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Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography–negative chemical ionization–mass spectrometry

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ABSTRACT

An analytical method for phenolic endocrine disrupting chemicals and acidic pharmaceuticals in river water was developed using gas chromatography mass spectrometry (GC–MS) coupled with negative chemical ionization (NCI) technique, and used for the determination of these compounds in the Pearl Rivers (Liuxi, Zhujiang and Shijing Rivers). Derivatization using pentafluorobenzoyl chloride (PFBOCl) and pentafluorobenzyl bromide (PFBBr) before GC–MS analysis were applied and optimized for phenolic compounds and acidic compounds, respectively. The target compounds were analyzed for river waters from the upstream to downstream of the Pearl Rivers. Phenolic compounds 4-*tert*-octylphenol (4-t-OP), 4-nonylphenol (4-NP), bisphenol-A (BPA), estrone (E1), estradiol (E2) and triclosan (TCS) were detected at trace or low levels in the water samples from Liuxi River and Zhujiang River. Diethylstilbestrol (DES) was not detected in the Pearl Rivers. The highest concentrations of the phenolic compounds were found in Shijing River, and they were 3150 ng/L for 4-t-OP, 11,300 ng/L for 4-NP, 1040 ng/L for BPA, 79 ng/L for E1, 7.7 ng/L for E2 and 355 ng/L for TCS, respectively. Only a few acidic pharmaceuticals were detected at low concentrations in water from Liuxi River and Zhujiang River, but the highest concentrations for the acidic pharmaceuticals were also found in Shijing River. The highest concentrations detected for clofibric acid, ibuprofen, gemfibrozil, naproxen, mefenamic acid and diclofenac were 17 ng/L, 685 ng/L, 19.8 ng/L, 125 ng/L, 24.6 ng/l and 150 ng/L, respectively. The results suggest Liuxi and Zhujiang Rivers are only slightly contaminated and can be used as drinking water sources, but Shijing River is heavily polluted by the wastewater from nearby towns.

1. Introduction

Many evidences have emerged showing that some chemicals (e.g. nonylphenol, bisphenol-A and natural estrogens) in effluents and river water at certain concentrations can cause disruption to endocrine systems and can also affect hormonal control of development in aquatic organisms and wildlife (Sumpter and Jobling, 1995; Damstra et al., 2002; Hayes et al., 2002; Kirk et al., 2002; Lintelmann et al., 2003). These chemicals are often described as endocrine disrupting chemicals (EDCs). Pharmaceuticals and personal care products (PPCPs) are another group of compounds which include pharmaceutical drugs, ingredients in cosmetics, food supplements, and other personal care products, as well as their respective metabolites

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Table 1 – Details of the phenolic endocrine disrupting chemicals and their characteristic ions and retention times

Compound	Supplier	M.W.	R.T.	Ions [a]			
4-*n*-NP (I.S.)	Dr Ehrenstorfer	220	20.78	414.2	415.2		
4-t-OP	Supelco	206	17.13	400.2	401.2		
4-NP	Dr Ehrenstorfer	220	18.15	414.2	415.2		
BPA-d16 (I.S.)	Supelco	244	34.15	630.2	420.2		
BPA	Supelco	228	34.34	616.1	406.1		
E1-d4 (I.S.)	Cambridge	274	36.69	468.2	450.1		
DES	RDH	268	35.41	656.1	446.1		
E1	RDH	270	36.77	464.2	418.1		
E2	Dr Ehrenstorfer	272	49.59	660.0	661.1		
$^{13}C_{12}$-TCS (I.S.)	Cambridge	301.5	25.25	494.0	496.0	299.0	301.0
TCS	Dr Ehrenstorfer	289.5	25.26	482.0	484.0	287.0	289.0

I.S.: internal standard; M.W.: molecular weight; R.T.: retention time.
4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.
[a] The underlined ions are the ones used for quantification.

and transformation products (Daughton and Ternes, 1999). Many of the PPCPs are designed to be biologically active; therefore wide detection of these compounds in sewage effluents and in the environment has generated some concern about their potential impact on ecosystem and human health (Andersen and Sandaa, 1994; Purdom et al., 1994; Hartmann et al., 1998; Reinthaler et al., 2003). In order to assess their potential ecological and healthy impacts, it is necessary to investigate these EDCs and PPCPs in the aquatic environment.

Gas chromatography–mass spectrometry (GC–MS) coupled with electron impact ionization (EI) has previously been used in the analysis of EDCs and PPCPs (Kojima et al., 2005; Chen et al., 2006; Nakada et al., 2006; Gibson et al., 2007; Kosjek et al., 2007). In most cases, derivatization steps are needed in order to decrease the polarity of target compounds before GC–MS analysis. The silylation reagents such as bis-(Trimethylsilyl)trifluoroacetamide (BSTFA), *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) and *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetimide (MTBSTFA) were the most commonly used ones for phenolic compounds and acidic compounds coupled with GC–EI–MS analysis. For phenolic compounds, BSTFA with or without trimethylchlorosilane (TMCS) was often used (Liu et al., 2004; Tan et al., 2007), while MTBSTFA and MSTFA were usually used for acidic pharmaceuticals (Kosjek et al., 2007; Yu et al., 2007). However, more fragment ions caused by EI could potentially decrease the response of quantification ions; hence the sensitivity of target compounds could decrease too. Serious matrix interferences were reported when analyzing wastewater and surface water (Tan et al., 2007; Ying et al., 2008). An alternative technique is using chemical ionization to analyze some EDCs and PPCPs to improve detection limits and reduce matrix interferences (Xiao and McCalley, 2000; Kuch and Ballschmiter, 2001; Nakamura et al., 2001; Boitsov et al., 2007). NCI is especially suited because of its selectivity and sensitivity, compared to both EI and PCI. However, it is always a challenge to develop an analytical method to analyze simultaneously a range of compounds with different physiochemical properties at trace levels.

EDCs and PPCPs have been detected in sewage effluents and in aquatic environments at concentrations ranging from ng/L to μg/L (Ternes, 1998; Kuch and Ballschmiter, 2001; Heberer, 2002a,b; Kolpin et al., 2002; Kosjek et al., 2007; Nakada et al., 2006; Nebot et al., 2007; Tan et al., 2007; Ying and Kookana, 2007; Ying et al., 2008). Commonly reported EDCs in waste waters include xenoestrogen compounds such as 4-*tert*-octylphenol (4-t-OP), 4-nonylphenol (4-NP) and Bisphenol-A (BPA), synthetical estrogens such as diethylstilbestrol (DES) and natural estrogens mainly estrone (E1) and estradiol (E2). Various PPCPs detected in wastewater include nonsteroidal anti-inflammatory drugs such as diclofenac and ibuprofen, lipid-regulating agents such as gemfibrozil and clofibric acid, and disinfectants such as triclosan (TCS).

Pearl River Delta is one of the most developed and populated regions in China. With the rapid development of industry and agriculture, the water quality of the Pearl Rivers has been deteriorated in the last years due to discharge of treated and untreated domestic and industrial wastewaters (Li et al., 2007). The Pearl Rivers are the important drinking water sources for the city of Guangzhou and surrounding towns. There have been only few reports on EDCs and PPCPs in the Pearl Rivers (Duan et al., 2004; Chen et al., 2006; Peng et al., 2006 and 2008). Peng et al. (2006) reported 4-NP and BPA in sediments of Pearl River estuary at concentrations of 660 ng/g and 4 ng/g, respectively. Chen et al. (2006) detected alkylphenols with concentrations of 628 ng/L for 4-NP and 68 ng/L for 4-t-OP in water from the downstream of Pearl River. Antibiotics have also been reported in wastewaters from sewage treatment plants in Hong Kong and Guangzhou (Richardson et al., 2005; Xu et al., 2007). Unfortunately, there has been no detailed investigation into various estrogens, nonsteroidal anti-inflammatory drugs and disinfectants in surface water of the Pearl Rivers used as drinking water sources.

The objectives of this study are to develop simultaneous extraction and analysis methods for two groups of emerging contaminants (phenolic and acidic compounds) in aqueous samples and to apply the methods for the determination of these target compounds in surface water of the Pearl Rivers (Liuxi River, Zhujiang River and Shijing River) using gas chromatography–mass spectrometry with negative chemical ionization (GC–NCI–MS). The first group of phenolic compounds includes seven estrogenic phenolic compounds 4-t-OP, 4-NP, BPA, TCS, E1, E2 and DES, and the second group includes 14 acidic compounds: acidic pharmaceuticals (clofibric acid, ibuprofen, gemfibrozil, fenoprofen, naproxen, ketoprofen, mefenamic acid, tolfenamic acid, diclofenac, meclofenamic acid and indomethacin) and three acidic herbicides (MCPA, 2,4-D and bentazone). The water quality of the Pearl Rivers as drinking water sources was also assessed by analyzing the

Table 2 – Details of the acidic pharmaceuticals and herbicides and their characteristic ions and retention times

Compound	Supplier	M.W.	R.T.	Ions [a]		
Mecoprop (I.S.)	RDH	214	22.84	213.1	214.1	215.1
Clofibric acid	Dr Ehrenstorfer	214	20.99	213.1	215.1	214.1
Ibuprofen	Dr Ehrenstorfer	206	21.98	205.2	206.2	
2,4-DCPA (S.A.)	Dr Ehrenstorfer	204	23.97	203.0	205.0	207.0
MCPA	Dr Ehrenstorfer	200	25.47	199.0	201.0	200.0
2,4-D	Dr Ehrenstorfer	220	27.54	219.0	221.0	223.0
Fenoprop (I.S.)	RDH	267.5	28.30	266.7	268.7	270.6
Gemfibrozil	Dr Ehrenstorfer	250	28.85	249.2	250.2	
Bentazone	Dr Ehrenstorfer	240	29.93	239.1	240.1	241.1
Fenoprofen	Dr Ehrenstorfer	242	30.48	241.1	242.1	
Naproxen	Dr Ehrenstorfer	230	32.25	229.1	230.1	
Ketoprofen	Dr Ehrenstorfer	254	34.86	253.1	254.1	
Mefenamic acid	Dr Ehrenstorfer	241	34.71	240.1	241.1	
Tolfenamic acid	Dr Ehrenstorfer	261	36.49	260.1	262.1	261.1
Diclofenac	Dr Ehrenstorfer	295	36.76	294.0	296.0	298.0
Meclofenamic acid	Sigma	295	38.40	294.0	296.0	298.0
Indomethacin	Dr Ehrenstorfer	357	47.32	356.1	358.1	357.1

I.S.: internal standard; S.A.: surrogate standard; M.W.: molecular weight; R.T.: retention time.
2,4-DCPA: 2,4-Dichlorophenoxyacetic acid.
[a] The underlined ions are the ones used for quantification.

distribution and levels of these EDCs and PPCPs in the surface waters.

2. Experimental

2.1. Chemicals and materials

High purity standards of seven phenolic compounds, eleven acidic pharmaceuticals and three herbicides as well as the recovery standard 2,4-dichlorophenylacetic acid (2,4-DCPA) and internal standards 4-*n*-nonylphenol (4-*n*-NP), [$^2H_{16}$]bisphenol-A (BPA-d_{16}), estrone-2,4,16,16-D_4 (E1-d_4), ^{13}C-labelled triclosan ($^{13}C_{12}$-TCS), mecoprop and fenoprop were purchased from Dr. Ehrenstorfer GmbH (Germany), Supelco (USA), Riedel-de Haën (RDH, Germany), Sigma-Aldrich (USA) or Cambridge Isotope Laboratories Incorporation (Massachusetts, USA). Detailed information of the target compounds, surrogate standard and internal standards are listed in Tables 1 and 2. The derivatization reagents pentafluorobenzoyl chloride (PFBOCl, purity >99%) and pentafluorobenzyl bromide (PFBBr, purity >99%) were obtained from Aldrich and Fluka, respectively. HPLC-grade methanol, triethylamine (TEA), acetonitrile, *n*-hexane, toluene, dichloromethane (DCM) and pyridine were purchased from Merck Corporation (Shanghai, China). The cartridges used for solid phase extraction (SPE) were Oasis HLB cartridges (*N*-vinylpyrrolidone and divinylbenzene copolymer, 500 mg, 6 mL) which were obtained from Waters Corporation (Milford, MA, USA).

Stock solution of individual compounds as well as surrogate and internal standards were prepared in methanol at the concentrations of 100 mg/L in amber glass bottles, and stored at −18 °C for later use. Mixture standards of the target compounds were serially diluted with methanol. 2% (V/V) of PFBOCl and 10% (V/V) of PFBBr were prepared by diluting pure derivatization reagents with toluene. The diluted derivatization reagents were kept in a glass desiccator to prevent the quality deterioration caused by moisture in air.

2.2. Sampling sites

Pearl River system is quite complex with the river nets usually interweaved with each other (Fig. 1). Usually, the river flows through Guangzhou city with a population of about 12 million is called Zhujiang River. Zhujiang River splits into two main streams at the turning point of Bai'etan, which merge into one river at Huangpu Port after flowing through Guangzhou city. East River merges with Zhujiang River and flows south into the South China Sea. Liuxi River starts from Liuxi Reservoir, which is located to the northeast of Guangzhou city. Liuxi River flows through Conghua city in southwest direction and is connected with Zhujiang River. Shijing is a small tributary stream connected with Zhujiang River. All three rivers (Liuxi, Zhujiang and Shijing Rivers) are important drinking water sources for metropolitan Guangzhou city and surrounding towns.

The reservoir water at the upstream of Liuxi River was selected as the control site (S0), which was also used as spike water for recovery tests. Water samples were collected at 3 sites of Liuxi River (S1–3), 7 sites of Zhujiang River (S4–10) and 4 sites of Shijing River (S11–14) (Fig. 1). Generally, grab samples (1 L) in amber glass bottles were taken from 0.5 m below the water surface. Two parallel samples were collected from each site. About 50 mL of methanol was added to each bottle and the pH was adjusted to 3 using 4 M H_2SO_4 in the field. Water samples were transported in coolers to the laboratory and stored in a cold room at 4 °C. The collected water samples were normally processed within 48 h using solid phase extraction (SPE). All water samples were collected in November and December, 2007, which were the dry season in South China.

2.3. Solid phase extraction

Solid phase extraction (SPE) was used to extract water samples. Briefly, 1 L of water samples was filtered through glass fiber filters (Whatman GF/F, 0.7 μm effective pore size, UK). Exactly 100 μL each of 1 mg/L of 4-*n*-NP, BPA-d16, E1-d4, $^{13}C_{12}$-TCS, mecoprop and fenoprop was added to each sample as the

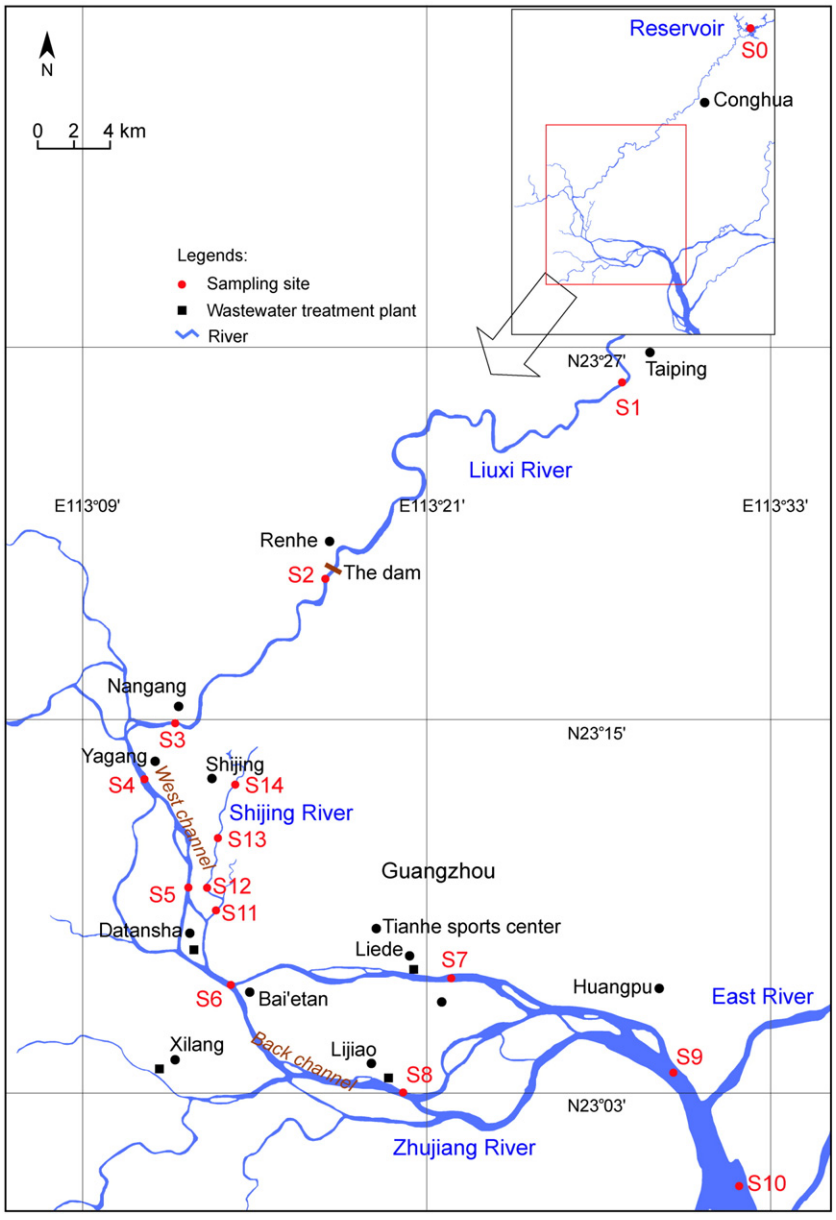

Fig. 1 – Location of sampling sites in the Pearl Rivers, South China.

internal standards, while 100 μL each of 1 mg/L 2,4-DCPA was added as the surrogate. Solid phase extraction cartridge (Oasis HLB, 6 mL, 500 mg) was conditioned by using 10 mL of methanol followed by 10 mL of re-distilled water. The filtered water samples passed through the pre-conditioned SPE cartridges at an approximate speed of 5 to 10 mL/min. The sample bottle was rinsed twice with two aliquots of 50 mL of 5% (v/v) methanol in re-distilled water, which passed through the cartridge. After passing through the air for at least 1 h, the target compounds were eluted from the cartridges using 7 mL methanol and 5 mL dichloromethane. The extracts were mixed and dried under a gentle nitrogen stream, then redissolved in 1 mL of methanol. Each final extract was then filtered through a 0.45 μm membrane filter into a 2 mL amber glass vial. The vials were kept at −18 °C until analysis.

The water from Liuxi Reservoir was collected and used for the recovery tests. Three spike concentrations of 5 ng/L, 100 ng/L and 200 ng/L were used with four replicates. The internal standards and surrogates were also added just after filtration to control all extraction process. The steps for solid phase extraction were the same as described above. Two blanks were carried out simultaneously. Recoveries of each target compound were calculated by comparing measured concentrations by internal standard methods to the spike concentrations.

2.4. *Derivatization*

2.4.1. *Derivatization of phenolic compounds*

The derivatization method for the phenolic compounds was developed based on the information in the literature using pentafluorobenzoylation (Kuch and Ballschmiter, 2001; Xiao et al., 2001; Boitsov et al., 2004). The steps used in the derivatization were introduced briefly as follows. First, 100 μL of an extract in methanol was transferred to a 10 mL glass tube (KIMAX, USA)

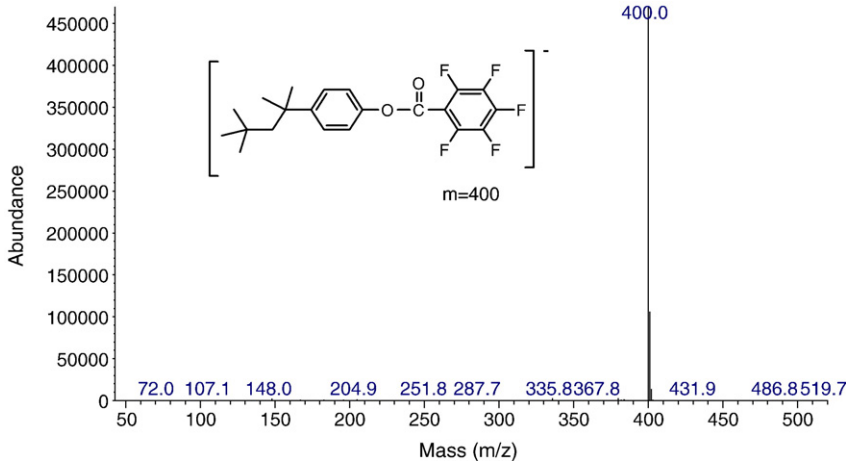

Fig. 2 – The mass spectrum of the pentafluorobenzoyl derivative of 4-t-octylphenol in negative chemical ionization (NCI) mode.

with polytetrafluoroethylene (PTFE) screw cap. Then the solvent methanol was dried under a gentle nitrogen stream. Two mL of 1 M $NaHCO_3$ aqueous solution and 1 mL of 1 M NaOH aqueous solution were added to the tube, which was vortex mixed for 10 s to dissolve the extract well. Two mL of *n*-hexane, 50 μL of 10% pyridine in toluene and 50 μL of 2% PFBOCl in toluene were added in sequence. The tube was tightly capped and manually shaken violently for 1 min, then left at the room temperature for 30 min. Then, the supernatant of *n*-hexane phase was transferred carefully to a 5 mL glass centrifugal tube using a glass pipette. Another 2 mL of *n*-hexane was added to the 10 mL tube again, and manually shaken for 1 min. After separated, the supernatant was transferred to the 5 mL glass centrifugal tube and combined with the previous one. The *n*-hexane mixture was dried under a gentle nitrogen stream. The final extract was re-dissolved in 100 μL of *n*-hexane, which was transferred to a 2 mL amber glass vial with a 250 μL flat-bottomed insert. Finally, the sample was ready for GC–NCI–MS analysis.

2.4.2. *Derivatization of acidic compounds*

The derivatization method for acid compounds was modified from the literature (Reddersen and Heberer, 2003). Briefly, 100 μL of an extract in methanol was transferred to a 2 mL vial with a 250 μL flat-bottomed insert tube. The methanol was dried under a gentle nitrogen stream. Then, 40 μL of toluene, 40 μL of 10%TEA in toluene and 20 μL of 10% PFBBr in toluene were added. The vial was well capped and mixed well on a vortex mixer. The vial was placed in a heating block and reacted for 60 min at 100 °C. The vial cap was fastened and mixed again after reaction for 15 min for fear that the solvent might volatilize from the vial. Then, the vial was cooled to the room temperature and the solvent in the vial was dried again under a gentle nitrogen stream. Finally, the derivatized sample was re-dissolved in 100 μL of toluene and ready for GC–NCI–MS analysis.

2.5. *Analytical procedure*

The target compounds were analyzed using an Agilent 6890N gas chromatograph (Agilent, USA) connected to an Agilent 5975B MSD mass spectrometer with a chemical ionization (CI) source. Separation of the compounds was achieved by using a DB35-MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (J&W, USA). Helium was used as the carrier gas and maintained at a constant flow rate of 1.0 mL/min for the analysis of both phenolic

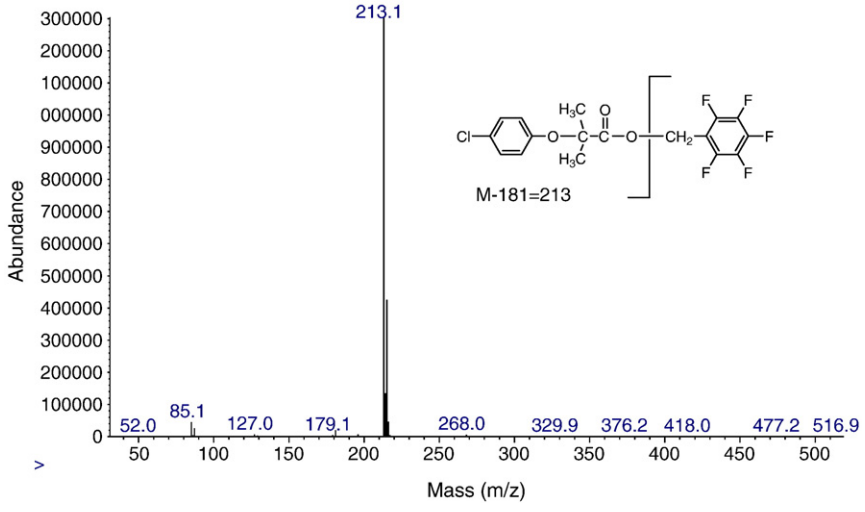

Fig. 3 – The mass spectrum of the pentafluorobenzyl derivative of clofibric acid in negative chemical ionization (NCI) mode.

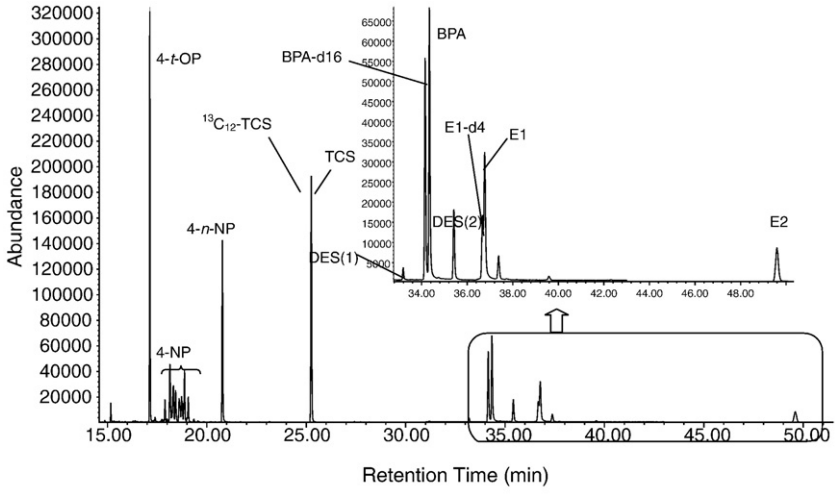

Fig. 4 – The total ion current chromatogram of the phenolic compound under the selected ion mode (SIM) of GC–NCI–MS. The concentration for each compound was 100 ng/mL. 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.

compounds and acidic compounds. Negative chemical ionization (NCI) mode was applied with methane (a purity >99.999%) as the reaction gas in the CI source. The flow rate of methane was always kept at 2.0 mL/min. The ion source temperature and the quadrupole temperature were both kept at 150 °C during the analysis when using NCI mode.

For the analysis of phenolic compounds, a sample volume of 2 μL was injected in splitless mode at an inlet temperature of 300 °C. The column temperature was programmed as follows: from 80 °C (1 min) to 220 °C at 10 °C/min, from 220 to 260 °C at 4 °C/min, and from 260 to 300 °C (8 min) at 5 °C/min, then to the temperature 310 °C (15 min) at 20 °C/min. The MS interface temperature was maintained at 310 °C.

For the analysis of acidic pharmaceuticals and herbicides, a sample volume of 2 μL was injected in splitless mode at an inlet temperature of 260 °C. The column temperature was programmed as follows: from 100 (1 min) to 150 °C (2 min) at 20 °C/min, from 150 to 205 °C (1 min) at 3 °C/min, and from 205 to 260 °C (5 min) at 10 °C/min, then at the same rate to 280 °C (3 min), and to the final temperature 310 °C at 15 °C/min, hold 10 min. The MS interface temperature was kept at 280 °C.

Quantitative analysis was carried out using selected ion monitoring (SIM) mode. The characteristic ions selected for the phenolic compounds and acidic compounds were listed in Tables 1 and 2. Data analysis was carried out on Agilent dada analysis software MSD ChemStation D.03.00.611.

3. Results

3.1. *Mass spectra in GC–NCI–MS*

The derivatives of the selected phenolic and acidic compounds were analyzed using negative ionization mode in GC–

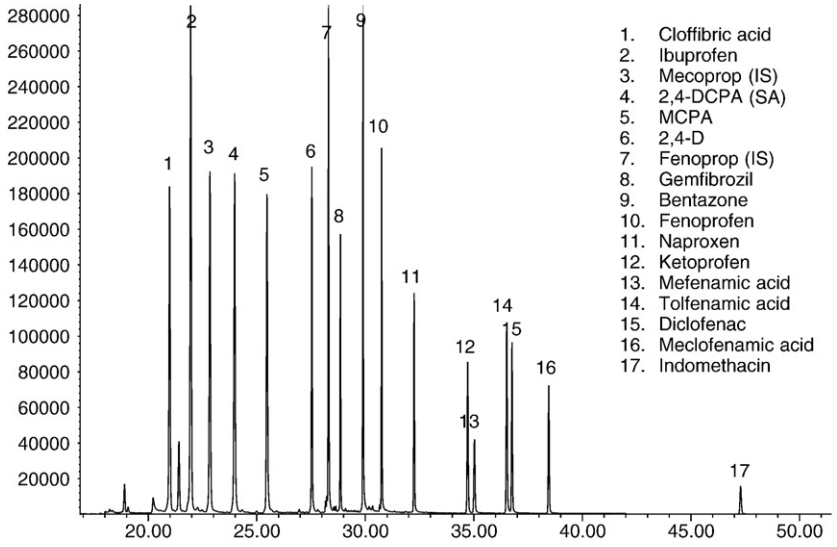

Fig. 5 – The total ion current chromatogram of the acidic compounds under the selected ion mode (SIM) of GC–NCI–MS. The concentration for each compound was 100 ng/mL.

MS. Similar NCI ionization patterns were observed to those in the literature (Kuch and Ballschmiter, 2001; Reddersen and Heberer, 2003). Derivatives of the phenolic compounds had intact molecular ion peak $[M]^-$ as the dominant ion in mass spectra, while derivatives of the acidic compounds broke the ester bond by removing the pentafluorobenzyl group and had the fragment ion peak of acidic molecular fragment ion $[M-181]^-$. The mass spectra of 4-t-OP and clofibric acid are shown here as two examples (Figs. 2 and 3). The total ion current (TIC) chromatograms of the phenolic compounds and acidic compounds were shown in Figs. 4 and 5, respectively.

Negative chemical ionization (NCI) in GC–MS gives soft ionization with less fragmentation than positive EI or CI because of the lower electron energy used in the analysis, thus has higher sensitivity. The dominant ions observed in NCI mass spectra were M^- or negative ions with a specific group being lost. Meanwhile, the ^{13}C isotopic ion or ^{37}Cl isotopic ion of the dominant ion often presents as the second most intensive ion. Thus, for the quantitative analysis these characteristic ions were chosen as the quantitative ion and qualifier ions, respectively (Tables 1 and 2).

A simultaneous derivatization step for phenolic compounds and acidic pharmaceuticals was reported by heating in an alkali solution using PFBBr, with the ester formed for acidic compounds and ether formed for phenolic compounds (Nakamura et al., 2001; Möder et al., 2007). However, perfluorotolyl derivatives of phenolic compounds had worse instrumental responses in GC–NCI–MS than their pentafluorobenzyl derivatizatives formed by using PFBOCl (Xiao and McCalley, 2000). Therefore, PFBOCl was chosen as the derivatization reagent for the phenolic compounds, and PFBBr were chosen as the derivatization reagent for the acidic compounds in the study. The phenolic hydroxy group (including other hydroxy groups in molecular structures) in phenolic compounds formed ester with PFBOCl through acylation reaction, while the carboxyl group in acidic compounds also formed ester with PFBBr through esterification reaction.

3.2. *Derivatization optimization*

Derivatization method for the phenolic compounds was optimized in the present study. $NaHCO_3$ and NaOH buffer solution with pH at 9.2 was selected as derivatization reaction

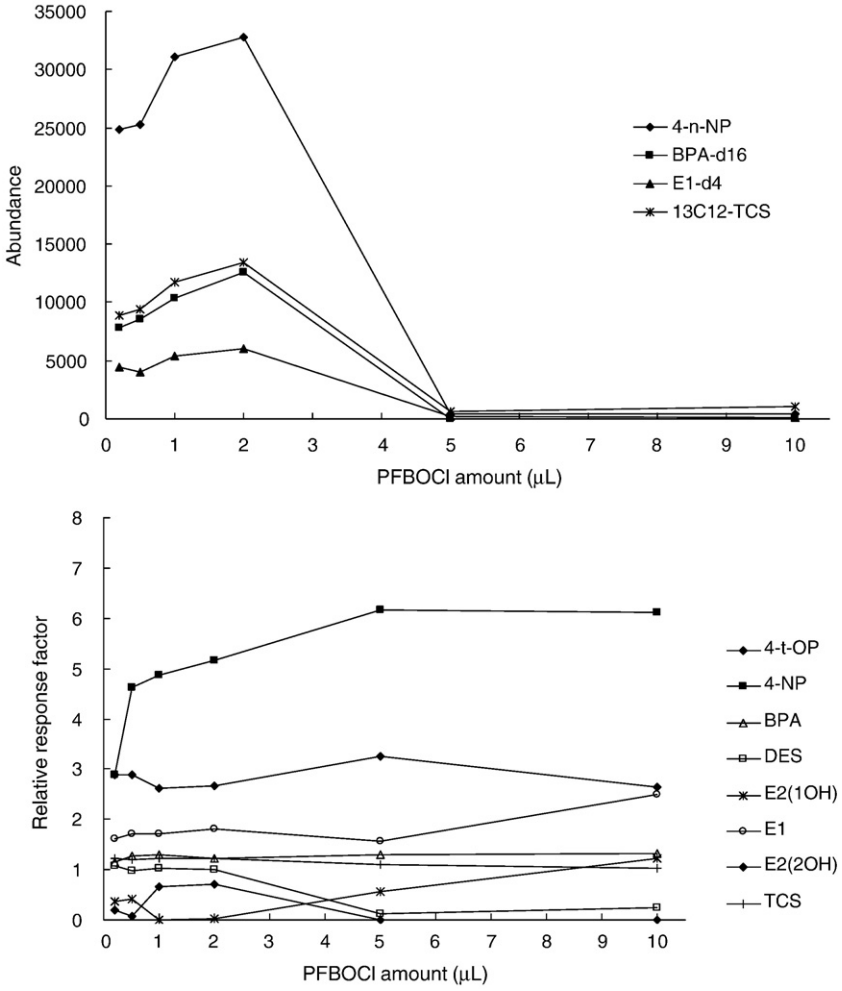

Fig. 6 – The abundance of internal standards and relative response factor (RRF) of the phenolic compounds versus the amounts of PFBOCl used in derivatization. The amount for each compound was 10 ng. E2 (1OH) represents the derivative with only one hydroxy group derivatized and E2 (2OH) represents the derivative with two hydroxy groups derivatized. 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.

Table 3 – Recoveries (%) and limit of detection of phenolic compounds

Compounds [a]	Spiked concentrations [b]			LOD (ng/L) [c]	LOQ (ng/L) [c]
	5 ng/L	100 ng/L	200 ng/L		
4-t-OP	99±4	75±4	74±11.4	0.3	1.0
4-NP	92±22	115±13	74±5	2.0	7.0
BPA	118±8	103±1	105±3	0.7	2.0
DES	73±4	71±3	71±2	0.2	0.5
E1	86±4	96±5	90±8	0.2	0.5
E2	135±12	145±5	163±34	0.3	1.0
TCS	105±7	98±2	97±3	0.2	0.5

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.
[b] Mean ± standard deviation (n=4).
[c] LOD: limit of detection; LOQ: limit of quantitation.

medium, and extraction step was carried out to removed superfluous PFBOCl and to avoid the interference from PFBOCl in matrix which was observed in the previous studies (Xiao and McCalley, 2000; Kuch and Ballschmiter, 2001; Xiao et al., 2001; Boitsov et al., 2004).

In most case, compounds with one hydroxy group (4-t-OP, 4-NP, 4-*n*-NP, TCS, $^{13}C_{12}$-TCS, E1 and E1-d_4) and some with two hydroxy groups (BPA, BPA-d16 and DES) would be derivatized to their corresponding pentafluorobenzoyl derivatives on all of the hydroxy groups. But for E2, two forms of the derivatives could be formed without addition of pyridine. One form was the derivative with only one hydroxy group derivatized (at retention time of 36.5 min and its dominant ion at m/z 466), and the other form was the derivative with both hydroxy groups derivatized completely (at retention time of 50 min and its dominant ion at *m*/z 660). Pyridine could promote the derivatization reaction of the phenolic compounds and enhance the responses of the derivatives. E2 was fully converted into a derivative with its two hydroxyl groups derivatized under the catalysis of pyridine. Therefore, a fixed amount of 5 μL (50 μL of 10% pyridine in toluene) was used in the derivatization of the phenolic compounds.

The responses of all phenolic compounds were also related to the amounts of PFBOCl used. The responses to different amount of PFBOCl added in the reaction were shown in Fig. 6. The relative response factor (RRF) defined by Zhang et al. (2006) was used to examine the appropriate amount of PFBOCl. Interestingly, the responses of all compounds decreased to almost zero when the amount of PFBOCl added was more than 2 μL. The reason behind this could be that a larger amount of PFBOCl increased the concentrations of Na^+ and Cl^- in the reaction medium, which made the extraction efficiency decrease dramatically. The concentrations of Na^+ and Cl^- increased due to the reaction of superfluous PFBOCl with NaOH in the medium. The inhibition effect by NaCl on extraction was confirmed by adding NaCl into the reaction medium on the condition that the amount of PFBOCl was 1 μL. Thus, the amount of PFBOCl added should not exceed 2 μL. Fig. 6 also showed that the RRFs for most compounds reached stable conditions when 1 μL of PFBOCl was used, and E2 with two hydroxy groups was also derivatized completely under this condition. Therefore, 1 μL of PFBOCl (50 μL of 2% PFBOCl in toluene) was used in the derivatization of the phenolic compounds.

The derivatization method for acidic compounds produced good responses for all acidic pharmaceuticals and herbicides in chromatograms (Fig. 5). Superfluous PFBBr left in vials after derivatization also can be removed by extraction as described of phenolic compounds, but unlike PFBOCl, PFBBr left in the vial almost has no interference with the target compounds during GC–NCI–MS analysis, so a further extraction step was not carried out in this study.

3.3. *Validation of the methods*

A simultaneous extraction of the phenolic EDCs and acidic pharmaceuticals and herbicides was developed using Oasis HLB cartridges. The SPE method generated very good recoveries for these compounds (Tables 3 and 4). Among the phenolic compounds analyzed, BPA, 4-t-OP, 4-NP, DES, E1 and TCS had good recoveries varying between 70% and 120% for the three concentrations, except for E2 having higher recoveries (>120%). The recoveries for most acidic pharmaceuticals and herbicides ranged between 70% and 120% at the spike concentrations of 5 ng/L, 100 ng/L and 200 ng/L with several compounds having recoveries lower than 70% for ibuprofen, 2,4-D, ketoprofen and indomethacin when spiked at a concentration of 5 ng/L.

The limit of detection (LOD) and limit of quantitation (LOQ) of the target compounds were calculated based on the standard derivations (SD) of seven replicates of spiked reservoir water at the concentration of 5 ng/L. LOD is defined as three times of SD, and LOQ is 10 times of SD. The results of LOD and LOQ for the two groups of compounds were listed in Tables 3 and 4, respectively. From the data, it can be seen that the analytical methods for the phenolic compounds and acidic pharmaceuticals and herbicides were very sensitive and could detect as low as a few ng/L for most target compounds in water.

Table 4 – Recoveries (%) and limit of detection of acidic pharmaceuticals

Compounds	Spike concentrations [a]			LOD (ng/L) [b]	LOQ (ng/L) [b]
	5 ng/L	100 ng/L	200 ng/L		
Clofibric acid	91±7	99±3	102±3	1.0	3.3
Ibuprofen	44±4	84±3	88±5	0.7	2.2
MCPA	88±4	95±2	98±4	0.8	2.6
2,4-D	57±2	96±3	98±3	0.2	0.7
Gemfibrozil	84±14	95±5	99±4	1.8	6.1
Bentazone	100±4	101±1	102±2	1.5	5.0
Fenoprofen	88±8	100±2	101±3	1.4	4.7
Naproxen	91±6	107±3	107±2	1.3	4.2
Ketoprofen	60±8	67±6	73±4	1.2	4.1
Mefenamic acid	127±15	139±4	145±5	2.2	7.5
Tolfenamic acid	114±8	104±4	103±6	0.8	2.8
Diclofenac	109±9	111±1	109±3	1.1	3.6
Meclofenamic acid	95±7	102±2	103±2	1.1	4.4
Indomethacin	43±5	76±7	78±5	1.0	3.3

[a] Mean ± standard deviation (n=4).
[b] LOD: limit of detection; LOQ: limit of quantitation.

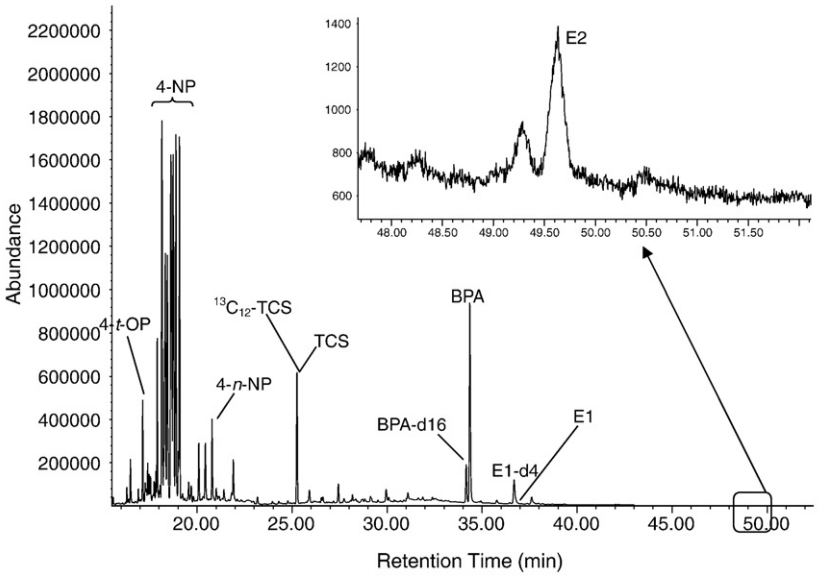

Fig. 7 – The total ion current chromatogram of phenolic compounds at sampling site 6. The internal standards were at the concentrations of 100 ng/L. 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.

3.4. *Analysis of river water*

The analytical method developed in the study was applied in the determination of the phenolic EDCs and acidic pharmaceuticals in surface water from the Pearl Rivers (Liuxi River, Zhujiang River and Shijing River). The chromatograms of the phenolic compounds and acidic pharmaceuticals from the sampling site S6 were shown in Figs. 7 and 8, respectively. The phenolic compounds detected in S6 exhibited a good chromatogram with little interference from matrix, while acidic pharmaceuticals suffered from some matrix interference. The concentrations for the phenolic compounds and acidic compounds in the surface water samples from S0 to S14 were listed in Tables 5 and 6. The water samples from Liuxi Reservoir had little contamination of the target compounds, with only trace levels of 4-t-OP, 4-NP, BPA and TCS.

4-t-OP, 4-NP, BPA and TCS were detected in all of the 15 sampling sites, while E1 was detected in all sites excepting the reservoir site (S0). E2 was detected in waters only from some of the sampling sites. DES was not detected in any sample. The highest concentrations for 4-t-OP, 4-NP, BPA, E1, E2 and TCS in water from Liuxi River were 7.1 ng/L, 269 ng/L, 140 ng/L, 3.1 ng/L, 2.1 ng/L and 12.4 ng/L, respectively, while those in Zhujiang River were 14.6 ng/L, 496 ng/L, 215 ng/L, 3.5 ng/L, 1.3 ng/L and

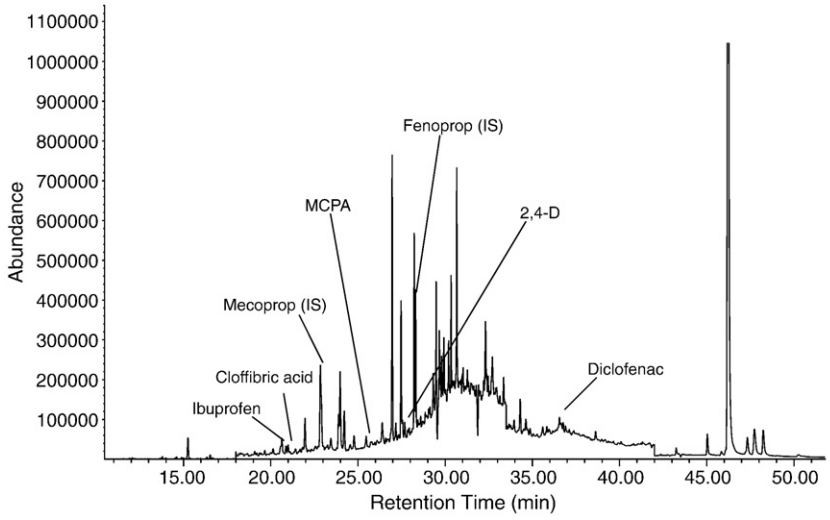

Fig. 8 – The total ion current chromatogram of acidic compounds at sampling site 6. The internal standards were at the concentrations of 100 ng/L.

26 ng/L, respectively. In Shijing River (site 11–site 14), the detected concentrations for most phenolic compounds were much higher, except for E2 being detected only at site S11 with a concentration of 7.5 ng/L. The highest concentrations were found in the upstream of Shijing River, and they were 3150 ng/L for 4-t-OP, 11300 ng/L for 4-NP, 1040 ng/L for BPA, 79 ng/L for E1, 7.7 ng/L for E2 and 355 ng/L for TCS, respectively.

Only some of the acidic compounds were detected in water from the Pearl Rivers. The acidic pharmaceuticals detected in the Pearl Rivers were clofibric acid, ibuprofen, gemfibrozil, naproxen, mefenamic acid and diclofenac. The distribution pattern of the acidic pharmaceuticals was similar to the phenolic compounds, with the maximum concentrations being detected in the water samples of Shijing River. The maximum concentrations detected for clofibric acid, ibuprofen, gemfibrozil, naproxen, mefenamic acid and diclofenac were 17 ng/L, 685 ng/L, 19.8 ng/L, 125 ng/L, 24.6 ng/l and 150 ng/L, respectively. In addition to the acidic pharmaceuticals, two acidic herbicides MCPA and 2,4-D were also found in the Pearl Rivers at very low concentrations. The herbicide 2,4-D was only observed in water from Zhujiang River, not found in Liuxi River and Shijing River.

4. Discussion

Liuxi River, the west channel and back channel of Zhujiang River are important drinking water sources for Guangzhou city and surrounding towns. Liuxi River and Zhujiang River were classified as level "II" and level "III" (slightly contaminated water) according to the Chinese national standards (China, 2002). The present study found no or trace amounts of the phenolic and acidic compounds in the water samples from Liuxi River and Zhujiang River. Detection of two herbicides suggests application of these two chemicals in the catchment; they could be used for the control of weeds in agricultural land and urban area. From the data (Tables 5 and 6), it is obvious that Liuxi Reservior with little human activities is the cleanest site among the three rivers. The levels of the phenolic compounds slightly increased from the upstream (S0) to the downstream (S3) of Liuxi River. This suggests possible contamination of domestic wastewater in the downstream of Liuxi River. It is also reflected in the detection of three drugs clofibric acid, ibuprofen and diclofenac at the site S3 as these human drugs are mainly from domestic sewage (Boyd et al., 2003; Gibson et al., 2007; Nakada et al., 2006).

Estrogens (E1 and E2) and xenoestrogens (4-t-OP, 4-NP and BPA as well as TCS) were detected in all sites of Zhujiang River. The main sources of these compounds are sewage effluents (Ying and Kookana, 2007; Ying et al., 2002a,b, 2008). In fact, Zhujiang River receives discharge of treated effluents from four municipal wastewater treatment plants in Guangzhou. The annual discharge of treated effluents was estimated to be more than 700 million tonnes (Kuang et al., 2006). However, the tidal wave has a significant influence on the water quality of Zhujiang River, and the water in the section of Guangzhou city is diluted by the water from the downstream through tidal action (Wang et al., 1997). In general, the levels of the phenolic compounds and acidic drugs found in the water samples of Liuxi and Zhujiang Rivers are comparable to those found in surface waters of other countries in the literature (Table 7).

Table 5 – Concentrations of phenolic compounds in surface water from the Pearl Rivers

Sites	Phenolic compounds (ng/L) [d]					
	4-t-OP	4-NP	BPA	E1	E2	TCS
S0	1.0±0.04 [a]	28.1±2.1	2.2±0.7	ND [b]	ND	0.6±0.0
S1	3.7±0.04	185±7	8.2±0.7	3.0±0.2	2.0±0.1	7.9±0.2
S2	2.7±0.4	66.9±3.0	43.5±3.5	0.7±0.1	<LOQ [c]	8.3±0.2
S3	6.9±0.3	244±35	132±11	3.0±0.0	0.9±0.1	12.4±0.1
S4	7.9±0.5	241±2	132±10	1.4±0.1	0.7±0.0	9.3±1.5
S5	12.8±2.5	481±21	166±6	3.3±0.3	1.3±0.0	22.3±0.1
S6	6.5±1.3	249±28	104±8	1.9±0.3	1.2±0.1	17.3±2.3
S7	6.6±0.4	290±12	164±9	2.6±0.0	1.0±0.1	23.7±3.7
S8	9.6±0.8	222±4	123±5	1.7±0.1	<LOQ	14.7±0.5
S9	6.8±0.3	207±5	109±1	1.6±0.3	<LOQ	13.1±0.3
S10	5.7±0.1	221±2	211±7	1.9±0.1	<LOQ	6.6±0.3
S11	65.5±1.3	1800±29	412±10	20.1±0.3	7.5±0.4	111±2
S12	195±24	3220±342	811±3	43.3±1.2	ND	202±11
S13	312±8	4600±226	832±64	49.1±3.4	ND	288±6
S14	2470±968	8890±3410	1030±21	75.0±5.3	ND	347±12

[a] Mean±standard deviation (n=4, replicate samples taken at the same time).
[b] ND: not detected.
[c] <LOQ: below the limit of quantitation.
[d] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol; DES: diethylstilbestrol.

Shijing River was also a drinking water resource for Guangzhou before 2000, but it has been heavily polluted in recent years due to the discharge of untreated domestic and industrial wastewater from the nearby towns along the river. The annual discharge of wastewater into the small river amounts to more than 100 million tonnes, of which is mainly domestic wastewater (Luo, 2002). The water quality was classified as level "V" (most polluted water) (China, 2002; Luo, 2002). In fact, the water quality parameters such as chemical oxygen demand (COD), NH_3-nitrogen, total phosphorus and dissolved oxygen (DO) were 2–12 times higher than the regulation standards for level "V" water (Li et al., 2008). The detected concentrations of EDCs and PPCPs also reflected its heavy contamination. Concentrations of the phenolic compounds (4-t-OP, 4-NP, BPA, TCS and E1) and some anti-inflammatory drugs (ibuprofen, naproxen and diclofenac) in Shijing River were very high, especially in the upstream of Shijing River. Since most of the phenolic compounds and acidic drugs were originated from wastewater, this study suggests that Shijing River is heavily contaminated by untreated wastewater from the nearby towns. The levels of the phenolic and acidic compounds in

Table 6 – Concentrations of acidic compounds in surface water from the Pearl Rivers

Sites	Acidic compounds in river water (ng/L)							
	Clofibric acid	Ibuprofen	MCPA	2,4-D	Gemfi Brozil	Naproxen	Mefenamic acid	Diclofenac
S0	ND	ND[b]	ND	ND	ND	ND	ND	ND
S1	4.8±0.4[a]	<LOQ[c]	ND	ND	ND	ND	ND	ND
S2	ND	ND	18.8±0.5	ND	ND	ND	ND	ND
S3	6.1±1.8	10.7±0.8	19.4±1.1	ND	ND	ND	ND	10.1±1.4
S4	10.1±4.0	13.1±1.4	17.8±5.8	ND	ND	ND	ND	8.4±0.8
S5	14.4±0.4	30.8±0.4	19.2±1.4	ND	ND	ND	<LOQ	25.1±0.2
S6	12.3±4.0	18.4±3.7	14.7±1.2	6.0±0.2	ND	ND	<LOQ	25.5±1.5
S7	16.6±2.5	20.3±5.6	8.9±1.3	5.9±1.3	ND	ND	<LOQ	30.0±3.8
S8	12.3±0.2	15.1±0.5	12.6±0.8	6.2±0.9	ND	ND	<LOQ	16.6±1.0
S9	9.7±2.2	6.1±0.8	7.1±1.2	5.7±1.0	ND	ND	<LOQ	11.2±1.5
S10	8.6±2.1	4.9±1.1	8.6±0.9	5.6±1.0	ND	ND	<LOQ	9.4±1.3
S11	<LOQ	171±6	20.0±0.2	ND	ND	24.7±3.8	11.6±1.3	58.5±0.8
S12	<LOQ	490±275	30.1±12	ND	17.4±3.5	42.9±9.7	17.7±1.8	105±14
S13	<LOQ	293±114	23.1±0.3	ND	14.4±0.6	55.2±4.8	22.4±3.1	116±1
S14	<LOQ	477±98	29.5±1.3	ND	ND	118±10.1	11.4±4.4	147±5

[a] Mean±standard deviation (n=4, replicate samples taken at the same time).
[b] ND: not detected.
[c] <LOQ: below the limit of quantitation.

Shijing River were much higher than those in surface waters of other countries (Table 7). Such high levels of these EDCs and PPCPs might affect the organisms living in the river, and might also have human health impacts if exposed to the water.

The phenolic compounds analyzed in the present study are estrogenic compounds. These estrogenic compounds have been suspected to be responsible for the estrogenic activity and fish reproductive disorders in rivers receiving effluent discharges (Aerni et al., 2004; Folmar et al., 1996; Nakada et al., 2004; Sumpter, 1995). The reported lowest observed effective concentration (LOEC) values for estrogenic responses in medaka, trout and roach are 10 ng/L for E1 and E2 (Metcalfe et al., 2001; Routledge et al., 1998). Expected estrogenicity for each compound in a water sample, expressed as 17β-estradiol

Table 7 – Comparison of the concentrations (ng/L) of EDCs and PPCPs in surface water with other regions since 2000

Compounds[a]	Asia					Europe		America
	Liuxi (S0–S3)	Zhujiang (S4–S10)	Shijing (S11–S14)	Japan	Korea	Germany	U.K.	USA
4-t-OP	3.3 (7.1)[b]	7.2(14.6)	259(3151)	24(47)	ND[c]	3.8(54)	0.3–30	
4-NP	124 (269)	256(496)	3950 (11296)	92(144)	137(327)	234(433)	<0.2	800
BPA	24.9 (140)	133(215)	811(1040)	57(150)	45(213)	156(417)	0.1–12	0–147
TCS	8.1 (12.4)	14.7(26.2)	247(356)		ND			8.8–26.3
E1	2.9(3.1)	1.8(3.5)	45.4(78.7)	34(47)	3.6	0.4(4)	7(17)	0–4.7
E2	0.9(2.1)	0.9(1.3)	ND(7.7)	3.5(7.7)	ND	0.3(3.6)	3(7.1)	0–4.5
Clofibric acid	2.3(7.3)	12.3(18.3)	ND			15(119)	ND	ND
Ibuprofen	1.0 (11.3)	15.1(31.1)	335(685)	50(180)	28	6.8(34)	ND	ND
Gemfibrozil	ND	ND	7.0(19.8)		6.6	5.9(35)	ND	51(107)
Naproxen	ND	ND	50.8(125)	50(120)	11	15.8(93)	ND	
Diclofenac	ND (11.3)	12.6(32.6)	115(150)		3.0	32.4(245)	ND	
References	This study	This study	This study	Furuichi et al., 2004; Kojima et al., 2005; Nakada et al., 2006	Ko et al., 2007; Kim et al., 2007	Kuch and Ballschmiter, 2001; Möder et al., 2007	Xiao et al., 2001; Zhang et al., 2006; Nebot et al., 2007	Kolpin et al., 2002; Boyd et al., 2003; Zhang et al., 2007.

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone; E2: estradiol.
[b] Median concentration (maximum concentration).
[c] ND: not detected.

equivalency (EEQ), can be calculated from the chemical analysis data and relative potency of the compound from *in vitro* yeast estrogen screen bioassay (Johnson and Sumpter, 2001). Based on the worst case scenario, the total highest EEQ for the water samples from Liuxi River, Zhujiang River and Shijing River were 3.7 ng/L, 3.1 ng/L and 48 ng/L, respectively. Therefore, these estrogenic compounds in water of Shijing River could have potential estrogenic effects on organisms in the river.

5. Conclusions

A sensitive analytical method was developed for determination of some phenolic endocrine disrupting chemicals and acidic pharmaceuticals as well as acidic herbicides in aquatic environment using gas chromatography–mass spectrometry coupled with negative chemical ionization technique. The target compounds (phenolic and acidic compounds) were extracted simultaneously using solid phase extraction. Then the extracts were converted into pentafluorobenzoyl derivatives for the phenolic compounds and into pentafluorobenzyl derivatives for the acidic compounds, respectively. Appropriate amounts of pyridine and pentafluorobenzoyl chloride needed for derivatization of the phenolic compounds were optimized. The developed method gave good recoveries for most target compounds and sensitive detection with LOQ at ng/L levels. The analytical methods were successfully applied to the determination of these compounds in the Pearl Rivers (Liuxi River, Zhujiang River and Shijing River). Liuxi and Zhujiang Rivers contained relatively lower levels of these target compounds. However, much higher concentrations of the phenolic estrogens and acidic drugs were found in the water samples from the sites of Shijing River. This study demonstrates that Shijing River is heavily polluted by wastewater from the nearby towns. Further studies are needed to investigate the potential ecological and healthy effects on organisms living in the river.

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