Determining estrogenic steroids in Taipei waters and removal in drinking water treatment using high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry

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Abstract

River water and wastewater treatment plant (WWTP) effluents from metropolitan Taipei, Taiwan were tested for the presence of the pollutants estrone (E_1), estriol (E_3), 17β -estradiol (E_2), and 17α -ethinylestradiol (EE_2) using a new methodology that involves high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry. The method was also used to investigate the removal of the analytes by conventional drinking water treatment processes. Without adjusting the pH, we extracted 1-L samples with PolarPlus C_{18} Speedisks under a flow rate exceeding 100 mL/min, in which six samples could be done simultaneously using an extraction station. The adsorbent was washed with 40% methanol/60% water and then eluted by 50% methanol/50% dichloromethane. The eluate was concentrated until almost dry and was reconstituted by 20 μ L of methanol. Quantitation was done by LC-MS/MS-negative electrospray ionization in the selected reaction monitoring mode with isotope-dilution techniques. The mobile phase was 10 mM *N*-methylmorpholine aqueous solution/acetonitrile with gradient elution. Mean recoveries of spiked Milli-Q water were 65–79% and precisions were within 2–20% of the tested concentrations (5.0–200 ng/L). The method was validated with spiked upstream river water; precisions were most within 10% of the tested concentrations (10–100 ng/L) with most RSDs < 10%. LODs of the environmental matrixes were 0.78–7.65 ng/L. A pre-filtration step before solid-phase extraction may significantly influence the measurement of E_1 and EE_2 concentrations; disk overloading by water matrix may also impact analyte recoveries along with ion suppression. In the Taipei water study, the four steroid estrogens were detected in river samples (ca. 15 ng/L for E_2 and EE_2 and 35–45 ng/L for E_1 and E_3). Average levels of 19–26 ng/L for E_1 , E_2 , and EE_2 were detected in most wastewater effluents, while only a single effluent sample contained E_3 . The higher level in the river was likely caused by the discharge of untreated human and farming waste into the water. In the drinking water treatment simulations, coagulation removed 20–50% of the estrogens. An increased dose of aluminum sulfate did not improve the performance. Despite the reactive phenolic moiety in the analytes, the steroids were decreased only 20–44% of the initial concentrations in pre- or post-chlorination. Rapid filtration, with crushed anthracite playing a major role, took out more than 84% of the estrogens. Except for E_3 , the whole procedure successfully removed most of the estrogens even if the initial concentration reached levels as high as 500 ng/L.

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1. Introduction

Feminizing pollutants 17 α -ethinylestradiol (EE₂) from oral contraceptives, 17 β -estradiol (E₂), as well as their metabolites estrone (E₁) and estriol (E₃) have been detected at ng/L levels in natural water and wastewater treatment plants (WWTP) influents/effluents (Desbrow et al., 1998; Ying et al., 2002). Even at these low concentrations, these pollutants can adversely affect production of vitellogenin in male fish and the sex ratio of fish populations (Jobling et al., 1998; Vethaak et al., 2005). Glucuronides or sulfates of these chemicals can be deconjugated by microorganisms in WWTP or the environment, and form biologically-active parent compounds (Desbrow et al., 1998; Ternes et al., 1999).

The most extensively used method to detect trace amounts of estrogenic steroids in water is solid-phase extraction (SPE) coupled to GC–MS/(MS), and detection limits can reach below 1 ng/L (Quintana et al., 2004; Xiao et al., 2001). However, the chemical derivatization process needed to prepare the analytes for injection into GC is time-consuming and labor-intensive. Recently, solid-phase microextraction (SPME) with on-fiber derivatization has been reported to accelerate this process (Carpinteiro et al., 2004). Another analytical equipment, LC-MS/MS, is as sensitive as GC–MS and does not require as much pre-treatment (Benijts et al., 2004; Vanderford et al., 2003).

There are some problems involved in the detection of E₁, E₂, EE₂, and E₃ in water. Due to the weak acidity of the four chemical pollutants, water samples need to be acidified for better retention of the analytes for reversed-phase SPE (Isobe et al., 2003; Quintana et al., 2004). This acidification, however, may co-extract more humic or fulvic acids and interfere with the analysis. In addition, a disk-type adsorbent, which allows much faster flow than a cartridge, has obvious benefits when it is necessary to extract a large volume of water.

The possibility that particulates may bind fractions of these moderately hydrophobic chemicals has not been adequately studied in water treatment systems (Snyder et al., 2003; Ying et al., 2002). Moreover, to avoid blocking the adsorbent, the currently-used SPE procedure pre-filters water samples before extraction to eliminate particulates and may underestimate the total concentration of the estrogens.

There is little information about the removal of estrogenic steroids when treating drinking water. Because of the phenolic moieties of the estrogens, oxidation can reduce the estrogenic activities (Deborde et al., 2004; Westerhoff et al., 2005). They have been reported to react more rapidly with ozone than with

chlorination (Alum et al., 2004; Zwiener and Richardson, 2005). On the contrary, Itoh et al. (2000) found a 2.3-fold increase in estrogenic effect in lake water after it was chlorinated. While estrogens have been removed efficiently by such advanced treatments as activated carbon, manganese oxide, and reverse osmosis (Westerhoff et al., 2005), it is not well established how efficiently they are removed by more conventional processes such as coagulation/sedimentation and filtration. Finally, some of the studies investigating the removal of estrogens from drinking water started with initial concentrations at tens of μ g/L, concentrations much higher than usually found in the environment and may, therefore, not be relevant (Carlile et al., 1996; Lee et al., 2004).

In this paper, we report a faster and easier method for quantifying the low E₁, E₂, EE₂, and E₃ levels typically found in water samples using LC-MS/MS with isotope-dilution techniques. Without adjusting the pH, river water, WWTP effluents and treated drinking water were extracted by a disk-type adsorbent at a flow rate greater than 100 mL/min. We found the detection limits of this method for these three matrixes to be 0.8–7.7, 1.3–7.2 and 0.06–0.15 ng/L, respectively. Pre-filtration of particulates before SPE might cause the underestimation of the total concentrations of E₁ and EE₂; disk overloading by the water matrix influenced the analyte recoveries in addition to ion suppression. We also investigated the removal of the analytes in drinking water treatment processes and found that rapid filtration eliminated much more analytes than other water treatment units, while both chlorination and coagulation/sedimentation removed between 20 and 40%. Conventional drinking water treatment removed over 88% of E₁, E₂, and EE₂, but less E₃.

2. Materials and methods

2.1. Chemicals

Estrone, estriol, 17 β -estradiol, and 17 α -ethinylestradiol were purchased from Sigma (Saint Louis, MO, USA; purity >98%). 3,4-¹³C₂-17 β -estradiol was obtained from Cambridge Isotope Laboratories (Andover, MA, USA; purity 99%). 2,4,16,16-²D₄-estrone, 2,4,17-²D₃-estriol, and 2,4,16,16-²D₄-17 α -ethinylestradiol were bought from C/D/N Isotope (Pointe-Claire, Quebec, Canada; purity 98%). Solvents (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). *N*-methylmorpholine was provided by Aldrich (Saint Louis, MO, USA; purity >99.5%). All standards were prepared in methanol and stored at 4 °C.

2.2. Sample collection

Grab samples from the Dan-Shui River, the major river flowing through Taipei City, were collected from the Taipei Bridge (E 121°30.433 and N 25°03.829) during June and September, 2004. Effluents of Nei-Hu WWTP in Taipei City (a secondary treatment facility with activated sludge units and a capacity of 240,000 CMD (cubic meter per day)) were collected during July and October, 2004. The samples had pH between 6.3 and 6.6 and temperature between 26 and 29 °C. Samples were sealed in 1-L pre-cleaned amber glass bottles with Teflon-liner caps and were stored at 4 °C until analysis; 1% formaldehyde was added as preservative (Fine et al., 2003; Petrovic and Barcelo, 2000). Twenty ng of each stable isotope-labeled internal standard (IS) were spiked into a 1-L sample, which was filtered using 90-mm PVDF (polyvinylidene fluoride) filter (pore size 0.45 µm). The sample was shaken for 30 min at 130 rpm before extraction.

Milli-Q water was used as a matrix for analytical method optimization. The raw water source of Chang-Hsing drinking water treatment plant (WTP) from Sin-Dian Creek, which supplies drinking water for most of the population of Taipei City, was used as the matrix for

method validation and for investigating the removal of estrogenic steroids from the water treatment processes. One-liter filtered samples were spiked with 100 or 500 ng of native compounds and with 50 ng of each IS; the samples were shaken as described above before following treatment. The raw water only contained trace levels of analytes that were relatively insignificant to the spiked amount. All the sampling sites are shown in Fig. 1.

2.3. Simulation of drinking water treatments

During the pre-chlorination, 1 L of spiked raw water (pH 7.2–7.6) was shaken with 1 mg/L sodium hypochlorite for 10 min at 110 rpm. The turbidities of samples after treatment were all lower than 10 NTU (measured by a Hach 2100P turbidimeter, Loveland, CO, USA). Coagulations were conducted as jar tests using a six-place gang stirrer (Fargo Model MS-121); 1 L of spiked raw water was subjected to 1 min of rapid mixing (100 rpm) with aluminum sulfate octadecahydrate (alum, 5 mg/L), 30-min flocculation at 20 rpm, and 30-min settling while the pH was controlled at 6.2–7.1. The supernatant was collected for analysis.

Rapid filtration simulated the design of Shui-An Rapid Sand Filter using a Wheeler filter bottom. A 150-

Fig. 1. Sampling locations of Taipei Bridge (A), Nei-Hu WWTP (B) and Chang-Hsing WTP (C). The black spot (D) is the intake for the WTP. The discharge of the WWTP enters the nearby Chi-Lung River. The yellow line indicates the area of the Taipei City. The map was modified from the web sites of Taiwan EPA (<http://edb.epa.gov.tw/gis/func1/frame.asp> and http://www.epa.gov.tw/attachment_file/).

cm glass column (10-mm i.d.) was packed with 30-cm filter gravel (grain size 2–50 mm), 51-cm filter sand (grain size 0.3–1.2 mm) and 25-cm crushed anthracite

(grain size 0.6–2.4 mm). The flow rate was controlled at 20 mL/min (flow velocity $\approx$ 296 m/day) using a Masterflex HV-77601-00 I/P-tubing pump (Vernon

Fig. 2. The chromatogram of the analytes and their internal standards (SRM Mode; m/z : precursor ion $\rightarrow$ product ion; DT: dwelling time; CE: collision energy; injection 4- μ L standard of 10 ng/ μ L E_3 and E_2 and 5 ng/ μ L of E_1 and EE_2).

Hills, IL, USA). One liter of raw water was pre-chlorinated and coagulated, and then spiked and treated. Additional 15-mL Milli-Q water was used to flush the column and was collected together with the spiked sample for analyzing estrogens.

The post-chlorination procedure (at pH 6.8–7.1) was similar to that of pre-chlorination; however, the raw water was treated through pre-chlorination, coagulation and rapid filtration before spiking the analytes. Free chlorine residuals were 0.5–0.7 ppm (measured by a Hach pocket colorimeter) after the treatment. The entire experimental process treated 1-L spiked raw water through the four steps as described above.

2.4. Extraction and analysis

Samples were passed through 50-mm Bakerbond PolarPlus C18 Speedisks (J.T. Baker, Phillipsburg, NJ, USA, conditioned with 10 mL of 50% methanol/50% dichloromethane (v:v), methanol, and Milli-Q water sequentially) at a flow rate of 110–130 mL/min. Six samples could be handled simultaneously using a Diskmate II rotary extraction station (J.T. Baker). After extraction, the sample reservoirs were rinsed with 20 mL of Milli-Q water; the disks were flushed with the rinsed water, followed by 10 mL of 40% methanol/60% water (v:v). The disks were moved to a vacuum manifold (J.T. Baker) and dried for 10 min using a vacuum system (about 25 kPa) before being eluted with three portions of 5-mL 50% methanol/50% dichloromethane. The collected eluate was filtered through 25 mm PTFE syringe filters (pore size 0.2- μ m), combined with two rinses of 0.5-mL methanol, and concentrated to barely dry at 45 °C in a 1.8-mL vial by a vacuum centrifuge (Thermo Savant SPD 1010 SpeedVac, Holbrook, NY, USA). Residues were reconstituted with 20 μ L of methanol and 4 μ L was injected.

A DU 2001 in-line solvent degasser (Sanwa Tsusho, Tokyo, Japan) and a Hitachi L-7100 HPLC pump (Tokyo, Japan) were coupled to a Thermo Finnigan TSQ 7000 triple-quadrupole mass spectrometer in negative electrospray ionization (ESI). The voltages of source, capillary, and tube lens were 4.51 kV, –52 V and –143 V, respectively. The capillary temperature was 250 °C. A BetaBasic C18 column was used for HPLC (150 $\times$ 2.1 mm, 3 μ m, Hypersil–Keystone, Bellefonte, PA, USA) and the temperature was kept at 60 °C using an ECO-1 column oven (Analab, Taipei, Taiwan). Eluent A was acetonitrile and eluent B was 10 mM aqueous *N*-methylmorpholine in Milli-Q water (pH 9.6) at a flow rate of 0.2 mL/min. The elution started with 10% of eluent A, followed by a 2-min gradient to 60%

of eluent A and a 10.5-min gradient to 100% of eluent A and then kept 100% for one more minute.

Data were obtained by selected reaction monitoring (SRM). The collision gas was argon and the cell pressure was 3 mTorr. The electron multiplier voltage was 1740 V. The sheath gas and auxiliary gas were nitrogen at 70 PSI and 20 PSI, respectively. There were two sections in a run: only the channels of E_3 and E_3 - 2D_3 were monitored at the first 11 min (one data point every 1.1 s); the retention time of E_2 , EE_2 , and E_1 was too close and had to be monitored together with their IS during the remaining 2.5 min (2.4 s/cycle). With the dwelling time described in Fig. 2, we optimized both the intensity and stability of the signals.

2.5. QA/QC, quantification, and data analysis

All glassware was rinsed with acetone, hexane, dichloromethane and methanol after it was washed and before being used for experiments. A Milli-Q water sample and two duplicates of real samples spiked with IS were run with each batch of samples to check for experimental contamination and access method reproducibility (all agreed within 16%). Isotope-dilution techniques were used to correct for variations in sample recovery and instrumental performance. Peak areas of analytes were normalized to those of their IS. Five specific concentrations of native analytes (ranging 0.05–5 ng/ μ L for E_1 and EE_2 and 0.05–10 ng/ μ L for E_2 and E_3) with a fixed 1 ng/ μ L of their stable isotope-labeled chemicals in methanol were injected for each analysis to build up a calibration curve (r^2 values were at least 0.98). Recoveries of the analytes and IS were calculated based on the standards added; therefore, both their losses in the sample preparation and matrix effects on the instrument influenced recoveries. LODs and LOQs were set at heights of threefold and tenfold over noise, respectively. Recovery differences of IS between spiking before and after filtration of water samples were compared with two-sample one-tailed Student's *t*-test. One-tailed *t*-test and One-Way ANOVA (Scheffe method) were used for comparing the removal percentage in different treatment processes.

3. Results and discussion

3.1. Analytical method development and validation

PolarPlus C18 adsorbent (14–18% carbon content, not end-capped) was able to extract the four estrogenic steroids at a high-flow rate without acidifying water samples. It performed better than the usual C18 (10–

Table 1
Quantification of the spiked Milli-Q water samples (1 L, $n=5$)

Spiked level (ng/L)	5.0	50	200 ^a
Analyte	Measured concentration (ng/L) ^b		
E ₁	4.9±1.6 (−1.4%)	39.5±9.7 (−21%)	170±36 (−15%)
E ₂	5.2±1.3 (+3.2%)	47.9±13 (−4.2%)	176±53 (−12%)
EE ₂	5.9±0.2 (+19%)	46.3±6.3 (−7.5%)	195±38 (−2.6%)
E ₃	6.0±1.2 (+21%)	52.8±9.8 (+5.5%)	233±12 (+17%)

^a $n=4$.

^b Shown as mean±SD (relative deviation from the spiked level).

14% carbon content). The C18 Speedisk cartridges (50 mg) retained 88–93% of the four chemicals from 1 mL of 16 µg/mL Milli-Q water ($n=3$), but PolarPlus C18 retained over 99% of each analyte. Moreover, no breakthrough was detected in 1-L samples using PolarPlus C18 50-mm disks containing up to 200 ng/L except for E₁ ($\approx 31\%$ was not trapped at the last 300 mL of the samples). We further investigated various solvents or their combinations (Sole et al., 2000; Xiao et al., 2001) and the best eluting solution, 15 mL of 50% methanol/50% dichloromethane, gave recoveries of E₁, E₂, EE₂, and E₃ at $95.0\pm 10.4\%$, $90.2\pm 6.9\%$, $87.7\pm 15.8\%$, and $95.1\pm 11.9\%$, respectively (mean±SD, $n=3$).

The washing process with 40% methanol/60% water, a cleanup procedure followed by Xiao et al. (2001), did not significantly reduce the recovery. The loss of E₁, E₂, EE₂ and E₃ resulting from this step were 1.7–1.9%, 0.9–1.2%, 1.4–1.6%, and 2.4–3.4%, respectively ($n=2$).

The method recovery, accuracy, and linearity were demonstrated with replicates of spiked Milli-Q water. The recoveries of E₁, E₂, EE₂, and E₃ were $75.5\pm 16.6\%$, $78.6\pm 18.0\%$, $67.8\pm 13.2\%$, and $65.2\pm 17.0\%$, respectively (spiked at 20 ng/L, mean±SD, $n=6$). Average LODs of E₁, EE₂, and E₃ were 0.16–0.24 ng/L and their method detection limits at 99% confidence level (MDL_{99%}) were 0.29–0.39 ng/L, respectively;

those of E₂ were higher (0.48 and 1.2 ng/L, respectively). The discrepancies from three spiked concentrations (5.0, 50, and 200 ng/L, $n=5$) were most within 15%, which verified the accuracy and linearity of the method (Table 1).

The method was validated with the spiked samples of raw water from Sin-Dian Creek. Average LODs and LOQs of the four chemicals were 0.47–1.3 ng/L and 1.6–4.3 ng/L, respectively (Table 2). The reported values of the spiked samples did not deduct the endogenous amount of the analytes (ND–5.6 ng/L) in the raw water; therefore, at the lowest spiked level (10 ng/L), the measured concentrations were somewhat higher than the added amount, and the RSDs were up to 23%. On the other hand, for the spiked samples at 50 and 100 ng/L, the measured levels were comparable to the fortified amount with small variations (RSDs $\leq 7\%$).

Addition of *N*-methylmorpholine to the mobile phase achieved a higher dissociation of the phenolic analytes ($pK_a \approx 10.4$) and resulted in a stronger signal in ESI without influencing the chromatography. Literature also demonstrated that addition of a weak base directly or post-column to mobile phase can increase the ESI response of estrogenic phenolic chemicals (Baronti et al., 2000; Chen et al., 2001; Johnson et al., 2000). In a neutral solution, their dissociation fraction is only about 0.04%; when the pH is elevated to pH 9.6 in the 10-mM

Table 2
Quantification of the spiked samples of raw water from Sin-Dian Creek (1 L, $n=4$)

Spiked level (ng/L)	No spike ^a	10	50	100	Mean of LOD	Mean of LOQ
Analyte		Measured concentration (ng/L) ^b			(ng/L) ^c	(ng/L) ^d
E ₁	4.18–5.57	15.2±0.9 (6.2%)	59.2±1.4 (2.4%)	116±12 (11%)	0.47	1.57
E ₂	ND–2.85	13.2±3.1 (23%)	49.5±2.9 (5.8%)	99.2±1.6 (1.7%)	0.80	2.67
EE ₂	ND	13.1±0.5 (3.4%)	47.6±3.4 (7.1%)	98.3±6.7 (6.8%)	0.55	1.83
E ₃	ND–5.20	17.9±4.1 (23%)	47.1±2.6 (5.5%)	95.2±5.7 (5.9%)	1.29	4.30

^a $n=3$; ND:<LOD.

^b Shown as mean±SD (RSD).

^c LOD: S/N ≥ 3 .

^d LOQ: S/N ≥ 10 .

Table 3

Concentrations in river water and WWTP effluents (ng/L, $n=10$)^a

Matrix	Analyte	Recovery of IS (%)	Mean of LOD	$n>\text{LOD}^b$	Min	Max	Median	Mean
River water	E ₁	35.7±6.1	0.78	10	22.4	66.2	31.1	34.7
	E ₂	23.7±12.4	3.11	10	1.40	33.9	14.5	14.4
	EE ₂	25.0±8.8	1.16	8	7.53	27.4	14.1	15.3
	E ₃	9.64±6.03	7.65	10	12.4	73.6	58.1	44.5
WWTP effluents	E ₁	53.1±22.7	1.31	10	10.2	48.6	22.7	26.0
	E ₂	21.6±10.6	7.22	8	4.50	44.5	17.8	23.0
	EE ₂	36.9±12.2	1.80	10	2.25	37.9	17.1	19.0
	E ₃	16.3±9.8	5.86	1	—	39.1	—	—

^a Samples were filtered prior to extraction.^b Number in the ten samples that were detectable ($S/N \geq 3$). Statistical data shown were calculated based on detectable samples.

N-methylmorpholine(aq), the dissociation fraction is raised to 13.7% and the signals were enhanced a lot. The authors tried a more basic ammonium hydroxide(aq) at 10 mM (pH 10.8); the peaks showed significant tailing, which may indicated less retention because of a higher dissociation.

3.2. Environmental water samples

3.2.1. Analysis of real samples

The levels of steroid estrogens in the Dan-Shui River (Table 3) were higher than most other reports on surface water (Adler et al., 2001; Mouatassim-Souali et al., 2003; Xiao et al., 2001); however, these previous investigations, which have ranged from <LOD to low ng/L levels, were from Japan and European countries, where most sewage is treated before discharge. We detected E₂ and EE₂ ≈ 15 ng/L on an average in the river water; E₁ and E₃ were in the range of 35–45 ng/L. The chromatograms of real samples (Figs. 3 and 4) indicated that background noise may interfere with the quantification at levels below 10 ng/L; however, this does not necessarily comprise the data reported because the concentrations observed were most significantly above 10 ng/L. The Dan-Shui River has been classified as seriously polluted by the Taiwan EPA (2006). About 47% of the total discharge from the basin is untreated domestic sewage and livestock-breeding wastewater, though amounts are decreasing; if based on NH₃–N measurements, the contribution of these two categories is around 79%. The DO, BOD, COD, NH₃–N, and total phosphorus during our sampling were 0.5–0.8, 2.9–9.0, 20.4–24.2, 2.81–4.96, and 0.471–0.534 mg/L, respectively (TDEP (Taipei Department of Environmental Protection), 2004), which indicated poor water quality. Since Taipei is the most crowded area in Taiwan (9649 persons/km² in Taipei vs the average number 627 in Taiwan at the end of year 2004, based on the National Statistics of Taiwan), it was not surprising to discover a higher concentration of these chemicals from our

sampling location. A USGS study (Kolpin et al., 2002a) reveals higher levels of estrogenic steroids in streams relative to most reports because they targeted sites susceptible to contamination from human, industrial, and agricultural wastewater, although the data in that study were criticized because GC/MS detection is subject to artifacts from organic matter (Ericson et al., 2002; Kolpin et al., 2002b). Kolodziej et al. (2004) also demonstrated that sources other than municipal wastewater (such as farming wastes) can contribute the level of steroid hormones in surface waters.

Previous studies indicate that EE₂ emissions are usually lower than one-tenth of E₂, but can reach equal levels in water because of persistence (Jurgens et al., 1999; Spengler et al., 2001), which is consistent with our results. We also found higher concentrations of E₁ and E₃ than E₂ and EE₂ in the river water, and this agrees with previous reports (Baronti et al., 2000; Xiao et al., 2001). E₁ is one of the major metabolites of E₂ and EE₂; it degrades slowly in the environment mainly into E₃ (Arcand-Hoy et al., 1998). Adlercreutz et al. (1994) also reported that daily urine excretions of E₁, E₂, and E₃ in oriental pre-menopausal women were 2.66, 1.09, and 5.68 μg. These would explain the higher levels of E₁ and E₃.

Concentrations of E₁, E₂, and EE₂ in WWTP effluents have varied significantly in previous investigations, which were influenced by operational conditions of WWTP, climates, and composition of influents. The medians of E₁, E₂, and EE₂ in the effluents in our investigation were 17–23 ng/L (Table 3 and Fig. 4), which are comparable with some reports (Mouatassim-Souali et al., 2003; Ying et al., 2002), but higher than those from Germany (Adler et al., 2001). E₃ was detected only in one of the ten samples (39 ng/L, LOD 5.9 ng/L). Some investigations stated that E₃ had disappeared after wastewater treatment (Quintana et al., 2004; Sole et al., 2000); conversely, Johnson and Sumpter (2001) reported a typical effluent concentration of E₃ was 20 ng/L, which was the highest among the four chemicals.

Fig. 3. The chromatogram of a river water sample (SRM Mode).

3.2.2. Matrix effects

Significant matrix effects were seen on real samples, and were more prominent in the river water samples. Use of isotope-dilution techniques is the best way to

tackle the effects (Van De Steene et al., 2006). LODs of real samples were much higher and their IS recoveries were lower (Table 3) than those of Milli-Q water. Higher background noises, ion suppression or breakthrough on

Fig. 4. The chromatogram of a WWTP effluent water sample (SRM Mode).

the disks could be the cause of this. To evaluate the matrix effect, we re-injected river water and effluent final extracts and their 10×dilutions. For E_1 , E_2 , and

EE_2 , recoveries of the diluted river water and effluent extracts were 2.3–3.7 and 1.5–2.5 times higher (mean, $n=4$) than those of original ones, respectively. The

differences in recoveries were more apparent for E_3 (7.1 and 5.0-fold, respectively), which could be explained by its earlier elution on HPLC.

The organic matter and other chemicals present in matrixes could compete for binding sites and reduce the capacity of the disks, resulting in a breakthrough of analytes (Xiao et al., 2001) and lower recoveries. Because the breakthrough tests were done using Milli-Q water, this factor cannot be excluded. To determine the possible effects of ion suppression and disk overloading, we spiked 50 ng of the four analytes into 1-L filtered raw water from Sin-Dian Creek before (set A, $n=4$) and after the extraction (set B, $n=4$). The raw water samples contained <2 ng/L of the analytes, with levels insignificant compared with the spiked amount. The recoveries of E_1 , E_2 , EE_2 , and E_3 in set B were $89.8 \pm 11.6\%$, $95.5 \pm 9.3\%$, $67.7 \pm 10.2\%$ and $66.6 \pm 9.2\%$, respectively, calculated using the analyte standards (external calibration). We found ion suppression to be considerable in EE_2 and E_3 . The recoveries of E_1 , E_2 , EE_2 , and E_3 in set A were $41.9 \pm 6.4\%$, $38.9 \pm 5.6\%$, $56.0 \pm 4.5\%$ and $39.3 \pm 2.7\%$, respectively, calculated using the recoveries of set B. The data cancelled out the ion suppression effect and revealed the influence of matrix to extraction efficiency. Compared with the recoveries in Milli-Q water, the matrix notably reduced the extraction efficiencies of E_1 , E_2 , and E_3 , though the impact was less on EE_2 , possibly because of its hydrophobicity; consequently, it would be better to use less sample volume in future studies. We demonstrated that disk overloading also influenced recoveries in addition to ion suppression for the raw water from Sin-Dian Creek, which is used as a drinking water source. However, the relative impact of these two factors on recoveries may not be the same in the Dan-Shui River water and WWTP effluents because of their inferior water quality, which, due to the high concentrations of the analytes, were not suitable for this evaluation.

3.2.3. Effects of pre-filtration of samples

Removal of particulates before extraction caused an underestimation of total concentrations of E_1 and EE_2 in river water and WWTP effluents, and the effects were stronger in river water. To avoid the clog of the adsorbent with particulates in water, there is a filtration step before SPE; therefore, existing methods actually extract soluble estrogenic steroids. To clarify the issue of particulate adsorption, we separated the river and WWTP effluent samples into two batches: one spiked with the isotope-labeled IS (20 ng/L) before filtering the particles and the other spiked after the filtration. In both matrixes, there were no significant differences in IS recoveries of E_2 and E_3 ($p=0.16$ – 0.43 , $n=10$) regard-

less of when IS was added. Furracker et al. (1999) reported that E_2 in wastewater at ng/L levels mainly remains in the aqueous phase, which is consistent with our findings. In contrast, the IS recoveries of E_1 and EE_2 spiked after filtration were significantly higher in both the river water (3–4 times higher, $p \ll 0.01$, $n=10$) and WWTP effluents (1.6- to 1.9-fold higher, $p < 0.01$, $n=10$). The water solubilities of E_1 , E_2 , and E_3 are around 13 mg/L, and that of EE_2 is only 4.8 mg/L (Xiao et al., 2001; Ying et al., 2002); the log K_{ow} of E_3 , E_2 , E_1 , and EE_2 are 2.5–2.8, 3.1–4.0, 3.1–3.4, and 3.7–4.2, respectively (Westerhoff et al., 2005; Ying et al., 2002). The relative hydrophobicity of EE_2 would explain its higher influence by particulate binding. Although the observed difference of particulate binding effect on E_1 and E_2 could not be explained based on their water solubility and log K_{ow} , the replacement of the hydroxyl group on the 17th carbon of E_2 with a ketone on E_1 may reduce the polarity of E_1 and increase the binding. Moreover, the total suspended solids in the river water (56–73 mg/L) were higher than those in the effluents (18–27 mg/L), possibly explaining the stronger effects of particulate binding in river water.

3.3. Removal of steroid estrogens from drinking water treatment

3.3.1. Chlorination

Normal chlorination processing in drinking water treatment may degrade estrogens inefficiently. Our results showed that only 20–40% of estrogenic steroids were eliminated by chlorination (Table 4). The matrix for post-chlorination contained considerably fewer particulates and organics after the pre-treatment of pre-chlorination, coagulation/sedimentation and rapid filtration. However, no significant differences in removal rates were observed between pre-chlorination and post-chlorination. One Japanese study (Snyder et al., 2003) also reported on the ineffectiveness of chlorination. Our tested concentrations of analytes did not significantly impact the removal rates except for E_2 in pre-chlorination and EE_2 in post-chlorination. There was no significant difference in the percentage removed among individual analytes, except for E_2 , which was degraded more than E_3 at 500 ng/L in pre-chlorination ($p=0.03$).

Some studies have demonstrated rapid or complete consumption of estrogenic steroids by chlorination; however, these studies were done with Milli-Q water or buffer solutions (Hu et al., 2003; Moriyama et al., 2004), used increased doses of total chlorine (Deborde et al., 2004), elongated contact time (Westerhoff et al., 2005) or utilized much higher initial concentrations of analytes

Table 4

Percentage removal in drinking water treatment processes (mean $\pm$ SD, $n=4$)

Process	Initial concentration (ng/L)	Estrone (E ₁)	17 β -estradiol (E ₂)	17 α -ethinyl estradiol (EE ₂)	Estriol (E ₃)
Pre-chlorination	100	33.4 $\pm$ 9.2	19.1 $\pm$ 16.5 ^a	23.7 $\pm$ 5.1	28.0 $\pm$ 7.0
	500	34.8 $\pm$ 5.6	39.5 $\pm$ 4.8 ^b	28.3 $\pm$ 10.7	23.2 $\pm$ 6.0
Coagulation/sedimentation	100	37.3 $\pm$ 14.9	52.1 $\pm$ 4.1 ^{a, c}	17.3 $\pm$ 15.3	26.7 $\pm$ 9.2
	500	29.8 $\pm$ 3.0	35.7 $\pm$ 10.8	27.1 $\pm$ 7.9	20.0 $\pm$ 7.5
Rapid filtration	100	94.1 $\pm$ 5.6	96.3 $\pm$ 5.5	94.9 $\pm$ 5.4	92.4 $\pm$ 4.1
	500	98.1 $\pm$ 2.2 ^b	98.7 $\pm$ 1.0 ^b	98.4 $\pm$ 1.7 ^b	83.6 $\pm$ 7.6
Post- chlorination	100	27.7 $\pm$ 17.2	22.4 $\pm$ 14.4	44.0 $\pm$ 5.8 ^a	31.5 $\pm$ 4.8
	500	25.3 $\pm$ 10.1	35.9 $\pm$ 12.9	16.7 $\pm$ 10.2	22.3 $\pm$ 8.4
Whole process	100	94.2 $\pm$ 4.8	88.4 $\pm$ 10.1	92.9 $\pm$ 4.3	84.7 $\pm$ 6.3 ^a
	500	90.5 $\pm$ 6.7 ^b	89.3 $\pm$ 3.6 ^b	93.3 $\pm$ 1.7 ^b	64.4 $\pm$ 2.9

^a Significantly different with the removal percentage of the 500 ng/L.^b Significantly higher than E₃.^c Significantly higher than EE₂.

(Carlile et al., 1996) irrelevant to environmental levels. The enhanced removal can be explained by a lack of competing organics or by the second-order reaction of HOCl with phenolic steroid estrogens (Deborde et al., 2004), though formation of more disinfection by-products may cause another concern.

3.3.2. Coagulation and sedimentation

Alum coagulation mainly removes chemicals adsorbing to particulates. This process eliminated 20–50% of the four analytes in our study (Table 4). There were no significant differences in percentage removed among the four analytes despite their different Log K_{ow} , with the exception of E₂ which was precipitated significantly more than EE₂ at 100 ng/L ($p=0.028$), which conflicted with its hydrophobicity. Our results are consistent with previous investigations that show only relatively high- K_{ow} chemicals (e.g. $>10^5$) can be removed appreciably during coagulation (Snyder et al., 2003; Westerhoff et al., 2005).

The tested initial concentrations did not perceptibly affect the removal rates except that more E₂ was removed at 100 ng/L than that of 500 ng/L ($p=0.024$); Westerhoff and colleagues (2005) also indicated that spiked concentration did not change the removal percentage at 10–250 ng/L.

We found that filtering out particulates before SPE may reduce some E₁ and EE₂ in river water and WWTP effluents, but not in the drinking water. The low turbidity of our raw water (3.6–7.7 NTU) would explain the difference. To further clarify the issue, we treated raw water spiked at 100 ng/L with 50-mg/L alum and found an average removal percentage of 31–38% ($n=3$, SD 3.6–7.8%), showing no significant increase compared with that of 5-mg/L alum.

3.3.3. Rapid filtration

The rapid filtration process removed most estrogenic steroids and performed significantly better than all other steps ($p<0.001$). 92–98% of the analytes were eliminated excluding a lower removal of E₃ than other analytes at 500 ng/L (84%, $p=0.001$) (Table 4). The spiked concentration did not notably affect the removal percentage.

The removal was unexpectedly high if the filtration process only removed particulates and adhering chemicals. To elucidate the removal mechanism, we packed two types of columns for further investigations: in addition to 30-cm of filter gravel for support in both, one was packed with 76-cm crushed anthracite and the other with 76-cm of filter sand. For the columns packed with the anthracite, the average removal percentage at 100 ng/L was 97–98% ($n=4$, SD 1.9–2.6%), which was similar to that of the original packed filtration columns. The columns packed with filter sand eliminated 34–46% of the analytes ($n=4$, SD 5.4–6.3%). Milli-Q water spiked at 100 ng/L were also treated with the columns packed with filter sand and had a removal percentage consistent with that of raw water ($n=4$, mean 31–42%, SD 5.2–10%). Consequently, the primary mechanism behind the removal was the adsorption of the organics by the anthracite rather than the filtering out of analytes adhering to particulates. Powdered activated carbon has been shown to be a good adsorbent for estrogenic compounds in advanced treatment (Snyder et al., 2003; Westerhoff et al., 2005), and it is interesting to find that crushed anthracite in conventional rapid filtration gave a comparable performance. Surprisingly, filter sand itself removed over 30% of the analytes not only from the raw water treated through pre-chlorination and coagulation but also from the Milli-Q water, which contained almost no particulates.

3.3.4. The whole process

The whole treatment eliminated 88–94% of E_1 , E_2 , and EE_2 and the spiked levels did not apparently influence their removal percentage (Table 4); the removal rate of E_3 was similar at 100 ng/L ($84.7 \pm 6.3\%$) but was significantly lower than other analytes at 500 ng/L ($64.4 \pm 2.9\%$).

Changes in concentrations after one treatment would have some minor effect on the removal of the next unit. Based on the removal percentage of each unit, the four analytes should be almost completely removed after the whole procedure. We did not observe apparent effects of the initial spiked levels to the performance in a single unit for most analytes; however, the combination of the four steps could not remove all estrogens at our study concentrations. A possible explanation for this observation is because of the lower concentrations of estrogens in the filtration unit. Wang (1994) demonstrated that lower initial atrazine concentrations lead to lower adsorption capacity in activated carbon because of the competition of natural organic matter in the water. When the spiked estrogens went through the pre-chlorination and coagulation/sedimentation units, their concentrations would have decreased 30–50% and resulted in a lower adsorption onto the anthracite. However, further studies are necessary to elucidate this phenomenon.

No estrogenic steroids were detected in the treated water from Chang-Hsing WTP ($n=6$, LOD 0.06–0.15 ng/L). The concentrations of the four analytes in the raw water were ND–5.9 ng/L, which were much lower than those in Dan-Shui River. In the detectable water samples, the average concentrations were 1.6–2.4 ng/L. Consequently, a conventional drinking water treatment should be able to handle the low concentrations of estrogenic steroids in the water source. While investigations report the absence of estrogenic steroids after conventional treatment (Fawell et al., 2001; Rodriguez-Mozaz et al., 2004), this may be because most drinking water sources are cleaner than usual surface water. A conventional drinking water treatment cannot guarantee complete removal of the analytes in re-used water in which initial concentrations are higher.

4. Conclusion

Our study demonstrates that PolarPlus C18 adsorbent can effectively be used to extract steroid estrogens from a large volume of water under a high-flow rate with no need to acidify samples. This saves a lot of time and labor. However, extracting less volume of water is suggested for dirty samples to alleviate the matrix

effects and breakthrough. Our data indicates that pre-filtration before SPE to avoid the clog of the adsorbent might significantly influence the estimation of total E_1 and EE_2 in river water and WWTP effluents; therefore, isotope-labeled IS should be spiked before rather than after the filtration step to correct for the loss of analytes due to partitioning to particulates. The use of isotope-labeled IS would also compensate the variations of SPE efficiencies caused by matrixes; extensive cleanup will decrease ion suppression but will not reduce the competition for adsorbent binding sites from soluble organics present in water.

Rapid filtration gave the best removal percentage (over 84%); the chlorination processes did not perform as well in our study as other reports (Snyder et al., 2003). While a conventional WTP should be able to eliminate trace levels of estrogenic steroids, we observed incomplete removal at higher concentrations, especially for the more hydrophilic E_3 . Advanced treatment will be needed if reclaimed water is directly used for drinking water.

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