Talanta 89 (2012) 237–245

Contents lists available at SciVerse ScienceDirect

Talanta

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

Ultra-high performance liquid chromatography/tandem mass spectrometry determination of feminizing chemicals in river water, sediment and tissue pretreated using disk-type solid-phase extraction and matrix solid-phase dispersion

Wen-Ling Chen[a], Gen-Shuh Wang[a,b], Jin-Chywan Gwo[c], Chia-Yang Chen[a,b,*]

[a] Institute of Environmental Health, National Taiwan University, 17 Hsu-Chou Rd., Taipei 10055, Taiwan
[b] Department of Public Health, National Taiwan University, 17 Hsu-Chou Rd., Taipei 10055, Taiwan
[c] Department of Aquaculture, National Taiwan Ocean University, 2 Beining Rd., Keelung 20224, Taiwan

ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 6 December 2011
Accepted 7 December 2011
Available online 14 December 2011

Keywords:
Feminizing chemicals
Ultra-high performance liquid chromatography/tandem mass spectrometry
Disk-type automated solid-phase extraction
Matrix solid-phase dispersion

ABSTRACT

This study developed and validated a method of measuring the feminizing chemicals 4-*tert*-octylphenol, 4-nonylphenol, nonylphenol monoethoxycarboxylate (NP_1EC), nonylphenol monoethoxylate (NP_1EO), nonylphenol diethoxylate (NP_2EO), estrone, 17β-estradiol, estriol, 17α-ethinyl estradiol and bisphenol A in river water, sediment, and tissue using ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) and isotope-dilution techniques. Water samples were pretreated using disk-type automated solid-phase extraction (SPE). Solid samples of sediment, fish, and clams were treated with matrix solid-phase dispersion (MSPD) using C_8 adsorbent. Eluents were directly passed following alumina cartridges for cleanup. The signal intensity of analytes on electrospray ionization (ESI) was compared with that of atmospheric pressure photoionization (APPI). The analytes were separated on a UHPLC C_{18} column with aqueous 10-mM ammonium acetate for NPEOs and aqueous 10-mM *N*-methylmorpholine for the other compounds. On-line cleanup was evaluated using two-dimensional liquid chromatography (2-D LC).

ESI could provide satisfactory response for all of the analytes. Though APPI did not offer suitable response for NP_1EO, NP_2EO and NP_1EC, it provided better signal intensities for the steroid estrogens (1.0-2.4 times) and the phenols (3.2–4.4 times) than ESI. UHPLC shortened chromatographic time to less than 10 min. Disk-type automated SPE and MSPD dramatically increased the throughput of sample preparation. The extraction efficiency on surface water samples ranged from 10% to 91%. The extraction efficiency of MSPD on sediment, fish, and clams was 51-101%, 36-109%, and 30-111%, respectively. Acidic alumina cleanup was essential for the analysis of the tissue sample, and reduced matrix effects better than 2-D LC on-line cleanup. The limits of detection (LODs) in water ranged from 0.81 ng/L to 89.9 ng/L. The LODs in sediment and tissue ranged from tens of pg/g wet weight to only a few ng/g wet weight. This method proved to be accurate and reproducible, as both quantitative biases and relative deviations remained smaller than 20% at three spiked levels.

1. Introduction

Alkylphenolic substances, bisphenol A (BPA), and steroid estrogens are feminizing chemicals and are known to elevate plasma vitellogenin, decrease gonadosomatic index and sperm motility, as well as delay the first reproduction in aquatic life [1–6]. The bioaccumulation of these chemicals and the induction of vitellogenin have been found in fish near wastewater treatment plants [7,8]. Alkylphenol ethoxylates may degrade into products with higher estrogenic potencies [9], become more persistent once they are partitioned into sediment and bioconcentrate in invertebrates and fish [10,11]. BPA is widely employed in the creation of polycarbonate oligomers, epoxy resins, and plasticizers for industrial purposes. Although BPA is more degradable and less bioaccummulative than alkylphenolic substances, its massive production and continuous discharge keep it ever present in environmental waters [12]. The concentrations of alkylphenolic substances and BPA in surface water are reported to range from tens ng/L to tens μg/L [13–17]. Nonylphenol (NP), which is the most abundant of these chemicals in sediment and tissue, has been

* Corresponding author at: Institute of Environmental Health, National Taiwan University, 17 Hsu-Chou Rd., Taipei 10055, Taiwan. Tel.: +886 2 3366 8101; fax: +886 2 2351 9557.
E-mail address: dbms@ntu.edu.tw (C.-Y. Chen).

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doi:10.1016/j.talanta.2011.12.020

reported to be found at low μg/g dry weight (d.w.) in invertebrates [13,18–20]. Steroid estrogens, on the other hand, are much more estrogenic than other feminizing chemicals, though until now they are less often detected in treated municipal wastewaters [21]. However, as the human population grows and livestock industry increases, there will be an increase in the discharge of synthetic and natural estrogens [22]. The estrogen concentrations in surface water are reported to range from <0.1 ng/L to tens of ng/L [23–25]. To date, up to 1 μg/L of estrone (E_1) has been detected in river water near a livestock farm in Taiwan [26].

Because of the complexity of environmental and tissue matrixes and the trace levels of feminizing chemicals, multi-step sample preparation is often needed to enrich analytes and reduce interferences, processes which significantly decrease throughput of the analysis. Most water samples are treated using solid-phase extraction (SPE) [1,27]. Disk-type adsorbents have been successfully used in SPE when analyzing feminizing chemicals in water [28,29], as they allow a flow rate up to 100 mL/min and reduce sample loading time. Solid samples are usually extracted using an organic solvent, a time-consuming method that often requires a large amount of organic solvent, or needs specific apparatus for processing and more energy [30]. Matrix solid-phase dispersion (MSPD), a combination of extraction and concentration into one step, reduces potential loss or contamination of analytes. For biological tissues, 0.1–5 g of samples are blended with an adsorbent which destructs tissues and releases the analytes. As performed in SPE, the mixture is then packed into a cartridge and eluted with a few milliliters of solvent. The eluent can be further separated by liquid chromatography (LC) or gas chromatography (GC). MSPD has been used to analyze alkylphenols, herbicides and mycotoxins in food [31–36].

Cleanup is often needed to prevent co-extracts from interfering with separation by chromatography or detection by instrument, for which SPE by octadecyl (C_{18}), alumina, silica or Florisil is commonly used [31,32,37–40]. Restricted access materials (RAMs) are adsorbents designed to separate small molecules from macromolecules [41], which make on-line cleanup possible. In a tandem-column approach with a connection valve, a portion of the extracts is loaded by the first pump onto the RAM pre-column where analytes are retained and macromolecular matrixes are flushed out by the mobile phase. Thereafter, the valve is switched and the analytes are desorbed into the analytical column for further separation and detection by a stronger mobile phase from the second pump. Such two-dimensional (2-D) LC on-line cleanup techniques have been coupled with liquid chromatography/tandem mass spectrometry (LC/MS/MS) [42–44].

The use of LC/MS/MS to determining feminizing compounds is increasing because it provides good selectivity and detection sensitivity [14,27,45]. Ultra-high performance liquid chromatography (UHPLC) is an LC system that can tolerate pressures up to 19,000 psi and takes advantage of a small-particle (sub-2 μm) column which possesses a very flat Van Deemter curve and increases linear velocity without significantly affecting separation. UHPLC provides rapid chromatography, increased peak capacity and better sensitivity without sacrificing LC resolution [46–48].

Traditional wastewater treatment plants may not be able to remove feminizing chemicals completely, which allows some of these chemicals to be released into the water body [49], and tertiary treatments such as ozonation are costly and energy consuming and may still be inefficient in their removal of these chemicals [1,50]. The environmental fate of feminizing chemicals such as nonylphenol ethoxycarboxylates (NPECs), nonylphenol ethoxylates (NPEOs) and BPA remains unclear and little studied. Therefore, there is a great need for a high-throughput method that can enable the analysis of these chemicals in environmental and biological matrixes to facilitate further researches. For this study, a method was developed to measure these chemicals in environmental water, sediment, and tissue using LC/MS/MS. With the method, we were able to evaluate the performance of electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) as well as the matrix effects of sediment and tissue after the cleanup by an acidic alumina or a 2-D LC system.

2. Methods

2.1. Reagents and solutions

4-*tert*-Octylphenol (OP), estrone (E_1), 17β-estradiol (E_2), estriol (E_3), 17α-ethinyl estradiol (EE_2), BPA, and BPA-$^2D_{16}$ were obtained from Sigma-Aldrich (Saint Louis, MO, USA; purity >98%). The technical mixture of NP was supplied by Riedel-de Haën (Seelze, Germany; purity >94%). 2,4,16,16-$^2D_4$-E_1 (E_1-$^2D_4$), 2,4,16,16-$^2D_4$-17β-E_2 (E_2-$^2D_4$), 2,4,17-$^2D_3$-16α-hydroxy-17β-estradiol (E_3-$^2D_3$), 2,4,16,16-$^2D_4$-17α-EE_2 (EE_2-$^2D_4$) and 4-*n*-Octyl-$^2D_{17}$-phenol (4-*n*-OP-$^2D_{17}$) were bought from C/D/N Isotopes (Pointe-Claire, Quebec, Canada; purity >98%). Nonylphenol monoethoxylate (NP_1EO, mixture of branched isomers), nonylphenol diethoxylate (NP_2EO, mixture of branched isomers), nonylphenol monoethoxycarboxylate (NP_1EC, mixture of ring/chain isomers), 4-*n*-NP-$^{13}C_6$, 4-*n*-NP_1EO-$^{13}C_6$, 4-*n*-NP_2EO-$^{13}C_6$, 4-*n*-NP_2EO and 4-*n*-nonylphenol diethoxycarboxylate (4-*n*-NP_2EC) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA; purity >98%, 100 μg/mL in nonane). 4-*n*-NP was obtained from Wako Pure Chemical Industries (Chiu-ku, Osaka, Japan; purity >98%). Formic acid (analytical grade, 88%) was form J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate (98%) and *N*-methylmorpholine (>99.5%) were from Sigma–Aldrich. PolarPlus C_8 Speedisks were supplied by J.T. Baker. Silica C_8 was obtained from SiliCycle (Quebec City, Quebec, Canada). Acidic alumina cartridges were supplied by Sigma-Aldrich. Solvents, including methanol, acetone, heptane, and dichloromethane, were all HPLC grade (J.T. Baker). Methanol and acetonitrile for LC mobile phases were LC/MS grade (J.T. Baker).

The individual stock solutions of OP, NP, E_1, E_2, E_3, EE_2, BPA, 4-*n*-OP-$^2D_{17}$, E_1-$^2D_4$, E_2-$^2D_4$, E_3-$^2D_3$, BPA-$^2D_{16}$ and 4-*n*-NP were prepared at 1 mg/mL in methanol. The working standard solutions were prepared by diluting stock solutions with acetone before use.

2.2. Apparatus

Water samples were extracted using a Horizon SPE-DEX 4790 automated solid-phase extractor (Horizon Technology, Salem, NH, USA). The eluents were concentrated using a SpeedVac concentrator (Thermo Savant SPD 1010, Holbrook, NY, USA). Analyte separation and detection was performed on a Waters ACQUITY UPLC system coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). A BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm) was purchased from Waters (Waters Corporation). The first pump for 2-D LC was an isocratic pump purchased from Jasco (PU-2080, Tokyo, Japan), and RAM pre-column, LiChrospher ADS RP-4 (25 mm × 2 mm i.d., 25 μm), was purchased from Merck (Darmstadt, Germany). Data acquisition and processing were performed using MassLynx V4.1 (Waters Corporation).

2.3. Sample collection and preparation

River water was taken from a tributary (Hsin-Dian Creek) of the Dan-Shui River, acidified to pH 2.5 with formic acid and refrigerated at 4° to prevent biodegradation. Fish (Oreochromis mossambicus) and clams (*Corbicula fluminea*) were purchased from supermarkets in Taipei City. Sediment was grabbed from an ecological pool at National Taiwan University and filtered through a 60-mesh (0.25 mm) standard sieve.

The analytes in water were extracted using automated SPE. 500-mL water samples were spiked with isotope-labeled internal standards (levels at 100 ng/L of 4-*n*-NP_2EC, 4-*n*-NP_2EO-$^{13}C_6$, E_1-$^2D_4$ and E_2-$^2D_4$; 200 ng/L of 4-*n*-OP^2D_{17} and 4-*n*-NP-$^{13}C_6$; 400 ng/L of 4-*n*-NP_1EO-$^{13}C_6$, E_3-$^2D_3$ and BPA-$^2D_{16}$). They were shaken for 30 min at 130 rpm and filtered through a PVDF membrane (pore size 0.45 μm) before SPE. The Speedisk was prewashed with 10-mL methanol/dichloromethane (50:50, v/v) and sequentially conditioned with 10-mL methanol and reagent water. The water sample was passed through the Speedisk at a flow rate of 80–90 mL/min. After drying for 10 min, the Speedisk was eluted twice with 5-mL methanol and acetone, respectively. The eluate was filtered through a PTFE syringe filter (pore size 0.2 μm) and concentrated using a SpeedVac to near dryness and was reconstituted with 100-μL acetone/methanol (1:1, v/v) containing recovery standards (0.5 ng/μL 4-*n*-NP_2EO and 1 ng/μL 4-*n*-NP).

Sediment and tissue samples were processed with MSPD. A one-gram sample (wet weight, w.w.) was spiked with isotope-labeled internal standards (levels at 100 ng/g w.w. of 4-*n*-NP_2EC, 4-*n*-NP_2EO-$^{13}C_6$, E_1-$^2D_4$, and E_2-$^2D_4$; 200 ng/g w.w. of 4-*n*-$OP\ ^2D_{17}$ and 4-*n*-NP-$^{13}C_6$; 400 ng/g w.w. of 4-*n*-NP_1EO-$^{13}C_6$, E_3-$^2D_3$, and BPA-$^2D_{16}$) and homogenized with 4 g of C_8 adsorbent by an IKA ULTRA-TURRAX Tube Drive (Wilmington, NC, USA) and then packed into a 12-mL SPE polypropylene cartridge with a polyethylene frit at the bottom and the top. The homogenization tube was rinsed with 5-mL methanol, and then it was added into the SPE cartridge as the first elution. The analytes were eluted twice with 5-mL methanol and acetone, respectively. The eluent was cleaned up by passing it through an acidic alumina cartridge attached to the bottom of the 12-mL cartridge, and filtered through a PTFE syringe filter (pore size 0.2 μm) before being concentrated to 1 mL. The concentrated extract was centrifuged at 3000 rpm (1411 × *g*) for 5 min. The clear supernatant was further concentrated to 100 μL, added with 80-μL acetone/methanol (1:1, v/v) and spiked with 20-μL recovery standards (5 ng/μL 4-*n*-NP_2EO and 10 ng/μL 4-*n*-NP in acetone).

2.4. Liquid chromatography

The BEH C_{18} column was used as the analytical column. For NPEOs on ESI positive mode, the mobile phases were 10-mM ammonium $acetate_{(aq)}$ (A, pH 6.4) and methanol (B) and the flow rate was 0.5 mL/min. The gradient started with 30% A and 70% B and was then increased to 100% B linearly over 2-min period, at which point it was held at 100% B for 1 min before being returned to the initial condition. The column was re-equilibrated for 2 min. The chromatographic time was 5.5 min. The column temperature was set at 55 °C. The mobile phases on ESI negative for the other analytes were composed of 10-mM *N*-$methylmorpholine_{(aq)}$ (A, pH 9.6) and methanol (B) and the flow rate was 0.5 mL/min. The linear gradient started with 70% A and 30% B and was increased to 90% B in 5 min, at which point it was held for 1 min before being returned to the initial condition. The column was re-equilibrated for 2 min. The chromatographic time was 8.5 min. The column temperature was set at 55 °C.

A 2-D LC system, which consisted of a RAM pre-column and BEH C_{18} column, was evaluated for its efficacy at on-line cleaning up of sediment and tissue samples. Sixteen micro-liters of the MSPD extract was flushed into the pre-column by water-acetonitrile (95:5, v/v) at a flow rate of 1.0 mL/min for four min. The six-port valve was then switched and the pre-column was back flushed using the initial compositions of mobile phases for the BEH C_{18} analytical column at flow rates of 0.25 mL/min and 0.2 mL/min on ESI positive and negative modes, respectively. The valve was switched back to the original position at the end of chromatography and the RAM pre-column was re-equilibrated for 1 min before next injection. The BEH C_{18} analytical column was re-equilibrated during the first four minutes of the next injection.

Table 1
The selected reaction monitoring transitions, cone voltage and collision energy on electrospray ionization.

Analyte	MW	SRM	CV	CE
IS: internal standard				
4-*tert*-Octylphenol (OP)	206.2	(−) 205.0 > 132.9	40	25
IS: 4-*n*-OP-$^2D_{17}$	223.3	(−) 222.1 > 107.6	40	25
4-Nonylphenol (NP)	220.2	(−) 219.0 > 133.0	35	30
		(−) 219.0 > 146.9	35	25
IS: 4-*n*-NP-$^{13}C_6$	226.2	(−) 225.0 > 111.8	35	30
Nonylphenoxyacetic acid (NP_1EC)	278.2	(−) 277.0 > 219.0	20	20
		(−) 277.0 > 132.9	20	40
IS: 4-*n*-NP_2EC	322.2	(−) 321.0 > 219.0	20	15
Nonylphenol monoethoxylate (NP_1EO)	264.2	(+) 282.1 > 126.9	15	10
		(+) 282.1 > 84.9	15	10
IS: 4-*n*-NP_1EO-$^{13}C_6$	270.2	(+) 288.2 > 271.4	15	10
Nonylphenol diethoxylate (NP_2EO)	308.2	(+) 326.2 > 182.9	15	10
		(+) 326.2 > 89.0	15	20
IS: 4-*n*-NP_2EO-$^{13}C_6$	314.2	(+) 332.1 > 315.3	15	10
Estrone (E_1)	270.2	(−) 269.0 > 144.8	60	40
		(−) 269.0 > 158.9	60	40
IS: E_1-$^2D_4$	274.2	(−) 274.0 > 146.8	60	40
17β-estradiol (E_2)	272.2	(−) 271.1 > 183.0	60	40
		(−) 271.1 > 144.8	60	40
IS: E_2-$^2D_4$	276.2	(−) 275.4 > 147.0	60	40
Estriol (E_3)	288.2	(−) 287.1 > 170.8	60	40
		(−) 287.1 > 145.0	60	40
IS: E_3-$^2D_3$	291.2	(−) 290.1 > 173.0	60	40
17α-ethinyl estradiol (EE_2)	296.2	(−) 295.0 > 145.0	55	45
		(−) 295.0 > 159.0	55	35
IS: E_2-$^2D_4$	276.2	(−) 275.4 > 147.0	60	40
Bisphenol A (BPA)	228.1	(−) 227.0 > 133.0	40	30
		(−) 227.0 > 211.0	40	30
IS: BPA-$^2D_{16}$	244.2	(−) 241.0 > 142.0	40	30

MW, molecular weight (nominal mass, Da); SRM, selected reaction monitoring; CV, cone voltage (V); CE, collision energy (V).

2.5. Tandem mass spectrometry

A triple-quadrupole mass spectrometer set at selected reaction monitoring (SRM) mode was used to detect the two most abundant ion pairs for quantification and confirmation, respectively. The suitability of ESI and APPI was tested. We infused standard solutions of individual analytes into the MS/MS using a syringe pump for optimizing the parameters. We also connected the flow of standard solutions from the syringe pump with the mobile phases from the LC pump using a Tee connector and optimized the parameters of the ESI probe. The ESI SRM transitions and relating parameters for each analyte are shown in Table 1. The capillary voltage and extractor voltage for NPEOs, which were detected on positive ESI mode, were set at 3 kV and 5 V. The source temperature and desolvation temperature were set at 150 °C and 500 °C. The cone gas flow and desolvation gas flow were set at 50 L/h and 1000 L/h. The other analytes were detected on negative ESI mode with the capillary voltage, extractor voltage, source temperature, desolvation temperature, cone gas flow and desolvation gas flow set at 3 kV, 5 V, 120 °C, 500 °C, 50 L/h and 1100 L/h, respectively. The collision gas was argon added to a pressure of 3.6×10^{-3} mbar. The APPI conditions are shown in Table S1 in the Supplementary Data.

2.6. Evaluation of extraction efficiency, matrix effects and backgrounds

To evaluate extraction efficiency and matrix effects, we pre- and post-spiked three levels of analytes to the samples before and after sample preparation, respectively. The alkylphenolic chemicals and

BPA levels were 400, 1000, 2000 ng/L and steroid estrogens were 200, 1000, 2000 ng/L of in river water. The alkylphenolic chemicals and BPA levels were 100, 200, 500 ng/g w.w. and the steroid estrogen levels were 50, 200, 500 ng/g w.w. for sediment, fish, and clams, respectively. Extraction efficiency was defined as the ratio of the analyte peak area in the pre-spiked sample to that in the post-spiked sample. The matrix effect factor was defined as the peak area ratio of analytes in the post-spiked sample to the same amount of standards in acetone/methanol (50:50, v/v) [51]. In addition to the matrix effect factor, we compared the slope of the matrix-matched calibration curve that was prepared in post-spiked sediment and tissue matrixes to that of the standard calibration curve [52,53]; the backgrounds in the samples for making matrix-matched calibration curves were deducted. To compare the matrix effects using alumina cleanup and 2-D LC, we injected samples that were not cleaned up by alumina into 2-D LC/MS/MS and alumina-cleanup samples into both UHPLC/MS/MS and 2-D LC/MS/MS.

We measured the reagent blank levels of analytes in Milli-Q water and in the river water used for spiking. We also evaluated the background contribution from the plastic ware using in the MSPD. A homogenization tubes were rinsed with 5-mL methanol, an SPE cartridge with frits was soaked thoroughly with the elution solvents (5-mL methanol twice and then 5-mL acetone twice), and a PTFE syringe filter was passed through 5-mL methanol and 5-mL acetone. These solvents were collected separately and were concentrated to barely dry, then were reconstituted with 100-μL acetone/methanol (1:1, v/v) for analysis. The elution solvents were also concentrated and reconstituted.

2.7. Method validation

The methods were validated using river water, fish, clams and sediment at three spiking levels ($n=4$). The quantification bias was calculated by comparing the measured level with the spiked level. The backgrounds were deducted if they were detected. Intra- and inter-day accuracy and precision were evaluated by duplicate measurements of a sample at each spiked level in different runs within the same day and on different days, respectively ($n=3$).

2.8. QA/QC and data analysis

Glassware was rinsed with acetone, heptane, dichloromethane and methanol before use. Homogenization tubes, cartridges, and frits were prewashed with methanol. After use, the glassware was washed with tap water and sonicated with tap water and Milli-Q water sequentially, and then rinsed with the four solvents. The detergent was free of neutral surfactants to avoid the contamination of alkylphenolic compounds. The C_8 adsorbent was sonicated with acetone and was dried before use.

We built calibration curves of analytes by normalizing their peak areas to their stable isotope-labeled chemicals as the internal standards, which the concentrations were at three levels based on their detection sensitivity: 0.5 μg/mL of 4-*n*-NP_2EC, 4-*n*-NP_2EO-$^{13}C_6$, E_1-$^2D_4$ and E_2-$^2D_4$; 1 μg/mL of 4-*n*-OP-$^2D_{17}$ and 4-*n*-NP-$^{13}C_6$; 2.0 μg/mL of 4-*n*-NP_1EO-$^{13}C_6$, E_3-$^2D_3$ and BPA-$^2D_{16}$. The linear dynamic ranges of all calibration curves were at least 200 times the magnitude with r^2 larger than 0.99 using $1/x^2$ weighting (Table S2 in the Supplementary Data). The signal intensity of EE_2-$^2D_4$ was weak and was, therefore, not suitable as the internal standard of EE_2. Instead, E_2-$^2D_4$ was used because it has a similar recovery and retention time to EE_2.

The instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) were defined as signal-to-noise ratio (S/N ratio) of the confirmatory ion at 3 and that of the quantitative ion at 10, respectively, for the analytes in the mixture standard solution that were injected into the UHPLC/MS/MS. We evaluated limits of detection (LODs) and limits of quantification (LOQs) using the confirmatory ions at S/N ratio = 3 and the quantitative ions at S/N ratio = 10, respectively [54,55], using the samples at the lowest spiked levels for method validation. The estimation of S/N ratio was to divide the signal intensity by the root-mean-square of the noise intensity. When calculated LODs were larger than LOQs based on the above definitions, the LOQs were reported as the same as the LODs.

3. Results and discussion

3.1. Performance of ESI and APPI

ESI provided suitable response in either negative or positive ion modes. The precursor ions of OP, NP, NP_1EC, E_1, E_2, E_3, EE_2, and BPA were the deprotonated molecular ions $[M-H]^-$ (Table 1). OP was only able to form a stable product ion (m/z 205 > 133). NPEOs tended to form sodium adducts $[M+Na]^+$, which are unable to produce stable product ions. The 10-mM ammonium acetate aqueous mobile phase in our study made NP_1EO and NP_2EO prone to forming ammonium adducts $[M+NH_4]^+$ rather than $[M+Na]^+$ [45,56–58].

APPI did not provide a suitable response for NP_1EO, NP_2EO, or NP_1EC under the source conditions used, and thus, it was not further investigated. However, it should be mentioned that it did provide better signal intensities than ESI on the steroid estrogens (1.0–2.4 times) and the phenols (3.2 times for OP and 4.4 times for NP) (Fig. 1), findings consistent with those reported by Lien et al. [29]. Bos et al. also reviewed and summarized APPI being efficient to low-polar compounds with aromatic structure such as steroids and polycyclic aromatic hydrocarbons [59]. We monitored $[M-H]^-$ for all compounds and $[M+H]^+$ for NP_1EO and NP_2EO, and tested mobile phase compositions including methanol and acetonitrile combined with aqueous phases of water, ammonium acetate and *N*-methylmorpholine. None of them worked for NPEOs or NP_1EC on APPI.

3.2. Effects of mobile phase compositions on signal intensities

Use of 10-mM *N*-methylmorpholine$_{(aq)}$ as the aqueous mobile phase enhanced signal intensities of steroid estrogens and phenols (1.6–6.8 times) and provided similar signal intensities of NP_1EC and BPA compared to that of 10-mM ammonium acetate$_{(aq)}$. We detected them separately from NPEOs which used 10-mM ammonium acetate$_{(aq)}$. *N*-methylmorpholine was reported to be able to increase the dissociation of some feminizing compounds and perfluorinated chemicals at negative ESI and thus enhance their signal intensities [28,55]. Shao et al. found that addition of ammonium acetate in the mobile phase strongly decreased the signals of NP, OP and BPA [60]. Due to different viscosity of solvents and different retention time of the analytes, it was suggested that phenols would respond better on ESI when methanol was used as the organic mobile phase, and estrogens and BPA would respond better when acetonitrile was used [45,61]. We obtained the best signal intensity of the analytes using methanol as the organic mobile phase combined with aqueous mobile phases of 10-mM ammonium acetate$_{(aq)}$ and *N*-methylmorpholine$_{(aq)}$, respectively, on ESI positive and negative mode. When acetonitrile was used, the signal intensities of steroid estrogens and NP_1EO were much weaker compared with those observed when methanol was used. Under these optimized conditions, the UHPLC/MS/MS provided good sensitivity, with IDLs ranging from 0.26 pg to 39 pg and the IQLs ranging from 0.51 pg to 29 pg (except for EE_2 127 pg) (Table S2 in the Supplementary Data).

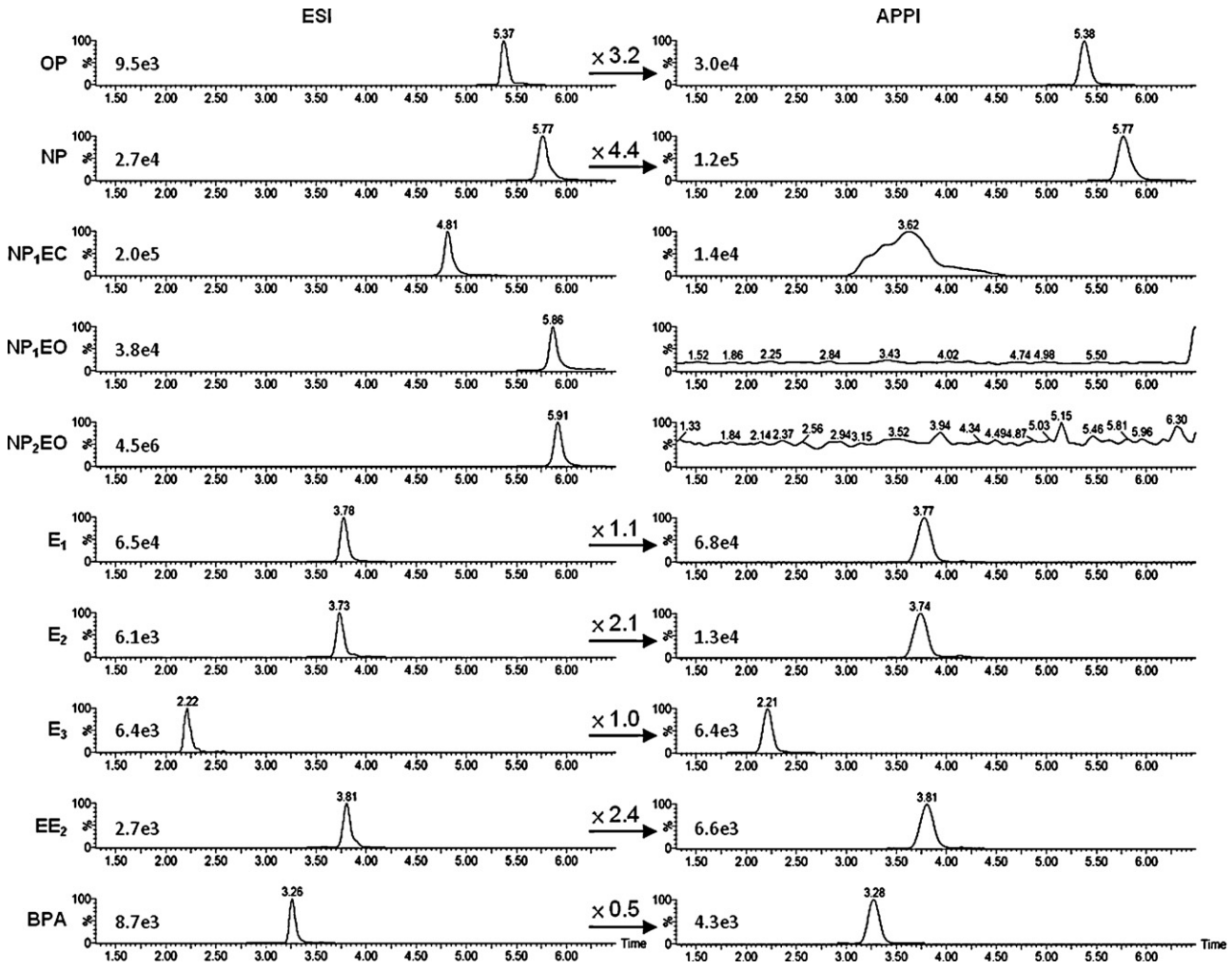

Fig. 1. Chromatograms on eletrospray ionization (ESI) and atmospheric pressure photoionization (APPI). 1 ng/μL of standards, 4-μL injection; 0.4-mL/min flow rate; mobile phase: 10-mM ammonium acetate$_{(aq)}$ and methanol on ESI (left); water and methanol on APPI (right).

3.3. Optimization of sample preparation

SPE was most efficient when water samples were adjusted to pH 2.5, extracted using C_8 adsorbent, and eluted with methanol and acetone sequentially without a wash step before the elution. The tested pH of water samples were 2.5 and 3.5. Water/methanol (60:40, v/v) was tested for the wash step. The tested elution solvents included methanol, methanol with 0.1% ammonium hydroxide (v/v), methanol/dichloromethane (50:50, v/v), acetonitrile, acetone, *n*-heptane, and acetone/*n*-heptane (50:50, v/v). The tests were performed on C_8 and C_{18} Speedisk cartridges. The finalized condition was scaled up to PolarPlus C_8 Speedisks using an automated solid-phase extractor. The extraction efficiency in spiked Milli-Q water ranged between 53.8% and 93.1%, with most RSDs below 20% (Table 2). The SPE efficiency of OP was low (8.6%), so OP was excluded from the analysis of river water. Although many methods use C_{18} or Oasis HLB for SPE [30,37], only 4.3–49.3% of alkylphenolic substances in spiked Milli-Q water could be eluted from C_{18} Speedisk cartridges. The extraction efficiency of NP, NP_1EC and NPEOs in river water was <30% (Table 2). These alkylphenolic compounds have been reported to significantly adsorb onto the particles in water [62,63]. More than 60% of NP are partitioned to the particle phase in sewage [64]. To evaluate the influence of suspended particles in river water on efficiency of extraction, we spiked analytes in pre-filtered (particle-free) river water and found that the extraction efficiency for alkylphenolic substances improved significantly (from 10–25% increasd to 66–104%). The majority of alkylphenolic substances may have been adsorbed to the particles and were removed at the filtration step before the SPE; consequently their internal standards should be added before the SPE to cancel out the influence on quantification.

The extraction efficiency of MSPD using C_8 adsorbent and eluted with methanol and acetone in spiked sediment, fish, and clams was 51.3–101%, 35.8–109%, and 30.0–111%, respectively. The RSDs were below 15% (Table 3). The efficiency for alkylphenolic chemicals was lower in sediment (≤71%) and that for NPEOs was even lower in

Table 2
Extraction efficiency (%) of analytes in water using automated SPE. The samples were spiked with 400 ng/L of alkylphenolic substances and bisphenol A and 200 ng/L of steroid estrogens (n = 4).

Analyte	Milli-Q water	River water	Particle-free river water
	Mean (RSD%)	Mean (RSD%)	Mean (RSD%)
NP	56.7 (40.2%)	25.0 (10.4%)	104 (8.34%)
NP_1EC	93.1 (8.02%)	10.1 (14.9%)	89.9 (13.4%)
NP_1EO	60.8 (14.9%)	12.2 (12.9%)	65.8 (4.40%)
NP_2EO	73.6 (15.3%)	14.7 (14.5%)	97.5 (6.05%)
E_1	73.2 (27.5%)	59.9 (9.09%)	108 (15.3%)
E_2	67.4 (18.7%)	78.6 (12.3%)	105 (15.8%)
E_3	66.1 (12.7%)	91.0 (6.43%)	92.7 (10.0%)
EE_2	53.8 (25.1%)	74.7 (8.89%)	65.9 (13.1%)
BPA	78.3 (14.2%)	76.6 (10.0%)	103 (14.6%)

Table 3
Extraction efficiency (%) of analytes in sediment, fish and clams processed with matrix solid-phase dispersion and acidic alumina cleanup.

Analyte	Spiked concentration (ng/g w.w.)	Sediment	Fish	Clam
		Mean (RSD%)	Mean (RSD%)	Mean (RSD%)
OP	100	54.9 (10.6%)	83.1 (6.43%)	99.6 (14.7%)
	200	51.3 (7.77%)	75.2 (7.72%)	94.8 (11.7%)
	500	44.3 (9.02%)	83.5 (7.43%)	92.2 (8.63%)
NP	100	66.6 (13.5%)	107 (4.49%)	98.4 (12.8%)
	200	61.8 (7.14%)	107 (15.4%)	97.2 (11.4%)
	500	60.6 (13.5%)	103 (6.65%)	98.5 (13.9%)
NP_1EO	100	59.5 (13.9%)	40.6 (8.70%)	30.0 (14.7%)
	200	54.7 (11.3%)	35.8 (8.37%)	30.0 (14.7%)
	500	47.4 (15.8%)	41.8 (13.5%)	32.9 (17.5%)
NP_2EO	100	71.2 (7.91%)	43.7 (10.0%)	37.7 (9.84%)
	200	59.1 (9.89%)	41.4 (7.38%)	41.6 (13.3%)
	500	53.6 (13.7%)	46.2 (11.0%)	40.8 (13.8%)
E_1	50	99.6 (2.91%)	84.3 (3.96%)	95.4 (10.7%)
	200	95.7 (7.78%)	84.0 (12.3%)	92.2 (9.03%)
	500	102 (5.54%)	85.4 (6.41%)	95.6 (10.0%)
E_2	50	90.5 (0.98%)	93.0 (3.96%)	83.1 (11.6%)
	200	89.2 (10.2%)	98.5 (8.91%)	81.4 (12.3%)
	500	91.8 (4.21%)	89.3 (4.97%)	90.5 (3.96%)
E_3	50	101 (0.92%)	99.2 (5.58%)	111 (11.3%)
	200	100 (8.60%)	96.8 (3.22%)	101 (8.67%)
	500	99.6 (5.03%)	95.4 (5.29%)	105 (4.19%)
EE_2	50	86.3 (4.56%)	93.1 (1.79%)	88.6 (7.26%)
	200	90.4 (9.72%)	96.8 (8.01%)	81.1 (9.64%)
	500	88.7 (8.45%)	90.2 (6.63%)	82.3 (8.67%)
BPA	100	99.3 (11.9%)	106 (14.2%)	84.2 (14.4%)
	200	99.8 (2.97%)	109 (10.0%)	83.6 (15.2%)
	500	97.5 (5.37%)	104 (12.8%)	86.6 (12.1%)

fish and clams (30–46%). More than 80% of steroid estrogens and BPA were extracted in the three matrixes. NP_1EC was lost during the acidic alumina cleanup and was, therefore, excluded from the analysis of sediment and tissue, although its extraction efficiency using MSPD was $\geq$39.0% in three matrixes. Using a vacuum manifold, we were able to complete the extraction of 12 samples within 3 h, and only used 20 mL of solvent on each sample. The elution occurred as result of gravity; thus, no heat or electric power was needed except for when drying cartridges for 10 min using vacuum pumping.

3.4. Matrix effects

The matrix effects were lower in the river water samples of the lowest spiked levels than those at the two higher spiked levels except for BPA, which had an insignificant matrix effect (Table S3 in the Supplementary Data). The matrix effect factors at post-spiked river water were above 50% for most compounds at the lowest spiked level but were mostly lower than 50% at those samples spiked at 1000 ng/L and 2000 ng/L. At higher concentrations, the analyte molecules may compete with each other for charges in the ESI source and suppress the signal intensities.

Alumina cleanup was essential for tissue samples before LC/MS/MS detection. Signal suppression was less significant in fish and clams that were treated with alumina compared with those were cleaned up using RAM pre-column. The two cleanup methods provided similar matrix effects for most of the analytes in sediment (Table 4). Alkylphenolic substances were more susceptible to matrix effects than other analytes, especially in clams (matrix effect factors $\leq$30%, Table S4 in the Supplementary Data). For 2-D LC, we reduced the mobile-phase flow rates to 0.2 (ESI−) and 0.25 (ESI+) mL/min because the isocratic pump could only tolerate a lower back pressure (about 400 bars) compared to UHPLC. The peaks of alkylphenolic substances were broader (0.2–0.3 min) than those found by UHPLC (0.1–0.2 min). The alkylphenolic substances were eluted at 100% and 80–90% methanol on 2-D LC and UHPLC, respectively. The total ion intensities of MS full scans on blank calm samples at the retention time of NPEOs and OP/NP using 2-D LC (Figure S1 in the Supplementary Data) were about 7-fold and 3-fold higher, respectively, than those using alumina cleanup and UHPLC (Figure S2 in the Supplementary Data). The co-eluted substances at higher organic portion of the mobile phase might explain the reason that the matrix effect was more significant in those samples using 2-D LC cleanup compared with those using alumina cleanup. They might also explain the reason that the earlier eluted steroid estrogens and BPA were less susceptible to matrix effects. Regarding the alumina cleanup samples, the slope ratio% of matrix-matched calibration curves to that of standard calibration curves were mostly similar to the matrix effect factors for most analytes (Table S4 in the Supplementary Data), demonstrating that either of the two methods of evaluating matrix effects provided comparable information. Moreover, 2-D LC was not able to further reduce the matrix effects on alkylphenolic substances for those samples that had already cleaned up using acidic alumina after re-analyzing these samples using 2-D LC (details not shown), and therefore 2-D LC was not used in the final method.

3.5. Background levels and method validation

Most of the analytes were not detected in Milli-Q water and in river water for spiking except for NP_2EO (66.4 ± 7.8 ng/L in Milli-Q water and 39.3 ± 7.1 ng/L in river water) and BPA (41.6 ± 14.3 ng/L in Milli-Q water and 84.0 ± 12.3 ng/L in river water, Table S5 in the Supplementary Data). The background NP_2EO in Milli-Q was higher than that in the river water for spiking. It has been reported that Milli-Q water containing more alkylphenolic substances than some surface waters and may not be suitable for background estimation [56]. No native steroid estrogens were detected in Milli-Q water that had been spiked with deuterium-labeled estrogens; this demonstrated that hydrogen-deuterium exchange on the deuterium-labeled internal standards was insignificant and did not result in background signals.

The method for determining the feminizing compounds in river water was accurate and reproducible (Table 5). Quantitative biases

Table 4
Comparison of matrix effect factors (%) on 2-D LC and acidic alumina cleanup after the matrix solid-phase dispersion ($n = 4$, spiked level at 200 ng/g w.w.).

Analyte	Sediment		Fish		Clam	
	Acidic alumina	2-D LC	Acidic alumina	2-D LC	Acidic alumina	2-D LC
	Mean (RSD%)	Mean (RSD%)	Mean (RSD%)	Mean (RSD%)	Mean (RSD%)	Mean (RSD%)
OP	57.1 (5.24%)	61.0 (8.75%)	54.1 (9.70%)	4.40 (9.85%)	26.5 (13.9%)	3.64 (12.7%)
NP	82.1 (16.3%)	50.4 (8.51%)	51.7 (15.8%)	2.02 (8.16%)	28.7 (11.9%)	2.06 (9.65%)
NP_1EO	84.4 (9.37%)	71.8 (10.2%)	43.1 (9.48%)	11.3 (12.3%)	26.2 (6.81%)	7.33 (10.2%)
NP_2EO	74.6 (13.9%)	101 (9.16%)	50.5 (3.05%)	12.7 (6.95%)	30.4 (9.44%)	17.7 (6.08%)
E_1	71.3 (4.94%)	71.6 (2.86%)	67.6 (13.7%)	39.3 (11.1%)	49.5 (5.13%)	33.3 (8.55%)
E_2	79.4 (3.66%)	73.8 (4.33%)	73.1 (10.7%)	49.2 (6.24%)	53.6 (5.98%)	47.4 (2.29%)
E_3	77.7 (3.45%)	79.9 (6.55%)	68.4 (8.76%)	44.3 (10.7%)	61.3 (3.94%)	35.2 (9.00%)
EE_2	81.6 (5.09%)	73.5 (2.62%)	62.8 (14.9%)	46.3 (6.95%)	60.3 (5.22%)	44.8 (3.25%)
BPA	96.6 (10.4%)	110 (16.6%)	66.3 (10.7%)	44.9 (7.51%)	66.0 (16.4%)	35.7 (17.6%)

Table 5
Accuracy and precision of the spiked samples ($n = 4$).

Analyte	Spiked concentration	River water		Sediment		Fish		Clam	
		RSD%	Bias %	RSD%	Bias %	RSD%	Bias %	RSD%	Bias %
OP	Low	–	–	10.7%	−2.81%	6.51%	−3.60%	6.92%	6.63%
	Medium	–	–	8.39%	13.0%	8.88%	−1.63%	6.98%	6.17%
	High	–	–	3.62%	3.59%	8.18%	6.18%	1.60%	3.02%
NP	Low	16.7%	−6.28%	4.28%	7.29%	4.00%	10.3%	4.83%	11.3%
	Medium	7.30%	3.53%	4.31%	5.81%	7.46%	12.4%	12.4%	14.4%
	High	4.50%	0.03%	4.12%	−4.77%	7.89%	−5.04%	13.4%	−6.93%
NP_1EC	Low	12.6%	8.69%	–	–	–	–	–	–
	Medium	15.5%	4.42%	–	–	–	–	–	–
	High	18.5%	−2.43%	–	–	–	–	–	–
NP_1EO	Low	18.5%	5.87%	4.33%	10.2%	4.75%	0.43%	7.49%	8.50%
	Medium	15.4%	12.0%	4.53%	4.10%	3.67%	11.1%	11.2%	−2.45%
	High	16.1%	7.99%	1.88%	2.31%	7.42%	5.71%	10.1%	−0.44%
NP_2EO	Low	14.7%	11.6%	11.2%	8.14%	13.3%	11.3%	10.7%	1.45%
	Medium	8.18%	7.09%	8.04%	15.3%	11.1%	10.9%	13.1%	13.2%
	High	12.8%	−5.40%	5.57%	6.91%	14.0%	4.79%	10.9%	−4.24%
E_1	Low	12.2%	−2.08%	5.70%	−0.60%	6.15%	−3.90%	3.88%	−4.70%
	Medium	19.1%	3.58%	3.21%	−6.08%	2.39%	−3.35%	1.12%	−2.60%
	High	18.4%	−4.44%	4.13%	0.21%	1.00%	−3.54%	2.04%	−1.41%
E_2	Low	14.9%	12.2%	6.74%	0.60%	2.62%	−1.20%	4.93%	−0.40%
	Medium	16.3%	7.64%	6.42%	−7.87%	6.76%	−0.25%	6.64%	−4.80%
	High	8.43%	7.80%	4.37%	−3.55%	5.07%	−4.21%	0.79%	−6.10%
E_3	Low	4.44%	−3.40%	1.30%	1.60%	2.17%	−2.56%	3.64%	2.08%
	Medium	3.66%	−8.97%	6.90%	−5.20%	1.28%	1.20%	4.34%	3.48%
	High	5.55%	1.71%	4.79%	−1.46%	3.80%	−6.74%	2.48%	−4.14%
EE_2	Low	15.9%	10.4%	9.53%	−4.30%	3.74%	−3.40%	5.86%	−2.70%
	Medium	7.99%	−4.83%	8.70%	−4.70%	7.38%	−8.42%	9.36%	−8.42%
	High	12.1%	11.5%	6.02%	−1.39%	3.17%	−13.1%	13.8%	−8.82%
BPA	Low	11.0%	19.3%	14.9%	12.6%	11.1%	14.4%	9.52%	6.98%
	Medium	18.9%	4.46%	3.16%	10.5%	8.10%	3.69%	9.76%	10.1%
	High	1.18%	3.87%	9.21%	−8.80%	7.51%	−7.83%	4.00%	−0.01%

The spiked concentrations in river water were 200 ng/L of steroid estrogens and 400 ng/L of alkylphenolic substances and bisphenol A at the low levels; they were 1000 ng/L and 2000 ng/L at the medium and high levels. The spiked concentrations in sediment and tissue were 50 ng/g w.w. of steroid estrogens and 100 ng/g w.w. of alkylphenolic substances and bisphenol A at the low levels; they were 200 ng/g w.w. and 500 ng/g w.w. at the medium and high levels.

Table 6
The limits of detection (LODs) and limits of quantification (LOQs) in four matrixes (mean ± SD, $n = 4$).

Analyte	River water		Sediment		Fish		Clam	
	LOD (ng/L)	LOQ (ng/L)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)
OP	–	–	0.27 ± 0.039	0.90 ± 0.13	0.19 ± 0.018	0.65 ± 0.059	0.41 ± 0.056	1.36 ± 0.19
NP	89.9 ± 23.4	115 ± 17.8	0.43 ± 0.056	0.43 ± 0.056	0.27 ± 0.026	0.27 ± 0.026	0.67 ± 0.067	0.67 ± 0.067
NP_1EC	18.1 ± 2.92	35.7 ± 10.0	–	–	–	–	–	–
NP_1EO	60.3 ± 9.24	60.3 ± 9.24	5.10 ± 0.38	8.46 ± 0.30	6.82 ± 0.81	11.5 ± 1.25	19.8 ± 3.40	21.7 ± 0.64
NP_2EO	2.39 ± 0.58	2.39 ± 0.58	0.065 ± 0.010	0.073 ± 0.010	0.12 ± 0.012	0.17 ± 0.026	0.17 ± 0.018	0.17 ± 0.018
E_1	4.77 ± 0.93	6.84 ± 0.76	0.069 ± 0.0067	0.17 ± 0.017	0.075 ± 0.011	0.17 ± 0.014	0.10 ± 0.015	0.21 ± 0.023
E_2	1.27 ± 0.074	2.94 ± 0.45	0.096 ± 0.012	0.12 ± 0.016	0.049 ± 0.0042	0.12 ± 0.0070	0.092 ± 0.0065	0.18 ± 0.0089
E_3	45.6 ± 5.45	45.6 ± 5.45	0.12 ± 0.0035	0.32 ± 0.044	0.097 ± 0.012	0.42 ± 0.035	0.20 ± 0.022	0.62 ± 0.054
EE_2	3.70 ± 0.60	8.17 ± 1.61	0.11 ± 0.012	0.25 ± 0.020	0.22 ± 0.019	0.40 ± 0.038	0.16 ± 0.017	0.41 ± 0.026
BPA	0.81 ± 0.15	0.81 ± 0.15	0.30 ± 0.050	0.69 ± 0.10	0.37 ± 0.046	0.99 ± 0.071	1.65 ± 0.21	2.92 ± 0.46

LOD, the S/N ratio of the confirmatory ion at 3; LOQ, the S/N ratio of the quantitative ion at 10.

Table 7
Concentrations in river water, sediment and tissue in Dan-Shui River.

	Water (ng/L, $n=3$)		Sediment (ng/g wet weight, $n=3$)		Fish (ng/g wet weight, $n=6$)	
	Mean ± SD	Geometric mean	Mean ± SD	Geometric mean	Mean ± SD	Geometric mean
OP	–	–	287 ± 273	203	36.3 ± 23.3	30.7
NP	1026 ± 326	990	817 ± 591	697	238 ± 39.9	235
NP_1EC	415 ± 157	391	–	–	–	–
NP_1EO	284 ± 247	223	161 ± 124	134	59.0 ± 21.3	56.0
NP_2EO	371 ± 216	331	76.6 ± 36.1	71.4	29.9 ± 4.44	29.6
E_1	9.20 ± 5.66	8.28	1.50 ± 0.42	1.47	0.80 ± 0.91	0.54
E_2	<LOD	<LOD	<LOD	<LOD	1.40 ± 0.40	1.36
E_3	<LOD	<LOD	<LOD	<LOD	0.45 ± 0.30	0.37
EE_2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BPA	808 ± 737	508	26.6 ± 18.0	22.9	30.8 ± 21.4	23.9

LOD, the S/N ratio of the confirmatory ion at 3.

were all smaller than 12.0% (except for BPA at the low level) and RSDs were all smaller than 19% ($n=4$). The intra- and inter-day quantitative biases and RSDs were all lower than 15%. The LODs and LOQs ranged from 0.81 ng/L to 89.9 ng/L and 0.81 ng/L to 115 ng/L, respectively (Table 6); Although we calculated the LODs based on the less abundant confirmatory ions, the LODs of this method were comparable to or just little higher than that of other SPE and LC/MS/MS based methods that reported LODs based on the S/N ratios of the most abundant product ions [28,37,45,64]. NP had the highest LOD among all of the analytes, which may result from its low extraction efficiency (25.0%) from river water due to the adsorption to particulates plus its higher instrumental noise level (1.4–10.4 times higher than other analytes). Regardless the similarity between NP_1EO and NP_2EO in the chemical structure, extraction efficiency of the sample preparation, and matrix effects, the LOD of NP_1EO was much higher than that of NP_2EO (Table 6), which could be primarily ascribed to NP_1EO's having a 13-fold higher IDL than NP_2EO.

NP, NP_2EO and BPA were detected in reagent blanks of MSPD (22.1 ± 1.3 ng, 2.74 ± 0.50 ng, and 18.6 ± 14.4 ng, respectively; $n=4$, Table S6 in the Supplementary Data). The primary contributions were from the homogenization tubes and SPE cartridges, which accounted for 83% of the NP, 24% of the NP_2EO, and 54% of the BPA.

The method for solid samples was accurate and reproducible when used to measure feminizing chemicals in fish, clams, and sediment (Table 5). The quantitative biases and RSDs of all analytes were less than 15% at the three spiked levels in three matrixes ($n=4$). The intra-day and inter-day measurements were also accurate and reproducible with most RSDs and biases smaller than 10%. The LODs ranged from 0.065 to 5.10 ng/g w.w. (equivalent to 0.12–9.7 ng/g d.w.) for sediment, 0.049–6.82 ng/g w.w. (equivalent to 0.22–30 ng/g d.w.) for fish, and 0.092–19.8 ng/g w.w. (equivalent to 0.60–129 ng/g d.w.) for clams, respectively (Table 6). The LODs of clam samples were higher than that in fish and sediment mainly resulting from more significant matrix effects, especially on alkylphenolic substances. Although the LODs in this study were evaluated using the less abundant confirmatory ions, and we only used one-gram sample in wet weight instead of dry weight, the LODs were comparable to most of the LC/MS/MS methods for sediment and tissue, which ranged from sub- to tens ng/g d.w. [45,57,58,65–67].

3.6. Concentrations in the field samples

We applied the proposed method to river water ($n=3$), fish (*Oreochromis mossambicus* and *Oreochromis niloticus*, $n=6$) and sediment ($n=3$) samples taken from Dan-Shui River in Taipei, Taiwan on April 27, 2011. For quality control purposes, an additional replicate and a spiked sample were included for each matrix. Quantification was reproducible and accurate with most of the relative percent deviations <10% and biases <20% in the duplicate and spiked real samples of the three matrixes, respectively. All of the alkylphenolic substances and BPA were detected in all of the samples (Table 7). NP was the most abundant (1026 ± 326 ng/L, 817 ± 591 ng/g w.w., and 238 ± 39.9 ng/g w.w. in water, sediment, and fish, respectively), followed by BPA in water (808 ± 737 ng/L), OP and NP_1EO in sediment (287 ± 273 ng/g w.w. and 161 ± 124 ng/g w.w, respectively) and NP_1EO in fish (59.0 ± 21.3 ng/g w.w.). The NP concentrations in river water were higher than most reports in North America, similar to that in some European countries and much lower than that in China; however, the BPA concentrations in river water was higher than that in Europe [68,69]. The concentrations of NP and NPEOs in sediment were within the ranges reported from USA, Canada, Japan and Korea [14,69]. Among the steroid estrogens, only low levels of E_1 was found in water and sediment (<LOD-13.2 ng/L and <LOD-1.8 ng/g w.w., respectively). Sub- to low-ng/g w.w. of E_1, E_2 and E_3 was found in fish.

4. Conclusions

An accurate and reproducible method (quantitative biases and RSDs smaller than 20%) was developed and validated for the determination of alkylphenolic substances, steroid estrogens and BPA in environmental water, sediment, and tissue. This method can save time, solvent and labor. The UHPLC shortened the chromatographic time to less than 10 min, and UHPLC/MS/MS on ESI provided IDLs at sub- to few pg levels for most analytes. APPI was suitable for ionizing phenols and estrogens and offered 1.0–4.4 times better signal intensities than ESI. However, we chose ESI because it was applicable to all of the analytes. To our knowledge, this is the first study to apply a disk-type adsorbent on the analysis of NPEOs and NP_1EC. Disk-type automated SPE and MSPD significantly improved the throughput of analyte extraction. The automated SPE device prevented potential variations from manual operations. The MSPD extraction for 12 samples took only 3 h. 2-D LC could be used on sediment samples or for analyzing estrogens and BPA in tissue, though acidic alumina cleanup was essential for the analysis of alkylphenolic substances in tissue samples.

Acknowledgements

The authors would like to thank the financial support from the National Science Council, Taiwan (NSC 98-2314-B-002-143-MY3).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.12.020.

References

[1] P. Burkhardt-Holm, Int. J. Water Resour. D. 26 (2010) 477–493.
[2] G.T. Ankley, K.M. Jensen, M.D. Kahl, E.J. Durhan, E.A. Makynen, J.E. Cavallin, D. Martinovic, L.C. Wehmas, N.D. Mueller, D.L. Villeneuve, Aquat. Toxicol. 99 (2010) 389–396.
[3] M.S. Dinizi, R. Mauricio, M. Petrovic, M.J.L. De Alda, L. Amaral, I. Peres, D. Barcelo, F. Santana, J. Environ. Sci. 22 (2010) 1613–1622.
[4] A. Jespersen, T.H. Rasmussen, M. Hirche, K.J.K. Sorensen, B. Korsgaard, J. Exp. Zool. Part A 313A (2010) 399–409.
[5] A. Hatef, S.M.H. Alavi, Z. Linhartova, M. Rodina, T. Policar, O. Linhart, J. Appl. Ichthyol. 26 (2010) 696–701.
[6] M. Beklioglu, S.B. Akkas, H.E. Ozcan, G. Bezirci, I. Togan, Ecotoxicology 19 (2010) 901–910.
[7] A.M. Al-Ansari, A. Saleem, L.E. Kimpe, J.P. Sherry, M.E. McMaster, V.L. Trudeau, J.M. Blais, Environ. Pollut. 158 (2010) 2566–2571.
[8] M.A. Kelly, A.M. Reid, K.M. Quinn-Hosey, A.M. Fogarty, J.J. Roche, C.A. Brougham, Ecotox. Environ. Safe. 73 (2010) 1658–1665.
[9] H.D. Zhou, X. Huang, X.L. Wang, X.H. Zhi, C.D. Yang, X.H. Wen, Q.H. Wang, H. Tsuno, H. Tanaka, Environ. Monit. Assess. 161 (2010) 107–121.
[10] X.L. Li, T.G. Luan, Y. Liang, M.H. Wong, C.Y. Lan, J. Environ. Sci. 19 (2007) 657–662.
[11] G.L. Huang, S.G. Hou, L. Wang, H.W. Sun, Water Res. 41 (2007) 4630–4638.
[12] J. Oehlmann, M. Oetken, U. Schulte-Oehlmann, Workshop on Plastic World, Academic Press, Inc. Elsevier Science, Erice, Italy, 2006, pp. 140–149.
[13] T. Mayer, D. Bennie, F. Rosa, G. Rekas, V. Palabrica, J. Schachtschneider, Environ. Pollut. 147 (2007) 683–690.
[14] M. Petrovic, E. Eljarrat, M.J.L. de Alda, D. Barcelo, Anal. Bioanal. Chem. 378 (2004) 549–562.
[15] R. Loos, G. Hanke, G. Umlauf, S.J. Eisenreich, Chemosphere 66 (2007) 690–699.
[16] C. Ribeiro, M.E. Tiritan, E. Rocha, M.J. Rocha, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 2729–2746.
[17] T.C. Chen, M.F. Shue, Y.L. Yeh, T.J. Kao, Environ. Monit. Assess. 161 (2010) 135–145.
[18] J.E. Loyo-Rosales, C.P. Rice, A. Torrents, Chemosphere 68 (2007) 2118–2127.
[19] C.Y. Cheng, L.L. Liu, W.H. Ding, Chemosphere 65 (2006) 2152–2159.
[20] S. Hong, E.J. Won, H.J. Ju, M.S. Kim, K.H. Shin, Mar. Pollut. Bull. 60 (2010) 308–313.
[21] W. Korner, U. Bolz, W. Sussmuth, G. Hiller, W. Schuller, V. Hanf, H. Hagenmaier, Chemosphere 40 (2000) 1131–1142.
[22] K.M. Lai, M.D. Scrimshaw, J.N. Lester, Crit. Rev. Toxicol. 32 (2002) 113–132.
[23] G.G. Ying, R.S. Kookana, Y.J. Ru, Environ. Int. 28 (2002) 545–551.
[24] V. Kumar, N. Nakada, N. Yamashita, A.C. Johnson, H. Tanaka, Environ. Pollut. 159 (2011) 2906–2912.
[25] A.C. Johnson, Environ. Sci. Technol. 44 (2010) 7865–7870.
[26] T.S. Chen, T.C. Chen, K.J.C. Yeh, H.R. Chao, E.T. Liaw, C.Y. Hsieh, K.C. Chen, L.T. Hsieh, Y.L. Yeh, Sci. Total Environ. 408 (2010) 3223–3230.
[27] V. Pacakova, L. Loukotkova, Z. Bosakova, K. Stulik, J. Sep. Sci. 32 (2009) 867–882.
[28] C.Y. Chen, T.Y. Wen, G.S. Wang, H.W. Cheng, Y.H. Lin, G.W. Lien, Sci. Total Environ. 378 (2007) 352–365.
[29] G.W. Lien, C.Y. Chen, G.S. Wang, J. Chromatogr. A 1216 (2009) 956–966.
[30] M.D. Vincent, J. Sneddon, Microchem. J. 92 (2009) 112–118.
[31] M. Petrovic, D. Barcelo, Chromatographia 56 (2002) 535–544.
[32] M. Zhao, F. van der Wielen, P. de Voogt, J. Chromatogr. A 837 (1999) 129–138.
[33] J. Rubert, C. Soler, J. Manes, Talanta 82 (2010) 567–574.
[34] B. Shao, H. Han, X.M. Tu, L. Huang, J. Chromatogr. B 850 (2007) 412–416.
[35] K. Stanley, S.M. Simonich, D. Bradford, C. Davidson, N. Tallent-Halsell, Environ. Toxicol. Chem. 28 (2009) 2038–2043.
[36] M. Garcia-Lopez, P. Canosa, I. Rodriguez, Anal. Bioanal. Chem. 391 (2008) 963–974.
[37] M.J.L. de Alda, S. Diaz-Cruz, M. Petrovic, D. Barcelo, J. Chromatogr. A 1000 (2003) 503–526.
[38] R.M. Garcinuno, L. Ramos, P. Fernandez-Hernando, C. Camara, J. Chromatogr. A 1041 (2004) 35–41.
[39] A. Arditsoglou, D. Voutsa, Environ. Sci. Pollut. R 15 (2008) 228–236.
[40] P. Navarro, J. Bustamante, A. Vallejo, A. Prieto, A. Usobiaga, S. Arrasate, E. Anakabe, E. Puy-Azurmendi, O. Zuloaga, J. Chromatogr. A 1217 (2010) 5890–5895.
[41] W.M. Mullett, J. Biochem. Biophys. Methods 70 (2007) 263–273.
[42] P.L. Kole, G. Venkatesh, J. Kotecha, R. Sheshala, Biomed. Chromatogr. 25 (2011) 199–217.
[43] E. Rodriguez-Gonzalo, D. Garcia-Gomez, R. Carabias-Martinez, Anal. Bioanal. Chem. 398 (2010) 1239–1247.
[44] R.N. Rao, D.D. Shinde, J. Pharmaceut. Biomed. 50 (2009) 994–999.
[45] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Mass Spectrom. Rev. 29 (2010) 776–805.
[46] L. Kortz, C. Helmschrodt, U. Ceglarek, Anal. Bioanal. Chem. 399 (2011) 2635–2644.
[47] K.M. Kalili, A. de Villiers, J. Sep. Sci. 34 (2011) 854–876.
[48] L. Novakova, H. Vlckova, Anal. Chim. Acta 656 (2009) 8–35.
[49] A. Navarro, R. Tauler, S. Lacorte, D. Barcelo, J. Hydrol. 383 (2010) 18–29.
[50] A.L. Filby, J.A. Shears, B.E. Drage, J.H. Churchley, C.R. Tyler, Environ. Sci. Technol. 44 (2010) 4348–4354.
[51] S. Kollipara, G. Bende, N. Agarwal, B. Varshney, J. Paliwal, Chromatographia 73 (2011) 201–217.
[52] S. Walorczyk, D. Drozdzynski, B. Gnusowski, Talanta 85 (2011) 1856–1870.
[53] K. Zhang, J.W. Wong, P. Yang, K. Tech, A.L. DiBenedetto, N.S. Lee, D.G. Hayward, C.M. Makovi, A.J. Krynitsky, K. Banerjee, L. Jao, S. Dasgupta, M.S. Smoker, R. Simonds, A. Schreiber, J. Agric. Food Chem. 59 (2011) 7636–7646.
[54] F. Hernandez, M. Ibanez, J.V. Sancho, O.J. Pozo, Anal. Chem. 76 (2004) 4349–4357.
[55] Y.C. Chang, W.L. Chen, F.Y. Bai, P.C. Chen, G.S. Wang, C.Y. Chen, Anal. Bioanal. Chem. (in press), doi:10.1007/s00216-011-5519-9.
[56] R. Loos, J. Wollgast, T. Huber, G. Hanke, Anal. Bioanal. Chem. 387 (2007) 1469–1478.
[57] J.E. Loyo-Rosales, I. Schmitz-Afonso, C.P. Rice, A. Torrents, Anal. Chem. 75 (2003) 4811–4817.
[58] I. Schmitz-Afonso, J.E. Loyo-Rosales, M.D. Aviles, B.A. Rattner, C.P. Rice, J. Chromatogr. A 1010 (2003) 25–35.
[59] S.J. Bos, S.M. van Leeuwen, U. Karst, Anal. Bioanal. Chem. 384 (2006) 85–99.
[60] B. Shao, H. Han, J.Y. Hu, J. Zhao, G.H. Wu, Y. Xue, Y.L. Ma, S.J. Zhang, Anal. Chim. Acta 530 (2005) 245–252.
[61] M. Di Carro, C. Scapolla, C. Liscio, E. Magi, Anal. Bioanal. Chem. 398 (2010) 1025–1034.
[62] E. Smith, I. Ridgway, M. Coffey, J. Environ. Monit. 3 (2001) 616–620.
[63] L.L. Sibali, J.O. Okwonkwo, R.I. McCrindle, Water SA 36 (2010) 229–238.
[64] T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, J. Hazard. Mater. 183 (2010) 701–711.
[65] F. Chen, G.G. Ying, J.F. Yang, J.L. Zhao, L. Wang, J. Environ. Sci. Heal. Part B 45 (2010) 682–693.
[66] B.J. Robinson, J.P.M. Hui, E.C. Soo, J. Hellou, Environ. Toxicol. Chem. 28 (2009) 18–25.
[67] R. Brix, C. Postigo, S. Gonzalez, M. Villagrasa, A. Navarro, M. Kuster, M.J.L. de Alda, D. Barcelo, Anal. Bioanal. Chem. 396 (2010) 1301–1309.
[68] V.K. Sharma, G.A.K. Anquandah, R.A. Yngard, H. Kim, J. Fekete, K. Bouzek, A.K. Ray, D. Golovko, J. Environ. Sci. Heal. A 44 (2009) 423–442.
[69] A. Soares, B. Guieysse, B. Jefferson, E. Cartmell, J.N. Lester, Environ. Int. 34 (2008) 1033–1049.