Hollow-fiber-supported liquid-phase microextraction using an ionic liquid as the extractant for the pre-concentration of bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol from water samples with HPLC detection

Yanmin Zou, Zhen Zhang, Xiaoling Shao, Yao Chen, Xiangyang Wu, Liuqing Yang, Jingjing Zhu and Dongmei Zhang

ABSTRACT

A new method for the determination of four endocrine disrupting compounds (EDCs) (bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol) in water samples has been developed using polypropylene hollow-fiber-supported ionic liquid (IL, 1-Octyl-3-methylimidazolium hexafluorophosphate, $[C_8MIM][PF_6]$) microextraction [HF-liquid-phase microextraction (LPME)] combined with high-performance liquid chromatography (HPLC)/UV. This method was used to investigate pollutants in surface water, on the Neijiang River, located at Zhenjiang, Jiangsu Province. Several parameters (sample pH, volume of accepter phase, ionic strength) were investigated. Under the optimum extraction conditions (sample pH, 2.0; volume of extraction solvent, 2.5 μL; ionic strength, 2.57) the proposed method offered: good linearity range, 0.15–100 μg L^{-1}, with the correlation coefficients (r^2) of 0.9996, 0.9994, 0.9990 and 0.9984, respectively; low limits of detection, 0.03, 0.05, 0.10, 0.05 μg L^{-1} (S/N = 3) for bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol, respectively; good reproducibility (relative standard deviation (RSD), 8.41, 7.61, 9.00, 7.22%, respectively, n = 5); satisfactory recoveries (80.2–107.1%, n = 5); and high enrichment factors, 5,240, 3,693, 2,425 and 2,086, were achieved, for the four chemicals, respectively. Using the proposed HF-LPME, among 15 sampling sites along Neijiang River, bisphenol A, diethylstilbestrol and 17-β-estradiol were detected in some sites, all of which were the near suburban sampling sites. The results indicate that the role of municipal sewage is an important source of EDC contamination.

Key words | EDCs, hollow-fiber-supported liquid-phase microextraction, HPLC/UV, ionic liquid

Yanmin Zou
School of Pharmacy,
Jiangsu University,
301 Xuefu Rd.,
212013 Zhenjiang,
Jiangsu, China

Zhen Zhang (corresponding author)
Xiaoling Shao
Yao Chen
Xiangyang Wu
Jingjing Zhu
Dongmei Zhang
School of the Environment,
Jiangsu University,
301 Xuefu Rd.,
212013 Zhenjiang,
Jiangsu, China
E-mail: zhangzhen@ujs.edu.cn

Liuqing Yang (corresponding author)
School of Chemistry & Chemical Engineering,
Jiangsu University,
301 Xuefu Rd.,
212013 Zhenjiang,
Jiangsu, China
E-mail: yangliuqing@ujs.edu.cn

INTRODUCTION

In recent decades, endocrine disrupting compounds (EDCs) have attracted increasing attention due to their adverse effects on aquatic organisms, ecosystem sustainability and human health (Ciocan *et al.* 2012). EDCs fall into two main categories: those which occur naturally, and those which are man-made and possess endocrine-disrupting properties even at trace levels (Koyama *et al.* 2013). Due to the incomplete removal of these EDCs from wastewater treatment plants, they are ubiquitous in environmental samples and ingested by humans and animals through food or water intake which can lead to bioaccumulation or biomagnification (Wang *et al.* 2010). Therefore, it is important to establish a convenient and reliable method to detect different classes of EDCs, especially the estrogenic compounds. The natural steroid hormones derived from excreta of humans and livestock (e.g. 17-β-estradiol, E2; estrone, E1), the synthetic estrogen added to foodstuffs for animals to promote their growth (e.g. diethylstilbestrol, DES) and some chemicals widely used as industrial material (e.g. bisphenol A, BPA) are phenolic compounds and endocrine disrupting EDCs have been identified from a range of investigations (Holmes *et al.* 2010; Meeker 2010).

Due to an environmental matrix effect and low concentration of EDCs, generally, occurring in water, the main step of the extraction process is isolation and enrichment. At present, trace enrichment is usually performed by conventional techniques such as liquid–liquid extraction and solid-phase extraction (Jiang *et al.* 2011). Those procedures require large volume of organic solvents, after which the extracts have to undergo further pre-concentration prior to analysis. In recent years, to eliminate or minimize these disadvantages, solid-phase microextraction (Basheer *et al.* 2007) and liquid-phase microextraction (LPME) (Xu *et al.* 2007) have been used for the extraction of a variety of EDCs. Especially, various modes of LPME have been developed such as single drop microextraction (SDME) (Psillakis & Kalogerakis 2002) and headspace SDME (Sun *et al.* 2008). Headspace SDME has been used mainly for the more volatile organic compounds. Although high enrichment factors (EF) can be achieved, a single drop of organic solvent is potentially unstable and may be easily dislodged from the syringe needle during the extraction process. To overcome the drawback of microdrop LPME, Pedersen-Bjergaard & Rasmussen (1999) developed a hollow-fiber-based LPME technique (HF-LPME). HF-LPME uses a porous-walled polypropylene hollow fiber to stabilize and protect the organic phase, and to enhance the mechanical robustness and the extraction efficiency (Ho *et al.* 2007). This technique represents a simultaneous extraction, cleanup and pre-concentration approach. The polypropylene fiber used for LPME is cheap, and a fresh piece is used for each extraction to avoid contamination. Recently, studies were also reported in detecting EDCs using HF-LPME, which were proved to be successful. Chen *et al.* employed three phase HF-LPME-high-performance liquid chromatography (HPLC)-UV simultaneous analysis of eight environmental estrogens (include E1, E2, BPA and DES) in environmental and biological samples (sediment and fish). The acceptor phase is 0.15 mol/L NaOH with 0.015 mol/L β-CD and supported phase is organic solvent. The EF was ranged from 88–376. The limits of detection (LODs) of the proposed method for BPA, E2, E1 and DES were 0.055, 0.66, 0.51 and 0.20 μg/L, respectively (Chen *et al.* 2013). Tan introduced a novel three-phase HF-LPME-HPLC for BPA from water samples. In the study, n-octanol was used as supported phase, and it showed some excellent performances as: EF, 241; LODs, 0.2 μg/L (Tan *et al.* 2012). Villar-Navarro adopt three -phase HF-LPME-HPLC for analysis of n-octylphenol (OP) and n-nonyl-phenol in environmental waters, and some parameters of the methods were: supported phase, dihexyl ether; EF, 50; LODs, 0.54 and 0.52 μg/L, respectively (Villar-Navarro *et al.* 2013). All of which indicated that HF-LPME coupling with

HPLC was a reliable method for detection of EDCs in aquatic environments with traditional organic solvent as extracting agents.

Ionic liquids (ILs) are ionic media resulting from the combination of organic cations and various anions with a melting point below 100 °C. ILs possess many significant advantages, such as low vapor pressure, a wide range of miscibility with other organic solvents, good thermal stability and dual natural polarity. Furthermore, as a kind of green extraction solvent, ILs can be used in separation techniques, instead of conventional organic solvents, to develop an effective and environmental friendly method for detection of pollutants (Berthod *et al.* 2008).

The Neijiang River is an important river of Zhenjiang, Jiangsu Province of China, and belongs to the Yangtze River tributary. The Neijiang River lies in upper reaches of many big cities (Changzhou, Wuxi, Suzhou and Shanghai), and provides millions of people with raw drinking water. Therefore, it is necessary to investigate the typical EDCs in this area.

The objective of this study was monitor the pollutants in the river using an adopted ionic liquid (IL, 1-Octyl-3-methylimidazolium hexafluorophosphate, $[C_8MIM][PF_6]$) to set up a hollow-fiber-supported ionic liquid microextraction method coupled with HPLC/UV to determine BPA, E2, E1 and DES in environmental water samples. Depending on the proposed analytical method (as the introduction in Procedure for Hollow Fiber Liquid Phase Microextraction), 15 sampling sites were selected along the Neijiang River to investigate the four organic containments. The results provide important background data for risk assessment of pollutants in the aquatic environment of this area.

EXPERIMENTAL

Reagents and materials

Polypropylene hollow fibers (pp1, inner diameter 600 μm, wall thickness 200 μm, pore size 0.2 μm; pp2, inner diameter 280 μm, wall thickness 50 μm, pore size 0.1 μm) were obtained from Membrana GmbH (Wuppertal, Germany). Micro syringe (needle OD: 0.3 mm, long: 8 mm, cylinder volume: 0.5 mL) was obtained from BD Consumer Healthcare company (Franklin Lakes, NJ, USA), used to flush out the acceptor into a small glass vial (200 μL, Alltech, Deerfield, IL, USA) after extraction. Individual standard BPA, E1, E2, DES, with purity of (or above) 99% was bought from Sigma-Aldrich (Steinheim, Germany). 1-Octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$)

with purity of 99% was obtained from Shanghai Barium Strontium Magnesium Industry Co. Ltd. Humic acid (contain 35.1% dissolved organic carbon) was purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade methanol and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA). Ultrapure water was prepared by a Milli-Q SP reagent water system (Millipore, Milford, MA, USA) and used throughout. Stock solutions of E2, E1, DES and BPA (100 mg L^{-1}) were prepared with methanol and stored at 4 °C. Working standard solutions were prepared by diluting the standard solutions with ultrapure water just before use.

HPLC analysis

Samples were analyzed by HPLC on a Waters Breeze 2 instrument consisting of a dual pump (1525), an autosampler (2707), a column oven, a column (150 mm × 4.6 mm, i.d. 5 μm, Eclipse XDB-C18) and UV-detector (2489). The mobile phase consisted of A (methanol: acetonitrile (50:50, v/v))/B (water). The four EDCs were separated within 15 min at flow rate of 1 mL min^{-1}. The column oven was set at 30 °C. and the UV detector was set at 280 nm. The gradient elution program was as follows: A increasing from 40 to 60% in 0–7.0 min, then increasing to 70% in 7.0–13.0 min and decreasing to 40% in 13.0–14.0 min.

Procedure for hollow-fiber liquid-phase microextraction

Segments of the microporous polypropylene hollow fiber were precleaned with acetone ultrasound for 20 min then dried and both ends were heat-sealed by sintering, which was used as the carrier of [C$_8$MIM][PF$_6$] for LPME. Before extraction, the hollow fiber segment was immersed in [C$_8$MIM][PF$_6$] ultrasound for 6 hours, consequently the lumen of the hollow fiber segment was filled with [C$_8$MIM][PF$_6$] as the extraction solvent (acceptor phase). Subsequently, the fiber segment was taken out and submerged in water and simultaneously oscillated for one minute to remove the excess [C$_8$MIM][PF$_6$] on the surface of the fiber. Then the hollow fiber segment was submerged into the sample solution (donor phase). The aqueous sample was stirred at 200 rpm at room temperature (25 °C) for several hours. After extraction, the hollow fiber was removed from the sample. The closed ends were cut and one end put in a glass vial and [C$_8$MIM][PF$_6$] (volume calculated by the segment length and inner diameter) was pushed out with a syringe with 100 μL of

methanol from the other end; and finally 20 μL of the analyte-enriched solvent was injected into the HPLC for analysis directly without further manipulation. Each segment of hollow fiber was used only for single extraction to avoid contamination. All experimental results were the average of three or more parallel experimental results. The operation of the extraction was evaluated by the concentration EF (EF, the ratio of concentration after and before extraction that was measured under the optimal conditions then multiplied by the dilution multiple).

Sample collection

All water samples were collected from Neijiang River in June 2012. Details of the sampling sites are described in detail in the Supporting Information (Figure A1; available online at http://www.iwaponline.com/wst/069/824.pdf). Samples were filtered through 0.45 μm of glass fiber micropore membranes and adjusted to pH 2 using hydrochloric acid and stored at 4 °C, in a brown glass bottle, before extraction.

RESULTS AND DISCUSSION

Optimization of microextraction conditions

pH of donor phase

The extraction process involves pH adjustment of the sample solution to a pH where molecules of the solution are uncharged, because uncharged molecules have a good tendency to be attracted to [C$_8$MIM][PF$_6$]. For phenolic compounds belonging to weak acid (weakly acidic phenolic compounds), the extraction efficiency can be influenced by the pH of the aqueous sample because the existing form of phenolic compounds (molecular state and ion state) depends on it. Phenolic compounds in the molecular state are more soluble compared with the ion state in the extraction agent, and this increases extraction efficiency, so in the donor phase often suitable amounts of acid are added (Andersen *et al.* 2002). Therefore, considering the instability of phenolic compounds under alkaline conditions, the pH was in the range of 1–5 by adding an appropriate amount of hydrochloric acid to the water samples (see Figure 1). The results show that the extraction efficiency is at the highest when the pH to 2. So pH = 2 was chosen in the following study.
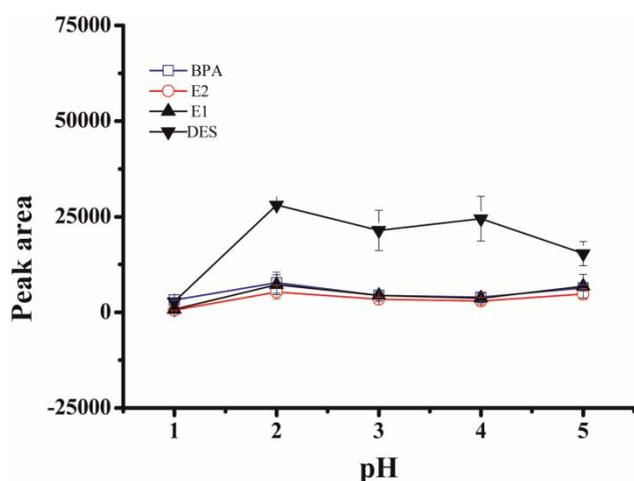
Figure 1 | Effect of donor phase pH on extraction efficiency donor phase (DP): 50 mL; spiked concentration: 50 µg L^{-1}; acceptor phase (AP): 5 µL; extraction time: 3 h.

Extracting solvent volume

In the HF-LPME procedure, the extracting solvent volume is a critical factor in obtaining high extraction efficiency of the analyte. For optimization of the extraction solvent volume, an experiment was conducted to test the dependence of peak area of four EDCs on [C$_8$MIM][PF$_6$] volume. The results shown in Figure 2 indicate that the peak area reached the maximum when the volume of [C$_8$MIM][PF$_6$] was 2.5 µL. It was observed that the extraction efficiency was remarkably affected by the volume of [C$_8$MIM][PF$_6$]. By increasing the volume of [C$_8$MIM][PF$_6$] from 2.5 to 20 µL, the peak area of four EDCs were decreased. Therefore, the volume of 2.5 µL was chosen as the optimum extracting solvent volume.

Ionic strength

The addition of salt to the sample solution can decrease the solubility of analytes and extraction solvent in the aqueous phase and thus improve the extraction performance by increasing the ionic strength of sample solution (Baghdadi & Shemirani 2009). To study the salt addition effect, the experiments were conducted by adding sodium chloride (ionic strength 0–3.42). Based on the results shown in Figure 3, it can be concluded that the extraction efficiency increased with ionic strength up to 2.57 and then decreased with further addition of the salt. Except at the peak area of BPA the other analytes almost remained unchanged with ionic strength 1.71–2.57. This phenomenon can be explained as follows: the addition of salt has dual influences on the extraction efficiency in HF-LPME technique. At the beginning of extraction, a salting out effect played a predominant role, resulting in a decrease of dissolubility of four EDCs in aqueous phase and, therefore, extraction efficiency increased. On the other hand, with the increasing NaCl concentration, the salt addition can suppress the extraction by electrostatic interaction (Fu *et al*. 2009). In addition, the occurrence of ion exchange could enhance the solubility of [C$_8$MIM][PF$_6$] in the aqueous phase and decrease the extraction efficiency (He *et al*. 2010). Considering the experimental results, ionic strength 2.57 was chosen in the subsequent work.

Extraction time

Two phase liquid–liquid microextraction is based on equilibria governing: the partitioning of analytes among donor

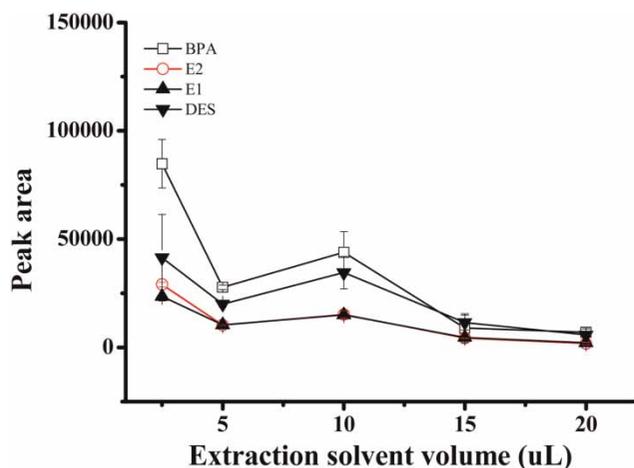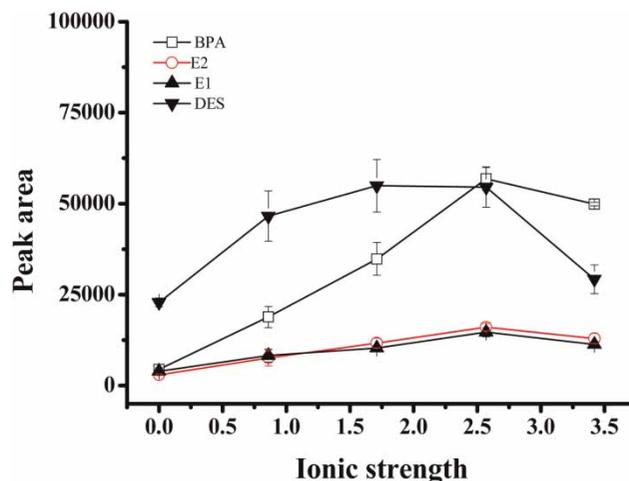**Figure 2** | Effect of extracting solvent volume on extraction efficiency. DP: 50 mL; pH 2; spiked concentration: 50 µg L^{-1}; AP: 2.5–20 µL; extraction time: 3 h.

Figure 3 | Effect of salinity on extraction efficiency. DP: 50 mL; pH 2; spiked concentration: 50 µg L^{-1}; AP: 2.5 µL; extraction time: 3 h.

and acceptor phases (Zhao & Lee 2002). The amount of analytes transferred into the ionic liquid will reach the maximum value when this equilibrium is established. Therefore, an appropriate extraction time during which equilibrium between the two phases is reached should be chosen. To determine how the extraction time affects the extraction efficiency, HF-LPME was performed for extraction times of 1, 2, 4, 6, 8 and 10 h. As shown in the Supporting Information (Figure A2; available online at http://www.iwaponline.com/wst/069/824.pdf), four EDCs reached equilibrium after 8 h, and then the extraction efficiency decreased with longer extraction time. Thus, an extraction time of 8 h was chosen for subsequent experiments.

Effect of humic acid

For most of the environmental sample analyses, the influence of humic acid is one of the factors which must be considered (Stevenson 1994). This study examined the effect of humic acid (0–25 mg L^{-1}) on the extraction efficiency of four EDCs during application of the HF-LPME method. The results shown in Figure 4 indicate that the
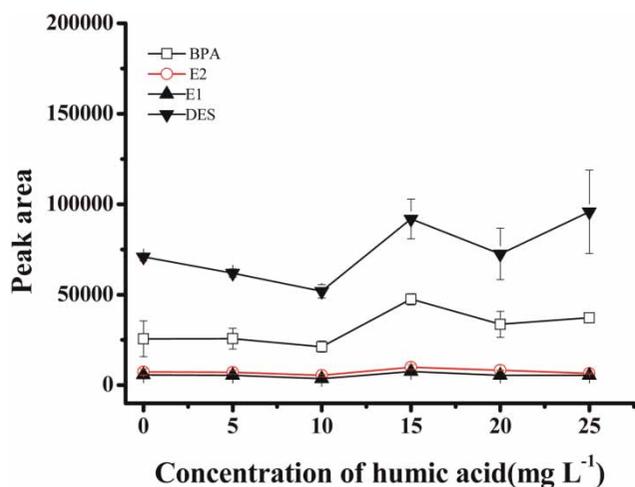

Figure 4 | Effect of humic acid on extraction efficiency, DP: 50 mL; pH 2; salinity: ionic strength 2.57 (NaCl); spiked concentration: 50 μg L^{-1}; AP: 2.5 μL; extraction time: 8 h.

extraction efficiency of E1 and E2 had no obvious change. DES and BPA had a certain change, with the increasing concentration of humic acid. The recoveries of the four EDCs were still kept at 80–110%. It was shown that humic acid had no obvious influence on the extraction efficiency of four EDCs during this method.

Method validation

To validate the applicability of the proposed HF-LPME procedure, calibration curves were plotted using six spiking levels of water in the concentration range 0.15–100 μg L^{-1} for four EDCs. For each level, five replicate extractions were performed under the optimal conditions. The linear dynamic range, correlation coefficient (r^2), the LOD, limit of quantity (LOQ) and the relative standard deviation (RSD) were calculated and are summarized in Table 1. Representative chromatograms of calibration standard solution of four EDCs at 10 mg L^{-1} and water sample fortified with four EDCs at 50 μg L^{-1} are shown in Figure 5. Furthermore, in comparison with the existing methods for extraction of EDCs (shown in Table 2), the established two phases HF-LPME (HF-LLME) indicated relatively better EF.

Monitoring of EDCs in Neijiang River region

The Yangtze River is the largest river in China. The Neijiang River is a branch of The Yangtze River in East China and provides millions of people with water. Zhenjiang is an industrialized city in the Yangtze River Delta, which is the most developed area in China. In Zhenjiang, a great deal of effluent from wastewater treatment plants (WWTPs), untreated sewage (city sewage and industrial waste) and upstream wastewater enters the Neijiang River and then flows into the Yangtze River. Concerns about health risks prompted us to monitor the EDCs residues in the Neijiang River and adjacent areas by this newly developed method during the rainy season (seen in Table 3).

Among the total of 15 sampling sites, DES was found in two sites and concentrations were 1.10 and 1.42 μg L^{-1},

Table 1 | The linear range, precision, detection limit and EFs of the method

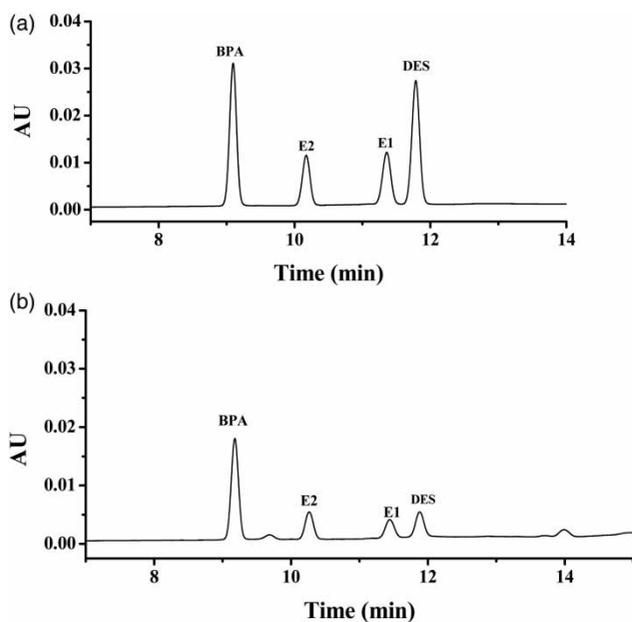| Analyte	Linear range (μg L^{-1})	Calibration curve	Precision (RSD, %, $n = 5$)	Correlation coefficient, r^2	EF	LOQ (μg L^{-1})	LOD (μg L^{-1})
BPA	0.15–100	$y = 2028.1x + 557.33$	8.41	0.9996	5,240	0.12	0.03
E2		$y = 566.51x + 119.91$	7.61	0.9994	3,693	0.14	0.05
E1		$y = 369.77x + 112.90$	9.00	0.9990	2,425	0.15	0.10
DES		$y = 806.37x - 305.78$	7.22	0.9984	2,086	0.12	0.05

Figure 5 | Representative chromatograms. (a) calibration standard solution of four EDCs at 10 mg L^{-1}; (b) water sample fortified with four EDCs at 50 μg L^{-1}.

respectively; E2 was found in one site with a concentration of 1.79 μg L^{-1}. Compared with studies reported in the scientific literatures (Wang et al. 2011), concentrations of the two EDCs (DES and E2) were the highest in the Neijiang River. BPA were detected in six sites with concentration in the range 0.25–0.92 μg L^{-1}, which is consistent with the other areas, such as the Yangtze River at Nanjing section (60.69 ng L^{-1}) (Lu et al. 2010); Huangpu River (0.21 μg L^{-1}) (Tan et al. 2012); Pearl River (6–881 ng L^{-1}) (Peng et al. 2008); rivers of Portugal (98.4 ng L^{-1}–10.7 μg L^{-1}) (Ribeiro et al. 2009; Niels et al. 2010); waters in North America (12 ng L^{-1}) and Europe (43 ng L^{-1}) (Klecka et al. 2009).

BPA was detected, in our study, as a main pollutant among the four investigated EDCs in the Neijiang. In fact, the production and consumption of BPA were estimated at 1.05×10^5 t and 3.2×10^4 t in 2001 and increased every year in China (Zeng et al. 2006). Effluents from WWTPs

are responsible for rises in estrogenic activity of aquatic environments (Coors et al. 2004). As seen by the six sampling sites, untreated industrial and municipal wastewaters are important sources of estrogens in surface waters, which is due to more and more wastes from industry, agriculture, and daily life in Neijiang River and its adjacent rivers.

CONCLUSIONS

In the present work, [C$_8$MIM][PF$_6$] was used as an extracting solvent in HF-LPME coupled with HPLC/UV, for the extraction and enrichment of four EDCs at trace levels from the Neijiang River (a branch of the Yangtze River), Zhenjiang, in East China. It was demonstrated that BPA was the main EDCs in the Neijiang. Our study also showed that higher levels of target EDCs were found near suburban, rather than urban, areas. The results of our study were due to significant human and industrial impact, and detailed reasons were listed as following:

(a) There are more factories in suburban sites than urban sites, and wastewater could be discharged directly into the environment through some hidden drain-outlets.
(b) Most wastewaters from aquaculture and domestic sewage in suburban sites were not treated.

The results of our study indicates that industrial wastewater and domestic sewage discharges are important sources of EDCs along the Neijiang Rive, and more attention should be given to the environmental risk of EDCs, especially BPA.

ACKNOWLEDGEMENTS

Table 2 | Characteristic performance data obtained by using the established method and other pre-concentration techniques for determination of EDCs in environment water samples

Method	Analyte	LOD (μg L^{-1})	RSD (%)	EF	Sample volume (mL)	Reference
HF-LLME-GC/MS	APs, CPs and BPA	0.012–0.026	5.9–13.9	162	10	Basheer & Lee (2004)
LLLME-HPLC	APs, BPA	0.017–0.0048	2.0–5.8	3–432	14	Lin et al. (2011)
DLLME-HPLC-MS/MS	APs, BPA	0.005–0.03[a]	9.0–19.0	–	30	Salgueiro-González et al. (2012)
HF-LLME-HPLC/UV	BPA,E1, E2,DES	0.03–0.10	7.22–9.00	2,086–5,240	50	The established method

[a]MQL

Table 3 | Concentrations of four EDCs in water samples collected from Neijiang River by the proposed method ($n = 3$)

Water sample no.	Average concentration ($\mu g\ L^{-1}$)			
	BPA	E2	E1	DES
1	N.D.	1.79	N.D.	N.D.
2	0.40	N.D.	N.D.	N.D.
3	0.92	N.D.	N.D.	1.10
4	N.D.	N.D.	N.D.	N.D.
5	0.45	N.D.	N.D.	N.D.
6	0.43	N.D.	N.D.	N.D.
7	0.25	N.D.	N.D.	N.D.
8	N.D.	N.D.	N.D.	N.D.
9	N.D.	N.D.	N.D.	N.D
10	N.D.	N.D.	N.D.	N.D
11	N.D.	N.D.	N.D.	N.D
12	0.34	N.D.	N.D.	1.42
13	N.D.	N.D.	N.D.	N.D
14	N.D.	N.D.	N.D.	N.D
15	N.D.	N.D.	N.D.	N.D

N.D.: not detected.

REFERENCES

Andersen, S., Halvorsen, T. G., Pedersen-Bjergaard, S. & Rasmussen, K. E. 2002 Liquid-phase microextraction combined with capillary electrophoresis, a promising tool for the determination of chiral drugs in biological matrices. *Journal of Chromatography A* **963** (1–2), 303–312.

Baghdadi, M. & Shemirani, F. 2009 In situ solvent formation microextraction based on ionic liquids: a novel sample preparation technique for determination of inorganic species in saline solutions. *Analytica Chimica Acta* **634** (2), 186–191.

Basheer, C. & Lee, H. K. 2004 Analysis of endocrine disrupting alkylphenols, chlorophenols and bisphenol-A using hollow fiber-protected liquid-phase microextraction coupled with injection port-derivatization gas chromatography–mass spectrometry. *Journal of Chromatography A* **1057**, 163–169.

Basheer, C., Alnedhary, A. A., Rao, B. S. M. & Lee, H. K. 2007 Determination of organophosphorous pesticides in wastewater samples using binary-solvent liquid-phase microextraction and solid-phase microextraction: A comparative study. *Analytica Chimica Acta* **605** (2), 147–152.

Berthod, A., Ruiz-Ángel, M. J. & Carda-Broch, S. 2008 Ionic liquids in separation techniques. *Journal of Chromatography A* **1184** (1–2), 6–18.

Chen, B. B., Huang, Y. L., He, M. & Hu, B. 2013 Hollow fiber liquid-liquid-liquid microextraction combined with high performance liquid chromatography-ultraviolet detection for the determination of various environmental estrogens in environmental and biological samples. *Journal of Chromatography A* **1305**, 17–26.

Ciocan, C. M., Cubero-Leon, E., Peck, M. R., Langston, W. J., Pope, N., Minier, C. & Rotchell, J. M. 2012 Intersex in Scrobicularia plana: Transcriptomic Analysis Reveals Novel Genes Involved in Endocrine Disruption. *Environmental Science and Technology* **46** (23), 12936–12942.

Coors, A., Jones, P. D., Glesy, J. P. & Ratte, H. T. 2004 Assessing the elimination of estrogenic activity in advanced wastewater treatment with a reporter gene-based bioassay. *Water Science and Technology* **50** (5), 181–188.

Fu, L. Y., Liu, X. J., Hu, J., Zhao, X. N., Wang, H. L. & Wang, X. D. 2009 Application of dispersive liquid–liquid microextraction for the analysis of triazophos and carbaryl pesticides in water and fruit juice samples. *Analytica Chimica Acta* **632** (2), 289–295.

He, L. J., Luo, X. L., Jiang, X. M. & Qu, L. B. 2010 A new 1,3-dibutylimidazolium hexafluorophosphate ionic liquid-based dispersive liquid–liquid microextraction to determine organophosphorus pesticides in water and fruit samples by high-performance liquid chromatography. *Journal of Chromatography A* **1217** (31), 5013–5020.

Ho, T. S., Vasskog, T., Anderssen, T., Jensen, E., Rasmussen, K. E. & Pedersen-Bjergaard, S. 2007 25,000-fold pre-concentration in a single step with liquid-phase microextraction. *Analytica Chimica Acta* **592** (1), 1–8.

Holmes, M., Kumar, A., Shareef, A., Doan, H., Stuetz, R. & Kookana, R. 2010 Fate of indicator endocrine disrupting chemicals in sewage during treatment and polishing for non-potable reuse. *Water Science and Technology* **62** (6), 1416–1423.

Jiang, L., Hu, X. L., Yin, D. Q., Zhang, H. C. & Yu, Z. Y. 2011 Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China. *Chemosphere* **82** (6), 822–828.

Klecka, G. M., Staples, C. A., Clark, K. E., Van der Hoeven, N., Thomas, D. E. & Hentges, S. G. 2009 Exposure analysis of bisphenol a in surface water systems in North America and Europe. *Environmental Science & Technology* **43** (16), 6145–6150.

Koyama, J., Kitoh, A., Nakai, M., Kohno, K., Tanaka, H. & Uno, S. 2013 Relative contribution of endocrine-disrupting chemicals to the estrogenic potency of marine sediments of Osaka Bay, Japan. *Water Air and Soil Pollution* **224** (5), 1570–1578.

Lin, C. Y., Fuh, M. R. & Huan, S. D. 2011 Application of liquid–liquid–liquid microextraction and high-performance liquid

chromatography for the determination of alkylphenols and bisphenol-A in water. *Journal of Separation Science* **34**, 428–435.

Lu, G. H., Song, W. T., Wang, C. & Yan, Z. H. 2010 Assessment of in vivo estrogenic response and the identification of environmental estrogens in the Yangtze River (Nanjing section). *Chemosphere* **80** (9), 982–990.

Meeker, J. D. 2010 Exposure to environmental endocrine disrupting compounds and men's health. *Maturitas* **66** (3), 236–241.

Niels, J., Ana, S., Susana, G. O., Carlos, M. B., Hans-Peter, E. K. & Walter, G. 2010 Occurrence and sources of selected phenolic endocrine disruptors in Ria de Aveiro, Portugal. *Environmental Science and Pollution Research* **17** (4), 834–843.

Pedersen-Bjergaard, S. & Rasmussen, K. E. 1999 Liquid-liquid-liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis. *Analytical Chemistry* **71**, 2650–2656.

Peng, X. Z., Yu, Y. Y., Tang, C. M., Tan, J. H., Huang, Q. X. & Wang, Z. D. 2008 Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. *Science of the Total Environment* **397** (1–3), 158–166.

Psillakis, E. & Kalogerakis, N. 2002 Developments in single-drop microextraction. *Trends in Analytical Chemistry* **21** (1), 53–63.

Ribeiro, C., Pardal, M. Â., Martinho, F., Margalho, R., Tiritan, M. E., Rocha, E. & Rocha, M. J. 2009 Distribution of endocrine disruptors in the Mondego River estuary, Portugal. *Environmental Monitoring and Assessment* **149** (1–4), 183–193.

Salgueiro-González, N., Concha-Graña, E., Turnes-Carou, I., Muniategui-Lorenzo, S., López-Mahía, P. & Prada-Rodríguez, D. 2012 Determination of alkylphenols and bisphenol A in seawater samples by dispersive liquid–liquid microextraction and liquid chromatography tandem mass spectrometry for compliance with environmental quality standards. *Journal of Chromatography A* **1223**, 1–8.

Stevenson, F. J. 1994 *Humus Chemistry: Genesis, Composition, Reactions*. 2nd edn, Wiley-VCH, New York.

Sun, S. H., Xie, J. P., Xie, F. W. & Zong, Y. L. 2008 Determination of volatile organic acids in oriental tobacco by needle-based derivatization headspace liquid-phase microextraction coupled to gas chromatography/mass spectrometry. *Journal of Chromatography A* **1179** (2), 89–95.

Tan, X. W., Song, Y. X., Wei, R. P. & Yi, G. Y. 2012 Determination of trace bisphenol a in water using three-phase hollow fiber liquid-phase microextraction coupled with high performance liquid chromatography. *Chinese Journal of Analytical Chemistry* **40** (9), 1409–1414.

Villar-Navarro, M., Ramos-Payán, M., Fernández-Torres, R., Callejón-Mochón, M. & Bello-López, M. Á. 2013 A novel application of three phase hollow fiber based liquid phase microextraction (HF-LPME) for the HPLC determination of two endocrine disrupting compounds (EDCs), n-octylphenol and n-nonylphenol, in environmental waters. *Science of the Total Environment* **443**, 1–6.

Wang, L., Ying, G. G., Zhao, J. L., Liu, S., Yang, B., Zhou, L. G., Tao, R. & Su, H. C. 2011 Assessing estrogenic activity in surface water and sediment of the Liao River system in northeast China using combined chemical and biological tools. *Environmental Pollution* **159** (1), 148–156.

Wang, L. Y., Zhang, X. H. & Tam, N. F. Y. 2010 Analysis and occurrence of typical endocrine-disrupting chemicals in three sewage treatment plants. *Water Science and Technology* **62** (11), 2501–2509.

Xu, J., Liang, P. & Zhang, T. Z. 2007 Dynamic liquid-phase microextraction of three phthalate esters from water samples and determination by gas chromatography. *Analytica Chimica Acta* **597** (1), 1–5.

Zeng, G. M., Zhang, C., Huang, G. H., Yu, J., Wang, Q., Li, J. B., Xi, B. D. & Liu, H. L. 2006 Adsorption behavior of bisphenol A on sediments in Xiangjiang River, Central-south China. *Chemosphere* **65** (9), 1490–1499.

Zhao, L. M. & Lee, H. K. 2002 Liquid-phase microextraction combined with hollow fiber as a sample preparation technique prior to gas chromatography/mass spectrometry. *Analytical Chemistry* **74** (11), 2486–2492.