng L^{-1}); **c** spiked sediment sample (25 ng g^{-1} dw) (*1*) 4-*t*-OP, (*2*) 4-CP, (*3*) 4-NP, (*4*) 5α-androstane, (*5*) NP1EO, (*6*) BPA-d_{16}, (*7*) BPA, (*8*) NP2EO derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

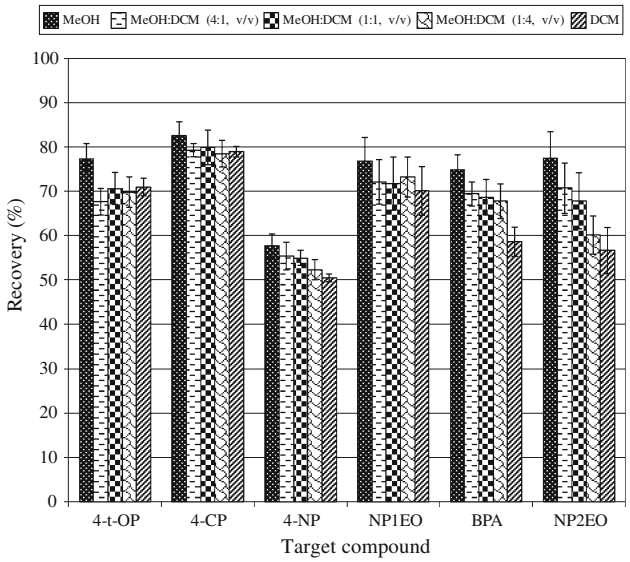

Fig. 2 The effect of extraction solvent on the recovery of target compounds from spiked sediment samples ($n = 3$). *Note* MAE conditions: 4 g sample extracted with 25 mL of different solvents at 80 °C for 20 min; SPE conditions: Sep-Pak C-18 cartridge, elution with 10 mL DCM; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

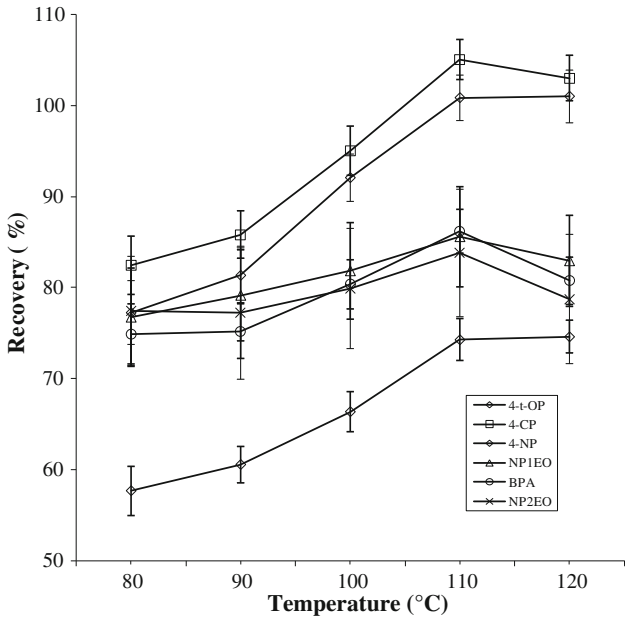

Fig. 3 The effect of extraction temperature on the recovery of target compounds from spiked sediment samples ($n = 3$). *Note*: MAE conditions: 4 g sample extracted with 25 mL of methanol at different temperature for 20 min; SPE conditions: Sep-Pak C-18 cartridge, elution with 10 mL DCM; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

injected in splitless mode at an inlet temperature of 280 °C. The column temperature was programmed as follows: from 60 to 150 °C at 15 °C min^{-1}, from 150 to 220 °C at 8 °C min^{-1} and 220 °C isothermal for 1 min, from 220 to

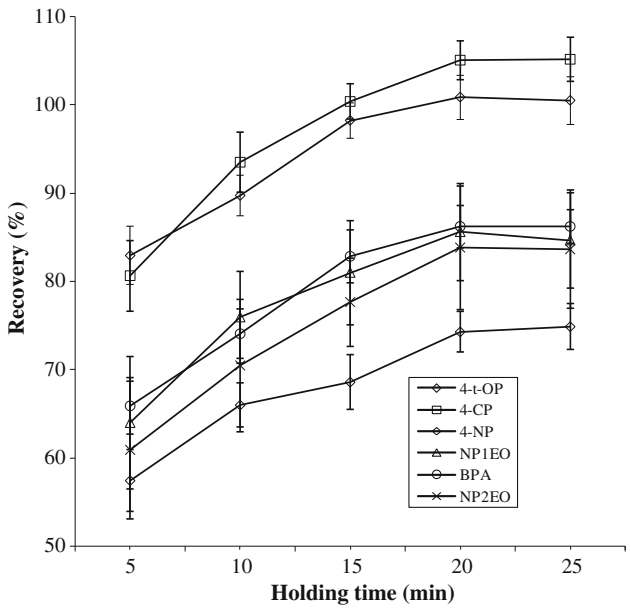

Fig. 4 The effect of extraction holding time on the recovery of target compounds from spiked sediment samples ($n = 3$). *Note* MAE conditions: 4 g sample extracted with 25 mL of methanol at 110 °C for different extraction holding time; SPE conditions: Sep-Pak C-18 cartridge, elution with 10 mL DCM; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

290 °C at 15 °C min^{-1} and 290 °C isothermal for 5 min. The MS transfer line temperature was maintained at 280 °C, whereas the ion source temperature was 250 °C. Mass spectra were operated in full-scan mode from *m/z*, 50–600 for qualitative analysis or selected ion monitoring (SIM) mode for quantitative analysis. Electron impact ionization energy was 70 eV. Examples of chromatograms for the identification of target compounds in standard solutions, spiked water and sediment samples were shown in Fig. 1.

Results and Discussion

Optimization of MAE

In order to obtain the efficient extraction of phenols from sediment samples by MAE, three key process factors, such as extraction solvent, extraction temperature and holding time, were investigated. Sediment samples collected from the Aquaculture Base were used as model matrix. The concentrations of 4-CP, NP1EO, NP2EO, BPA, 4-*t*-OP and 4-NP were <0.40, 8.9, 10.3, 2.2, 0.15 and 1.0 ng g^{-1} dry weight in these sediment samples, respectively.

The effect of extraction solvent on the recovery of target compounds from spiked sediment samples were shown in Fig. 2. The recoveries of every compound were very low and similar among different extraction solvents, with

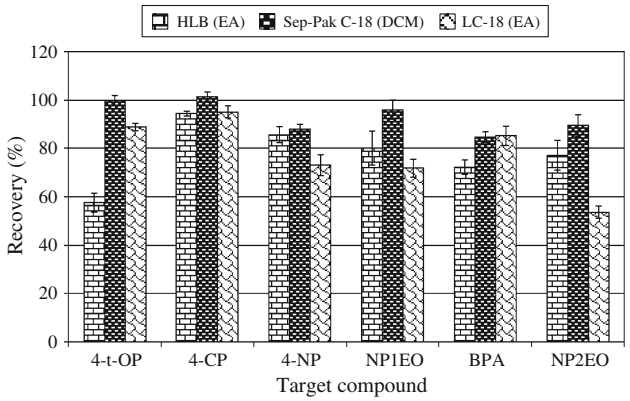

Fig. 5 The effect of different kinds of SPE cartridge on the recovery of target compounds from spiked water samples ($n = 3$). *Note* 500 mL of water sample was adjusted to pH 4.5; Oasis® HLB cartridge, elution with 10 mL EA; Sep-Pak C-18 cartridge, elution with 10 mL DCM; LC-18 cartridge, elution with 10 mL EA; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

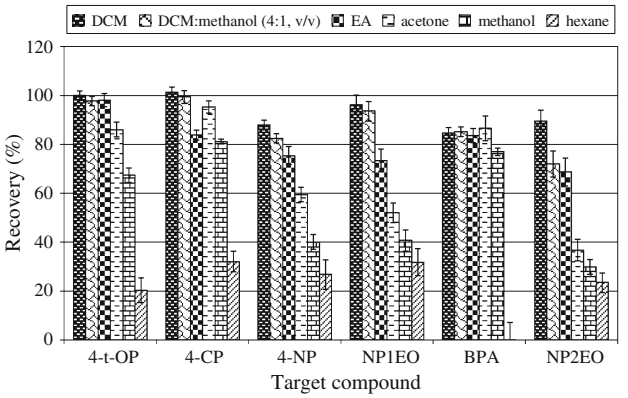

Fig. 6 The effect of elution solvent on the recovery of the target compounds from spiked water samples ($n = 3$). *Note* SPE conditions: water sample was adjusted to pH 4.5; Sep-Pak C-18 cartridge, elution with 10 mL different elution solvents; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

25 mL of different solvents at 80 °C for 20 min. The recoveries ranged from 50 to 82%. According to the MAE operating guide, the power constantly varied with extraction temperature, which must not exceed about two times than boiling point of extraction solvent. Boiling point of DCM is 39.8 °C, whereas that of methanol is 64.5 °C. That means solvent with higher boiling point will be more suitable to be used at higher temperature during the extraction. In addition, methanol can improve extraction efficiency for target compounds by efficiently absorbing microwave energy. Thus, methanol was chosen as the best solvent for further studies in order to obtain the higher recovery.

The mean recovery of all analytes significantly increased with the temperature range from 80 to 110 °C, as shown in Fig. 3. But, this increase leveled off when temperature increased from 110 to 120 °C except for 4-*t*-OP and 4-NP, which had slight increases. The effects of holding time on the recoveries of target compounds were shown in Fig. 4. All of the mean recoveries were dramatically improved with the increase of holding time from 5 to 20 min, whereas they had no significant changes between 20 and 25 min. Therefore, 20 min was chosen as the holding time of extraction.

In addition, the work volume of extraction vessel (100 mL) for Milestone Ethos 1 is >10 mL and <30 mL, so 25 mL was chosen as the solvent volume. Sulfur present in sediment samples from Dianchi Lake did not significantly interfere with GC determination because of the effective removal by copper.

As it is apparent from the above discussion, 25 mL of methanol as extraction solvent at 110 °C for 20 min were selected as the best operating conditions for the extraction

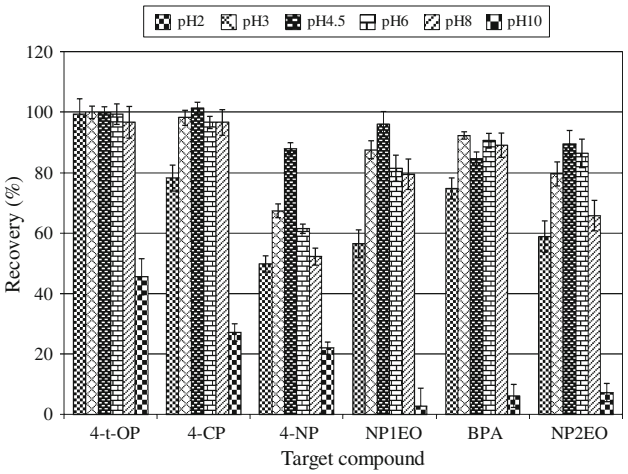

Fig. 7 The effect of water sample pH on the recovery of the target compounds from spiked water samples ($n = 3$). *Note* SPE conditions: Sep-Pak C-18 cartridge, elution with 10 mL DCM; derivatization conditions: 50 μL of BSTFA at 70 °C for 50 min; injection solvent: hexane

of the target compounds with the recovery ranging from 74 to 105%.

Optimization of SPE

In order to find the optimum conditions for the SPE, SPE phase cartridge, elution solvent and pH of water samples were investigated. An appropriate SPE cartridge with different sorbent materials plays a key role in the achievement of high and reproducible recovery for the target compounds. Using recovery experiments in spiked water samples, three types of cartridges (Sep-Pak C-18, Oasis® HLB and LC-18 cartridges) were tested for their extraction efficiencies. The SPE cartridge was eluted by the optimal

Table 1 Chromatogram information of target EDCs, surrogate and internal standard

Compounds	Retention time (min)	Molecular mass	Quantification ions (*m/z*)	Confirmation ions (*m/z*)
4-*t*-OP	10.88	206	207	278
4-CP	14.09	212	269	284
4-NP	14.44	220	179	292
5α-Androstane	15.91	260	203, 245	260
NP1EO	16.19–16.89	264	207, 251, 265, 279, 293, 307	336
BPA-d_{16}	17.86	244	368	386
BPA	17.94	228	357	372
NP2EO	18.84–19.44	308	295, 309, 323, 337, 351	380

elution solvent in order to achieve the highest recovery. The cartridges were eluted as follows: (1) Sep-Pak C-18 cartridges were eluted with 10 mL of DCM; (2) Oasis HLB® and LC-18 cartridges were separately eluted with 10 mL of ethyl acetate. The pH value of water samples was adjusted to 4.5 by hydrochloric acid or sodium hydroxide. The results were shown in Fig. 5. For 4-*t*-OP and BPA, the recoveries of the target compounds were very low when Oasis HLB cartridges were used. For NP, NP1EO and NP2EO, low recoveries (<73%) were obtained with LC-18 cartridges. Sep-Pak C-18 cartridges achieved the best recoveries (84–101%) with their lower cost, were therefore chosen for further testing.

Solvents were used to desorb the target compounds from Sep-Pak C-18 cartridges. The target compounds spanned a large polarity range using different elution solvents, such as DCM, DCM: MeOH (4:1, *v/v*), ethyl acetate, acetone, methanol and hexane, which have different elution strength and polarity. The mean recoveries ($n = 3$) of the analytes with elution solvents are shown in Fig. 6. Hexane produced poor recoveries (between 0.13 and 32%) for the target compounds, which may be due to the relatively polar nature of these compounds. Except for 4-CP (81%) and BPA (76%), recoveries of other compounds were also low with methanol as elution solvent. Low recoveries of 4-NP, NP1EO and NP2EO were obtained with acetone as the elution solvent. Recoveries (between 69 and 98%) were achieved with ethyl acetate as the elution solvent. Better recoveries of the target compounds ranged from 72 to 99% using DCM:MeOH as the elution solvent. But, the best recoveries were achieved using DCM as the elution

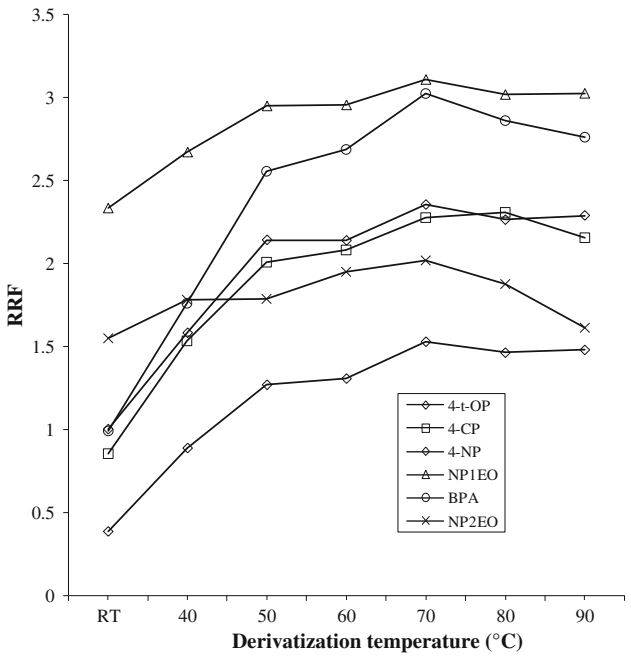

Fig. 8 The effect of different derivatization temperature on the RRFs value of derivatized target compounds; derivatization conditions: 50 μL of BSTFA for 50 min; injection solvent: hexane

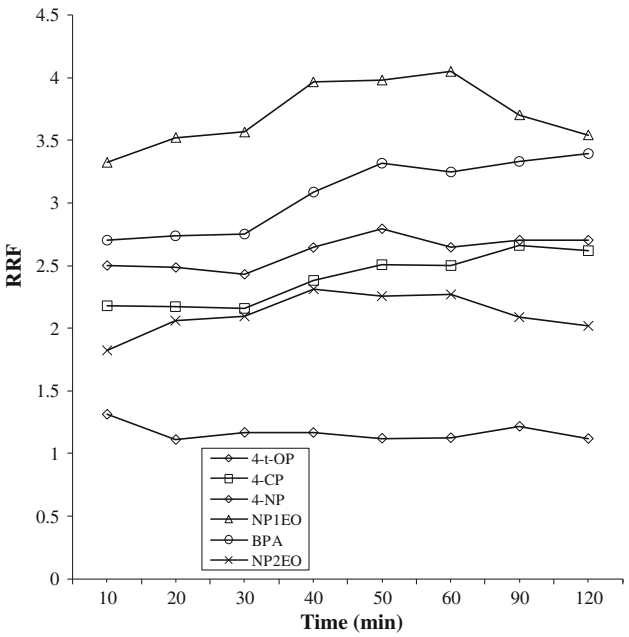

Fig. 9 The effect of different derivatization time on the RRFs value of derivatized target compounds; derivatization conditions: 50 μL of BSTFA at 70 °C; injection solvent: hexane

Table 2 Mean recovery, RSD, LOQ and linear range of the target compounds in spiked water and sediment samples

Compounds	Recovery (%)				LOQ in water (ng L^{-1})	LOQ in sediment (ng g^{-1} dw)	Linear range (ng μL^{-1})
	In water	RSD (%) $n = 3$	In sediment	RSD (%) $n = 3$			
4-*t*-OP	99.8	2.0	100.8	2.5	0.20	0.31	0.005–5
4-CP	101.4	1.8	105.0	2.0	0.47	0.40	0.005–5
4-NP	87.9	2.2	74.3	3.1	0.40	0.52	0.005–5
NP1EO	96.0	4.1	85.6	6.4	8.50	7.40	0.05–5
BPA	84.7	2.3	86.2	2.8	0.55	0.35	0.005–5
NP2EO	89.4	5.0	83.8	8.4	11.50	9.50	0.05–5

solvent. The results suggested that not only could DCM as elution solvent obtain sufficient recoveries and pure extracts, but also it evaporated more easily than other solvents.

The effect of pH on extraction efficiency was studied by adjusting the pH value of water sample with diluted solutions of sodium hydroxide and hydrochloric acid. As shown in Fig. 7, the extraction recoveries at pH 4.5 were better for all target compounds than under other acid–base conditions. Excess of acid or base led to impurity peaks and lower recoveries, in particular for NP, NP1EO, NP2EO and BPA. For example, the recoveries of NP1EO, NP2EO and BPA were close to zero at pH 10.

Optimization of Derivatization

Derivatization reactions are affected by many possible factors, such as derivatization reagent, reaction temperature, reaction time and injected solvent. Preliminary experiments were conducted in order to select the derivatization reagent between BSTFA and PTA-OH. During the derivatization procedure, BSTFA and PTA-OH were investigated with the same condition. Except for NP1EO and NP2EO, 4-NP, 4-*t*-OP, 4-CP and BPA can be all derivatized by PTA-OH. At the same time, the reaction of target compounds with PTA-OH can produce unexpected derivatization byproducts, such as water, which led to signal suppression of the compound during GC–MS analysis. But, BSTFA can react very fast with the six target compounds, and produce derivatized compounds with high volatility, adequate stability and solubility. In addition, although BSTFA and PTA-OH both improved chromatographic performance through derivatization of 4-NP, 4-*t*-OP, 4-CP and BPA, BSTFA derivatives had a higher response than PTA-OH derivatives. Therefore, BSTFA was chosen as the optimal derivatization reagent for the target compounds and the ions monitored for each compound were listed in Table 1.

The reaction temperature and time for BSTFA were also investigated. As shown in Fig. 8, RRFs of all analytes increased significantly from room temperature to 50 °C, and gradually increased from 50 to 70 °C, but decreased suddenly between 70 and 90 °C except for 4-*t*-OP and 4-NP. As shown in Fig. 9, the best reaction time was 50 min, but RRFs of NP1EO and NP2EO would decrease when exceeded this time. These results may be also attributed to the degradation of the compounds at higher temperature and/or longer time. For the injected solvent, four solvents (hexane, pyridine, acetone and ethyl acetate) were tested in order to obtain the best result for the GC–MS. Hexane eventually was chosen as the injected solvent because it produced higher RRFs and lower base-line of chromatogram than other solvents.

In order to improve the degree of derivatization of the target compounds, different volumes of BSTFA (from 50 to 200 μL) were investigated. Results showed that the degree of derivatization was not affected by the volume of the derivatization reagent. In addition, SIM was also conducted to prove the degree of derivatization, and the result suggested that the derivatization was complete because the characteristic peaks of underivatized compounds were not observed in the derivatization standard solutions. Thus, the smallest volume 50 μL of BSTFA had achieved complete derivatization of the target compounds. The derivatives at 2 °C could be measured unchanged even after 10 days.

The optimized derivatization procedure was: 50 μL of BSTFA was added to 2 mL glass vial and the mixture reacted at 70 °C for 50 min. After derivatization, the final sample volume was adjusted to 100 μL using hexane with 100 ng of internal standard.

Method Validation

The analytical method was validated by limit of detection (LOD), the limit of quantification (LOQ), the linear range, mean recovery and precision. The LOD, calculated as the concentration corresponding to three times the standard deviation of blanks, was measured by integrating blank peak area for each analyte in ten independent

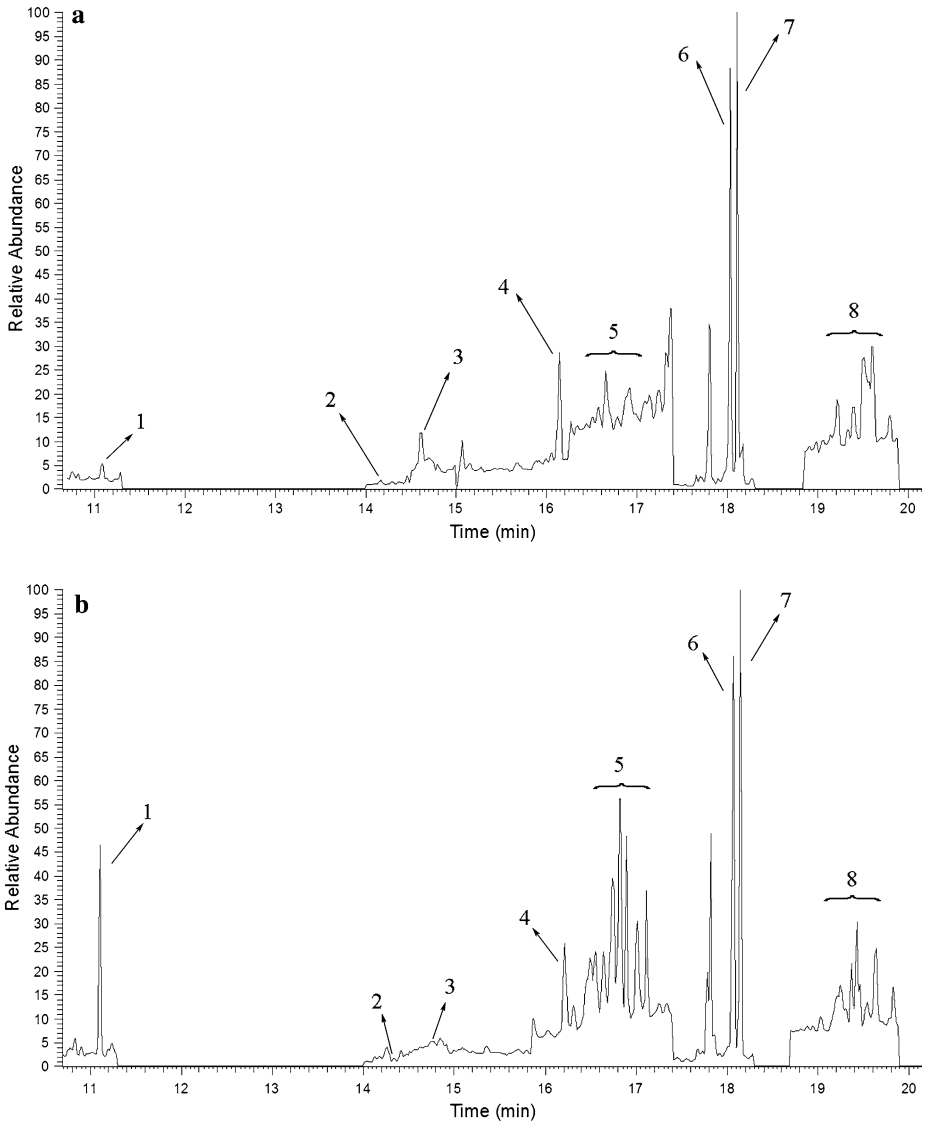

Fig. 10 SIM chromatogram of target compounds in environmental samples **a** water sample; **b** sediment sample (*1*) 4-*t*-OP, (*2*) 4-CP, (*3*) 4-NP, (*4*) 5α-androstane, (*5*) NP1EO, (*6*) BPA-d_{16}, (*7*) BPA, (*8*) NP2EO

performances. LOQ was determined as the analyte concentration corresponding to ten times the standard deviation. The LOD values ranged from 0.10 (4-*t*-OP) to 2.3 ng L^{-1} (NP2EO) in water, while 0.15 (4-*t*-OP) to 2.9 ng g^{-1} dw (NP2EO) in sediments. Similarly, the LOQs varied from 0.20 (4-*t*-OP) to 11.50 ng L^{-1} (NP2EO) for water samples and from 0.31 (4-*t*-OP) to 9.50 ng g^{-1} dw (NP2EO) for sediment samples. For both water and sediment samples, the LODs and LOQs achieved in the present work were at similar levels or lower than those obtained in previous studies with GC–MS [1, 8, 17–19]. A series of injections of target compounds in the concentration ranged from 0.005 to 5 ng μL^{-1} with 1.0 ng μL^{-1} of internal standards were used to determine the linear concentration range. The calibration curves were linear with coefficients of determination (R^2) higher than 0.992. The precision of the method was estimated with triplicate analyses of the same spiked experiments. The method resulted in good repeatability and reproducibility with relative standard deviations (RSDs) <13% for all target EDCs in both types of samples. Mean recovery, RSD, LOQ and linear range of the target compounds in spiked water and sediment samples were given in Table 2. These results indicate the high performance of the developed analytical method.

Analysis of Environmental Samples

The improved method was successfully applied to the analysis of phenolic EDCs in surface water and sediment samples, collected from Caohai site (the state key surface water quality monitoring section) of Dianchi Lake, on July 5, 2010. SIM chromatograms of target compounds in water and sediment samples from Caohai site were showed in Fig. 10. As a result, the mean concentrations of 4-*t*-OP,

4-CP, 4-NP, BPA, NP1EO and NP2EO were 5.5, 4.6, 16.1, 149, 114 and 97 ng L^{-1} in surface water, whereas 15.6, 1.1, 3.1, 178, 444 and 186 ng g^{-1} dw in surface sediment, respectively. NP1EO, NP2EO and BPA were the three dominant phenolic EDCs in the Caohai site of Dianchi Lake.

Conclusions

The integrated method for the analysis of six phenolic EDCs (4-NP, NP1EO, NP2EO, 4-*t*-OP, BPA and 4-CP) in surface water and sediment was developed by optimizing the MAE, SPE and derivatization procedure. For sediment samples, sufficient extraction was performed by using 25 mL of methanol as extraction solvent at 110 °C for 20 min in the MAE, reducing the time and cost of analysis. For water samples, sufficient isolation of the target compounds from the matrix was obtained by using Sep-Pak C-18 cartridges with 10 mL of dichloromethane as the elution solvent when pH value of water samples was adjusted to 4.5. The final sample extracts were derivatized by using 50 μL BSTFA at 70 °C for 50 min. The method achieved good repeatability and reproducibility with RSDs ranged from 4.1 to 12.8% for all target EDCs in both types of samples. Satisfactory recoveries ranged from 85 to 101% and 74 to 105% in spiked water and sediment samples, respectively. LODs ranged from 0.10 (4-*t*-OP) to 2.3 ng L^{-1} (NP2EO) for surface water, and from 0.15 (4-*t*-OP) to 2.9 ng g^{-1} dw (NP2EO) for sediment samples. At the same time, the LOQs varied from 0.20 (4-*t*-OP) to 11.50 ng L^{-1} (NP2EO) for water samples and from 0.31 (4-*t*-OP) to 9.50 ng g^{-1} dw (NP2EO) for sediment samples.

The developed method has been successfully demonstrated to determine the concentrations of the target EDCs in surface water and sediment samples from Dianchi Lake. Thus, the method could be a promising approach for analysis of six phenolic endocrine disrupting chemicals in water and sediment samples.

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